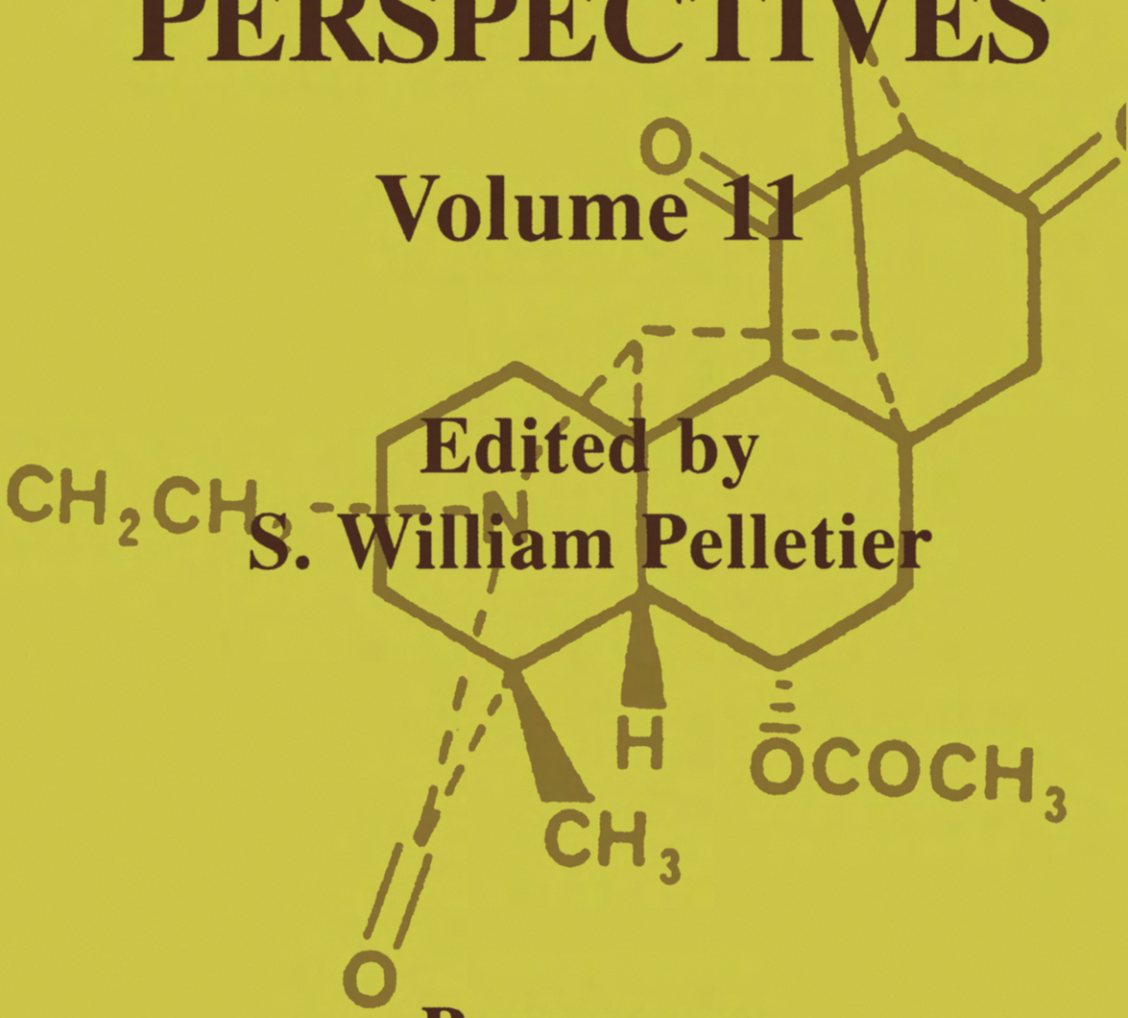
ALKALOIDS: CHEMICAL & BIOLOGICAL PERSPECTIVES

Volume 11



Edited by
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Alkaloids: Chemical and Biological Perspectives

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ALKALOIDS: CHEMICAL AND BIOLOGICAL PERSPECTIVES

Volume Eleven

Edited by
S. WILLIAM PELLETIER
Institute for Natural Products Research

and

Department of Chemistry
The University of Georgia, Athens

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Dedicated to
the memory of

Léo Edmond Marion
(1899 – 1979)

Leo Marion, starting from humble beginnings, showed rare ability, courage and determination in obtaining a Ph.D. in organic chemistry. His illustrious natural products career really began with a year of research with the renowned Ernst Späth in Vienna, followed by fruitful collaboration with R.H.F. Manske at the National Research Council, Ottawa. His major research on identification, structure and stereochemistry of the alkaloids of Fumariaceae, Papilionaceae, Lycopodiaceae and Ranunculaceae won him international recognition. But his most pioneering work was on alkaloid biosynthesis, begun in 1948 with S. Kirkwood. This research led to flourishing schools in that discipline (Leete, Spenser). His initiative in recruiting and encouraging M. Pryzybylska gave impetus to the use of X-ray crystallography in determining the structure and stereochemistry of complex alkaloids.

Leo Marion's role as author (in *The Alkaloids*, reviews) was overshadowed by his vital role as editor-in-chief in reorganizing and establishing high standards for the Canadian Journals of Research. This, his functions as administrator at the National Research Council, as a teacher in helping establish chemistry departments at the University of Montreal and Carleton University, and finally as Dean of Science at the University of Ottawa, crowned a most distinguished career.

O. E. Edwards

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Preface

Volume 11 of this series presents five timely reviews on current research on alkaloids. Chapter 1 by Paul L. Schiff, Jr. is a monumental survey of research that has been carried out over the past decade on the *Thalictrum* alkaloids. Forty-six new alkaloids are described from fifteen species of the genus *Thalictrum*, as well as 116 alkaloids of known structure from thirty-six species and subspecies of this genus. The chapter includes discussions of isolation and structure elucidation, analysis, biosynthesis, cell culture, and pharmacology. Also featured are inclusive compilations of botanical sources, alkaloids by alkaloid types, and calculated molecular weights of the *Thalictrum* Alkaloids.

Chapter 2 by Giovanni Appendino provides a fascinating treatment of Taxine, a collective name referring to a mixture of diterpenoid alkaloids from the yew tree (genus: *Taxus*). Taxine is responsible for the toxic properties of the yew tree that has been documented in historical and fictional literature, from Julius Caesar to Shakespeare, and from Agatha Christie to T. S. Eliot. The chapter treats the history, isolation techniques, structure elucidation, chemistry, and pharmacology of Taxine.

Chapter 3 by Mary D. Menachery surveys the alkaloids of South American Menispermaceae (moonseed family). Many different structural types are included in this family. The alkaloid-bearing plants are woody-vines, shrubs, or small trees. Several of these species possess potent curare activity. The chemistry as well as pharmacology of these alkaloids is summarized.

Chapter 4 by Russell J. Molyneux, Robert J. Nash, and Naoki Asano treats the chemistry and biological activity of the calystegines and related *nor*tropane alkaloids. These polyhydroxylated bicyclic alkaloids represent another class of compounds that inhibit glycosidases, producing profound effects in biological systems by disrupting the essential cellular function of glycoprotein processing.

Chapter 5, a related chapter by Robert J. Nash, Naoki Asano, and Alison A. Watson, reviews polyhydroxylated alkaloids that inhibit glycosidases. Topics covered include distribution, ecological significance and toxicity, isolation, synthesis, and biosynthesis.

Each chapter in this volume has been reviewed by at least one expert in the field. The editor thanks these reviewers for the important contributions they have made to this volume. Indexes for both subjects and organisms are provided.

The editor invites prospective contributions to write him about topics for review in future volumes of this series.

S. William Pelletier
Athens, Georgia
June 13, 1996

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The *Thalictrum* Alkaloids: Chemistry and Pharmacology (1985-1995)

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Pittsburgh, Pennsylvania 15261

CONTENTS

1. INTRODUCTION*

In the last decade, no less than forty-six new alkaloids have been isolated and identified from some fifteen species of the genus *Thalictrum*. In addition, another 116 alkaloids of known structure have been reisolated from some thirty-six different species and/or subspecies of this same genus. Many of these alkaloids have interesting and important pharmacological properties, and a plethora of studies involving the biological evaluation of these compounds have been undertaken and published. The purpose of this work is to review the research that has been published in the last decade. This review begins in about 1985, at about the time in which the material in the previously published chapter [1] was concluded.

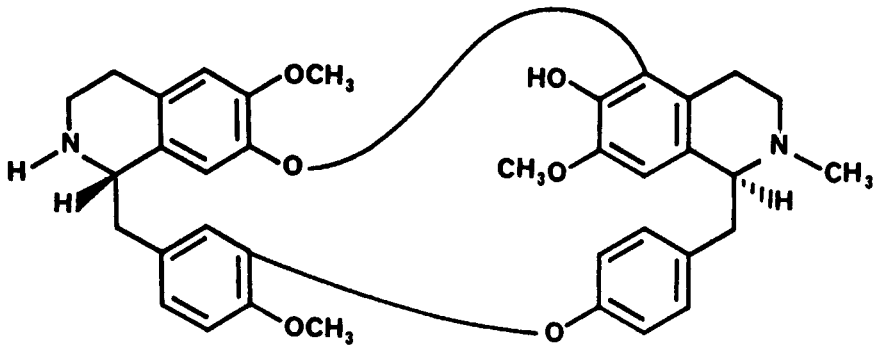
* This chapter is dedicated to two very special people. First, to my dear wife Denise, whose love as wife, mother, and grandmother permeates all those around her; and second, to Professor Raymond W. Doskotch, whose dedication to and love of science has so greatly enriched my life.

2. BOOK CHAPTERS AND REVIEWS

The following are book chapters or reviews that focus on the subject of *Thalictrum* alkaloids, and have been published within the last decade.

2.1. The Alkaloids of *Thalictrum cultratum* Wall. (1986) [2-4]

This is a review summarizing the isolation and identification of the alkaloids of *Thalictrum cultratum* Wall. The plant was collected in Azad Kashmir, Pakistan, and extracts of the whole plant proved to be a uniquely prolific source of benzylisoquinoline-derived alkaloids, including monomers of the aporphine, pavine, phenanthrene, and protoberberine groups, and dimers of the bisbenzylisoquinoline, secobisbenzylisoquinoline, and aporphine-benzylisoquinoline groups. Alkaloids of previously established structures that were isolated include the aporphine (+)-thalicsimidine, the benzylisoquinoline (+)-reticuline, the pavine (-)-thalisopavine, the phenanthrenes thaliglucine and thalflavidine, the protoberberine berberine, and the secobisbenzylisoquinoline (-)-revolutinone. Eighteen bisbenzylisoquinoline alkaloids were isolated, with these compounds belonging to the thalisopine, thalicberine, thalidasine, and thalmine series [3]. Bisbenzylisoquinoline alkaloids of previously established structures that were isolated from these extracts include (-)-thalisopine [(-)-thaligosine], (-)-thalrugosaminine, (+)-*O*-methylthalicberine, (+)-thaliphylline, (-)-thalmine, (-)-*O*-methylthalmine, (-)-thalictine, (-)-thalidasine, (-)-2-northalidasine, (-)-thalrugosidine, and (-)-thalirugosinone. Five of the new bisbenzylisoquinoline alkaloids were members of the thalmine-series including (-)-2-northalmine (**1**), (-)-5-hydroxythalmine (**2**), (-)-thalmiculine (**3**), (-)-thalmiculimine (**4**), and (+)-thalmiculatimine (**5**), while the remaining two new bisbenzylisoquinolines belonged to the thalidasine and thalicberine families, and included (-)-5-hydroxythalidasine (**6**), and (+)-thasivasine (**7**), respectively. Twelve alkaloids of the aporphine-benzylisoquinoline group were isolated, with six being of known structures [4]. These included four bases of the thalicarpine-type, namely (+)-adiantifoline, (+)-thalmelatidine, (+)-thalmineline, and (+)-thalilutine, and one alkaloid of the thalifaberine-type, (+)-thalifaberine. In addition, although (+)-thalifaroline was designated as a new alkaloid, it was later found to have been isolated and described two years earlier from extracts of *Thalictrum faberi* Ulbr., at which time it had been named thalifarapine [128]. Five new alkaloids of the thalifaberine-type were isolated and characterized, and include (+)-thalifaricine (**8**), (+)-thalifaramine (**9**), (+)-thalifarazine (**10**), (+)-thalifaretine (**11**), and (+)-thalifaronine (**12**). The final new alkaloid of the aporphine-benzylisoquinoline dimeric group that was isolated was (+)-thalibulamine (**13**), a member of the istanbulamine series of alkaloids.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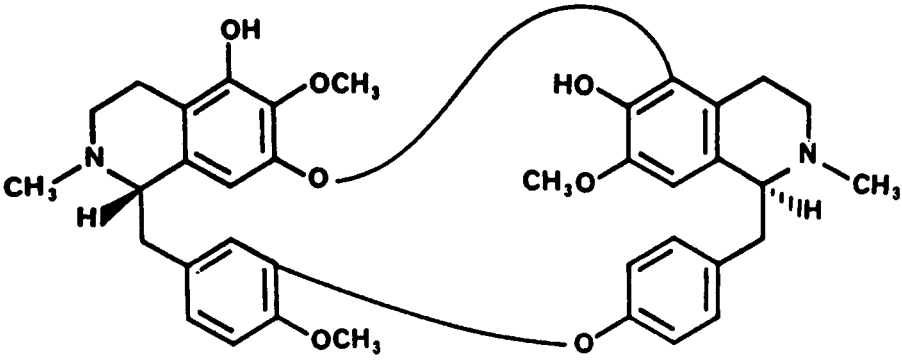
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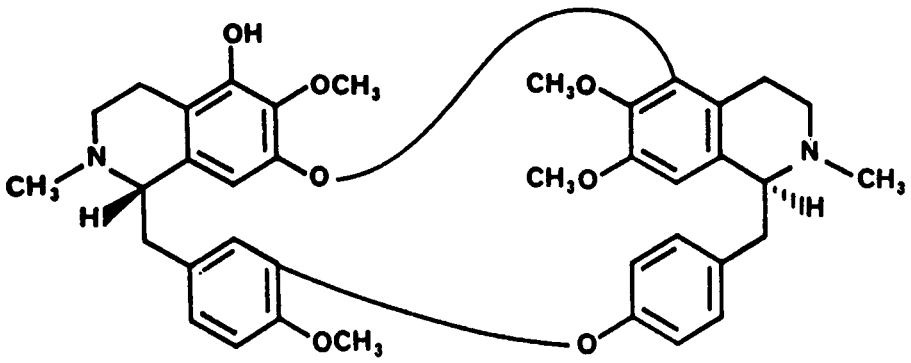
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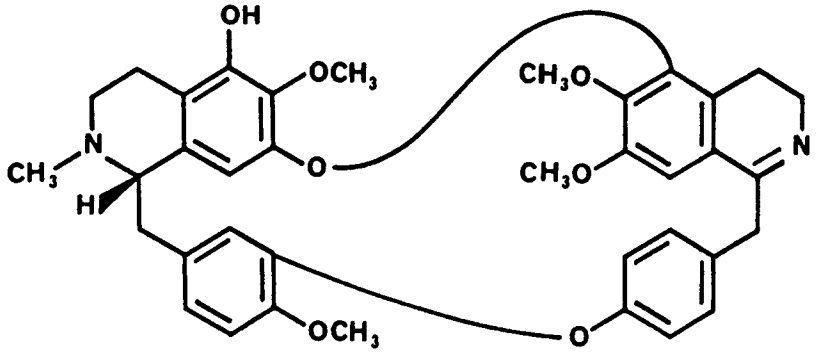
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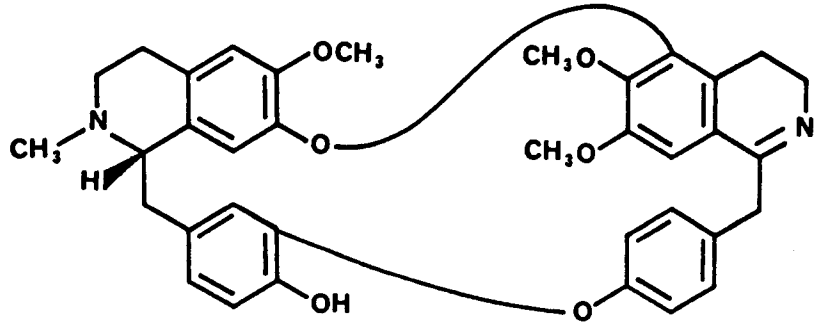
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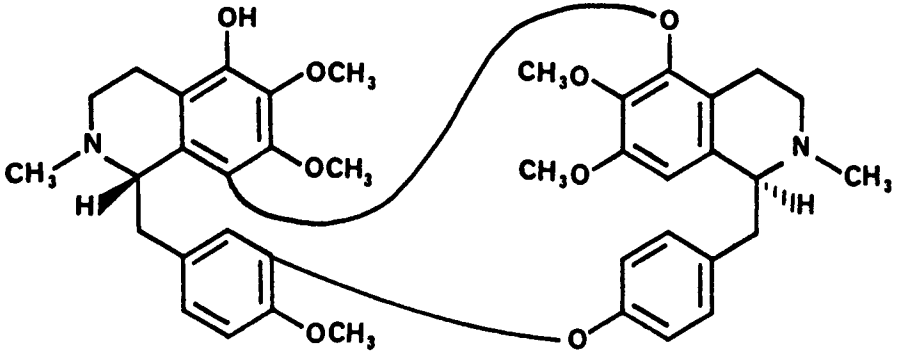
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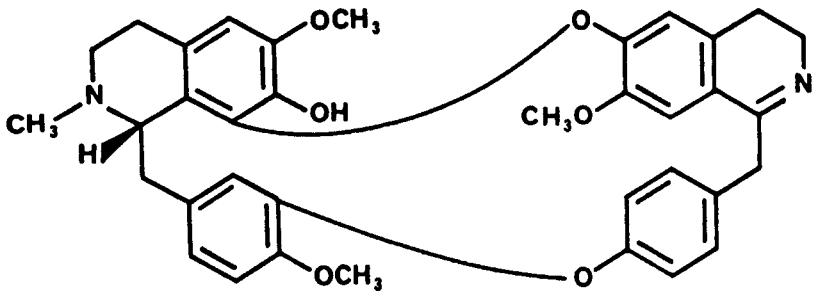

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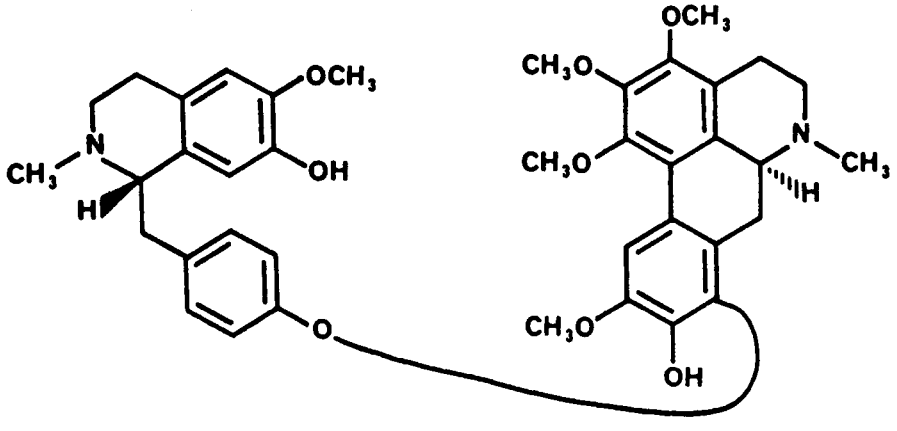

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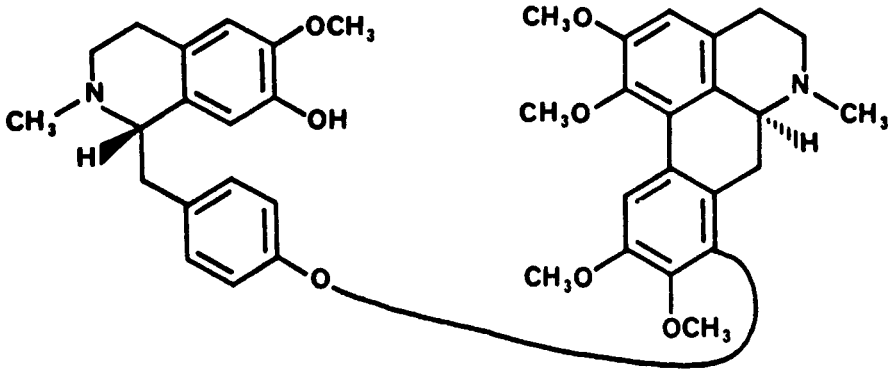

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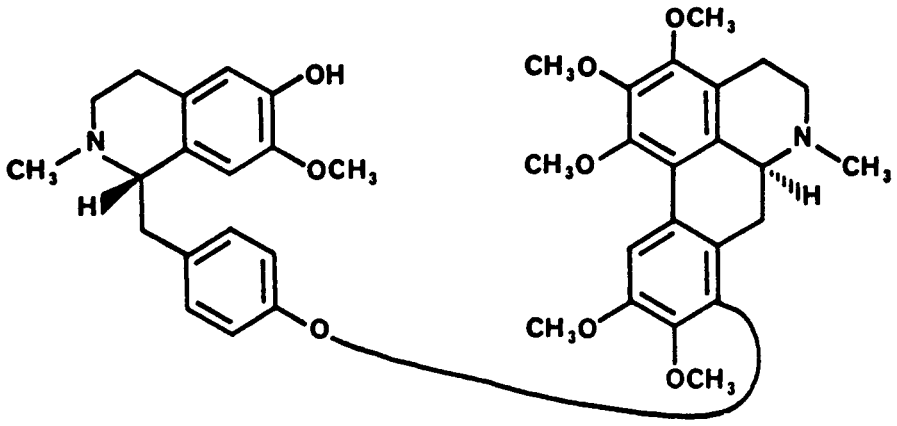
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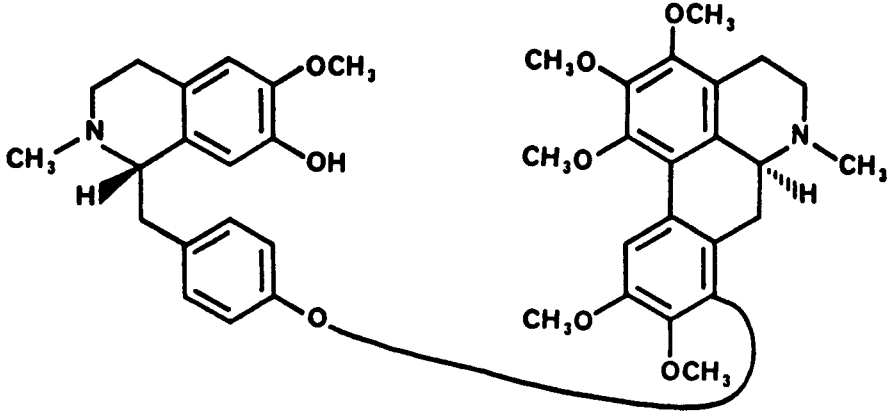
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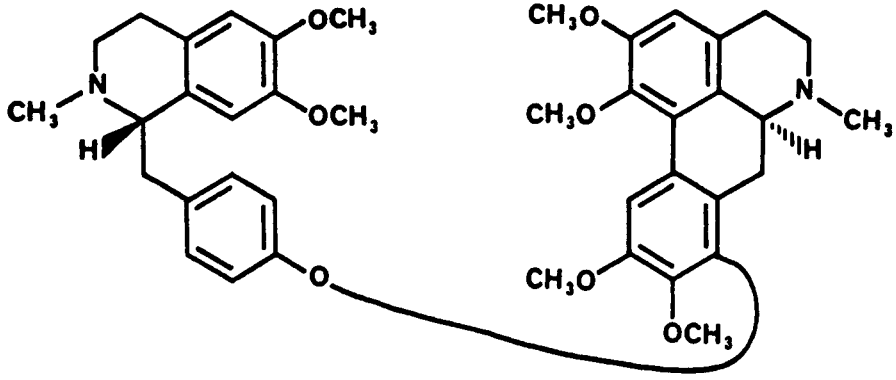
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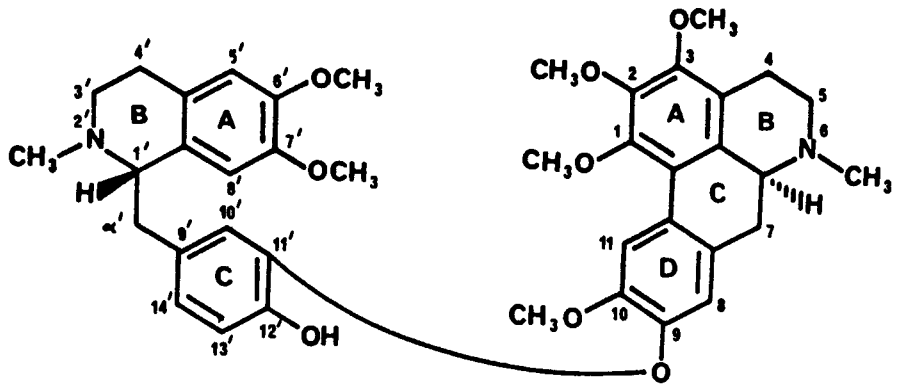

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2.2. The Alkaloids of Anatolian *Thalictrum* Species (1986)[5]

This review describes the alkaloids that have been isolated from five Turkish Anatolian taxa of *Thalictrum*, including *T. aquilegifolium*, *T. sultanabadense*, *T. minus* var. *majus*, *T. minus* var. *minus*, and *T. minus* var. *microphyllum*. In the decade of the 1980's, forty isoquinoline-derived alkaloids have been isolated from these plants, including ten new alkaloids. Nine species of *Thalictrum* have been described as being found in Turkey, including *T. aquilegifolium* L., *T. flavum* L., *T. foetidum* L., *T. isopyroides* C.A.Meyer, *T. lucidum* L., *T. minus* L. var. *majus* (Crantz) Crepin, *T. minus* L. var. *minus* L., *T. minus* L. var. *microphyllum* Boiss., *T. orientale* Boiss., *T. simplex* L., and *T. sultanabadense* Stapf. The three varieties of *T. minus* resemble each other greatly, and are only distinguished morphologically by the size of the terminal leaflets and the manner of branching.

Thalictrum minus var. *microphyllum* Boiss. is a plant of the Central Anatolian plateau, and is distinguished from the other two varieties of *T. minus* by its smaller leaflets. The alkaloids of the roots and rhizomes were isolated from an ethanolic extract via classic partitioning procedures, while the alkaloids of the leaves were isolated from the Mayer's Reagent alkaloid complex precipitate via ion-exchange procedures, followed by acid-base partitioning. Twenty-eight alkaloids were isolated from this plant, including twenty-four alkaloids from the roots and rhizomes, and six alkaloids from the leaves. Alkaloids that were isolated from extracts of the leaves included the monomers thalactamine (isoquinoline), takatonine and thalmicrinone (benzylisoquinolines), berberine (protoberberine), magnoflorine (aporphine), and the dimeric bisbenzylisoquinoline thalmethine. The predominance of monomers (5) to dimers (1) in the leaves was noted. As to extracts of the roots and rhizomes, the following monomers were isolated: berberine, jatrorrhizine, and palmatine (protoberberines); thaliglucinone (phenanthrene);

and magnoflorine (aporphine). The dimers obtained from extracts of the roots and rhizomes clearly outnumbered the monomers and included eleven bisbenzylisoquinolines and eight aporphine-benzylisoquinolines as follows: bisbenzylisoquinolines - aromoline, homoaromoline, obaberine, obamegine, *O*-methylthalicberine, thalicberine, thaligosine, thaligrisine, thaliphylline, thalirugine, and thalirugosine; aporphine benzylisoquinolines - adiantifoline, thaliadanine,thalmelatidine, bursanine, istanbulamine, iznikine, uskudaramine, and *N*(2')-noradiantifoline. Novel alkaloids isolated from these extracts include the benzylisoquinoline thalmicrinone, the bisbenzylisoquinolines thaligrisine and thaliphylline, and the aporphine-benzylisoquinolines bursanine, istanbulamine, iznikine, uskudaramine, and *N*(2')-noradiantifoline. Berberine and magnoflorine are the only alkaloids isolated from both the underground and aboveground parts of the plant. Administration of an ethanolic extract of the roots and rhizomes to anesthetized Wistar rats in an intravenous dosage of 30 mg/kg produced a decrease in both systolic and diastolic blood pressure, with systolic pressure responses generally recovering more than diastolic pressure increases over the hour following administration. These effects were postulated to be due to either an inhibitory effect on the responses of the sympathetic branch of the autonomic nervous system, or a direct relaxant effect on vascular smooth muscle.

Extraction of the roots and rhizomes of *T. minus* var. *minus* L. with EtOH and workup in the classical manner afforded three aporphine-benzylisoquinoline dimeric alkaloids including thalmelatidine (major alkaloid), thalmineline, and adiantifoline, as well as the monomeric alkaloids thaliglucinone, berberine and magnoflorine. Extraction of the leaves using ion-exchange procedures, followed by workup afforded the bisbenzylisoquinolines thalicberine, *O*-methylthalicberine, thaliphylline, thalmethine, *O*-methylthalmethine, thalivarmine and thalsivasine, the latter two being new compounds. In addition, the aporphine-benzylisoquinoline dimer thalmelatidine was isolated. (-)-*N*-methylcanadine (*N*-methyltetrahydroberberine) was determined to be the major alkaloid of the leaves.

Only three alkaloids have been isolated from extracts of *T. minus* var. *majus* (Crantz) Crepin including the bisbenzylisoquinolines obaberine (roots and rhizomes) and thalicberine (leaves), and the aporphine-benzylisoquinoline thalmelatidine (roots and rhizomes).

T. sultanabadense Stapf., a small plant that grows under the shade of rocks on the rocky mountain slopes of eastern Turkey, is a source of the dimeric bisbenzylisoquinoline alkaloids thalbadensine, hernandezine, and thalictine, and the monomeric alkaloids thalifoline (isoquinolone), berberine (protoberberine), and magnoflorine (aporphine).

T. aquilegifolium L., a species that grows in western Anatolia, is not a rich source of alkaloids, with only the monomeric aporphines magnoflorine, isoboldine, and isocorydine having been isolated.

2.3. Dimeric Isoquinoline Alkaloids from *Thalictrum minus* Populations of Southern Bulgaria (1986)[6]

This study reports the chromosome numbers and the dimeric alkaloids present in the flowering aerial parts of five additional Bulgarian populations of *T. minus* and attempts to interpret these facts in the light of biosystematics in the aggregate species. Within the hexaploid types bisbenzylisoquinoline alkaloids are predominantly produced, but aporphine-benzylisoquinolines are the major alkaloids generated by the decaploid types. Thalmethine, *O*-methylthalmethine, and oxyacanthine are found in the hexaploids, while within the decaploid cytotype two chemogeographic races (race A and race B) were distinguished according to the presence of aporphine-benzyisoquinoline alkaloids. Three populations from southern Bulgaria were classified as race A, on the basis of their tendency to produce thalmelatine and thalipine, aporphine-benzylisoquinoline dimers with one and two phenolic hydroxy groups, respectively. Race B populations, situated in the Eastern Stara Planina Mountains, principally contain the fully methylated thalicarpine, with a lesser amount of the monophenolic thalmelatine.

Some twenty-seven populations of Bulgarian *T. minus* have been studied for their chromosome number, and dimeric alkaloids have been detected in thirteen of them. It was noted that the hexaploid cytotype is more commonly found in the lowlands, while the decaploids are more typical to the mountains above 1500 meters. In addition, bisbenzylisoquinolines are found only in the hexaploids, while aporphine-benzylisoquinolines occur only in the decaploids. It may be assumed from this research that up to two cytotypes and two chemotypes have been detected in Bulgaria that show an attitudinal pattern of distribution.

2.4. The *Thalictrum* Alkaloids: Chemistry and Pharmacology (1987) [1]

This is a book chapter that embodies 669 references.

2.5. The Alkaloids of Tangsongcao (*Thalictrum* genus) (1988) [7]

This is a review published in Chinese that cites eighty-eight references.

2.6. Secretion of Secondary Products by Plant Cell Cultures (1988) [8]

This is a review containing fifty-three references on alkaloid secretion by plant cells, featuring the author's research on the secretion of berberine by cell cultures of *Thalictrum minus*.

2.7. Alkaloids of Wild-Growing Plants. II. *Thalictrum minus* L. (1991) [9]

This is a review published in Serbo-Croatian that contains ninety-two references.

2.8. Chemical and Cytological Investigation of *Thalictrum minus* from the Vojvodina Region of the former Yugoslavia (1992)[10]

It has been previously demonstrated that morphologically identical populations of *T. minus* may differ in the composition of their contained alkaloids. In addition, cytological research has demonstrated that *T. minus* may be diploid, hexaploid, or polyploid. It was assumed that a well-defined correlation existed between levels of polyploidy and the nature of the dimeric alkaloids that were produced by a given plant population, hence the types of dimeric alkaloids were taken as evolutionary markers within this aggregate species [11]. Earlier studies of *T. minus* from several localities within the Vojvodina region of Yugoslavia revealed that all of the plants were hexaploids and did not contain dimeric alkaloids of the aporphine-benzylisoquinoline group [12,13]. In a more recent study, the presence of dimers of the bisbenzylisoquinoline type (thalmethine, *O*-methylthalmethine, thalicberine, and *O*-methylthalicberine) and monomers of the isoquinoline type (thaliglucine, thaliporphine, thalactamine, thalflavine, and berberine) were found in extracts of the above ground portions of *T. minus* L. collected at two separate localities in Vojvodina. The populations of this species were divided into two chemotypes (one from the region of Fruska Gora and one from the region of Beocin) on the basis of the structure of the major alkaloids, and the plants were determined to be hexaploids. Specifically, thalmethine, *O*-methylthalmethine, thalicberine, *O*-methylthalicberine, thaliglucine, thaliporphine, and berberine were isolated from the plant obtained in the region of Fruska Gora, while the monomeric alkaloids thalactamine, thalflavine, and berberine were found in the plant obtained from the region of Beocin. The Beocin population of plants was found to be poor in alkaloids, likely a consequence of low concentrations of microelements and in the enzymic activators responsible for alkaloid biosynthesis [10].

3. DISSERTATIONS ABOUT THALICTRUM ALKALOIDS

3.1 Plant-Derived Alkaloids

3.1.1. Isolation and Identification and Structural Elucidation of Tertiary Alkaloids from the Root of *Thalictrum minus* L. Race C, by MOA ElSheikh, 223 pp., The Ohio State University, 1985 [14]

This dissertation describes the isolation and determination of structures of twenty-seven alkaloids from extracts of the roots of *Thalictrum minus* L. race C. Nine of these twenty-seven alkaloids were of novel structures including three aporphines (6a,7-dehydrothaliadine, 7'-dihydrodehydrothaliadine, and 7'-dihydrothaliadine), one isoquinolone (thalmirine), two oxoaporphines (7'-dihydrooxothaliadine, oxothaliadine), two aporphine-benzylisoquinoline dimers (6a,7-dehydroadiantifoline and 6-noradiantifoline), and one bisbenzylisoquinoline (squarosine). Alkaloids of previously established structure that were isolated and identified included three aporphines (delporphine, isoboldine, and thalisopynine); one benzylisoquinoline [(+)-laudanidine], one isoquinolone (*N*-methylcorydaldine), three aporphine-benzylisoquinoline dimers (adiantifoline, thalicarpine, and thalmineline), and seven bisbenzylisoquinolines (*O*-methylthalibrine, *O*-methylthalicberine, obaberine, thaliracebine, thalistine, thalmirabine, and thalrugosine). In addition, the structures of the aporphine-benzylisoquinoline dimer thalmelatidine and the bisbenzylisoquinolines thalfine and thalfinine were revised.

3.1.2. Chemical and Spectral Studies of *Thalictrum* Alkaloids, by SS Lee, 324 pp., The Ohio State University, 1985 [15]

This dissertation describes the study of the alkaloids of three species of *Thalictrum*, and resulted in the isolation and identification of thirty-seven alkaloids from these plants, including seventeen novel alkaloids.

Fourteen alkaloids were isolated from extracts of the whole plant of *Thalictrum fauriei* Hayata, among which were seven alkaloids of novel structure including three aporphines (3-*O*-demethyloconovine, faurine, and *O*-methylfaurine), three aporphine-benzylisoquinoline dimers (fauridine, faurithaline, and 3-methoxyfaurithaline), and one aporphine-pavine dimer (fauripavine). Alkaloids of previously established structure that were isolated and identified included five aporphines (corydine, isocorydine, isooconovine, oconovine, and thalisopynine), and two morphinans (ocobotrine and pallidine).

Seven alkaloids were isolated from extracts of the roots of *Thalictrum foetidum* L. including one new alkaloid, the bismorphinan bisocobotrine. Alkaloids of hitherto established structures that were isolated included one benzylisoquinoline (reticuline), two morphinans (ocobotrine and sinoacutine), and three bisbenzylisoquinolines (thalibrine, thalidasine, and thalirugosidine).

Sixteen alkaloids were isolated from extracts of the roots of *Thalictrum longistylum* DC among which were nine alkaloids of novel structure including four benzylisoquinoline-protoberberines (berberlongine, berberstyline, longiberine, and *O*-methyllongiberine), and five

bisbenzylisoquinolines (5-*O*-demethyllongine, 5'-hydroxylongine, 5'-hydroxythalidezine, longine, and *N*-norhernandezine). There were seven bisbenzylisoquinoline alkaloids of known structures that were isolated, including *N*-desmethylthalistyline, hernandezine, isothalidezine, *O*-methylthalibrine, *N*-northalidezine, thalibrine, and thalidezine. The most significant feature of this research was the discovery of a new class of alkaloids, the dimeric benzylisoquinoline-protoberberines, hitherto unknown in nature.

3.1.3. The Isolation and Identification of Alkaloids from Jordanian *Thalictrum isopyroides* C.A.M. (Ranunculaceae) - Part I. The Synthesis of Thalmicrinone, A Confirmation of Structure - Part II, by SMK Al-Khalil, 243 pp., University of Pittsburgh, 1986 [16]

This dissertation describes the isolation and identification of twelve alkaloids of known structures from an extract of the roots of the Jordanian *Thalictrum isopyroides* C.A.M. including seven aporphines (delporphine, isoboldine, magnoflorine, *N*-methylcassythine, *N*-methyllaurotetanine, preocoteine, and thaliporphine), one oxoaporphine (thalicminine), and two bisbenzylisoquinolines (thaligosinine and thalisopidine). In addition, the oxobenzylisoquinoline alkaloid thalmicrinone was synthesized via the use of a Reissert compound, similar to the method previously used in the synthesis of the oxobenzylisoquinoline alklaoid rugosinone. This synthesis constituted a confirmation of structure for the naturally occurring thalmicrinone. Two publications resulted from this research [17,18].

3.1.4. Isolation and Identification of Quaternary Alkaloids from the Roots of *Thalictrum cultratum* Wall. (Ranunculaceae) - Part I. Isolation and Identification of Alkaloids from the Roots and Stems of *Pycnarrhena manillensis* Vidal. (Menispermaceae) - Part II., by MC Lin, 263 pp., University of Pittsburgh, 1987 [19]

The first part of this dissertation documents the isolation and identification of seven quaternary alkaloids from extracts of the roots of *Thalictrum cultratum* Wall., including six protoberberines (berberine, columbamine, jatrorrhizine, palmatine, (+)-thalidastine, and thalifendine) and one aporphine (magnoflorine). This research resulted in one publication [20].

3.1.5. Alkaloids of *Thalictrum thalictroides*, by MS Hoard, 151 pp., University of Southern Mississippi, 1992 [21]

This dissertation describes a study of the alkaloidal constituents of *Thalictrum thalictroides*. Magnoflorine and either berberine or epiberberine were isolated and identified, as

well as sucrose and myo-inositol. A change in the crystal structure of magnoflorine was postulated to have occurred during grinding with potassium bromide, and on subsequent recrystallization from methanol.

3.2 Cell Culture-Derived Alkaloids

3.2.1. Process Strategies and Bioreactor Operation for Berberine Production in Cell Suspension Cultures of *Thalictrum rugosum*, by DI Kim, 169 pp., Rutgers, The State University, 1990 [22]

Seven publications resulted from this research dissertation [23-29].

3.2.2. Berberine Production in Suspended and Immobilized Cell Culture of *Thalictrum rugosum*: Permeabilization and *In Situ* Product Separation, by JW Choi, 256 pp., Rutgers, The State University, 1990 [30].

Two publications resulted from this research dissertation [31,32].

4. PATENTS

One patent was granted for extraction of alkaloids from *Thalictrum* species. Finely-powdered samples of *Thalictrum* species were first moistened with NH_4OH (10%), and then extracted with a nonpolar chlorinated organic solvent. The extracting solvent was treated with citric acid (0.1-0.5N), and the citric acid basified to pH 9-10 before extraction (2x) with chlorinated solvent. The solvent was removed via distillation, and the residue dissolved in a mixture of polar and nonpolar solvents to afford crystalline alkaloids, such as β-allocryptopine. The alkaloids have antitussive, antimicrobial, anti-inflammatory, anti-arrhythmic, and choleretic-cholagogue properties [33].

5. ALKALOIDS OF ESTABLISHED STRUCTURES THAT HAVE BEEN ISOLATED OR REISOLATED FROM THALICTRUM SPECIES (1985-1995)

The following abbreviations are used to indicate the plant part studied: ag = above-ground parts; fr = fruits; l = leaves; r = roots; rh = rhizomes; st = stems; wp = whole plant

5.1. Monomeric Alkaloids

5.1.1. Aporphines

Baicaline - *T. baicalense* Turcz. (st) [34]

Corydine - *T. fauriei* Hayata (wp) [15,35]

Delporphine - *T. isopyroides* C.A.M. (r) [17]; *T. minus* L. race C (r) [14]

Domesticine - *T. minus* L. var. *adiantifolium* Hort. (ag) [36]

Glaucine (*O*-Methylthalicmidine) - *T. baicalense* Turcz. (st) [34]; *T. collinum* Wallr. (ag,r) [37]; *T. flavum* L. (r) [38]; *T. ichengense* Lecoy et Oliv. (r) [39,40]; *T. longipedunculatum* E. Nikit. (r) [41]; *T. minus* L. [42]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]

Isoboldine (*N*-Methyllaurelliptine) - *T. aquilegifolium* L. (r) [43]; *T. collinum* Wallr. (ag) [37], (ag) [44]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]; *T. minus* L. race C (r) [14]

Isocorydine - *T. aquilegifolium* L. (r) [43]; *T. delavayi* Franch. (wp) [45]; *T. fauriei* Hayata (wp) [15,35]; *T. pedunculatum* Edgew. (wp) [46]

Isooconovine - *T. fauriei* Hayata (wp) [15]

Leucoxylonine - *T. delavayi* Franch. (wp) [45]; *T. simplex* L. (ag) [107]

Magnoflorine (Thalictrine) - *T. aquilegifolium* L. (r) [43]; *T. collinum* Wallr. (r) [37]; *T. cultratum* Wall. (r) [20]; *T. delavayi* Franch. (wp) [45]; *T. fauriei* Hayata (wp) [35]; *T. foetidum* L. (ag) [48], (r) [49]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [50]; *T. isopyroides* C.A.M. (r) [17]; *T. longipedunculatum* E. Nikit. (r) [41]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (ag,r) [51], (r) [52]; *T. minus* L. var. *minus* (r,rh) [53]; *T. sessile* Hayata (r) [54]; *T. sultanabadense* Stapf. (ag,r) [55]; *T. tuberiferum* Maximowicz [56]

N-Methylcassythine - *T. isopyroides* C.A.M. (r) [17]

O-Methylisoboldine - *T. minus* L. var. *adiantifolium* Hort. (ag) [36]

N-Methyllaurotetanine - *T. hazarica* R.R.S. (wp) [57]; *T. isopyroides* C.A.M. (r) [17]

Nantenine - *T. minus* L. var. *adiantifolium* Hort. (ag) [36]

Noroconovine - *T. pedunculatum* Edgew. (wp) [46]

Oconovine - *T. fauriei* Hayata (wp) [15,35]; *T. pedunculatum* Edgew. (wp) [46]

Ocoteine (Thalicmine, *N,O*-Dimethylcassyfiline) - *T. delavayi* Franch. (wp) [45]; *T. isopyroides* C.A.M. (r) [17]; *T. longipedunculatum* E. Nikit. (r) [41]

Preocoteine - *T. isopyroides* C.A.M. (r) [17]

Thaliadine (3-Methoxyhernandaline) - *T. minus L. ssp. majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [58,59]

Thalicsimidine (*O*-Methylpreocoteine, Purpureine) - *T. cultratum* Wall. (wp) [2]; *T. flavum* L. (r) [38]; *T. ichengense* Lecoy et Oliv. (r) [39,40]; *T. kuhistanicum* Ovcz. et Koczk. (ag) [60]; *T. pedunculatum* Edgew. (wp) [46]

Thaliporphine (Thalicmidine, *O*-Methylisoboldine) - *T. buschianum* (ag) [44]; *T. ichengense* Lecoy et Oliv. (r) [39,40]; *T. isopyroides* C.A.M. (r) [17]; *T. longipedunculatum* E. Nikit. (r) [41]; *T. minus* L. (ag) [10]

Thalisopynine (Thalisopinine) - *T. fauriei* Hayata (wp) [15]; *T. minus* L. race C (r) [14]

Thalphenine - *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [52]

Trilobinine - *T. acutifolium* (Hand.-Mazz.) Boivin (r) [61]

5.1.2. Dehydroaporphines

Dehydroglaucine - *T. ichengense* Lecoy et Oliv. (r) [39,40]

Dehydroocoteine (Dehydrothalicmine) - *T. isopyroides* C.A.M. (r) [17]

5.1.3. Proaporphines

Pronuciferine - *T. pedunculatum* Edgew. (wp) [46]

5.1.4. Oxoaporphines

Liriodenine - *T. sessile* Hayata (ag) [62]

Thalicminine - *T. isopyroides* C.A.M. (r) [17]

5.1.5. Benzylisoquinolines

Laudanidine - *T. minus* L. race C (r) [14]

Reticuline - *T. cultratum* Wall. (wp) [2]; *T. foetidum* L. (r) [15]; *T. pedunculatum* Edgew. (wp) [46]

Thalmeline - *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [59]

5.1.6. Oxobenzylisoquinolines

Rugosinone - *T. javanicum* Bl. (r) [63]

5.1.7. Isopavines

Thalisopavine - *T. cultratum* Wall. (wp) [2]

5.1.8. Isoquinolones

N-Methylcorydaldine - *T. minus* L. race C (r) [14]

Noroxyhydrastinine - *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [59]

Thalactamine - *T. foetidum* L. [64]; *T. minus* L. [65], (ag) [66], (ag) [10]

Thalflavine - *T. foetidum* L. (ag) [48]; *T. minus* L. [42], (ag) [66]; *T. minus* L. (ag) [10]

Thalifoline - *T. sultanabadense* Stapf. (r,ag) [55]

5.1.9. Morphinans

Ocobotrine - *T. fauriei* Hayata (wp) [15,35]; *T. foetidum* L. (r) [15]

Pallidine - *T. fauriei* Hayata (wp) [15]

Sinoacutine - *T. foetidum* L. (r) [15]

5.1.10.Pavines

Argemonine - *T. foetidum* L. (ag) [49]; *T. simplex* L. (ag) [67]

Argemonine-*N*-Oxide - *T. foetidum* L. [64]

Eschscholtzidine - *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [59]

Isonorargemonine - *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [59]

5.1.11.Phenanthrenes

Thalflavidine - *T. cultratum* Wall. (wp) [2]

Thalicthuberine - *T. delavayi* Franch. (wp) [45]; *T. hazarica* R.R.S. (wp) [57]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [58,59]

Thaliglucine - *T. cultratum* Wall. (wp) [2]; *T. flavum* L. (r) [38]; *T. minus* L. (ag) [10]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]

Thaliglucinone - *T. longipedunculatum* E. Nikit. (ag,r) [41]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]; *T. minus* L. var. *minus* (r,rh) [53]

5.1.12.Protoberberines

Berberine - *T. baicalense* Turcz. (st) [34]; *T. collinum* Wallr. (r) [37]; *T. cultratum* Wall. (r) [20], (wp) [2]; *T. delavayi* Franch. (r) [68]; *T. flavum* L. (ag) [69], (r) [38]; *T. foetidum* L. (r) [49,70,71], (ag) [48]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [50]; *T. honanense* W.T. Wang et S.H. Wang (r) [72]; *T. javanicum* Bl. (r) [63]; *T. lankesteri* Standl. (ag) [73]; *T. longipedunculatum* E. Nikit. (r) [41]; *T. minus* L. [42], (ag) [66], (ag) [10]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (ag,r) [51], (r) [52]; *T. minus* L. var. *minus* (r,rh) [53]; *T. purpurascens* DC (r) [74]; *T. sessile* Hayata (r) [54]; *T. sultanabadense* Stapf. (r) [55]

Berberrubine - *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [75]

Columbamine - *T. cultratum* Wall. (r) [20]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [50]; *T. lankesteri* Standl. (ag) [73]; *T. longipedunculatum* E. Nikit. (r) [41]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]

Coptisine - *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [50]

Dehydrodiscretine - *T. fauriei* Hayata (wp) [35]

Deoxythalidastine - *T. uchiyamai* Nakai (r) [76]

Groenlandicine - *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [50]

Jatrorrhizine - *T. cultratum* Wall. (r) [20]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [50]; *T. honanense* W.T. Wang et S.H. Wang (r) [72]; *T. lankesteri* Standl. (ag) [73]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [52]; *T. uchiyamai* Nakai (r) [76]

8-Oxocoptisine - *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [75]

Oxyberberine (Berlambine) - *T. acutifolium* (Hand.-Mazz.) Boivin (r) [61]; *T. foetidum* L. (r) [71]; *T. javanicum* Bl. (r) [63]

Palmatine - *T. cultratum* Wall. (r) [20]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [50]; *T. lankesteri* Standl. (ag) [73]; *T. minus* L. (ag) [48]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]

Thalidastine - *T. cultratum* Wall. (r) [20]

Thalifaurine - *T. fauriei* Hayata (wp) [35]

Thalifendine - *T. cultratum* Wall. (r) [20]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [50]; *T. honanense* W.T. Wang et S.H. Wang (r) [72]; *T. javanicum* Bl. (r) [63]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [52]; *T. uchiyamai* Nakai (r) [76]

5.1.13.Tetrahydroprotoberberines

N-Methylcanadine (*N*-Methyltetrahydroberberine) - *T. minus* L. (ag) [48]; *T. minus* var. *minus* L. (ag) [77]

5.1.14.Isohomoprotoberberines

Puntarenine - *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (rh,r) [75]

5.1.15.Protopines

Allocryptopine (Thalictrimine, β-Allocryptopine) - *T. minus* L. [42]

α-Allocryptopine - *T. minus* L. (ag) [48]

Cryptopine (Thalisopyrine) - *T. delavayi* Franch. (r) [68]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (r) [78], (rh,r) [75]

Izmirine - *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (r) [78]

Protopine - *T. foetidum* L. [64]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (r) [78], (rh,r) [75]

Protothalipine - *T. uchiyamai* Nakai (r) [76]

Pseudoprotopine - *T. delavayi* Franch. (r) [68]

5.1.16.Diterpenoids

Spiradine A - *T. sessile* Hayata (r) [54,79]

Spiredine - *T. sessile* Hayata (r) [54]

Spirasine I - *T. sessile* Hayata (r) [54]

Spirasine II - *T. sessile* Hayata (r) [54]

Spirasine III - *T. sessile* Hayata (r) [54]

5.1.17.Betaines

Choline - *T. minus* L. var. *adiantifolium* Hort. (ag) [36]

5.2. Dimeric Alkaloids

5.2.1. Aporphine-Benzylisoquinolines

Adiantifoline - *T. cultratum* Wall. (wp) [4]; *T. honanense* W.T. Wang et S.H. Wang (r) [72]; *T. minus* L. var. *adiantifolium* Hort. (ag) [36]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [51,59]; *T. minus* L. var. *minus* (r,rh) [53]; *T. minus* L. race C (r) [14]

O-Desmethyladiantifoline - *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [58,59]

Methoxyadiantifoline - *T. foetidum* L. (r) [71]

Thalicarpine - *T. minus* L. (ag) [6]; *T. minus* L. race C (r) [14]

Thalictrogamine - *T. foetidum* L. (ag) [49]

Thalifaberine - *T. cultratum* Wall. (wp) [4]

Thalifarapine (Thalifaroline) - *T. cultratum* Wall. (wp) [4]

Thalifarazine - *T. sessile* Hayata (ag) [62], (r) [54]

Thalilutine - *T. cultratum* Wall. (wp) [4]

Thalipine - *T. foetidum* L. (ag) [49]; *T. minus* L. (ag) [6]

Thalmelatidine - *T. cultratum* Wall. (wp) [4]; *T. honanense* W.T. Wang et S.H. Wang (r) [72]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [58,51,59]; *T. minus* L. var. *hypoleucum* [80]; *T. minus* var. *minus* L. (ag) [77], (r,rh) [53]; *T. minus* L. race C (r) [14]

Thalmelatine - *T. foetidum* L. (ag) [49]; *T. minus* L. (ag) [6]

Thalmineline - *T. cultratum* Wall. (wp) [4]; *T. minus* L. var. *minus* (r,rh) [53]; *T. minus* L. race C (r) [14]

5.2.2. Dehydroaporphine-Benzylisoquinolines

6a,7-Dehydromethoxyadiantifoline - *T. foetidum* L. (r) [71]

5.2.3. Bisbenzylisoquinolines

Aromoline (Thalicrine) - *T. cultratum* Wall. (wp) [81]; *T. fortunei* (wp) [82]

N-Desmethylthalistyline - *T. longistylum* DC (r) [15]

Hernandezine - *T. delavayi* Franch. (r) [68], (wp) [45]; *T. flavum* L. (ag) [69], (r) [38]; *T. foetidum* L. [64]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (r) [78], (rh,r) [75]; *T. lankesteri* Standl. (ag) [73]; *T. longistylum* DC (r) [15]; *T. sultanabadense* Stapf. (ag) [55]

Isothalidezine - *T. delavayi* Franch. (r) [68]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (r) [78]; *T. longistylum* DC (r) [15]

O-Methylthalibrine - *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (r) [78]; *T. longistylum* DC (r) [15]; *T. minus* L. race C (r) [14]

O-Methylthalicberine (Thalmidine) - *T. aquilegifolium* L. (wp) [329]; *T. buschianum* (ag) [44]; *T. collinum* Wallr. (ag,r) [37], (ag,r) [44]; *T. cultratum* Wall. (wp) [2,3]; *T. flavum* L. (ag) [69]; *T. foetidum* L. [64]; *T. kuhistanicum* Ovcz. et Koczk. (ag) [60]; *T. longipedunculatum* E. Nikit. (ag) [41]; *T. minus* L. (ag) [10,48], [42]; *T. minus* L. var. *hypoleucum* [80]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (ag) [51]; *T. minus* var. *minus* L. (ag) [77]; *T. minus* L. race C (r) [14]

O-Methylthalmethine - *T. minus* L. [42], (ag) [6,10]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (ag) [51]; *T. minus* var. *minus* L. (ag) [77]

O-Methylthalmine - *T. cultratum* Wall. (wp) [84]

Neothalibrine - *T. cultratum* Wall. (wp) [81]

2-Northalidasine - *T. cultratum* Wall. (wp) [2,84]

N-Northalidezine - *T. longistylum* DC (r) [15]

Obaberine - *T. cultratum* Wall. (wp) [81]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [51]; *T. minus* L. race C (r) [14]

Oxyacanthine - *T. cultratum* Wall. (wp) [81]; *T. minus* L. (ag) [6]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (r) [51]

Talcamine - *T. buschianum* (ag,r) [44]

Thalabadensine (Thalbadensine) - *T. sultanabadense* Stapf. (ag,r) [55]

Thalfine (Thalphine) - *T. minus* L. race C (r) [14]

Thalfinine (Thalphinine) - *T. minus* L. race C (r) [14]

Thalfoetidine - *T. fargesii* Fr. ex Fin. et Gagnep. (r) [85,86]; *T. flavum* L. (ag) [69], (r) [38]; *T. longipedunculatum* E. Nikit. (ag) [41]; *T. minus* L. [42]

Thalibrine - *T. foetidum* L. (r) [15]; *T. longistylum* DC (r) [15]

Thalicberine - *T. longipedunculatum* E. Nikit. (ag) [41]; *T. minus* L. (ag) [48], [42], (ag) [10]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (ag) [51]; *T. minus* var. *minus* L. (ag) [77]

Thalictine - *T. cultratum* Wall. (wp) [3]; *T. sultanabadense* Stapf. (r) [87], (r,ag) [55]

Thalidasine - *T. cultratum* Wall. (wp) [84]; *T. fargesii* Fr. ex Fin. et Gagnep. (r) [85,86]; *T. flavum* L. (ag) [69]; *T. foetidum* L. (r) [15,49]; *T. longipedunculatum* E. Nikit. (ag) [41]; *T. squarrosum* (r) [88,89]

Thalidezine - *T. delavayi* Franch. (r) [68]; *T. foetidum* L. [64]; *T. glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang (r) [78], (rh,r) [75]; *T. longistylum* DC (r) [15]

Thaligosinine - *T. fargesii* Fr. ex Fin. et Gagnep. (r) [86]; *T. foetidum* L. (r) [49]; *T. isopyroides* C.A.M. (r) [17]

Thaliphylline - *T. cultratum* Wall. (wp) [2,3]; *T. minus* var. *minus* L. (ag) [77]

Thaliracebine - *T. minus* L. race C (r) [14]

Thalirugine - *T. cultratum* Wall. (wp) [81]

Thalisopidine - *T. fargesii* Fr. ex Fin. et Gagnep. (r) [86]; *T. isopyroides* C.A.M. (r) [17]

Thalisopine (Thaligosine) - *T. cultratum* Wall. (wp) [3,81]; *T. foetidum* L. [64]; *T. javanicum* Bl. (r) [63]; *T. minus* L. ssp. *majus* (*T. minus* L. var. *elatum* (Jacq.) Stoj. et Stefanov) (ag) [51]

Thalistine - *T. minus* L. race C (r) [14]

Thalmethine - *T. minus* L. [42], (ag) [6,10]; *T. minus* var. *minus* L. (ag) [77]

Thalmine - *T. buschianum* (ag) [44]; *T. collinum* Wallr. (ag) [37], (ag,r) [44]; *T. cultratum* Wall. (wp) [2,84]; *T. kuhistanicum* Ovcz. et Koczk. (ag) [60]

Thalmirabine - *T. delavayi* Franch. (r) [68]; *T. minus* L. race C (r) [14]

Thalrugosaminine - *T. cultratum* Wall. (wp) [2,3]; *T. foetidum* L. (r) [49]; *T. javanicum* Bl. (r) [63]

Thalrugosidine - *T. cultratum* Wall. (wp) [2,3]; *T. foetidum* L. (r) [15]

Thalrugosine (Thaligine, Isofangchinoline) - *T. minus* L. race C (r) [14]

Thalrugosinone - *T. cultratum* Wall. (wp) [84]

5.2.4. Secobisbenzylisoquinolines

Revolutinone - *T. cultratum* Wall. (wp) [2]

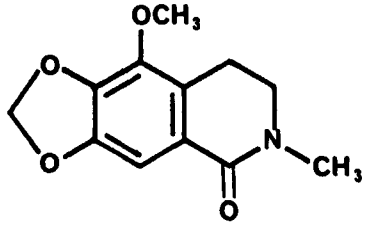
6. ALKALOIDS OF REVISED STRUCTURE

6.1. Thalflavine Tabular Review [90, Alkaloid No. 13], UV [90,91], IR [90,91], ^{1}H NMR [90,91], EIMS [90,91]

In 1970, an alkaloid named thalflavine (mp 132-133° [Me_2CO]) was isolated from *Thalictrum flavum* L., and assigned as 1-oxo-2-methyl-5-methoxy-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (**14**) via a consideration of physicochemical and spectral data [92]. Apparent confirmation of the validity of this assignment occurred in 1971 when oxidation ($KMnO_4/Me_2CO$) of what was then assigned as thalmelatidine (**15**), an aporphine-benzylisoquinoline dimeric alkaloid isolated from *Thalictrum minus* L. var. *elatum* Jacq. in 1970 [93] produced an isoquinolone, mp 137-139° ($CHCl_3$), identified as thalflavine [94]. In a subsequent study, oxidation ($KMnO_4/Me_2CO$) of thalistyline (**16**), a bisbenzylisoquinoline alkaloid isolated from *Thalictrum longistylum* DC. [95,96] and *Thalictrum podocarpum* Humb. [96,97] in 1977, apparently produced 1-oxo-2-methyl-5-methoxy-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline as colorless needles, mp 136-137° (MeOH)[95]. This isoquinolone was stated to be identical (UV, ^{1}HNMR, MS) to that obtained via oxidation of thalmelatidine [94] and to thalflavine [92]. By way of contrast, the structure of thalmelatidine was revised to **17** in 1985 [14] based on extensive ^{1}HNR studies, particularly double irradiation and nOe experiments, and would thus require the structure of thalflavine to be represented as 1-oxo-2-methyl-5,6-methylenedioxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (**18**), since the latter is a direct oxidation product of the former.

In order to address these ambiguities, isoquinolones **14** and **18** were synthesized via well-established pathways. Treatment of the appropriate aldehyde (either 2-methoxy-3,4-

methylenedioxybenzaldehyde or 2,3-methylenedioxy-4-methoxybenzaldehyde) with nitromethane and ammonium acetate provided the corresponding β-nitrostyrene which was reduced with $LiAlH_4$ in THF to afford the β-phenethylamine. *N*-Formylation of the amine with formic acid provided the *N*-formyl amide which was subsequently cyclized to the corresponding 3,4-dihydroisoquinoline via refluxing with $POCl_3$. Oxidation of the 3,4-dihydroisoquinolinemethiodide salt with alkaline $K_3Fe(CN)_6$ afforded the appropriate isoquinolone. A comparison of the physicochemical and spectral properties of synthetic isoquinolones **14** and **18** with those assigned to thalflavine strongly supported the revision of structure of thalflavine to **18**. Accordingly, it is appropriate to conclude that both the naturally occurring thalflavine [92] and the isoquinolone obtained as an oxidation product of the alkaloids thalmelatidine [94], thalistyline [95], thaliracebine [98], and thalistine [99] is isoquinolone **18**. Finally, the revision of structure of thalflavine as isoquinoline **18** would suggest that the structures of the following alkaloids be changed to reflect their 5,6-methylenedioxy-7-methoxy substitution as follows: thalmelatidine (**15**)[94] to **17**, thalistyline (**16**)[95] to **19**, *N*-desmethylthalistyline (**20**)[95] to **21**, *N*-methylthalistyline (**22**)[95] to **23**, thalirabine (*O*-desmethylthalistyline)(**24**)[95] to **25**, thaliracebine (**26**)[98] to **27**, and thalistine (**28**)[99] to **29**.



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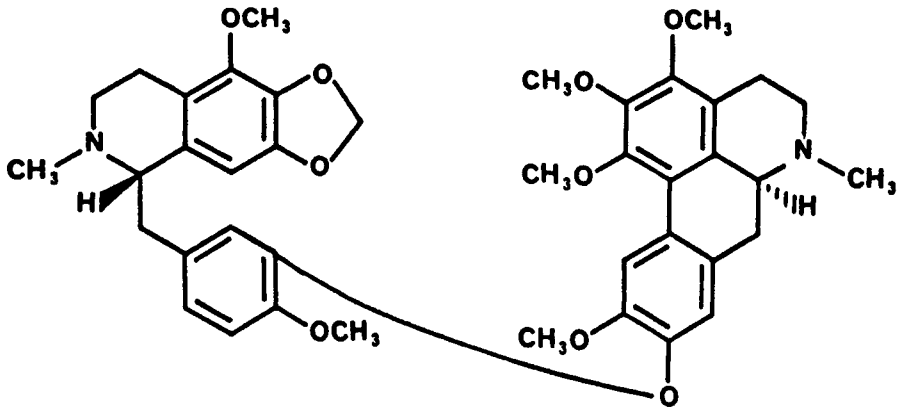

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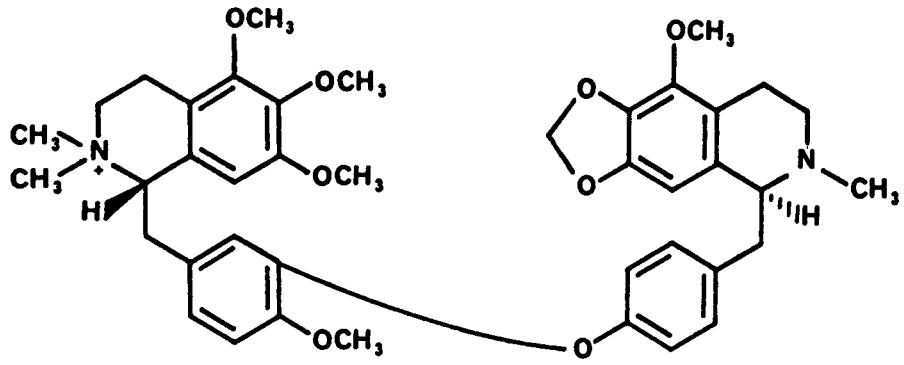

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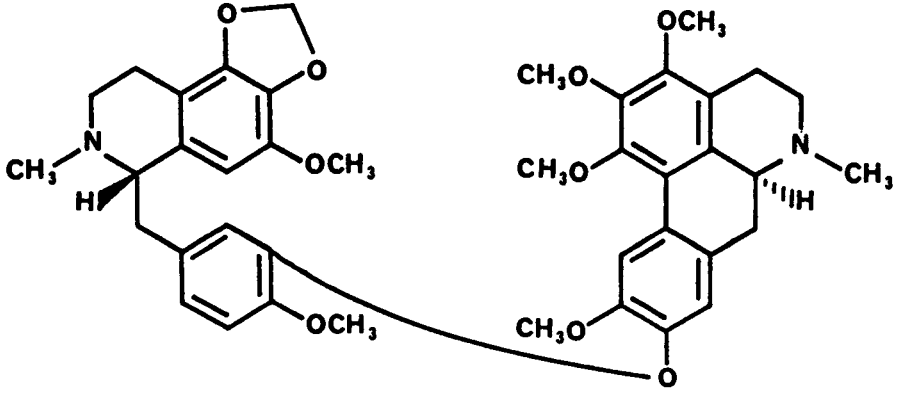

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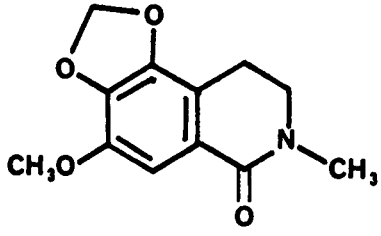

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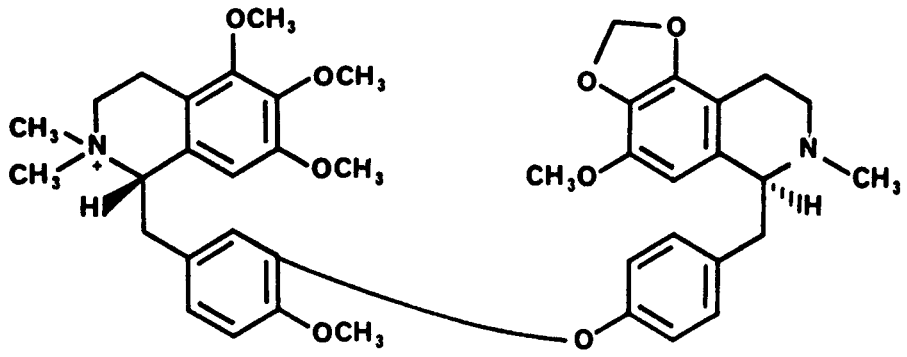

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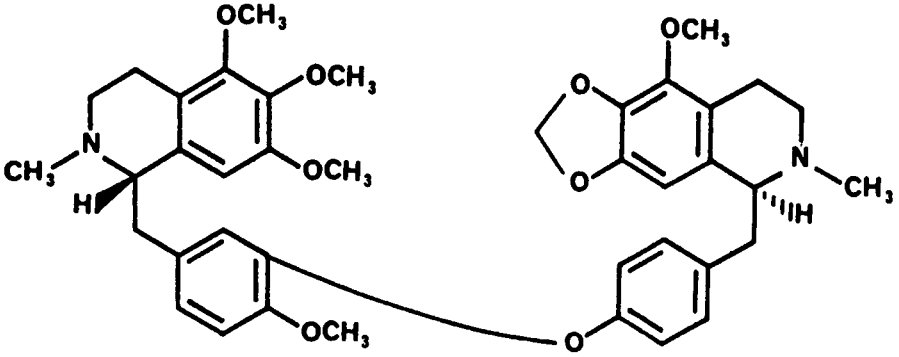

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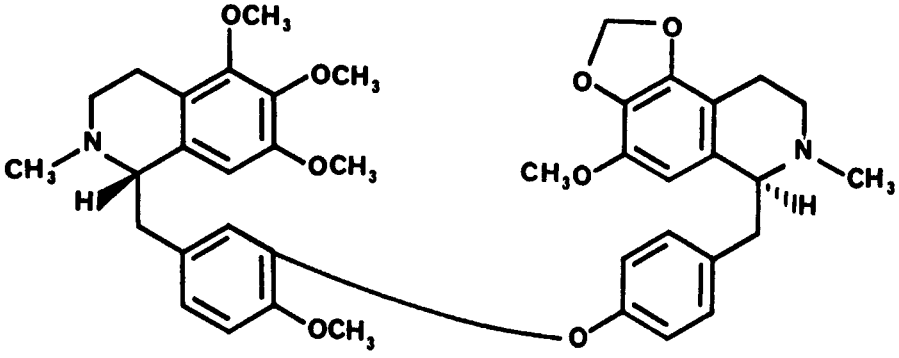

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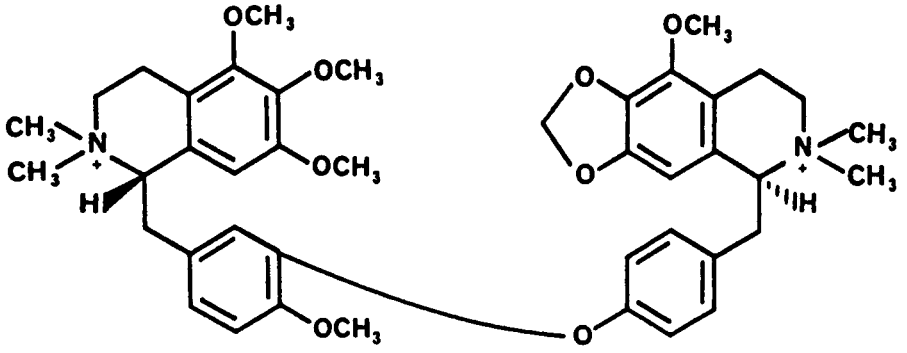

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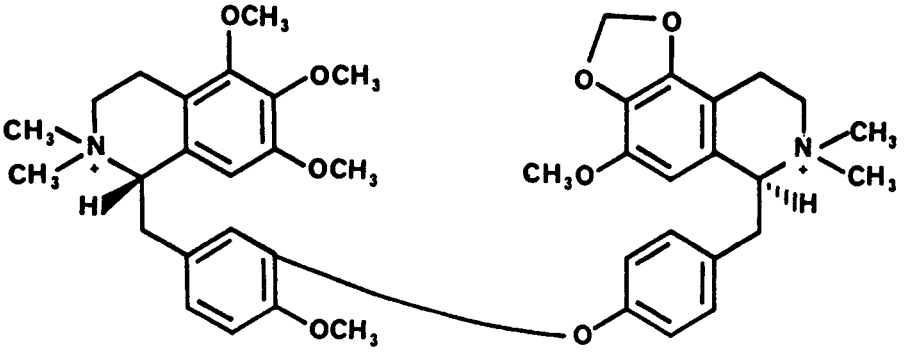

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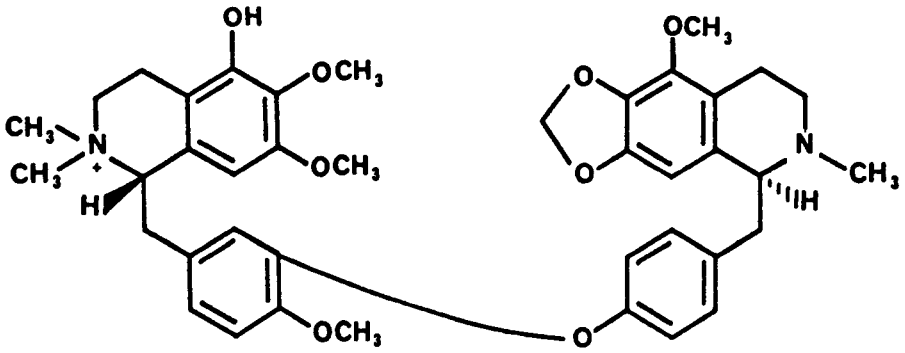

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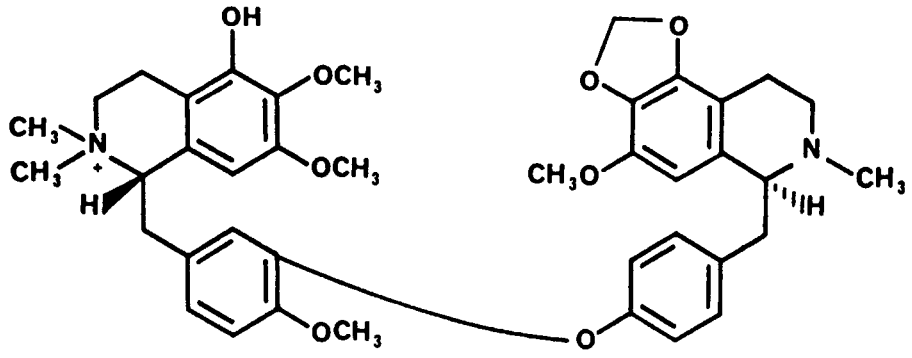
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N
CH3
+
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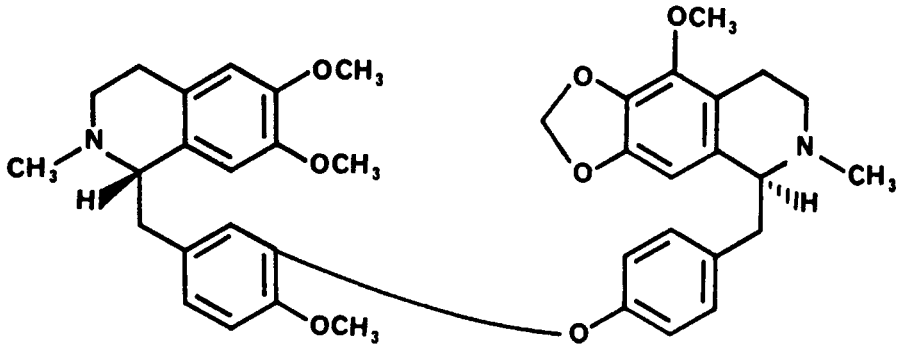
O
O
CH3O
N
CH3
H
OCH3
O

25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H
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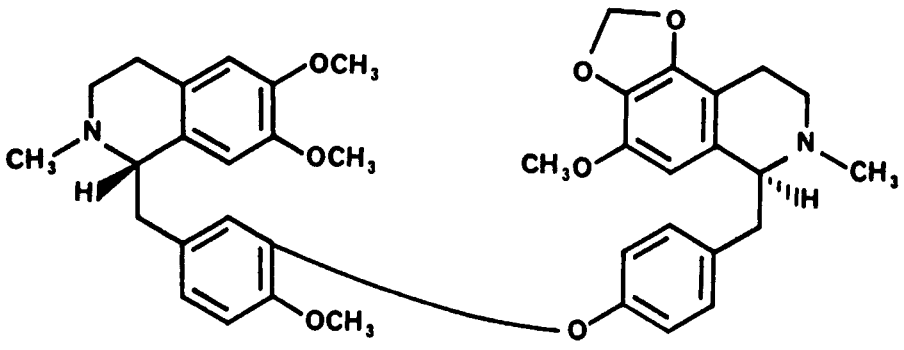
O
O
N
CH3
H
O

26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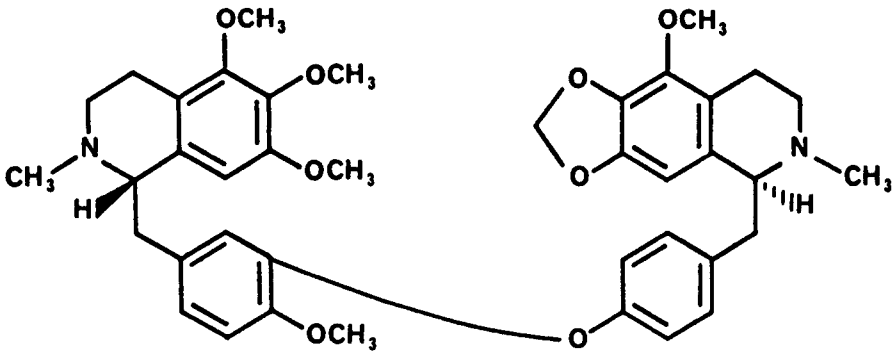
O

27



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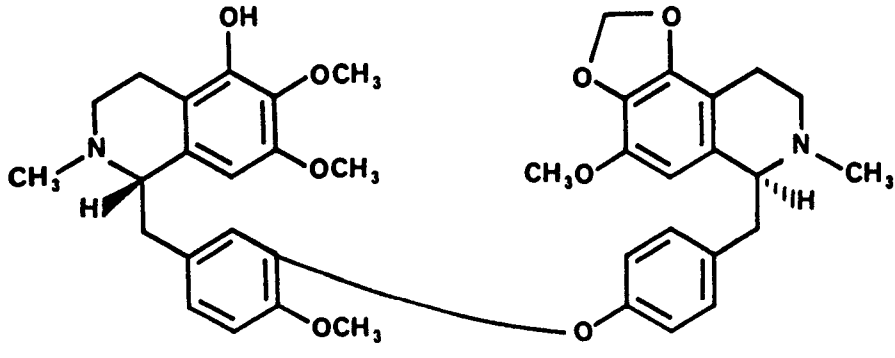

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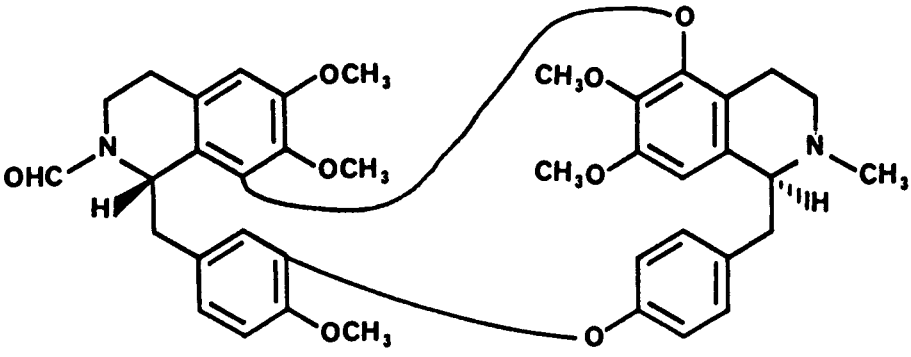
6.2. Thalrugosinone Tabular Review [100, Alkaloid No. 224], UV [84,100], ^{1}H NMR [84,100], EIMS [84,100], CD [84,100]

Thalrugosinone (**30**), $C_{38}H_{42}O_8N_2$ (654.2941), $[\alpha]_D^{22}$ -42° (c 0.30, MeOH), was reisolated as an amorphous solid via preparative TLC of fractions obtained from an extract (EtOH) of the whole plant of Pakistani *T. cultratum* Wall. in 1986 [84]. The alkaloid had been first isolated from extracts of *Thalictrum rugosum* in 1980 in very limited quantities, thereby handicapping the structural assignment. That assignment, which was based on ^{1}H NMR and EIMS data, resulted in the representation of thalrugosinone as **31** [101]. The reisolation of thalrugosinone in larger quantities (79 mg) in 1986 afforded the opportunity to study some of the chemistry of the alkaloid. Although no original and authentic sample of thalrugosinone was available, a comparison of the ^{1}H NMR and EIMS of the original isolate with that of the later isolate

demonstrated their identity as the same compound. The isolated alkaloid was characterized by a UV spectrum (MeOH) that displayed maxima at 241 nm (sh) (log ε 4.34) and 280 (3.62), while the CD spectrum (MeOH) exhibited extrema at Δε (nm) 0 (300), -3.9 (282), 0 (270), -0.2 (255), 0 (252), +11.8 (239), with a negative tail below 230 nm. The IR spectrum (KBr) showed a carbonyl absorption at 1660 cm^{-1}. Repeated attempts to reduce the alkaloid with $NaBH_4$ in MeOH were unsuccessful, despite the presence of two ketonic functions in **31**. However, treatment of an ethereal solution of the alkaloid with $LiAlH_4$ readily afforded (-)-thalidasine (**32**), which was also isolated from *T. cultratrum*. In addition, acidic hydrolysis (18% aqueous HCl) for 5 hours yielded (-)-2-northalidasine (**33**), an alkaloid that was also present in *T. cultratum*. These chemical transformations led to a revision in the structure of thalrugosinone, and its assignment as 2-formyl-2-northalidasine (**30**). A detailed ^1H NMR spectral analysis of the alkaloid, including the measurement of nOes, was used to support the structural revision. The ^1H NMR chemical shift assignments for the alkaloid were made as follows: δ4.48 [m, H(1)], 7.50 [s, *N*(2)CHO], 3.24 [m, H(3a)], 4.61 [m, H(3b)], 6.36 [H(5)], 6.12 [d, 1H, J = 1.9 Hz, H(10)], 6.78 [d, 1H, J = 8.2 Hz, H(13)] and 6.52 [dd, 1H, J = 1.9, 8.2 Hz, H(14)]; δ3.88 [m, H(1')], 2.65 [s, *N*(2')Me], 6.47 [s, 1H, H(8')], 6.42 [dd, 1H, J = 2.2, 8.3 Hz, H(10')], 6.58 [dd, 1H, J = 2.2, 8.3 Hz, H(11')], 6.99 [dd, 1H, J = 2.2, 8.3 Hz, H(13')], 7.62 [dd, 1H, J = 2.2, 8.3 Hz, H(14')]; 3.78 [s, C(6)OMe], 3.34 [s, C(7)OMe], 3.90 [s, C(12)OMe], 3.47 [s, C(6')OMe, and 3.88 [s, C(7')OMe]. Reciprocal nOe enhancements between the following were observed: *N*(2)CHO and H(1), H(1) and H(10), H(α) and H(10), H(5) and C(6)OMe, H(10) and H(10'), H(13) and C(12)OMe, H(1') and *N*(2')Me, H(8') and C(7')OMe, and H(8') and H(α'). In addition, other nOes were observed from H(5) to H(4a), H(α') to H(10'), H(α') to H(14'). Several observations were made by the authors concerning the influence of the *N*(2)-formyl function on the chemical shifts of the adjacent protons. First, in comparison with ^1H NMR spectrum of 2-northalidasine (**33**), the H(1) and one of the C(3) protons have undergone a substantial downfield shift. The H(1) has shifted from δ4.05 in 2-northalidasine (**33**) to δ4.48 in thalirugosinone because of its proximity to the neighboring *N*(2)-formyl group in the latter. In addition, because of the proximity of one of the H(3) protons in thalrugosinone (**30**) to the oxygen of the *N*(2)-formyl group in the compound, there is a downfield shift from δ3.36 to δ4.61. Second, the chemical shift of the *N*(2)-formyl proton is shifted upfield from the normal range of δ8.5-9.0 to δ7.50 because of the conformational shielding effects of the aromatic rings of the alkaloid. With regard to the ^{13}C NMR spectrum of the alkaloid, the chemical shift of the carbonyl carbon atom was observed at δ161.4, and its bonding to a hydrogen atom was further confirmed by a spin echo Fourier transform analysis. There was signal observed around δ199-200, where ketonic absorption is typically evident. Finally, the EIMS provided additional confirming evidence in favor of the structural revision. The molecular ion (74%) was observed at m/z 666.2882 (calcd. for $C_{39}H_{42}O_8N_2$, 666.2940), while the M-1 ion (54%) was observed at m/z 665.2821 (calcd. for $C_{39}H_{41}O_8N_2$, 666.2861). Other diagnostically important fragment ions include those at m/z 635 (100%), 439 (72)(**34**) [observed 439.1862; calculated for $C_{24}H_{27}O_6N_2$, 439.1866], 425 (18)(**35**) [observed 425.1704; calculated for $C_{23}H_{25}O_6N_2$, 425.1704], 411 (32)(**36**)

[observed 411.1896; calculated for $C_{23}H_{27}O_5N_2$, 411.1916], 409 (36), 211 (37)[observed 211.0762; calculated for $C_{14}H_{11}O_2$, 411.1916], 204 (49), and 190 (30). The authors observed that thalrugosinone was the first *N*-formyl containing bisbenzylisoquinoline alkaloid in nature, and that its biosynthesis likely proceeds via oxidation of the *N*(2)-methyl group of thalidasine (**32**) to a hydroxymethyl group which undergoes further oxidation to the formyl group. The stability of thalrugosinone (**30**) is consistent with its structure, being both a tertiary amine and an amide. Furthermore, the stability of **30** tends to stand in contrast with the apparent instability of the diaminoketone (**31**) that was portrayed in its original structural postulation [84].



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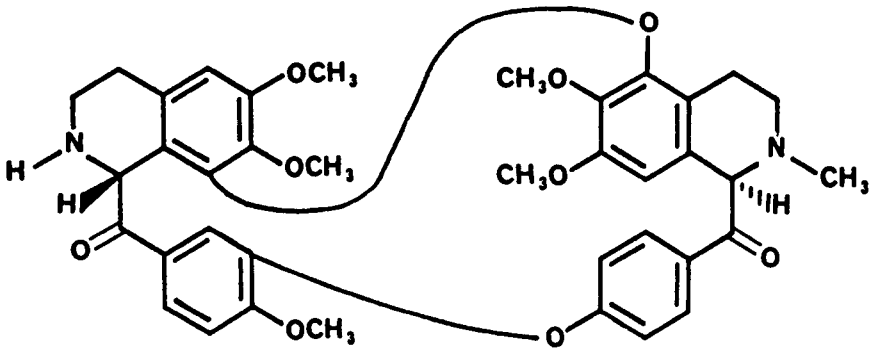

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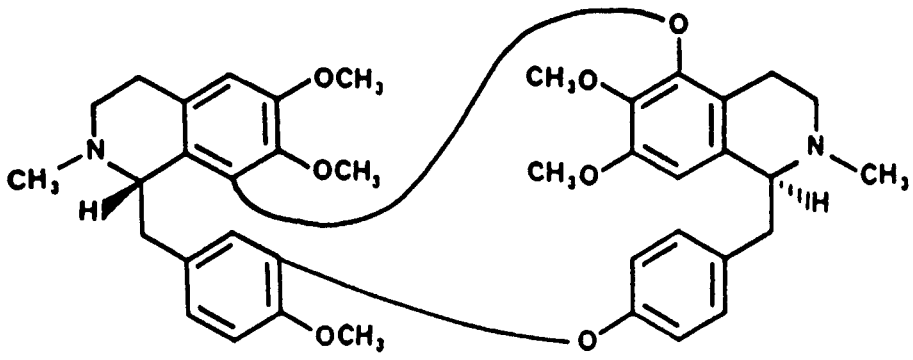

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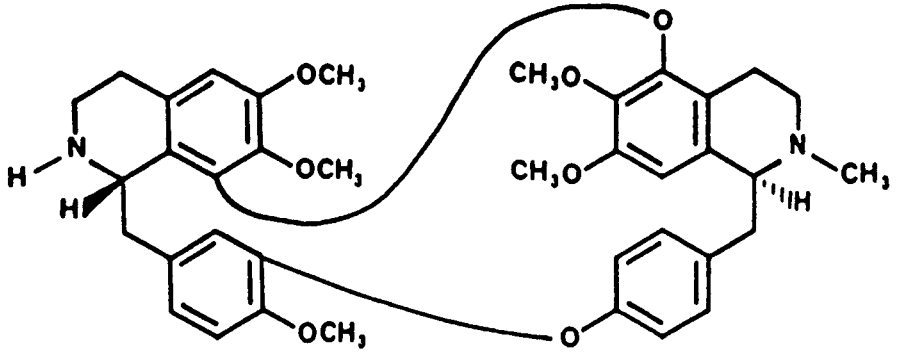

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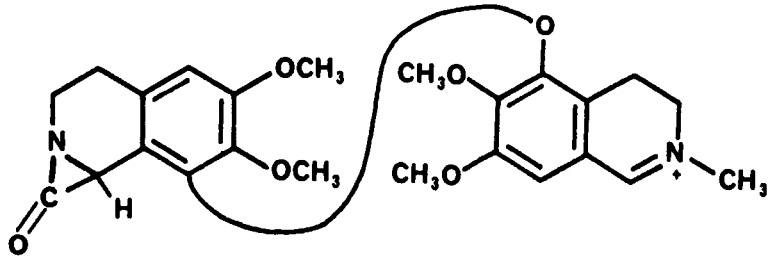

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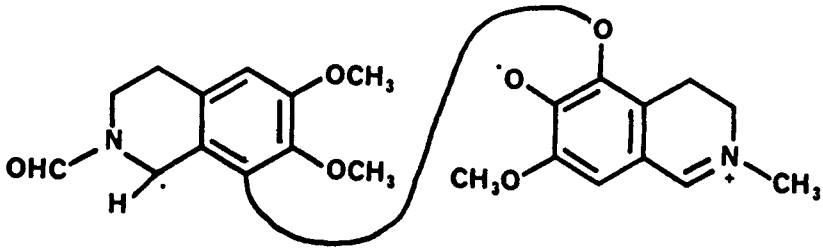

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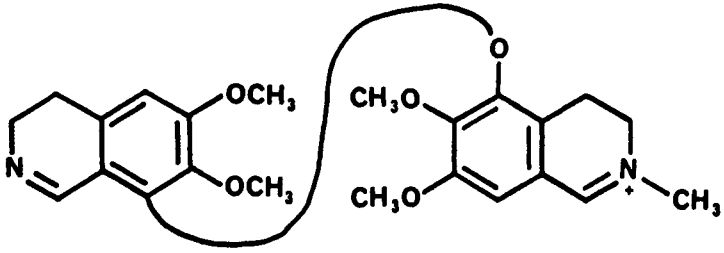

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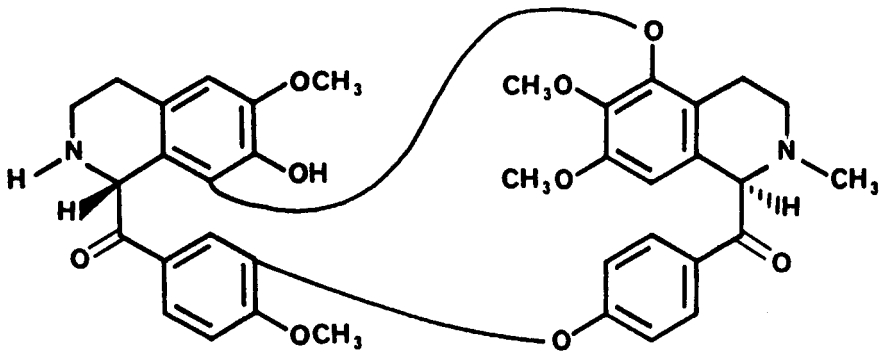
6.3. Thalpindione

With the structural revision of thalrugosinone to **30** [84], and with the knowledge that the monophenolic alkaloid thalpindione converts to thalrugosinone on methylation (CH_2N_2), revision of the structure of thalpindione from **38** to **39** was reasonable [84].



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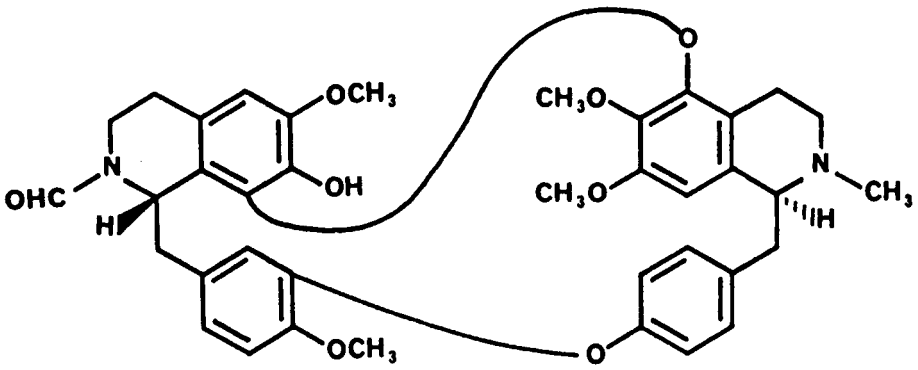

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7. NEW ALKALOIDS - MONOMERS

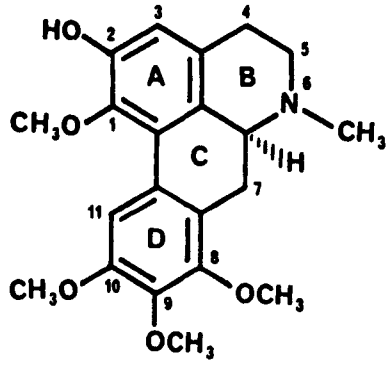
Some applicable generalizations in the following discussion of new *Thalictrum* alkaloids are: UV and CD spectra were recorded in MeOH; IR spectra were recorded in $CHCl_3$; and ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, unless otherwise noted.

7.1. Aporphines

The numbering of the aporphine ring in the following alkaloids (aporphines, dehydroaporphines, oxoaporphines) is according to accepted practice [1,102-106], and is illustrated in the structure of acutifolidine (**40**).

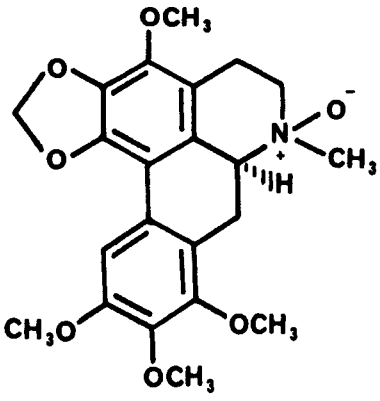
7.1.1. (+)-Acutifolidine. (UV [61], IR [61], ^{1}H NMR [61], ^{13}C NMR[61], EIMS [61]).

Acutifolidine (**40**), $C_{21}H_{25}O_5N$ (371.1731), mp 183-184°C, $[\alpha]_D^{27.5}$ +77.4° (c 1.033, MeOH), was isolated as colorless prisms from an extract of the roots of the Chinese *T. acutifolium* (Hand.-Mazz.) Boivin in 1989 [61]. The alkaloid showed a positive reaction with $FeCl_3$, and its UV spectrum was characterized by maxima at 280 nm (log ε 4.23), 301 (4.22), and 310 (sh), with a bathochromic shift in alkaline medium to 296 and 322 nm. The IR spectrum displayed absorption bands at 3080, 2830, 2800, 2720, 1608, 1516, 1370, 1260, and 1052 cm^{-1}. The ^{1}H NMR spectrum revealed the presence of one *N*-methyl group (s, δ2.53), four methoxy groups (δ3.71, 3.88, 3.90, and 3.95), and one low-field aromatic proton (s, δ7.92). A D_2O exchangeable broad singlet at δ5.50-6.00 was attributable to a phenolic hydroxy group. The high resolution EIMS showed the parent ion at m/z 371.1730 (calcd. 371.1731 for $C_{21}H_{25}O_5N$), with other important fragment ions at m/z 370.1676 (M-1), 356.1489 (M-15), 340.1535 (M-31), 328.1314 (M-43 [M-CH_2=NCH_3]), 313.1071 (M-15-43) and 180.0717. These spectral data were characteristic of a pentaoxygenated aporphine containing four methoxy groups and one phenolic hydroxy group [102-106]. In addition, the presence of a highfield methoxy signal (δ3.71) was characteristic of a C(1) methoxy group, while that of a lowfield proton absorption (δ7.92) was characteristic of H(11). NOe experiments revealed that irradiation of the methoxy groups with chemical shifts at δ3.88, 3.90, and 3.95 produced no discernible effect on the C(1)-methoxy group (δ3.71) nor on the proton at δ6.80. However, a 20% enhancement in the H(11) proton signal (δ7.92) was noted on irradiation of the methoxy signal at δ3.88. It was concluded that the phenolic hydroxy group must be located at C(2), while the four methoxy groups were placed at C(1), C(8), C(9), and C(10). The structural assignment was supported by the following ^{13}C NMR spectral data, with corresponding assignments: 149.3 [C(1)], 130.5 [C(1a)], 129.3 [C(1b)], 149.0 [C(2)], 114.2 [C(3)], 123.5 [C(3a)], 23.4 [C(4)], 52.8 [C(5)], 62.8 [C(6a)], 33.7 [C(7)], 123.0 [C(7a)], 145.5 [C(8)], 145.2 [C(9)], 144.7 [C(10)], 114.2 [C(11)], and 122.8 [C(11a)].



7.1.2. (+)-Leucoxylonine *N*-Oxide. UV [107], IR [107], ^{1}H NMR [107], EIMS [107], CIMS [107], CD [107].

Leucoxylonine *N*-oxide (**41**), $C_{22}H_{25}O_7N$ (415.1631), $[\alpha]_D^{21}$ +30.9° (c 0.06, MeOH), was isolated via size exclusion chromatography (Sephadex LH-20) of the alkaloid fraction of an extract of the aerial parts of *T. simplex* L. in 1995 [107]. The UV spectrum exhibited a single maximum at 280 nm (log ε 3.63), while the CD spectrum displayed extrema at Δε -2.17 (305), -2.40 (297), -2.40 (295), -3.43 (283), +20.03 (245), -8.63 (222), -6.93 (216)(sh), and -4.34 (209). The IR spectrum was characterized by bands at 3600, 3000, 2930, 2850, 1730, 1630, 1600, 1580, 1495, 1465, 1425, 1400, 1350, 1260, 1140, 1120, 1055, 1000, 949, 920, 850, and 660 cm^{-1}, and was very similar to that of leucoxylonine (**42**), an aporphine that had been isolated via column chromatography from the same extract. The CIMS showed the $(M+H)^+$ ion at m/z 416 (2.5%), while the EIMS typically failed to display a parent ion at m/z 415, but showed an important M-16 fragment ion at m/z 399 (91%) that is characteristic for *N*-oxides [102-106]. Other fragment ions reported include the following: 398 (100), 385 (11), 384 (22), 368 (13), 356 (18), 325 (6), 298 (4), 169 (3), 139 (10), 97 (20), 91 (12), 83 (51), and 58 (8). The ^{1}H NMR spectrum was also very similar to that of leucoxylonine (**42**), with the exception of the chemical shift of the *N*-methyl group. This signal was observed as a singlet at δ3.25 in the *N*-oxide, rather than the characteristic higher field position (δ2.58) in the parent leucoxylonine (**42**), and was thus consistent for an *N*-oxide [102-106]. Other salient features of the ^{1}H NMR spectrum included the presence of five singlets and one double doublet, the former being represented by four methoxy groups (δ3.89, 3.90, 3.92, 4.08) and one aromatic proton [δ7.40, H(11)], while the latter was assignable to one methylenedioxy group [δ5.91 and 6.16, 2H, J = 1.2 Hz]. A consideration of these spectral data, as well as the specific rotation, prompted the assignment of this new alkaloid as (+)-leucoxylonine *N*-oxide (**41**).



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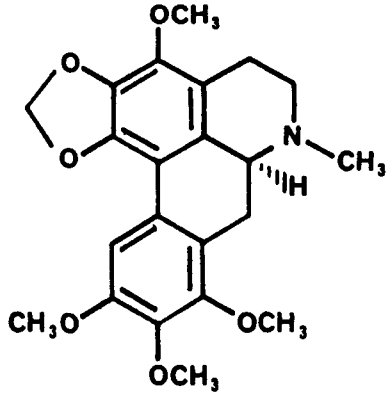

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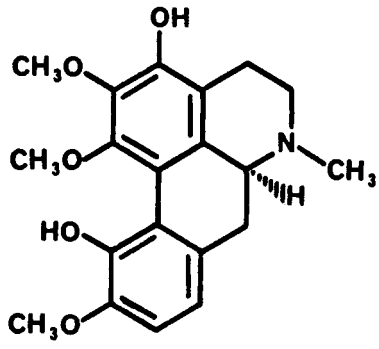
7.1.3. (+)-*N*-Methyldanguyelline. Tabular Review [106, Alkaloid No. 573], UV [46,106], ^{1}H NMR [46,106], EIMS [46,106].

N-Methyldanguyelline (**43**), $C_{20}H_{23}O_5N$ (357.1576), $[\alpha]_D$ +96° (c 0.25, $CHCl_3$), was isolated via preparative TLC of the basic fraction of an ethanolic extract of the whole plant of *T. pedunculatum* Edgew. in 1989 [46]. The UV spectrum of the alkaloid was characteristic of a 1,2,10,11- or a 1,2,3,10,11-oxygenated aporphine and displayed maxima at 219 nm (log ε 4.40), 277 (4.02), and 312 (sh) (3.72) [102-106]. Upon addition of alkaline solution, the spectrum exhibited a strong bathochromic shift with hyperchromic effect, suggesting the phenolic nature of the alkaloid. The EIMS showed a molecular ion at m/z 357, with the base peak at m/z 342, and other significant fragment ions at m/z 340 (37%), 326 (61), 311 (13), and 310 (313). The ^{1}H NMR spectrum showed four upfield singlets readily assignable to one *N*-methyl group (δ2.66) and three methoxy groups (δ3.74, 3.92, and 4.00). The aromatic region displayed a two-proton singlet at δ6.84, with a phenolic proton appearing as a sharp singlet (D_2O exchangeable) at δ8.52. The presence of a two-proton singlet at approximately δ6.8 was characteristic of a C(10)-C(11) disubstituted phenolic aporphine, with the phenolic group being present at C(11). In order to verify this observation, the ^{1}H NMR spectrum was determined in DMSO-d_6, followed by DMSO-d_6/NaOD. Examination of the first spectrum (neutral medium) revealed that the H(8) and H(9) protons appeared as a pair of doublets at δ6.80 and 6.83 (J=8.1 Hz), while in alkaline medium the H(8) proton had shifted upfield to δ5.82, with the H(9) proton being observed at δ6.31. This upfield shift of approximately 1 ppm is indicative of the para-relationship of the H(8) proton to the C(11) phenolic group [108,109]. The next problem was to unambiguously assign the placement of the methoxy groups. The high field methoxy (δ3.74) was readily assigned to C(1) based on long precedent [102-106], but nOe studies were undertaken in order to place the other methoxy groups. Irradiation of the H(9) proton at δ6.84 produced an enhancement of the C(10) methoxy singlet at δ3.92, while irradiation of the C(1) methoxy singlet (δ3.74) enhanced both the phenolic hydroxy singlet at δ8.52 and the C(2) methoxy singlet at δ4.00. Reciprocal enhancements were observed to each of these effects. It was therefore concluded that the second phenolic group in *N*-methyldanguyelline must be placed at C(3), as this is the only remaining position of aromaticity. The authors made the empirical observation that for 1,2,3-trisubstituted aporphines containing a phenolic group at C(3) and methoxy groups at C(1) and C(2), the C(2) methoxy resonance will be found near δ4.00. On the other hand, if the phenolic group is located at C(2) and the methoxy groups are found at C(1) and C(3), then the C(3) methoxy signal is observed near δ3.92. With this in hand, the authors postulated a conclusive structural assignment of danguyelline, the noraporphine parent alkaloid that had been isolated from extracts of *Xylopia danguyella* (Annonaceae) in 1981 [104,110]. This postulation fixed the ring A methoxylation to C(2) and the hydroxylation (phenol) to C(3).



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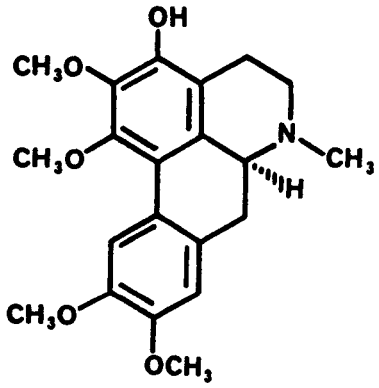
7.1.4. (+)-Thalbaicalidine. Tabular Review [105, Alkaloid No. 417], UV [105,111],^{1}H NMR [105,111], EIMS [105,111].

Thalbaicalidine (**44**), $C_{21}H_{25}O_5N$ (371.1733), mp 191-193°C (EtOAc), $[\alpha]_D$ +73° (MeOH), was isolated from an extract of the epigeal parts of *T. baicalense* Turcz. in 1984 [111]. The alkaloid was characterized by UV maxima (EtOH) at 282, 305, and 315 nm, with a bathochromic shift in alkaline medium to 326 nm. The EIMS displayed a parent ion at m/z 371 and other important fragment ions at m/z 370, 356 (M-Me), 341, and 328 (M-CH_2NCH_3). The ^{1}H NMR spectrum indicated the presence of one *N*-methyl group at δ2.46; four methoxy groups at δ3.62 (3H), 3.83 (6H), and 3.88 (3H); and two aromatic protons as uncoupled singlets at δ6.65 and 7.77. These data suggested that the alkaloid was a 1,2,3,9,10-pentaoxygenated aporphine [102-106], and comparison with the data for *N*-methylthalbaicaline [obtained by methylation (CH_2O/HCOOH) of thalbaicaline (**45**)] showed them to be identical. Hence, thalbaicalidine (*N*-methylthalbaicaline) was assigned as 3-hydroxy-1,2,9,10-tetramethoxyaporphine [111].

7.1.5. (+)-Thalbaicaline. Tabular Review [105, Alkaloid No. 416], UV [105,111], ^{1}H NMR [105,111], EIMS [105,111].

Thalbaicaline (**45**), $C_{20}H_{23}O_5N$ (357.1576), $[\alpha]_D$ +61° (MeOH), was first isolated from an extract of the epigeal parts of *T. baicalense* Turcz. in 1984 [111], and later from an extract of the stems in 1986 [34]. The alkaloid was characterized by UV maxima (EtOH) at 220, 285, 303, 313 nm, with a bathochromic shift in alkaline medium to 325 nm. The EIMS displayed a parent ion at m/z 357 and other important fragment ions at m/z 356, 342 (M-Me), 340, 328 (M-CH_2NH), 327, and 297. The ^{1}H NMR spectrum indicated the presence of four methoxy groups at δ3.63, 3.81, 3.83, and 3.88, and two aromatic protons as uncoupled singlets at δ6.36 and 7.81. Acetylation (Ac_2O/pyr) of thalbaicaline afforded an *N,O*-diacetyl derivative, characterized by two

strong IR maxima (KBr) at 1650 (amide) and 1760 cm^{-1} (phenolic ester). The EIMS displayed the parent ion at m/z 441, with other fragment ions at m/z 382, 381, 340, 339, 327, 310, and 293. The ^1H NMR spectrum showed the presence of one *N*-acetyl function as two singlets with a total intensity of 3 protons at δ2.09 and 2.13, one phenolic acetyl function as a singlet at δ2.30, four methoxy groups as two singlets at δ3.64 (3H) and 3.84 (9H), and two aromatic protons as two singlets at δ6.67 and 7.93. These data were consistent with the assignment of thalbaicaline was a new 1,2,3,9,10-pentasubstituted monophenolic noraporphine [102-106]. The position of the phenolic group was established via a consideration of the EIMS of *N,O*-diacetylthalbaicaline (**46**), *N*-acetylnorthalicmine (**47**), and *N*-acetylbaicaline (**48**). The EIMS of *N,O*-diacetylthalbaicaline and *N*-acetylnorthalicmine displayed peaks of medium intensity at m/z 381 (M-60) and 365 (M-32), respectively, while in the spectrum of *N*-acetylbaicaline, these peaks were not discernible. On the basis of this difference in these mass spectra, as well as a consideration of the other data, thalbaicaline was assigned as **45** [111].



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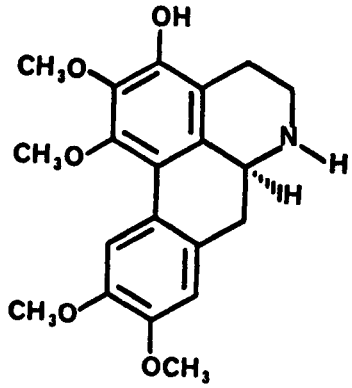

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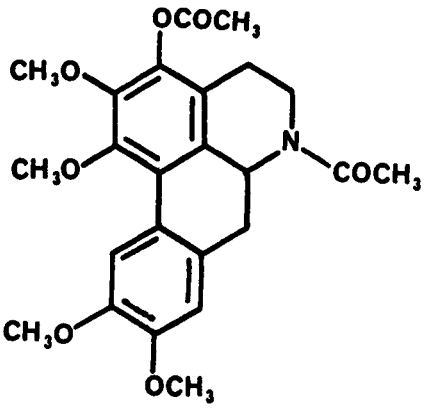

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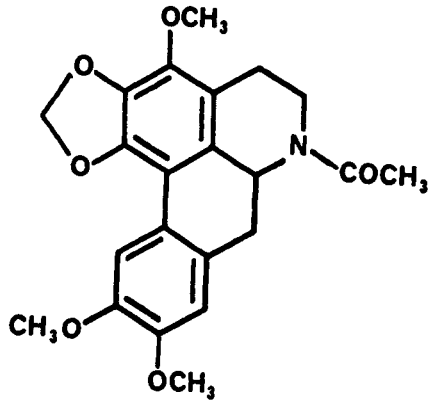

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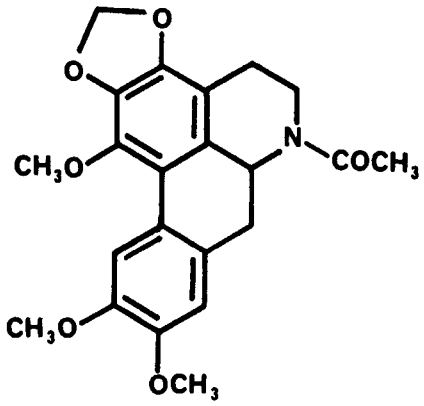

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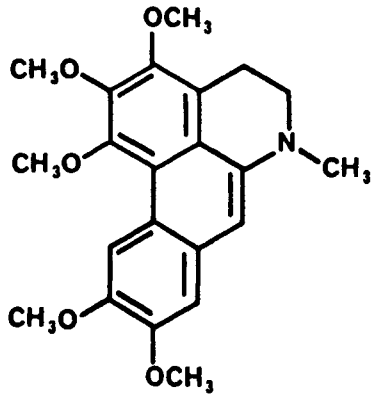
7.2. Dehydroaporphines

7.2.1. Dehydrothalicsimidine. Tabular Review [106, Alkaloid No. 619], UV [40,106], IR [40,106], ^{1}H NMR [40,106], ^{13}C NMR [40,106], EIMS [40,106], FABMS [40].

Dehydrothalicsimidine (**49**), $C_{22}H_{25}O_5N$ (383.1733), mp 127-128°C (petrol-EtOAc), was isolated as optically inactive yellow crystals from an extract (EtOH) of the roots of *T. ichengense* Lecoy et Oliv. in 1989 [40]. The UV spectrum (EtOH) was characteristic of a dehydroaporphine and showed maxima at 220 nm (log ε 4.21), 257 (4.55), 270 (4.53), and 335 (3.98) [102-106], while the IR spectrum (KBr) showed strong absorption in the 1540-1620 cm^{-1} region. The EIMS displayed the molecular ion and base peak at m/z 383, with the FABMS being supportive. The ^{1}H NMR spectrum exhibited six singlets that were readily assignable to one downfield *N*-methyl group (δ3.09), five methoxy groups [δ3.91 (6H) and 4.05 (9H)], and three singlet aromatic protons (δ6.66, 7.09, and 9.00), the last of which was downfield and characteristic of the H(11) proton in dehydroaporphines [102-106]. The H-4 and H-5 protons were observed as a multiplet at δ3.21-4.05. These data were supportive of a 1,2,3,9,10-pentamethoxylated aporphine [102-106]. The authors assigned the proton resonating at δ7.09 as H(7), based on a 1964 reference that cites the chemical shift of a proton located ortho- to a methoxy group being found at a higher field (0.4-0.7 ppm) than otherwise (δ7.2-7.4)[112]. Subsequent irradiation of the proton at δ7.09 resulted in a 10% nOe on the proton at δ6.66, thereby prompting the assignment of the latter proton as H(8). However, the premise proposed in the 1964 reference was made in reference to aporphines, not dehydroaporphines, and was proposed some four years prior to the discovery of dehydrodicentrine, the first naturally occurring dehydroaporphine [113]. There is a considerable amount of evidence that would suggest the assignment of the H(7) and H(8) protons should be reversed, thereby assigning the signal at 6.66 ppm to H(7) and that at 7.09 ppm

to H(8) [102-106]. The structural assignment was supported by the following ^{13}C NMR spectral data: 146.6 [C(1)], 120.3[C(1a)], 121.3 [C(1b)], 150.7 [C(2)], 147.8 [C(3)], 121.9 [C(3a)], 24.7 [C(4)], 50.0 [C(5)], 40.7 [NCH_3], 141.7 [C(6a)], 102.9 [C(7)], 129.3 [C(7a)], 106.7 [C(8)], 148.8 [C(9)], 146.2 [C(10)], 108.4 [C(11)], and 118.5 [C(11a)].



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7.3. Oxoaporphines

7.3.1. Oxobaicaline (7-Oxobaicaline). Tabular Review [105, Alkaloid No. 432], UV [34,105], IR [34,105], ^1H NMR [34,105], EIMS [34,105].

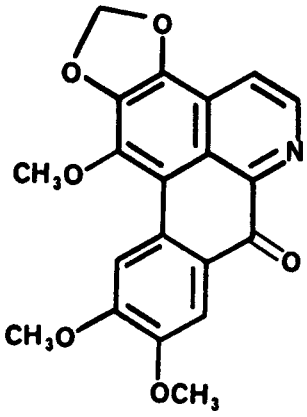
Oxobaicaline (**50**), $C_{20}H_{15}O_6N$ (365.0899), mp 240°C (dec.)(MeOH), was isolated in the form of large needles from an extract ($CHCl_3$) of the stems of *T. baicalense* Turcz. in 1986 [34]. The UV spectrum (EtOH) of the alkaloid showed maxima at 250, 289, 380, and 500 nm, with a bathochromic shift in acidic medium but no shift in alkaline medium, while the IR spectrum (KBr) displayed a strong band at 1650 cm^{-1}. These data were characteristic of an oxoaporphine alkaloid [102-106], and were supported by the EIMS that showed the parent ion and base peak at m/z 365, and other significant fragment ions at m/z 350, 349, 336, 320, 307, 279, 223, and 185.5. The ^1H NMR spectrum (TFA) indicated the presence of three methoxy groups as two singlets at δ3.73 (6H) and 3.80 (3H), and one methylenedioxy group as a singlet at δ6.18. Two one-proton singlets at δ7.60 and 8.45 were assigned to the C(8) and C(11) protons, respectively, while the protons at C(4) and C(5) were observed (but not described) in the δ8.20-8.40 region. Reduction of the alkaloid with Zn/H_2SO_4 afforded (+/-)-baicaline (**51**), an alkaloid previously isolated from this same plant in 1982 [114], while oxidation of baicaline with CrO_3 and $KMnO_4$ gave oxobaicaline (**50**), therefore confirming the structure of this new oxoaporphine [34].

7.4. Pavines

The numbering of the pavine ring in the following alkaloids is according to accepted practice [1], and is illustrated in the structure of 2-demethylthalimonine (**52**).

7.4.1. (-)-2-Demethylthalimonine. UV [67], IR [67], ^{1}H NMR [67], EIMS [67], CD [67].

2-Demethylthalimonine (**52**), $C_{20}H_{21}O_5N$ (355.1420), $[\alpha]_D^{22}$ -43.8° (c 0.02, MeOH), was isolated by preparative TLC from the alkaloid fraction of an extract (EtOH) of the aerial parts of *T. simplex* L. in 1992 [67]. The UV spectrum (EtOH) of the alkaloid displayed a single maximum at 284 nm (log ε 3.61), while the CD spectrum showed extrema at Δε -0.61 (313 nm), -0.57 (306), -0.43 (289), -0.34 (275), +1.37 (250), -2.37 (235), +4.50 (222), -26.25 (207). The IR spectrum was characterized by bands at 3400, 3025, 2956, 2855, 2815, 2401, 1518, 1465, 1377, 1230, 1215, 812, and 795 cm^{-1}. The EIMS displayed a parent ion at m/z 355 (19%), with other significant fragment ions at m/z 354 (13), 341 (4), 340 (4), 205 (13), 204 (100), and 190 (13). The ^{1}H NMR spectrum indicated the presence of one *N*-methyl group at δ2.64 (s), two methoxy groups as singlets at δ3.79 and 3.84, one methylenedioxy group as a doublet at δ5.88 and 5.95 (J=1.0 Hz), three aromatic protons as discrete singlets at δ6.49, 6.60, and 6.84, and one phenolic hydroxy group at δ4.40-4.50 (exchangeable with D_2O). These data were characteristic of a monophenolic pavine alkaloid containing two methoxy groups and one methylenedioxy group [115,116]. Treatment of 2-demethylthalimonine with a solution of ethereal CH_2N_2 afforded thalimonine (**53**), thereby fixing the skeletal structure and positions of oxygenation. Only the position of the phenolic hydroxy group at C(2), C(8), or C(9) remained to be established. The base peak at m/z 204 in the EIMS could be assignable to one of two fragment ions, either fragment ion **54** ($C_{12}H_{14}O_2N$) or fragment ion **55** ($C_{11}H_{10}O_3N$), and without high resolution measurements, this assignment was not possible. A previous study of the high resolution ^{1}H NMR spectrum (with nOe enhancements) of the parent alkaloid thalimonine (**53**) had demonstrated that the methoxy group at C(2) was found at δ3.86, while methoxy groups at the C(8) and C(9) positions were observed at δ3.84 and 3.78, respectively. On this basis, the methoxy groups of 2-demethylthalimonine were assigned to C(8)[δ3.84] and C(9)[δ3.79], leaving the placement of the phenolic hydroxy group at C(2), completing the assignment of 2-demthylthalimonine (**11**) as (-)-8,9-dimethoxy-2-hydroxy-3,4-methylenedioxypavinane. There were no nOe studies reported to corroborate these assignments. Additional signals in the ^{1}H NMR spectrum of 2-demethylthalimonine (**52**) include the following: δ2.64 [1H,s,H(5)], 2.74 [1H,s,H(11)], 3.19-3.26 [1H,m,H(5')], 3.43-3.48 [1H,m,H(11')], 4.04-4.10 [2H,t,H(6) and H(12)].



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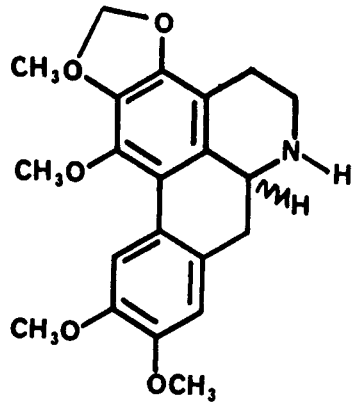

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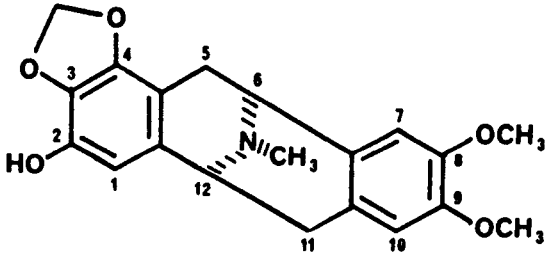

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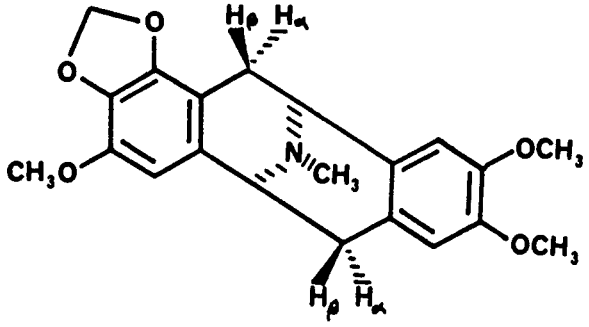

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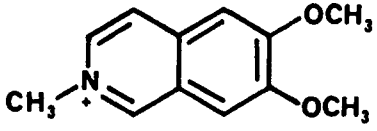

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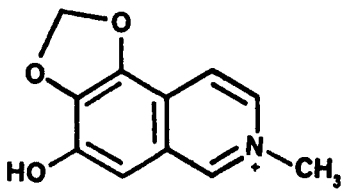

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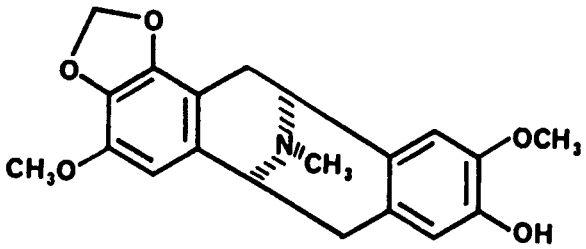
7.4.2. (-)-9-Demethylthalimonine. UV [67], IR [67], ^{1}H NMR [67], EIMS [67], CD [67].

9-Demethylthalimonine (**56**), $C_{20}H_{21}O_5N$ (355.1420), $[\alpha]_D^{22}$ -51.7° (c 0.067, MeOH), was isolated along with 2-demethylthalimonine (**52**) by preparative TLC of the basic fraction of an extract (EtOH) of the aerial parts of *T. simplex* L. in 1992 [67]. The UV spectrum (EtOH) of **56** was characterized by a single maximum at 283 nm (log ε 3.68), while the CD spectrum displayed extrema at Δε -0.08 (309 nm), -0.07 (305,sh), -0.22 (288), +0.41 (276), +0.05 (257,sh), -2.65 (234), +0.77 (220). The IR spectrum was characterized by signals at 3400, 3007, 2958, 2856, 1734, 1651, 1516, 1464, 1375, 1346, 1257, 1188, 1055, and 796 cm^{-1}. The EIMS displayed a parent ion at m/z 355 (46%), with other significant fragment ions at m/z 354 (25), 341 (4), 340 (8), 218 (63), 204 (20), and 190 (100). The ^{1}H NMR spectrum suggested the presence of one *N*-methyl group at δ2.59 (s), two methoxy groups as singlets at δ3.84 and 3.86, one methylenedioxy group as a double doublet at δ5.88 and 5.95 (J=1.0 Hz), three aromatic protons as two singlets at δ6.31 (1H) and 6.59 (2H), and one phenolic hydroxy group at δ4.40-4.50 (exchangeable with D_2O). These data were remarkably similar to the data reported for its positional isomer 2-demethylthalimonine (**52**), and were likewise characteristic of a monophenolic pavine alkaloid containing two methoxy groups and one methylenedioxy group [115,116]. As anticipated, treatment of 9-demethylthalimonine with a solution of ethereal CH_2N_2 gave thalimonine (**53**), thereby establishing fixing the skeletal structure and positions of oxygenation of **56**. Examination of the EIMS fragment ions at m/z 190 and 218, assigned as **57** and **58**, respectively, allowed the assignment of one of the methoxy groups of **56** to the ring bearing the methylenedioxy function. Thus, only the assignment of the phenolic hydroxy group to C(8) or C(9) remained to be established. Since one of the two methoxy groups of **56** has the same chemical shift (δ3.84) as that of the C(8) methoxy group of thalimonine (**53**), and the other methoxy group has the same chemical shift (δ3.86) as that of the C(2) of thalimonine (**53**), the phenolic group of **56** was placed at C(9), and 9-demethylthalimone (**56**) was characterized as (-)-2,8-dimethoxy-9-hydroxy-3,4-methylenedioxypavinane. As with its positional isomer 2-demethylthalimonine, no nOe experiments were undertaken to validify these assignments. Finally, additional signals in the ^{1}H NMR spectrum of 9-demethylthalimonine (**56**) include the

following: δ2.65 [1H,s,H(5)], 2.80 [1H,s,H(11)], 3.21-3.29 [1H,m,H(5')], 3.43-3.49 [1H,m,H(11')], 4.04-4.08 [2H,t,H(6) and H(12)].



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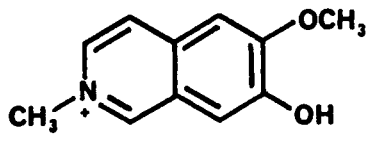

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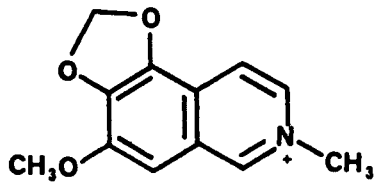

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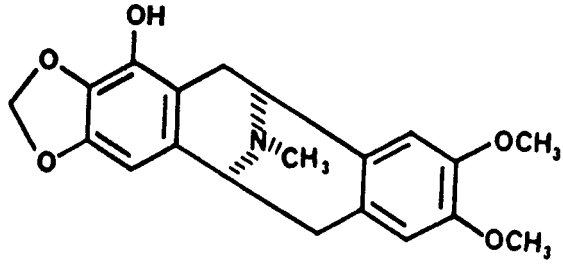
7.4.3. (-)-4-Hydroxyeschscholtzidine. UV [59], ^{1}H NMR[59], EIMS[59].

4-Hydroxyeschscholtzidine (**59**), $C_{20}H_{21}O_5N$ (355.1420), $[\alpha]_D^{25}$ -234° (c 0.09, $CHCl_3$), was isolated via column chromatography of the alkaloid fraction of an extract (MeOH) of the roots of *T. minus* L. ssp *majus* in 1992 [59]. The UV spectrum (EtOH) exhibited a single maximum at 287 nm. The EIMS showed the parent ion at m/z 355 (55%), with other intense fragment ions at 354 (25), 220 (60), and 188 (100). The ^{1}H NMR spectrum indicated the presence of one *N*-methyl group at δ2.74 (3H,s), two methoxy groups at δ3.83 (3H,s) and 3.84 (3H,s), six aliphatic protons at δ2.50-4.50 (m), one methylenedioxy group as a double doublet at δ5.86 and 5.90, and three aromatic protons as singlets at δ6.24, 6.46, and 6.61. The mass spectral fragment ions were similar to those of the parent alkaloid eschscholtzidine, and to other related pavine alkaloids, with the fragment ion at m/z 220 corresponding to an *N*-methylisoquinolinium ion bearing two methoxy groups and one hydroxy group, while that at m/z 188 corresponded to a similar ion bearing one methylenedioxy group. In order to determine the site of the hydroxy group, the proton chemical shifts in the aromatic regions of the ^{1}H NMR spectrum were determined in DMSO-d_6. It was observed that the signal at δ6.61 was shifted upfield by 0.70 ppm when the spectrum was obtained in DMSO-d_6 and base. The magnitude of

this shift is characteristic for an aromatic proton that is para- to a phenolic hydroxy group, and the presence of a positive Gibbs Test (for a phenol with a free para- position) further corroborated the observation. These data suggested the placement of the phenolic group at either C(1) or C(4). The placement of the hydroxy group at C(4) was postulated because of the paucity of benzylisoquinoline precursor alkaloids containing oxygen functional groups at C(8)(the carbon that corresponds to C(1) in the pavine ring). Two alternative biosynthetic pathways were proposed for the genesis of the pentaoxygenated 4-hydroxyeschscholtzidine as follows: benzylisoquinoline (reticuline) -> 5-hydroxyreticuline -> -> -> 4-hydroxyeschscholtzidine; or benzylisoquinoline (reticuline) -> isonorargemonine -> eschscholtzidine -> 4-hydroxyeschscholtzidine [59].



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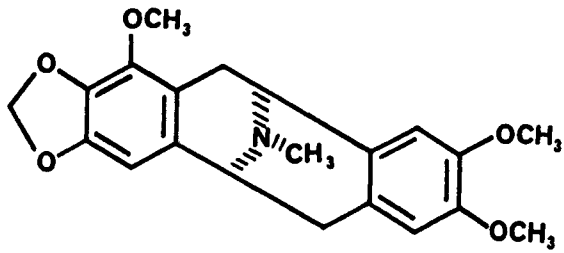
7.4.4. (-)-Thalimonine. UV [117], IR [117], ^{1}H NMR [117], ^{13}C NMR [117], EIMS [117], CD [117].

Thalimonine (**53**), $C_{21}H_{23}O_5N$ (369.1576), $[\alpha]_D^{22}$ -118° (c 0.20, MeOH), was isolated via preparative TLC of the alkaloid fraction of an extract (EtOH) of the aerial parts of *T. simplex* L. in 1991 [117]. The UV spectrum (EtOH) exhibited a single maximum at 287 nm (log ε 3.84), while the CD spectrum was characterized by extrema at Δε +0.16 (294), -0.46 (287), +1.94 (274), +0.47 (247), +8.10 (220), and -43.16 (206). The IR spectrum was typified by absorption maxima at 3009, 2962, 2872, 1600, 1517, 1465, 1450, 1373, and 1172 cm^{-1}. The EIMS displayed a parent ion at m/z 369 (67%), with other intense fragment ions at 368 (23), 204 (100), and 218 (63). The ^{1}H NMR spectrum was characterized by four singlets (3H each) suggesting the presence of one *N*-methyl group (δ2.53) and three methoxy groups (δ3.78 [C(9)], 3.84 [C(8)], and 3.86 [C(2)]). In addition, the presence of one methylenedioxy function was indicated by the appearance of a two-proton doublet at δ5.88 (J=0.98 Hz), as well as three aromatic protons as singlets at δ6.31 [H(1)], 6.45 [H(10)], and 6.61 [H(7)]. Finally, a series of aliphatic protons appeared as signals from δ2.56 to δ4.03 as follows: δ2.56 (s,1H)[H(11_β)], 2.63 (s,1H)[H(5_β)], 3.20 (dd,1H)[H(5_α)], 3.41 (dd,1H)[H(11_α)], 4.03 (m,2H)[H(6) and H(12)]. These data were similar to those reported for (-)-2,3-methylenedioxy-4,8,9-trimethoxypavinane (**60**), an alkaloid

isolated from extracts of *Thalictrum strictum* Ledeb. in 1976 [118]. The m/z 218 and 204 mass spectral fragment ions of thalimonine (**53**) were characteristic of pavine alkaloid fragmentation and could be represented by isoquinolinium ions **58** and **55**, respectively. In order to establish the positions of the varying groups in thalimonine, a series of nOe experiments were undertaken. Irradiation of the C(2) methoxy group (δ3.86) or the H(1) proton (δ6.31) produced a strong nOe between these groups, while irradiation of the aromatic singlet at δ6.45 [H(10)] effected the C(9) methoxy signal at δ3.78. In addition, irradiation of the C(8) methoxy group at δ3.84 produced a nOe on the H(7) aromatic proton singlet at δ6.61, while irradiation of the H(6)/H(12) multiplet at δ4.03 effected the H(1) and H(7) proton singlets at δ6.31 and 6.61, respectively. Irradiation of aromatic proton singlet at H(7)(δ6.61) produced a nOe effect on the C(8) methoxy singlet (δ3.84), as well as the H(6)/H(12) multiplet at δ4.03, while a similar irradiation of the aromatic proton singlet at δ6.31 [H(1)] effected the multiplet at δ4.03 [H(6) and H(12)] and the methoxy singlet at δ3.86 [C(2)]. As no authentic sample of the earlier isolated alkaloid [118] whose structure was assigned as **60** was available, the authors were not certain as to whether thalimonine was truly a new alkaloid, or simply represented a reisolation and subsequent correction of structure for this previously isolated base. In any event, thalimonine was characterized as a new alkaloid [(-)-3,4-methylenedioxy-2,8,9-trimethoxypavinane] and assigned as **53**. The structural assignment was supported by the following ^{13}C NMR spectral data ($CDCl_3$), with corresponding assignments: 106.3 [C(1)], 146.3 [C(2)], 142.3 [C(3) and C(4)], 132.5 [C(4a)], 27.6 [C(5)], 56.4 [C(6)], 129.8 [C(6a) and C(12a)], 110.4 [C(7)], 147.8 [C(8)], 148.2 [C(9)], 111.3 [C(10)], 123.9 [C(10a)], 34.2 [C(11)], 56.7 [C(12)], 55.9 [C(2)OMe], 55.4 [C(8)OMe], 55.7 C(9)OMe], 101.3 [OCH_2O], 40.8 [NMe].



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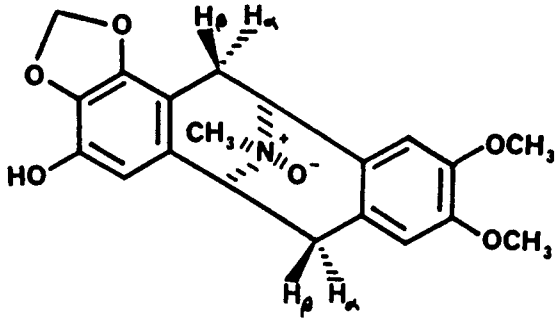
7.4.5. (-)-Thalimonine *N*-Oxides A and B. UV [107], IR [107], ^1H NMR [107], ^{13}C NMR [107], EIMS [107], CIMS [107], CD [107].

Thalimonine *N*-oxide A (**61**) [$C_{21}H_{23}O_6N$ (385.1524), $[\alpha]_D^{21}$ -53.3° (c 0.05, MeOH)] was isolated, along with its epimeric *N*-oxide thalimonine *N*-oxide B (**62**) [$C_{21}H_{23}O_6N$ (385.1524), $[\alpha]_D^{21}$ -80.0° (c 0.02, MeOH)], from a polar column chromatographic fraction of the alkaloid

fraction of a defatted extract (EtOH) of the aerial parts of *T. simplex* L. in 1995 [107]. Workup of this polar fraction via size-exclusion chromatography, followed by preparative reverse-phase HPLC, resulted in the isolation of two epimeric *N*-oxides, designated thalimonine *N*-oxide A (**61**) and thalimonine *N*-oxide B (**62**). The UV spectrum of epimer A displayed maxima at 251 nm (log ε 3.27) and 284 (3.28), while the CD spectrum exhibited extrema at Δε +0.10 (294), -0.14 (288), +1.61 (276), +0.45 (261)(sh), -8.12 (236), +15.22 (218) and -39.70 (205). The UV spectrum of epimer B was similar, and displayed maxima at 246 nm (log ε 3.34) and 277 (3.34), while the CD spectrum exhibited extrema at Δε +0.05 (294), -0.23 (287), +1.17 (274), +0.32 (262)(sh), -8.86 (237), +11.47 (219) and -36.25 (206). The IR spectra of these epimers were similar in all absorption bands with the exception of a band at 1080 cm^{-1} in the spectrum of epimer A (**61**), which appeared in at 1090 cm^{-1} in the spectrum of epimer B (**62**). Since both of these absorption bands were absent from the IR spectra of (-)-thalimonine (**53**), (-)-2-demethylthalimonine (**52**), and (-)-9-demethylthalimonine (**56**), these two bands were specifically assigned to epimer A and epimer B, respectively. Other absorption bands reported in both the IR spectra of epimer A and epimer B include the following: 3000, 2920, 2845, 1730, 1650, 1610, 1519, 1465, 1345, 1250, 1135, 1055, 1000, 980, 905, 870, 850, and 820 cm^{-1}. The EIMS of both epimers were very similar, with only slight differences in intensity of certain fragment ions, and were characterized by weak parent ions at m/z 385 (2-3%). Other fragment ions resemble those found in the spectrum of thalimonine (**53**), and include ions at m/z 383 (3-4%)(M-2)(dehydrogenation product), 370 (5-6%), 369 (21), 368 (9-12), 355 (6-7), 354 (8), 218 (48-53), 205 (13), 204 (100), 191 (5-10), 190 (29-32), 188 (6-8), 77 (10-13), 57 (6-11), and 55 (6-11). The CIMS of both epimers showed $M+H^+$ at m/z 386 (1-6%), with other common ions at m/z 371 (7-18%), and 370 (100).

The ^1H NMR spectra of these epimers was likewise very similar to that of the parent thalimonine (**53**), but there were notable differences that proved to be diagnostic. First, there was an obvious and characteristic downfield shift of the *N*-methyl absorption in the *N*-oxides (δ3.39 [*N*-oxide A] and δ3.45 [*N*-oxide B]) in comparison with the parent base thalimonine (δ2.53). Second, the two sets of aliphatic AMX spin systems [H(5a), H(5b), H(6), H(11a), H(11b), H(12)] were noticeably shifted, with the different downfield shifts between the H(5b) and H(11b) protons of epimers A and B being used to infer the influence of the neighboring oxide oxygen atom, and allowing the supposition of the relative configuration of the nitrogen atom in these epimers. The following chemical shifts were observed and assigned for those protons that were part of this aliphatic AMX system: H(5a) -[(δ3.33, d, J = 17.2 Hz, *N*-oxide A) and (δ2.87, d, J = 16.3 Hz, *N*-oxide B)] as compared with thalimonine [δ2.87, s]; H(5b) -[(δ3.10, d, J = 5.9 Hz, *N*-oxide A) and (δ3.95, dd, J = 5.8, 16.3 Hz, *N*-oxide B)] as compared with thalimonine [δ3.20, dd, J = 5.9, 16.5 Hz]; H(6) - [(δ4.65, d, J = 5.7 Hz, *N*-oxide A) and (δ4.97, t, 16.3 Hz, *N*-oxide B)] as compared with thalimonine [δ4.03, t]; H(11a) - [(δ2.73, d, J = 16.0 Hz, *N*-oxide A) and (δ3.19, d, J = 17.5 Hz, *N*-oxide B)] as compared with thalimonine [δ2.56, s]; H(11b) - [(δ4.24, dd, J = 5.8, 16.0 Hz, *N*-oxide A) and (δ3.61, dd, J = 6.1, 17.5 Hz, *N*-oxide B)] as compared with thalimonine [δ3.41, dd, J = 5.9, 16.2 Hz]; H(12) - [(δ4.54, d, J = 5.7 Hz, *N*-oxide A) and (δ4.91,

dd, 6.8, 16.3 Hz, *N*-oxide B)] as compared with thalimonine [δ4.03, t]. Other chemical shift assignments for these alkaloids are as follows: H(1) - [(δ6.37, s, *N*-oxide A) and (δ6.34, s, *N*-oxide B)] as compared with thalimonine [δ6.32, s]; H(7) - [(δ6.61, s, *N*-oxide A) and (δ6.63, s, *N*-oxide B)] as compared with thalimonine [δ6.61, s]; H(10) - [(δ6.53, s, *N*-oxide A) and (δ6.50, s, *N*-oxide B)] as compared with thalimonine [δ6.45, s]; C(2)OMe - [(δ3.90, s, *N*-oxide A) and (δ3.89, s, *N*-oxide B)] as compared with thalimonine [δ3.86, s]; C(8)OMe - [(δ3.85, s, *N*-oxide A) and (δ3.86, s, *N*-oxide B)] as compared with thalimonine [δ3.84, s]; C(9)OMe - [(δ3.80, s, *N*-oxide A) and (δ3.81, s, *N*-oxide B)] as compared with thalimonine [δ3.78, s]; C(3)+C(4)CH_2O_2 - [(δ5.95, dd, J = 1.4, 26.7 Hz, *N*-oxide A) and (δ5.91, dd, J = 1.4, 13.0 Hz, *N*-oxide B)] as compared with thalimonine [δ5.88, dd, J = 1.4, 23.2 Hz].



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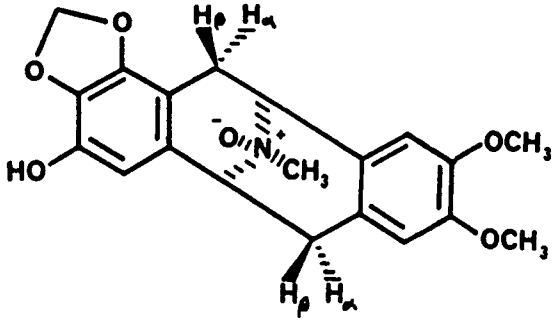

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A series of partial nOe experiments were undertaken in order to confirm the assignment of structure of the two oxides. In particular, irradiation of *N*-methyl group of epimer A produced

an enhancement of the H(5b), H(6), and H(12) protons, while irradiation of H(7) affected an enhancement of the adjacent H(5a) and H(6) aliphatic protons. With regard to epimer B, irradiation of the *N*-methyl group produced an enhancement of the H(6), H(11b), and H(12) protons, while irradiation of H(11a) afforded an enhancement of H(1) and H(10), as well as demonstrating strong coupling with H(11b). By contrast, irradiation of H(12) affected H(1) and the *N*-methyl group, but produced a reversed nOe on H(11b). The ^{13}C NMR spectrum of the epimers demonstrated the anticipated downfield shifts for *N*-methyl carbon atoms, as a result of the *N*-O dipole associated with *N*-oxides. In addition, downfield shifts involving the methine C(6) and C(12) atoms, as well as the C(5) and C(11) methylene atoms were also observed. The complete ^{13}C NMR chemical shift data for the oxides and the parent thalimonine (**53**) were assigned as follows: C(1) - [(δ106.0, *N*-oxide A); (δ106.2, *N*-oxide B); (δ106.3, thalimonine)]; C(2) - [(146.2, *N*-oxide A); (146.1, *N*-oxide B); (146.3, thalimonine)]; C(3) - [(143.4, *N*-oxide A); (143.2, *N*-oxide B); (142.3, thalimonine)]; C(4) - [(142.9, *N*-oxide A); (142.2, *N*-oxide B); (142.3, thalimonine)]; C(4a) - [(135.0, *N*-oxide A); (135.5, *N*-oxide B); (132.5, thalimonine)]; C(5) - [(31.5, *N*-oxide A); (29.9, *N*-oxide B); (27.6, thalimonine)]; C(6) - [(69.9, *N*-oxide A); (70.0, *N*-oxide B); (56.4, thalimonine)]; C(6a) - [(δ126.0, *N*-oxide A); (δ124.4, *N*-oxide B); (δ129.8, thalimonine)]; C(7) - [(111.1, *N*-oxide A); (111.0, *N*-oxide B); (110.4, thalimonine)]; C(8) - [(148.2, *N*-oxide A); (147.9, *N*-oxide B); (147.8, thalimonine)]; C(9) - [(148.6, *N*-oxide A); (148.1, *N*-oxide B); (148.2, thalimonine)]; C(10) - [(110.0, *N*-oxide A); (110.0, *N*-oxide B); (111.3, thalimonine)]; C(10a) - [(121.4, *N*-oxide A); (121.5, *N*-oxide B); (123.9, thalimonine)]; C(11) - [(35.0, *N*-oxide A); (35.7, *N*-oxide B); (34.2, thalimonine)]; C(12) - [(70.6, *N*-oxide A); (69.8, *N*-oxide B); (56.7, thalimonine)]; C(12a) - [(129.6, *N*-oxide A); (129.6, *N*-oxide B); (129.8, thalimonine)]; C(2)OMe - [(56.7, *N*-oxide A); (56.5, *N*-oxide B); (55.9, thalimonine)]; C(8)OMe - [(56.1, *N*-oxide A); (56.0, *N*-oxide B); (55.4, thalimonine)]; C(9)OMe - [(55.8, *N*-oxide A); (55.8, *N*-oxide B); (55.7, thalimonine)]; C(3)+C(4)CH_2O_2 - [(101.8, *N*-oxide A); (101.5, *N*-oxide B); (101.3, thalimonine)]; NMe - [(76.6, *N*-oxide A); (77.0, *N*-oxide B); (40.8, thalimonine)].

Authentic samples of the *N*-oxides were prepared via treatment of a solution of (-)-thalimonine (**53**) in EtOH with H_2O_2. The solution was stirred for 48 hours at 4°, treated with K_2CO_3, and stirred for the next 24 hours at 4°, then for 4 days at 22°. A subsequent experiment varied the conditions by stirring for only 1 hour at 4°, while all subsequent oxidative procedures were performed at 22° over 7 days. It was found that only 66% of the alkaloid was oxidized over a period of week, regardless of temperature, thereby prompting the observation that pavine oxidation at the *N*-atom is a difficult and time-consuming process, and thus likely excluding the artefactual formation of pavine *N*-oxides during extraction and workup processes. In this experiment, 16 mg of thalimonine was oxidized, affording 9.6 mg of *N*-oxides, which were separated via preparative HPLC, and leaving 5.5 mg of unoxidized free base.

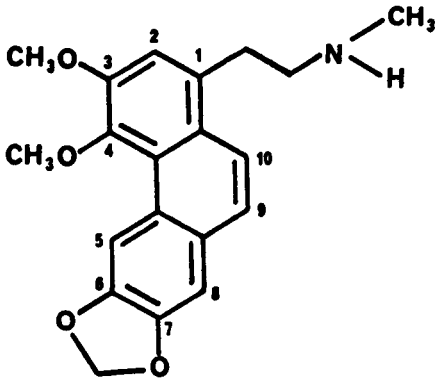
7.5. Phenanthrenes

The numbering of the phenanthrene ring in the following alkaloids is according to accepted practice [1,102-106], and is illustrated in the structure of northalicthuberine (**63**).

7.5.1. Northalicthuberine.

IR [119], ^{1}H NMR [119], EIMS [119].

Northalicthuberine (**63**), $C_{20}H_{21}O_4N$ (339.1470), was isolated from an extract (EtOH) of the aerial parts of *T. simplex* L. in 1993, along with the known phenanthrene alkaloids thalicthuberine (**66**) and thalicthuberine-*N*-oxide (**65**)[119]. The IR spectrum (KBr) of **63** displayed an absorption band at 3418 cm^{-1} that was characteristic of the stretching of a *N*H group, as well as other bands at 2925, 2853, 1716, 1600, 1508, 1465, 1421, 1384, 1367, 1288, 1272, 1126, 1104, 1065, 1039, 982, 946, 857, 809, 761, 701, and 630 cm^{-1}. The EIMS was very characteristic of phenanthrenes, displaying the parent ion at m/z 339 (92%), and other important fragment ions at m/z 338 (88)(M-H), 324 (100)(M-Me), 308 (31)(M-31)(M-OMe), 295 (75)(M-44)(M-CH_2=$NHCH_3$), 281 (38)(M-58)(M-$CH_2CH_2NHCH_3$), 265 (25), 58 (96), and 44 (67)[1,102-106]. The ^{1}H NMR spectrum resembled thalicthuberine (**64**), but lacked one of the *N*Me functions, and displayed signals at δ2.45 (s,3H)[NMe], 2.73 (m,2H)[αCH_2], 3.30 (m,2H)[βCH_2], 3.89 (s,3H)[C(4)OMe], 4.02 (s,3H)[C(3)OMe], 6.10 (s,2H)[C(6)-C(7) CH_2O_2], 7.19 (s,2H)[H(2) and H(8)], 7.51 (d,1H,J=9.2 Hz)[H(9)], 7.75 (d,1H,J=9.2 Hz)[H(10)], and 9.15 (s,1H)[H(5)]. The alkaloid was thus assigned as **63** based on the EIMS and ^{1}H NMR spectrum. No UV nor ^{13}C NMR spectra were reported for this base.



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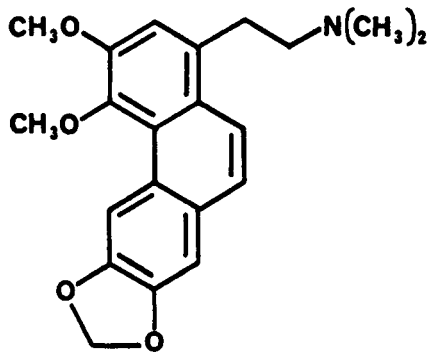

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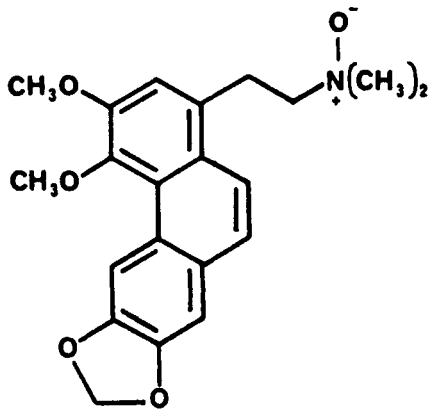
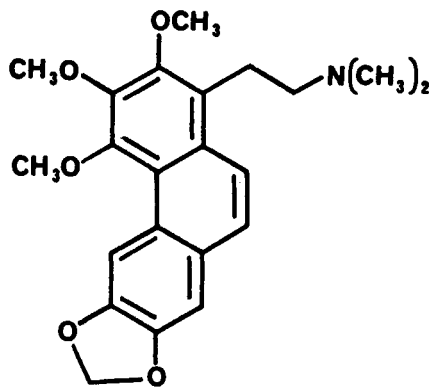


65



66

7.5.2. Thalihazine. UV [57], ^{1}H NMR [57], EIMS [57].

Thalihazine (**66**), $C_{22}H_{25}O_5N$ (383.1733), was isolated (along with the phenanthrene alkaloid thalicthuberine (**64**) and the aporphine alkaloid *N*-methyllaurotetanine) by preparative TLC of fractions obtained by column chromatography of the alkaloid fraction of an extract (EtOH) the whole plant of *T. hazarica* R.R.S. in 1987 [57]. The UV spectrum of the alkaloid showed maxima at 261 nm (log ε 4.30), 283 (3.77), 315 (3.37), and 344 (2.90) [1,102-106], while the EIMS was characterized by the facile loss of the dimethylaminomethylene ion (CH_2=$N^+(CH_3)_2$)(m/z 58), and displayed the parent ion at m/z 383 (7%), with other significant fragment ions at m/z 325 (5%)(M-58)(M-CH_2=$N(CH_3)_2$), 310 (1), 295 (2), 280 (1), 267 (3), and 58 (100). These spectral data were characteristic of phenanthrenes [1,102-106], and prior to the ^{1}H NMR spectral characterization of thalihazine (**66**), the authors undertook a complete nOe study on thalicthuberine (**64**), the companion alkaloid that had been isolated along with thalihazine. Significant and/or strong nOes were detected among various protons, allowing the following unambiguous assignments to be made for thalicthuberine (**64**): δ2.40 (s,6H)[$N(Me)_2$], 2.66 (m,2H)[αCH_2], 3.25 (m,2H)[βCH_2], 3.90 (s,3H)[C(4)OMe], 4.03 (s,3H)[C(3)OMe], 6.10 (s,2H)[C(6)-C(7) CH_2O_2], 7.19 (s,1H)[H(2)], 7.51 (d,1H,J=9.1 Hz)[H(9)], 7.76 (d,1H,J=9.1 Hz)[H(10)], and 9.16 (s,1H)[H(5)]. The ^{1}H NMR spectrum of thalihazine (**66**) was very similar to that of thalicthuberine (**64**), differing only by the presence of one additional methoxy group and the absence of one aromatic proton. A detailed nOe study permitted the unequivocal assignment of the ^{1}H NMR proton spectrum of thalihazine (**66**) as follows: δ2.45 (s,6H)[$N(Me)_2$], 2.59 (m,2H)[αCH_2], 3.31 (m,2H)[βCH_2], 3.96 (s,3H)[C(4)OMe], 4.00 (s,3H)[C(2)OMe], 4.06 (s,3H)[C(3)OMe], 6.10 (s,2H)[C(6)-C(7) CH_2O_2], 7.59 (d,1H,J=9.1

Hz)[H(9)], 7.80 (d,1H,J=9.1 Hz)[H(10)], and 9.06 (s,1H)[H(5)]. Four interesting observations were made by the authors following this study. First, the most downfield methoxy signal in the ^{1}H NMR spectrum of the two phenanthrene alkaloids cited in this paper corresponded to the C(3) methoxy, that methoxy that lay between the other two. The same was noted for aporphines bearing three methoxy groups in ring A [102-106]. Second, with regard to the ^{1}H NMR spectra of thalihazine (**68**) and aporphines, it was noted that whenever a methoxy group was present at the C(3) position of an aporphine, the H(11) proton signal shifts upfield by about 0.1 ppm [4]. In the case of thalihazine (**66**), the signal for the H(5) proton appears at δ9.06, some 0.1 ppm higher that the identical proton in thalicthuberine (**64**) which is observed at δ9.16. Third, it has been observed that aporphines that are unsubstituted at C(11) are usually more likely to undergo Hofmann β-elimination of their *N*-metho salt derivatives than are those corresponding aporphine *N*-metho salts in aporphines unsubstituted at C(11)[120]. A literature examination revealed that the classical phenanthrene-type alkaloids are typically unsubstituted at the C(5) position [the C(11) aporphine position][102-106,120]. The same observation may be made for thalihazine (**66**), which is unsubstituted at the corresponding C(5) position. Fourth, it was previously noted that the genus *Thalictrum* has a proclivity for the production of trioxygenated ring A aporphinoids [1,121], and the isolation of thalihazine (**66**) is one a further example of this phenomenon.

7.6. Diterpenes

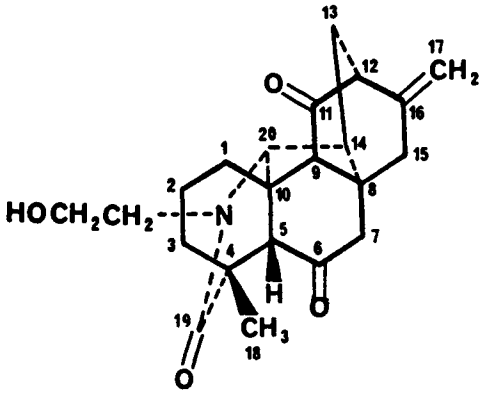
The numbering of the diterpene ring in the following alkaloids is illustrated in the structure of thalicessine (**67**).

7.6.1. (+)-Thalicessine. UV [54], IR [54,79], ^{1}H NMR [54,79], ^{13}C NMR [54,79], EIMS [54,79], CD [54].

Thalicessine (**67**), $C_{22}H_{27}O_4N$ (369.1940), mp 213-216°C (MeOH), $[\alpha]_D^{25}$ +113° (c 0.2, $CHCl_3$), was isolated as colorless prisms from an extract (EtOH) of the roots of *T. sessile* Hayata in 1987 [54,79]. An empirical formula of $C_{22}H_{27}O_4N$ was determined via a consideration of the EIMS parent ion (m/z 369), as well as the results of elemental analysis. The UV spectrum was characterized by a single maximum at 308 nm (log ε 1.65). A consideration of pertinent IR (nujol), ^{1}H NMR, and ^{13}C NMR spectral data suggested that the alkaloid was a diterpenoid compound containing the following functionality: two ketone carbonyl groups (ν 1710 cm^{-1}; δ 207.6 (s) and 208.9 (s) ppm); one lactam carbonyl group (ν 1630 cm^{-1}; δ 177.1 (s) ppm); one exocyclic methylene group (ν 1620 and 890 cm^{-1}; δ4.85 (d, 1H, J = 2.40 Hz) and 5.02 (d, 1H, J = 2.40 Hz); δ111.1 (t) and 141.9 (s) ppm); one methyl group (δ1.50 (s, 3H); 25.5 (q) ppm); and one hydroxyethyl group attached to nitrogen (ν 3350 cm^{-1}; δ2.85 (t,1H,J=5.12 Hz, lost on addition of D_2O). Other signals were observed at δ3.45 (1H, ddd, J = 14.16,5.13,3.41 Hz), 3.62

(1H, ddd, J = 14.16,8.05,3.41 Hz), 3.78 (1H , m, changed to ddd [J = 11.48, 5.13, 3.41 Hz] on addition of D_2O), 3.88 (1H, m, changed to ddd (J = 11.48, 8.05, 3.41 Hz) on addition of D_2O; and δ49.7 (t) and 60.9 (t). These spectral data, particularly the ^{13}C NMR spectrum, were very similar to the diketone **68**, a derivative of the diterpene alkaloid spiradine A (**69**)[122]. The notable differences between the spectra of diketone **68** and thalicessine (**67**) existed in the presence of the hydroxyethyl and lactam groups in the latter. The placement of one of the carbonyl groups of thalicessine (**67**) at C(6) was made via a consideration of the nature of the products formed by successive treatment of the alkaloid with NaOD/dioxane to form a trideuterio-product **70**, followed by reduction ($NaBH_4$/THF) and then acetylation (Ac_2O/pyr) to **71**. The trideuterio- compound **71** failed to demonstrate ^{13}C NMR spectral signals corresponding to those at δ51.5 (t)[C(7)] and 60.0 (d)[C(5)] in thalicessine (**67**), and was characterized by a mass spectral parent ion at m/z 372 ($C_{22}H_{24}D_3O_4N$). The reduced acetylated product **71** (M^+ m/z 458 [$C_{26}H_{30}D_3O_6N$]) was characterized by a ^1H NMR spectrum that showed a singlet at δ4.18 [H(6)] in comparison with the ^1H NMR spectrum of the diaceate derivative **72** (prepared in the same manner as **71**, but without initial deuteration) that displayed a multiplet at δ4.20 [H(6)]. These data aided in the placement of one of the two carbonyl groups at C(6). The second carbonyl group was placed at C(11) for at least two reasons [79]. First, thalicessine (**67**) and spiradine A (**69**) were both isolated from the same extract, and since spiradine A possessed both an aminoacetal group at C(6) and a carbonyl group at C(11), it was reasoned that this was logical precedent. Second, a compound that is deuterized at C(9) or C(12) could not be obtained and be in accord with Bredt's Rule. In a subsequent paper [54], the authors presented additional arguments for the placement of the carbonyl group at this position via the citation of the occurrence of a single UV spectral maximum at 308 nm (characteristic of that for a β,γ-unsaturated ketone), as well as the CD spectral Cotton effects at 314 nm (Δε +1.32) and 276 (Δε -0.75). These characteristics were also noted to be common in other diterpenes of the spirasine series [54]. Finally, the proposed structure of thalicessine as **67** was confirmed by ^1H-^1H and ^1H-^{13}C correlated spectroscopy (COSY). This paper is particularly significant, in that it represents the first reported isolation of diterpenoid alkaloids from the genus *Thalictrum*.

Complete ^{13}C NMR spectral assignments for the new spiradine-type diterpenoid alkaloid thalicessine (**67**) and spiradine A-derived ketone **68** follow: Thalicessine (**67**): δ39.8 (t)[C(1)], 20.6 (t)[C(2)], 34.2 (t)[C(3)], 46.5 (s)[C(4)], 60.0 (d)[C(5)], 207.6 (s) or 208.9 (s)[C(6)], 51.5 (t)[C(7)], 43.9 (s)[C(8)], 75.6 (d)[C(9)], 42.9 (s)[C(10)], 208.9 (s) or 207.6 (s)[C(11)], 63.7 (d)[C(12)], 33.3 (t)[C(13)], 47.0 (d)[C(14)], 35.1 (t)[C(15)], 141.9 (s)[C(16)], 111.1 (t)[C(17)], 25.5 (q)[C(18)], 177.1 (s)[C(19)], 53.9 (d)[C(20)], 49.7 (t)[NCH_2CH_2OH], 60.9 (t)[NCH_2CH_2OH]; Spiradine A-derived ketone **68**: δ40.6 (t)[C(1)], 18.7 (t)[C(2)], 30.2 (t)[C(3)], 47.0 (s)[C(4)], 60.3 (d)[C(5)], 204.0 (s) or 211.1 (s)[C(6)], 50.7 (t)[C(7)], 43.0 (s)[C(8)], 78.2 (d)[C(9)], 38.1 (s)[C(10)], 211.1 (s) or 204.0 (s)[C(11)], 65.3 (d)[C(12)], 33.6 (t)[C(13)], 45.6 (d)[C(14)], 35.1 (t)[C(15)], 143.5 (s)[C(16)], 110.2 (t)[C(17)], 30.7 (q)[C(18)], 61.0 (t)[C(19)], 53.4 (d)[C(20)], 43.1 (q)[NCH_3].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5
6
7
H
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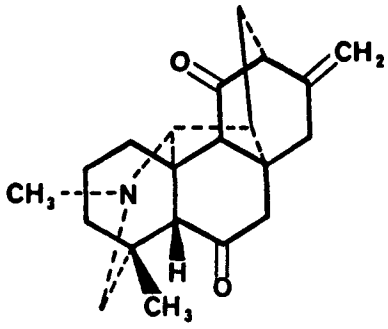
O

67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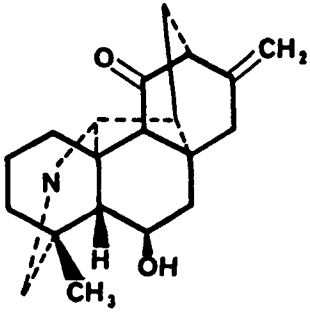
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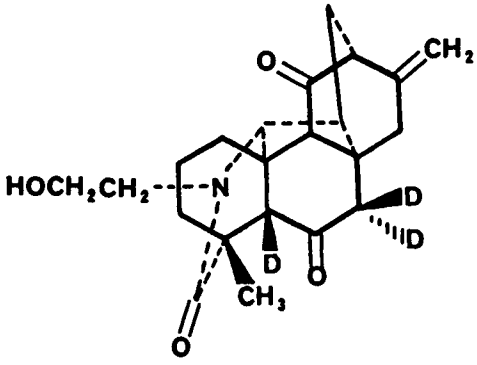
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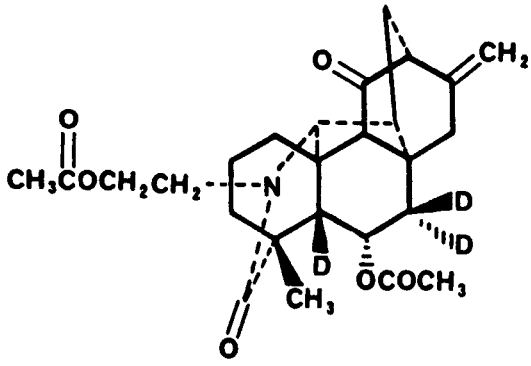
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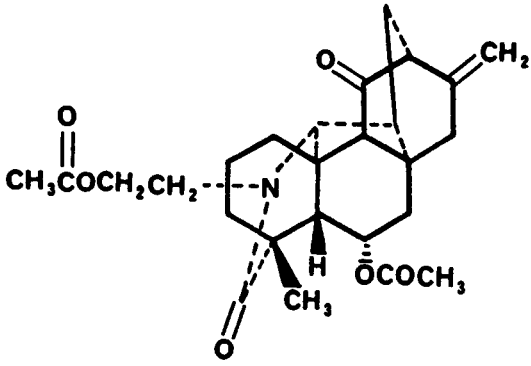
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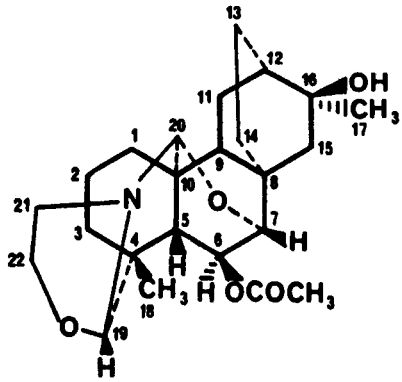
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4
6
H
H
22
CH3
18
H
OCOCH3
O
19
H

73

7.6.2. (-)-Thalicsiline. IR [54,123], ^{1}H NMR [54,123], ^{13}C NMR [54,123], EIMS [54,123], X-RAY [54,123].

Thalicsiline (**73**), $C_{24}H_{35}O_5N$ (417.2515), mp 183-186°C (MeOH), $[\alpha]_D^{20}$ -11.4° (c 0.1, MeOH), was isolated, along with thalicessine (**67**) [54,79] and five other spiradine-type alkaloids, as colorless prisms from an extract (EtOH) of the roots of *T. sessile* Hayata in 1987 [54,123]. The IR spectrum (nujol) exhibited bands at 3540 (-OH), 1720 and 1240 (CH_3CO-), and 1100 (ether) cm^{-1}, while the high resolution EIMS was characterized by a parent ion at m/z 417.2481 (417.2515, calculated for $C_{24}H_{35}O_5N$). An examination of the ^{13}C NMR spectral data and comparison with literature values [124,125] suggested the presence of an ajaconine-type [C(7)-C(20) carbinolamine ether type linkage) diterpene skeleton. A single-crystal X-ray analysis revealed the complete structure and relative stereochemistry of thalicsiline (**73**). The ^{1}H NMR spectrum of the alkaloid was characterized by certain characteristic signals, and was in agreement with the specified structure. The presence of an oxazolidine ring system was demonstrated via the appearance of a five-proton multiplet between δ3.00 and 4.10, in which the proton at C(19) is observed as an epimeric pair of signals at δ4.11 (S) and 3.86 (R), existing in a ratio of approximately 1:3. This behavior is also common in the spirasine series of diterpenoids [126]. Additional epimeric pairs of signals (all in a 1:3 ratio) were observed and assigned as follows: δ5.32 and 5.67 (dd, J = 2.40 Hz)[H(6)], δ4.82 and 4.58 (s)[H(20)], δ2.05 and 2.06 (s)[C(6)-$COOCH_3$), δ1.31 and 1.30 (s)[H(17)], δ0.94 and 1.11 (s)[H(18)]. A complete ^{13}C NMR spectral assignment ($CDCl_3$) for the thalicsiline (**73**) was made as follows: δ40.54 (t)[C(1)], 22.70 (t)[C(2)], 47.21 (t)[C(3)], 35.16 (s)[C(4)], 52.18 (d)[C(5)], 70.79 (d)[C(6)], 70.91 (d)[C(7)], 36.27 (s)[C(8)], 42.53 (d)[C(9)], 35.28 (s)[C(10)], 29.02 (t)[C(11)], 38.26 (d)[C(12)], 26.68 (t)[C(13)], 23.52 (t)[C(14)], 20.24 (t)[C(15)], 73.83 (s)[C(16)], 30.19 (q)[C(17)], 22.58 (q)[C(18)], 96.40 (d)[C(19)], 85.59 (d)[C(20)], 51.01 (t)[C(21)], 63.30 (t)[C(22)], 169.72 (s)[C(23)], 21.41 (q)[C(24)].

Thalicsiline (**73**) is notable in that it is the first ajaconine-type alkaloid bearing an oxazolidine ring, as well as an oxygen function at C(6), and is only the second ajaconine-type alkaloid found in nature. In addition, thalicsiline is only the second diterpene alkaloid to have been isolated from the genus *Thalictrum*. Thalicsiline was observed to possess anti-inflammatory activity as measured by a 42% reduction in carrageenan-induced inflammation in the hindfoot of Sprague Dawley rats when the alkaloid was administered at an intraperitoneal dosage of 20 mg/kg x 2 [123,127].

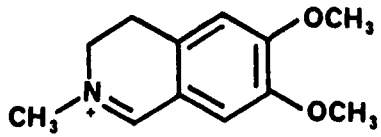
8. NEW ALKALOIDS - DIMERS

8.1. Aporphine-Benzylisoquinolines

The numbering of the aporphine-benzylisoquinoline ring in the following alkaloids is according to accepted practice [1,128,130,131], and is illustrated in the structure of thalibulamine (**13**).

8.1.1. (+)-Thalibulamine. Tabular Review [128, Alkaloid No. 67], UV [2,4,128], ^{1}H NMR [2,4,128], EIMS [2,4,128], CD [2,4,128].

Thalibulamine (**13**), $C_{40}H_{46}O_8N_2$ (682.3254), $[\alpha]_D$ +63° (c 0.2, MeOH), was isolated along with eleven other aporphine-benzylisoquinoline dimeric alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1986 [2,4]. The UV spectrum was typical of that of an aporphine-benzylisoquinoline dimeric alkaloid and displayed maxima at 225 nm (log ε 4.79), 270 (sh)(4.29), 281 (4.42), 301 (4.31), and 314 (4.24), while the CD spectrum exhibited extrema at Δε (nm) 0 (320), -5.6 (300), -8.0 (272), 0 (255), +80 (239), with a negative tail below 230 nm. The EIMS was characterized by the molecular ion at m/z 682 (0.07%), with other fragment ions at m/z 681 (0.2%), 680 (0.3), 476 (1), 475 (2), 206 (**74**)(100), and 190 (4). The ^{1}H NMR spectrum was very similar to that of istanbulamine (**75**), save for the appearance of an extra upfield methoxy signal at δ3.60, while the EIMS parent ion and base peak were both 14 amu higher than those for istanbulamine, suggesting the likelihood that thalibulamine was the *O*-methyl ether of istanbulamine. The structure of thalibulamine as **13** became evident upon an examination of the entire ^{1}H NMR spectrum that displayed signals for two *N*-methyl groups as singlets at δ2.29 [*N*(6) or *N*(2')] and 2.51 [*N*(2') or *N*(6)]; six methoxy groups as singlets at δ3.60 [C(7')], 3.75 [C(1)], 3.81 [C(6')], 3.89 [C(3)], 3.91 [C(10)], and 3.96 [C(2)]; and seven aromatic protons at δ6.06 [s, 1H, H(8')], 6.52 [s, 1H, H(5')], 6.74 [dd, 1H, J = 2,8.2 Hz, H(14')], 6.82 [br s, 2H, H(10') and H(8)], and 6.91 (d, 1H, J = 8.2 Hz, H(13')]. The H(1') proton was found as a multiplet at δ3.72.

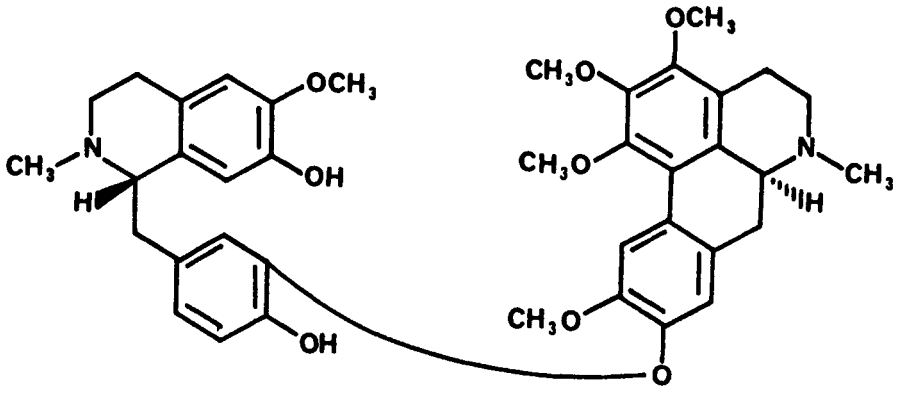


8.1.2. (+)-Thalifaberidine. UV [129], ^{1}H NMR [129], ^{13}C NMR [129], EIMS [129].

Thalifaberidine (**76**), $C_{39}H_{44}O_8N_2$ (668.3097), $[\alpha]_D$ +87° (c 0.01, MeOH), was isolated as a yellow solid from the phenolic alkaloid fraction of an extract (EtOH) of the roots of *T. faberi* Ulbr. in 1994 [129]. The UV spectrum was characterized by two maxima at 282 nm (log ε 4.10) and 314 (sh)(3.90), while the EIMS displayed the base peak at m/z 192. The high resolution EIMS showed a M^+-H ion at m/z 667.3012 (667.3020, calculated for $C_{39}H_{43}O_8N_2$). The structural solution of thalifaberidine proceeded via a detailed ^{1}H NMR and ^{13}C NMR spectral investigation, and included such contemporary techniques as DEPT, COSY, ROESY, HETCOR, FLOCK, and selective INEPT. Features that were immediately apparent included the following: First, magnetic resonance spectra displayed signals that were characteristic of two *N*-methyl groups, five methoxy groups, two aliphatic methine protons, seven aromatic methine protons, six methylene carbon atoms, and sixteen quaternary carbon atoms, and were thus characteristic of a benzyisoquinoline-aporphine dimer with five methoxy groups and two phenolic hydroxy groups. Second, the presence of a lower field one proton signal at δ7.86, as well as an AA'BB' four aromatic proton pattern at δ6.77 and 6.93, were characteristic for members of the thalifaberine (**77**) group [1,128,130-132]. Third, the presence of the EIMS base peak at m/z 192 (**78**) and the high field aromatic proton singlet at δ5.92 (H-8') suggested that one of the phenolic groups should be located at in the benzylisoquinoline system at C-(6'), while the other phenolic hydroxy group should be in the aporphine system.

An examination of the ROESY spectrum showed nOe contours between H-11 and both the C(10) and C(1) methoxy groups, as well as between H(8') both the H(1') and H(α'B) protons. In addition, other spatial relationships were observed between H(1') and NMe(2'), as well as between H(10') and H(1'). Selective INEPT and FLOCK experiments were used to confirm these assignments, and to assign all of the quaternary carbon atoms. The utilization of these and related spectral correlations permitted the assignment of all of the chemical shift values. The ^{1}H NMR signals were assigned as follows: 2.84 (m, 1H, H(4A) where A is used to designate one of the geminal protons), 2.75 (m, 1H, H(4B) where B is used to designate one of the geminal protons), 2.99 [m, H(5A)], 2.35 [m, H(5B)], 2.28 [s, *N*(6)Me], 2.81 [m, H(6a)], 3.20 [dd, J = 4.0,14.0 Hz, H(7A)], 2.00 [t, 14.0 Hz, H(7B)], 7.86 [s, H(11)]; 3.63 [m, H(1')], 2.48 [s, *N*(2')Me], 3.13 [m, H(3'A)], 2.72 [m, H(3'B)], 2.71 [m, H(4'A)], 2.50 [m, H(4'B)], 6.51 [s, H(5')], 5.92 [s, H(8')], 3.08 [dd, J = 4.5, 13.5 Hz, H(α'A)], 2.78 [m, H(α'B)], 6.93 [d, J = 8.5 Hz, H(10')], 6.77 [d, J = 8.5 Hz, H(11')], 6.77 [d, J = 8.5 Hz, H(13')], 6.93 [d, J = 8.5 Hz, H(14')]; 3.76 [s, C(1)OMe], 3.95 [s, C(2)OMe], 3.86 [s, C(3)OMe], 3.92 [s, C(10)OMe], and 3.50 [s, C(7')OMe]. The ^{13}C NMR chemical shift assignments were as follows: 149.14 [C(1)], 122.36 [C(1a)], 130.81 [C(1b)], 145.11 [C(2)], 149.61 [C(3)], 122.03 [C(3a)], 23.41 [C(4)], 52.69 [C(5)], 43.70 [C(6)NMe], 62.33 [C(6a)], 26.38 [C(7)], 122.03 [C(7a)], 138.49 [C(8)], 137.99 [C(9)], 146.37 [C(10)], 107.85 [C(11)], 123.55 [C(12)]; 67.45 [C(1')], 43.03 [*N*(2')Me], 46.11 [C(3')], 24.52 [C(4')], 125.67 [C(4'a)], 114.38 [C(5')], 144.02 [C(6')], 144.25 [C(7')], 110.58 [C(8')], 127.48 [C(8'a)], 40.19 [C(α')], 133.00 [C(9')], 114.58 [C(10')], 130.84 [C(11')], 156.55

[C(12')], 130.84 [C(13')], 114.58 [C(14')]; 60.54 [C(1)OMe], 60.90 [C(2)OMe], 60.26 [C(3)OMe], 56.10 [C(10)OMe], 55.38 [C(7')OMe]. Thalifaberidine was thus assigned as 6',9-demethylthalifaberine (**76**).



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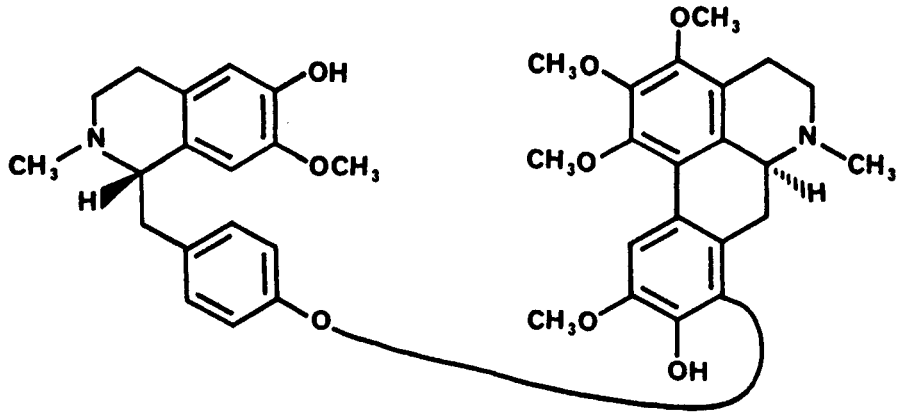

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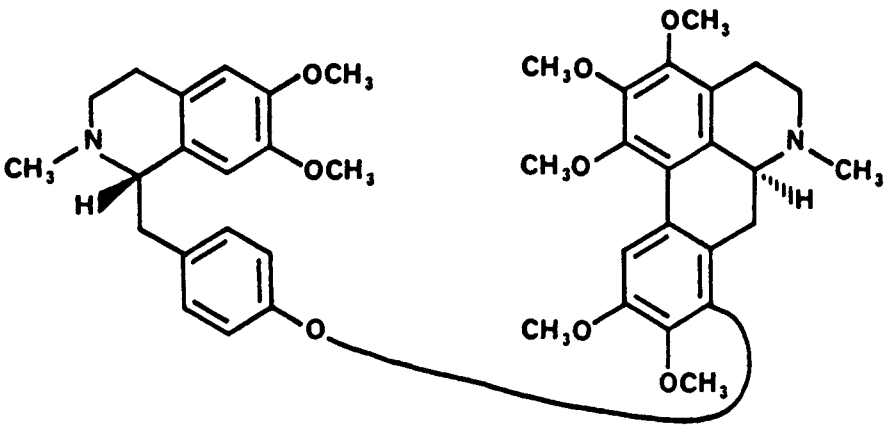

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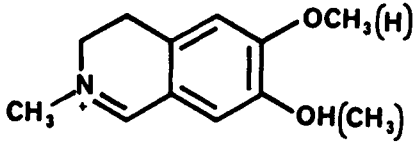

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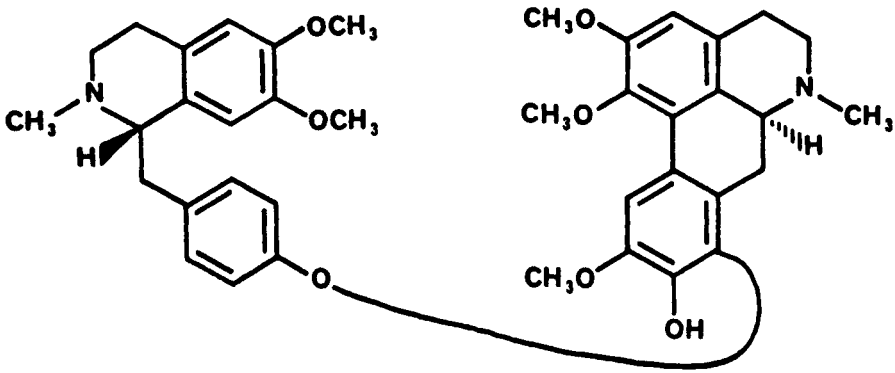
8.1.3. (+)-Thalifaboramine. Tabular Review [128, Alkaloid No. 69], UV [128,133], IR [128,133], ^{1}H NMR [128,133], EIMS [128,133], CD [128,133].

Thalifaboramine (**79**), $C_{39}H_{44}O_7N_2$ (652.3137), $[\alpha]_D$ +63° (c 0.2, MeOH), was isolated as a yellow amorphous solid after preparative TLC from selected fractions of an extract (EtOH) of the roots of *T. faberi* Ulbr. in 1987 [133]. The UV spectrum showed maxima at 283 nm (log ε 4.30) and 313 (sh)(3.98), with a bathochromic shift in alkali to 290 nm (log ε 4.14) and 330 (4.14)(hyperchromic shift), suggesting the phenolic nature of the alkaloid. The EIMS displayed a very small parent ion at m/z 652 (about 0.1%), with other important fragment ions at m/z 446 (M^+ - 206)(5%) and 206 (100%), the latter of which suggests the presence of isoquinolinium ion **74**, formed by the facile doubly benzylic cleavage of an benzylisoquinoline. The ^{1}H NMR spectrum was suggestive of a thalifaberine (**76**)-type alkaloid [1,128,130-132], and displayed the typical AA'BB' quartet that is characteristic of the H(10'), H(11'), H(13'), H(14') symmetrical protons of the benzyl-ring (ring C) of the benzylisoquinoline-portion of the alkaloid, leaving C(12') as the obvious terminus of the diarylether linkage to the aporphine-half. These data also

placed the phenolic group somewhere in the aporphine-portion of the molecule, and the 17 nm bathochromic shift in strong alkali, combined with notable hyperchromism, suggested the placement of the phenol at the C(3) or C(9) position [134]. Obvious significant features of the ^{1}H NMR spectrum included the presence of two *N*-methyl groups, five methoxy groups, four additional aromatic protons, and one phenolic group (D_2O exchangeable). The structure of thalifaboramine was firmly settled after a nOe difference study was undertaken. Most importantly, it was noted that there was a significant enhancement of the H(3) signal on irradiation of the C(2) methoxy group, serving to prove that the phenolic hydroxy group cannot be located at C(3). In addition, a significant enhancement of the H(11) signal by irradiation of the C(10) methoxy demonstrated that the diaryl ether terminus cannot be placed at C(10), and hence the phenolic group must be located at C(9). Other nOe enhancements included the H(3) onto the C(2) methoxy, the C(1) methoxy onto H(11), the H(5') onto the C(6') methoxy, the C(6') methoxy onto the H(5'), and the C(7') methoxy onto the H(8'). ^{1}H NMR chemical shift assignments are as follows: δ6.54 [s, H(3)], 2.26 [s, *N*(6)Me], 6.95 [s, H(9), D_2O exchanged), 7.97 [s, H(11)]; 2.54 [s, *N*(2')Me], 6.54 [s, H(5')], 5.89 [s, H(8')], 6.93 [d, 2H, J = 8.9 Hz, H(10') and H(14')], 6.73 [d, 2H, J = 8.9 Hz, H(11') and H(13')]; 3.63 [s, C(1)OMe], 3.84 [s, C(2)OMe], 3.87 [s, C(10)OMe], 3.81 [s, C(6')OMe], 3.41 [s, C(7')OMe. The CD curve of thalifaboramine (**79**) [Δε -3.29 (305), -3.99 (276), and +51.72 (243)] was very similar to that of thalifaberine (**77**)(*O*-methylthalifaboramine), and supported the assignment of the absolute configuration (**S,S**) for thalifaboramine (**79**).

Thalifaboramine is but one of over 16 new aporphine-benzylisoquinoline dimeric alkaloids that have been isolated from *Thalictrum faberi* Ulbr., and the crude basic fraction, as well as most of the new alkaloids, including thalifaboramine (**79**), have demonstrated cytotoxic effects against the P-388 carcinoma cell [133].



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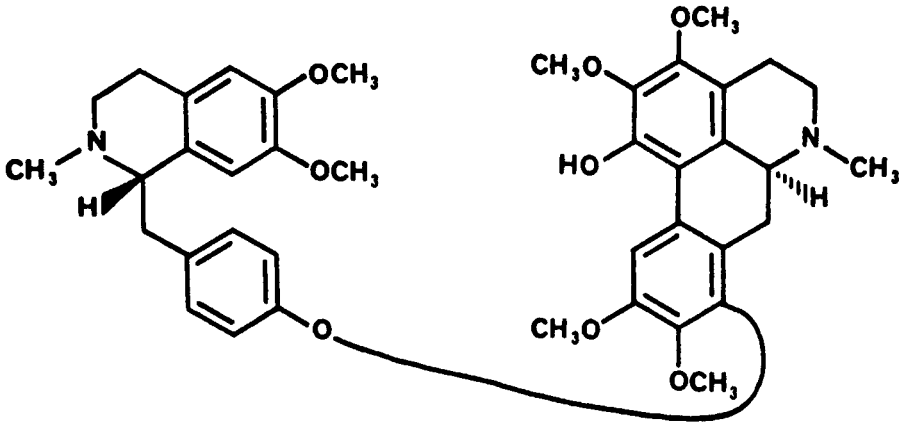
8.1.4. (+)-Thalifalandine. Tabular Review [128, Alkaloid No. 74], UV [83,128], IR [83,128], ^{1}H NMR [83,128], EIMS [83,128], CD [83,128].

Thalifalandine (**80**), $C_{40}H_{46}O_8N_2$ (682.3254), $[\alpha]_D^{14}$ +83.3° (c 0.375, MeOH), was isolated as an amorphous solid from an extract of the roots of *T. faberi* Ulbr. in 1986 [83]. The alkaloid displayed a UV spectrum with maxima at 205 nm (log ε 4.47), 225 (sh)(4.37), 285 (4.03), and 308 (sh)(3.89), with a bathochromic shift on the addition of strong alkali to 204 nm (log 4.37), 226 (sh)(4.30), 290 (3.83), 313 (3.95), and 325 (3.95). The IR spectrum showed an absorption at 3530 cm^{-1}. These data were characteristic of a phenolic aporphine-benzyltetrahydroisoquinoline dimeric alkaloid [1,128,130,131]. The ^{1}H NMR spectrum was similar to that of a thalifaberine (**77**)-type alkaloid [1,128,130-132], and displayed the typical AA'BB' quartet that is characteristic of the H(10'), H(11'), H(13'), H(14') symmetrical protons of the benzyl-portion of the benzylisoquinoline half of the alkaloid. The EIMS was characterized by the presence of a very small parent ion at m/z 682 (about 0.1%), and with other important fragment ions at m/z 476 (M^+ - 206) and 206 (100%). The base peak at m/z 206 (100%) may be represented by the isoquinolinium ion **74**, formed by the facile doubly benzylic cleavage of an benzylisoquinoline, and suggests the presence of two methoxy groups in the isoquinoline portion of the dimer. Treatment of thalifalandine with diazomethane afforded thalifaberine (**77**), thus fixing the skeletal framework of the new alkaloid, and establishing it as an *O*-demethylthalifaberine derivative. Furthermore, the CD curve of thalifalandine (**80**) [Δε -7.50 (304), -8.80 (278), and +74.97 (243.3)] was very similar to that of thalifaberine (**77**), thus permitting the assignment of an identical absolute configuration (**S,S**) for thalifalandine (**80**).

A comparison of thalifalandine (either directly or via spectral data) with thalifarapine (3-*O*-demethylthalifaberine) (**81**) demonstrated that the alkaloids were different, thereby eliminating the C(3) position as a potential site for the placement of the single phenolic hydroxy group. The position of this phenol in thalifalandine was established via a consideration of the complete ^{1}H NMR spectral data, including the results of nOe difference spectroscopy. Reciprocal nOe enhancements were observed between the following: the low field H(11)(δ7.79) and its neighboring methoxy group at C(10)(δ3.90); the high field H(8')(δ5.85) and its neighboring methoxy group at C(7')(δ3.49); the H(5')(δ6.55) and its neighboring methoxy group at C(6')(3.81). There was no significant nOe observed between H(11) and any other methoxy group except that at C(10), suggesting the absence of a methoxy group at C(1), and thus confirming the presence of the single phenolic group at C(1). Hence, thalifalandine was identified as 1-*O*-demethylthalifaberine, and assigned as **80**. The ^{1}H NMR chemical shift assignments are summarized as follows: δ2.65 [s, *N*(6)Me], 7.79 [s, H(11)]; 2.32 [s, *N*(2')Me], 6.55 [s, H(5')], 5.85 [s, H(8')], 6.95 [d, 2H, J = 8.9 Hz, H(10') and H(14')], 6.76 [d, 2H, J = 8.9 Hz, H(11') and H(13')]; 3.96 [s, C(2)OMe], 3.76 [s, 6H, C(3)OMe + C(9)OMe], 3.90 [s, C(10)OMe], 3.81 [s, C(6')OMe, 3.49 [s, C(7')OMe]. (Note: It is this author's observation that the authors of this paper have likely reversed the assignment of the *N*(2')- and *N*(6)- methyl groups, as there is

ample precedent for the assignment of the former at the lower field (about δ2.5-2.6), while the latter is at the higher field (δ2.3-2.4)[1,2,4,128,130-132]).



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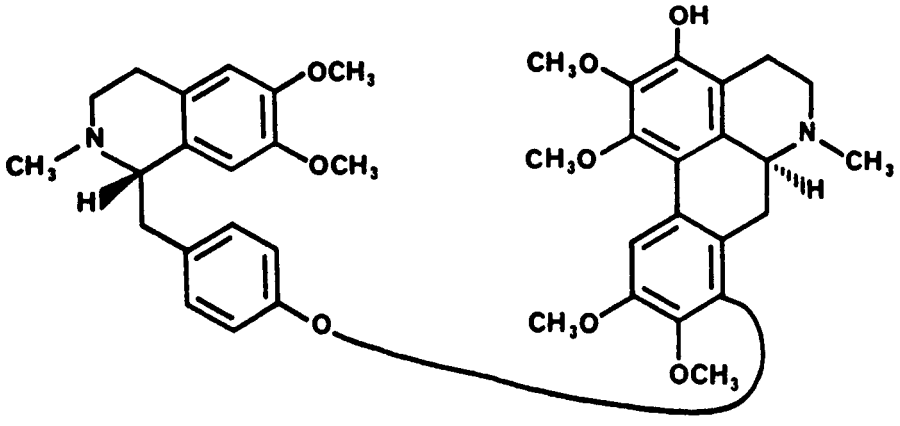
Thalifalandine was found to possess significant cytotoxicity against P_{388} and L_{1210} leukemic cells, with an IC_{50} of 0.7-1.8 μg/ml [83]. This same cytotoxic effect against P_{388} cells has been observed in eight other thalifaberine-type dimeric alkaloids that have been isolated from *T. faberi* [83].



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8.1.5. (+)-Thalifaramine. Tabular Review [128, Alkaloid No. 68], UV [2,4,128], ^{1}H NMR [2,4,128], EIMS [2,4,128], CD [2,4,128].

Thalifaramine (**9**), $C_{39}H_{44}O_7N_2$ (652.3137), $[\alpha]_D$ +76° (c 0.06, MeOH), was isolated along with eleven other aporphine-benzylisoquinoline dimeric alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1986 [2,4]. The UV spectrum (MeOH) of this phenolic alkaloid was similar to those of other aporphine-benzylisoquinoline dimers and displayed maxima at 228 nm (log ε 4.75), 270 (sh)(4.35), 280 (4.42), and 308 (4.04), while the CD spectrum exhibited extrema at Δε (nm) 0 (310), -4 (300), -8 (270), 0 (250), +87 (237), with a negative tail below 234 nm. The EIMS displayed the molecular ion at m/z 652 (0.8%), with other fragment ions at m/z 651 (1%), 637 (0.4), 460 (2), 459 (3), 192 (100), and 178 (8). The base peak at m/z 192 (100%) may be represented by the isoquinolinium ion **78** that is formed by the facile doubly benzylic cleavage of an benzylisoquinoline, and suggested the presence of one methoxy group and one hydroxy group in the isoquinoline portion of the dimer. The ^{1}H NMR spectrum was indicative of a thalifaberine (**77**)-type alkaloid [1,128,130-132], displaying the typical AA'BB' quartet that is characteristic of the H(10'), H(11'), H(13'), H(14') symmetrical protons in the benzyl ring (ring C) of the benzylisoquinoline-portion of the alkaloid. In addition, a comparison of the chemical shift values of thalifaramine with those of the nonphenolic thalifaronine (**12**) showed a remarkable similarity. Since the phenolic thalifaramine displayed a molecular ion and a base peak that were both 14 amu lower than those of thalifaronine (**12**), and since the ^{1}H NMR spectrum of thalifaramine lacked the presence of the characteristic high field methoxy singlet at δ3.53, thalifaramine was assigned as 7'-*O*-demethylthalifaronine (**9**). The CD curve of thalifaramine (**9**) was very similar to that of thalifaberine (**77**), thus permitting the assignment of an identical absolute configuration (S,S) for thalifaramine (**9**). The assignment of the chemical shifts of thalifaramine (**9**) were made as follows: δ6.63 [s, H(3)], 2.35 [s, *N*(6)Me], 8.05 [s, H(11)]; 2.57 [s, *N*(2')Me], 6.53 [s, H(5')], 5.93 [s, H(8')], 7.00 [d, 2H, J = 8.5 Hz, H(10') and H(14')], 6.80 [d, 2H, J = 8.5 Hz, H(11') and H(13')]; 3.71 [s, C(1)OMe], 3.89 [s, C(2)OMe], 3.80 [s, C(9)OMe], 3.91 [s, C(10)OMe], and 3.84 [s, C(6')OMe].

Thalifaramine (**9**) was most recently isolated from an extract of the roots of *T. faberi* Ulbr. in 1994 [129].

8.1.6. (+)-Thalifarazine. Tabular Review [128, Alkaloid No. 72], UV [2,4,128], ^{1}H NMR [2,4,128], EIMS [2,4,128], CD [2,4,128].

Thalifarazine (**10**), $C_{40}H_{46}O_8N_2$ (682.3254), $[\alpha]_D$ +72° (c 0.06, MeOH), was isolated along with eleven other aporphine-benzylisoquinoline dimeric alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1986 [2,4]. The UV spectrum of this phenolic alkaloid was similar to those of other aporphine-benzylisoquinoline dimers and displayed maxima at 228 nm (log ε 4.70), 270 (sh)(4.29), 283 (4.38), 297 (sh)(4.25), and 310 (sh)(4.02)while the CD spectrum

exhibited extrema at Δε (nm) 0 (315), -4.7 (297), -9.4 (272), 0 (255), +80 (240), with a negative tail below 232 nm. The EIMS displayed the molecular ion at m/z 682 (0.23%), with other fragment ions at m/z 681 (0.27%), 680 (0.41), 490 (2.2), 192 (100), and 177 (7). The base peak at m/z 192 (100%), as represented by the isoquinolinium ion **78** was formed by the facile doubly benzylic cleavage of an benzylisoquinoline, and suggested the presence of one methoxy group and one hydroxy group in the isoquinoline portion of the dimer. The ^{1}H NMR spectrum was indicative of a thalifaberine (**77**)-type alkaloid [1,128,130-132], displaying the typical AA'BB' quartet that is characteristic of the H(10'), H(11'), H(13'), H(14') symmetrical protons in the benzyl ring (ring C) of the benzylisoquinoline-portion of the alkaloid. It was observed that thalifarazine (**10**) had essentially the same EIMS as thalifaretine (**11**), including the base peak at m/z 192. A comparison of the ^{1}H NMR spectrum of thalifarazine (**10**) with that of thalifaretine (**11**) showed that one of the three methoxy absorptions near δ3.80 of the latter was missing while a characteristic high field methoxy singlet had appeared at δ3.57 in the spectrum of the former. Hence, the phenolic function in thalifarazine was placed at C(6'), and thalifarazine was assigned as **10**. The assignment was additionally corroborated via a consideration of reciprocal nOe enhancements that were observed between the following: the low field H(11)(δ7.89) and its neighboring methoxy group at C(1)(δ3.80); the low field H(11) and its neighboring methoxy group at C(10)(δ3.93); the H(11',13') to the C(9)-methoxy; and the H(8') to the C(7)-methoxy. Other nOe enhancements were the *N*(2')Me to the H(1') and H(1') to H(8'). A comparison of the CD curve of thalifarazine (**10**) with that of thalifaberine (**77**) showed a great similarity, thus permitting the assignment of an identical absolute configuration (**S,S**) for thalifarazine (**10**). The assignment of the chemical shifts of thalifarazine (**10**) was as follows: δ2.33 [s, *N*(6)Me], 7.89 [s, H(11)]; 2.54 [s, *N*(2')Me], 6.60 [s, H(5')], 5.93 [s, H(8')], 6.97 [d, 2H, J = 8.5 Hz, H(10') and H(14')], 6.77 [d, 2H, J = 8.5 Hz, H(11') and H(13')]; 3.80 [s, C(1)OMe], 3.97 [s, C(2)OMe], 3.90 [s, C(3)OMe], 3.80 [s, C(9)OMe], 3.93 [s, C(10)OMe] and 3.57 [s, C(7')OMe].

Thalifarazine (**10**) was most recently isolated from an extract of the roots of *T. faberi* Ulbr. in 1994 [129].

8.1.7. (+)-Thalifaretine. Tabular Review [128, Alkaloid No. 73], UV [2,4,128], ^{1}H NMR [2,4,128], EIMS [2,4,128], CD [2,4,128].

Thalifaretine (**11**), $C_{40}H_{46}O_8N_2$ (682.3254), $[\alpha]_D$ +61° (c 0.1, MeOH), was isolated along with eleven other aporphine-benzylisoquinoline dimeric alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1986 [2,4]. The UV spectrum (MeOH) of this phenolic alkaloid was similar to those of other aporphine-benzylisoquinoline dimers and displayed maxima at 222 nm (log ε 4.76), 272 (sh)(4.24), 283 (4.39), 293 (sh)(4.27), and 310 (sh)(4.04) while the CD spectrum exhibited extrema at Δε (nm) 0 (314), -8.4 (300), -10 (273), 0 (255), +81 (239), with a negative tail below 230 nm. The EIMS displayed the molecular ion at m/z 682 (0.8%), with other fragment ions at m/z 490 (1.2), 192 (100), and 177 (5). The base peak at m/z 192

(100%), as represented by the isoquinolinium ion **78**, was formed by the facile doubly benzylic cleavage of an benzylisoquinoline, and suggested the presence of one methoxy group and one hydroxy group in the isoquinoline portion of the dimer. The ^{1}H NMR spectrum was indicative of a thalifaberine (**77**)-type alkaloid [1,128,130-132], displaying the typical AA'BB' quartet that is characteristic of the H(10'), H(11'), H(13'), H(14') symmetrical protons in the benzyl ring (ring C) of the benzylisoquinoline-portion of the alkaloid. Both the parent ion and base peak of thalifaretine are 14 amu less than those of thalifaberine (**77**), suggesting that thalifaretine is either 6'-*O*-demethylthalifaberine or 7'-*O*-demethylthalifaberine. Since the high field δ3.56 methoxy signal representing the C(7')-methoxy group is missing in the spectrum of thalifaretine, it was concluded that thalifaretine was 7'-*O*-demethylthalifaberine, and the alkaloid was assigned as **11**. Additional confirmation for the structural assignment was via the observation of reciprocal nOe enhancements between the following: the low field H(11)(δ7.90) and its neighboring methoxy group at C(1)(δ3.80); the low field H(11) and its neighboring methoxy group at C(10)(δ3.93); the H(11',13') to the C(9) methoxy; H(5') to the C(6')OMe; H(1') to H(8'); H(10',14') to H(1'). In addition a nOe was observed from *N*(2')Me to H(1'). The CD curve of thalifaretine (**11**) showed an obvious similarity with that of thalifaberine (**77**), thus directing the assignment of an identical absolute configuration (S,S) for thalifaretine (**11**). The assignment of the chemical shifts of thalifaretine (**11**) were follows: δ2.34 [s, *N*(6)Me], 7.90 [s, H(11)]; 2.53 [s, *N*(2')Me], 6.51 [s, H(5')], 6.00 [s, H(8')], 6.98 [d, 2H, J = 8.5 Hz, H(10') and H(14')], 6.78 [d, 2H, J = 8.5 Hz, H(11') and H(13')]; 3.80 [s, C(1)OMe], 3.96 [s, C(2)OMe], 3.90 [s, C(3)OMe], 3.83 [s, C(9)OMe], 3.93 [s, C(10)OMe] and 3.80 [s, C(6')OMe].

8.1.8. (+)-Thalifaricine. Tabular Review [128, Alkaloid No. 75], UV [2,4,128], ^{1}H NMR [2,4,128], EIMS [2,4,128], CD [2,4,128].

Thalifaricine (**8**), $C_{39}H_{44}O_8N_2$ (668.3097), $[\alpha]_D$ +66° (c 0.1, MeOH), was isolated along with eleven other aporphine-benzylisoquinoline dimeric alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1986 [2,4]. The UV spectrum of this diphenolic alkaloid was like those of other aporphine-benzylisoquinoline dimers and showed maxima at 224 nm (log ε 4.74), 274 (sh)(4.31), 283 (4.40), 295 (sh)(4.31), and 310 (sh)(4.17), with a bathochromic shift in strong alkali to 282 (sh)(log ε 4.19) and 315 (4.29). The CD spectrum exhibited extrema at Δε (nm) 0 (315), -5.2 (300), -2 (288), -6.6 (275), 0 (255), +73 (240), with a negative tail below 236 nm. The EIMS was characterized by the molecular ion at m/z 668 (0.1%), with other fragment ions at m/z 667 (0.2%), 476 (2.2), 192 (100), and 177 (8). The base peak at m/z 192 (100%), represented by the isoquinolinium ion **78**, was formed by the facile doubly benzylic cleavage of an benzylisoquinoline, and suggests the presence of one methoxy group and one hydroxy group in the isoquinoline portion of the alkaloid. The ^{1}H NMR spectrum was indicative of a thalifaberine (**77**)-type alkaloid [1,128,130-132], displaying the typical AA'BB' quartet that is characteristic of the H(10'), H(11'), H(13'), H(14') symmetrical protons in the benzyl ring

(ring C) of the benzylisoquinoline-portion of the alkaloid. The placement of one of the phenolic hydroxy groups at C(7') was made because of the absence of the characteristic high field methoxy signal for a C(7') at around δ3.55. In addition, the presence of a bathochromic shift with hyperchromic effect in strong alkali in the UV spectrum directed that the second phenolic group was present at either C(3) or C(9)[134]. Finally, the presence of a methoxy group at δ3.89 and the absence of same in the δ3.82 range dictated that the placement of the second phenolic group should be at C(9), and the structure of thalifaricine was established as **8**. Further confirmation for the structural assignment was made via the observation of reciprocal nOe enhancements between the following: the low field H(11)(δ7.90) and its neighboring methoxy group at C(1)(δ3.77); the low field H(11) and its neighboring methoxy group at C(10)(δ3.96); H(5') to the C(6')OMe; *N*(2')Me to H(1'); H(1') to H(8'). In addition a nOe was observed from H(1') to H(13',14') and from H(4a) to C(3)OMe. The distinct similarity of the CD curve of thalifaricine (**8**) to that of thalifaberine (**77**) directed the assignment of an identical absolute configuration (**S,S**) for thalifaricine (**8**). The assignment of the chemical shifts of thalifaricine (**8**) were follows: δ2.32 [s, *N*(6)Me], 7.90 [s, H(11)]; 2.54 [s, *N*(2')Me], 6.52 [s, H(5')], 5.99 [s, H(8')], 6.98 [d, 2H, J = 8.5 Hz, H(10') and H(14')], 6.80 [d, 2H, J = 8.5 Hz, H(11') and H(13')]; 3.77 [s, C(1)OMe], 3.96 [s, C(2)OMe], 3.89 [s, C(3)OMe], 3.96 [s, C(10)OMe] and 3.80 [s, C(6')OMe].

Thalifaricine (**8**) was most recently isolated from an extract of the roots of *T. faberi* Ulbr. in 1994 [129].

8.1.9. (+)-Thalifaronine. Tabular Review [128, Alkaloid No. 70], UV [2,4,128], ^{1}H NMR [2,4,128], EIMS [2,4,128], CD [2,4,128].

Thalifaronine (**12**), $C_{40}H_{46}O_7N_2$ (666.3293), $[\alpha]_D$ +68° (c 0.1, MeOH), was isolated along with eleven other aporphine-benzylisoquinoline dimeric alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1986 [2,4]. The UV spectrum of this nonphenolic alkaloid was similar to those of other aporphine-benzylisoquinoline dimers and showed maxima at 227 nm (log ε 4.75), 268 (sh)(4.30), 280 (4.38), and 304 (sh)(4.08). The CD spectrum exhibited extrema at Δε (nm) 0 (310), -4.0 (297), -1.7 (287), -8.1 (270), 0 (255), +74 (236), with a negative tail below 232 nm. The EIMS displayed the molecular ion at m/z 666 (0.3%), with other fragment ions at m/z 665 (0.6%), 664 (0.5), 460 (1), 459 (1), 206 (100), and 190 (4). The base peak at m/z 206 (100%), represented by the isoquinolinium ion **74**, was formed by the facile doubly benzylic cleavage of an benzylisoquinoline, and suggested the presence of two methoxy groups in the isoquinoline portion of the alkaloid. The ^{1}H NMR spectrum was indicative of a thalifaberine (**77**)-type alkaloid [1,128,130-132], displaying the typical AA'BB' quartet that is characteristic of the H(10'), H(11'), H(13'), H(14') symmetrical protons in the benzyl ring (ring C) of the benzylisoquinoline-portion of the alkaloid. The molecular ion of thalifaronine is some 30 amu less than that of thalifaberine (**77**), indicating one less methoxy group in the former.

However, since both alkaloids have the same base peak at m/z 206, representing the isoquinoline ring of the dimer, the absence of the one methoxy group in thalifaronine must derive from the aporphine-half of the alkaloid. The major difference in the ^{1}H NMR spectrum of the two alkaloids is one less downfield methoxy absorption (δ3.89 - 3.96), indicating that the lacking methoxy must be at one of the three following positions: C(2), C(3), or C(10). A complete nOeds confirmed the absence of the methoxy group from C(3), and allowed the assignment of thalifaronine as **12**. Reciprocal nOe enhancements between the following were observed: the low field H(11)(δ8.03) and its neighboring methoxy group at C(1); the low field H(11) and its neighboring methoxy group at C(10); H(3) to the C(2)OMe; H(5') to the C(6')OMe; H(8') to C(7')OMe; H(11',13') to C(9)OMe; H(1') to H(8'). In addition a nOe was observed from H(1') to *N*(2')Me. A comparison of the CD curves of thalifaronine (**12**) and thalifaberine (**77**) showed a distinct similarity, and thus the assignment of an identical absolute configuration (**S,S**) for thalifaronine (**12**). The assignment of the chemical shifts of thalifaronine (**12**) were as follows: δ2.35 [s, *N*(6)Me], 8.03 [s, H(11)]; 2.61 [s, *N*(2')Me], 6.56 [s, H(5')], 5.92 [s, H(8')], 6.98 [d, 2H, J = 8.5 Hz, H(10') and H(14')], 6.78 [d, 2H, J = 8.5 Hz, H(11') and H(13')]; 3.71 [s, C(1)OMe], 3.90 [s, C(2)OMe], 3.80 [s, C(9)OMe], 3.90 [s, C(10)OMe], 3.83 [s, C(6')OMe], and 3.53 [s, C(7')OMe].

Thalifaronine (**12**) was most recently isolated from an extract of the roots of *T. faberi* Ulbr. in 1994 [129].

8.1.10. Aporphine-Benzyltetrahydroisoquinoline Dimeric *Thalictrum* Alkaloid Subgroups

Approximately ten years ago, it was observed that the *Thalictrum* aporphine-benzyltetrahydroisoquinoline dimeric alkaloids could be conveniently grouped into three subgroups, and that certain empirical observations could be drawn from these subgroups [1]. The alkaloids were drawn in a uniform fashion, with the benzyltetrahydroisoquinoline half on the left side (numbered 1' to 14' in a standard fashion) and the aporphine half on the right side (numbered 1 to 11 in a standard fashion). The following observations were made at that time:

a. The aporphine rings of these dimers are oxygenated at C(1), C(2), C(9), and C(10), with C(3) being an additional site in at least half of the alkaloids.

b. The benzyltetrahydroisoquinoline rings of these dimers are oxygenated at C(6'), C(7'), C(9), and C(12'), with C(5'), C(11'), and C(13') also being involved sites.

c. These dimers may belong to three different structural subgroups

Subgroup A: C(12') + C(13') oxygenation with a C(10') -> C(9) diphenyl ether bridge

Subgroup B:	C(11') + C(12') oxygenation with a C(10') -> C(9) diphenyl ether bridge
Subgroup C:	C(12') -> C(8) diphenyl ether bridge

d. All alkaloids of these three dimeric subgroups are of the (*S,S*) configuration and are dextrorotatory with specific rotations between +18° and +229°. The specific rotations of the various subgroups are:

Subgroup A	+18° to +135°
Subgroup B	+76° to +229°
Subgroup C	+61° to +99°

e. There are only two exceptions to the above guidelines, namely, istanbulamine which is linked via a C(11') -> C(9) diphenyl ether bridge, and uskudaramine, which is the only C(11') -> C(9) biphenyl alkaloid of the series.

Within the last decade, nine new aporphine-benzyltetrahydroisoquinoline dimeric alkaloids have been isolated from extracts of two *Thalictrum* species, *T. cultratum* Wall. (whole plant) [thalibulamine (**13**), thalifaramine (**9**), thalifarazine (**10**), thalifaretine (**11**), thalifaricine (**8**), thalifaronine (**12**)][2,4] and *T. faberi* Ulbr. (roots) [thalifaberidine (**76**)[129], thalifaboramine (**79**)[133], thalifalandine (**80**)[83]. A review of the structures and properties of these alkaloids demonstrates that there was complete concurrence with the observations that were made ten years earlier. In particular, eight of the nine alkaloids were members of Subgroup C [C(12') -> C(8) diphenyl ether bridge], while the ninth alkaloid thalibulamine (**13**), was the 7'-methylether of istanbulamine (one of the two exceptions described in section e above). All of the new alkaloids were of the (*S,S*) configuration, with specific rotations ranging from +61° to +87°.

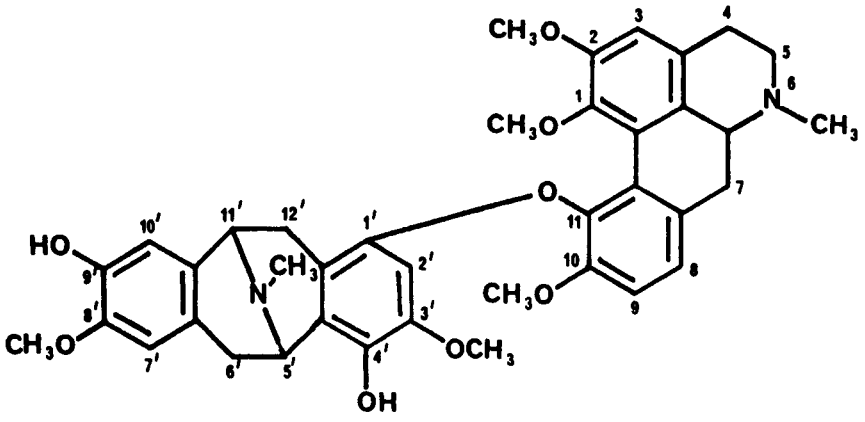
8.2. Aporphine-Pavines

8.2.1. EP-10. UV [35], ^{1}H NMR [35], ^{13}C NMR [35], EIMS [35]

EP-10 (**82**), $C_{39}H_{42}O_8N_2$ (666.2941), mp 205-210°, was isolated from an extract (EtOH) of the whole plant of the Taiwanese *T. fauriei* Hayata in 1986 [35]. The UV spectrum showed absorption maxima at 210, 270, 294, and 300 nm, and was similar to those of the aporphine-pavine alkaloids pennsylpavine (**83**) and pennsylpavoline (**84**) that had been isolated from *T. polygamum* Muhl. some twenty years earlier [135]. The specific rotation of the alkaloid was not reported, nor was any CD spectral data provided. The high resolution EIMS showed a parent ion and base peak at m/z 666.2953 (666.2641, calcd. for $C_{39}H_{42}O_8N_2$) with other important fragment

ions at m/z 665 (10%)(M-1), 651 (67)(M-15), 635 (63)(M-31), and 333 (28)(M/2). Fragment ions assignable to the aporphine part of the dimer included those found at m/z 340 (7%) and 296 (9), which were tentatively assigned as **85** and **86**, respectively. Fragment ions arising from the pavine portion of the molecule were observed at m/z 341 (15%) and 190 (100%), and were postulated as **87** and **57**, respectively. An important ion observed at m/z 529 (37%) was likely formed via the fragmentation of part of the pavine ring, and was represented as **88**. The ^1H NMR spectrum indicated the presence of two *N*-methyl groups as a singlet at δ2.55 and five methoxy groups as three singlets at δ3.41, 3.62 (9H) and 3.74. Six aromatic protons were observed as four singlets at δ5.62, 6.39, 6.62, and 6.68, as well as one AB quartet centered at δ6.75. The presence of an AB quartet was taken to represent H(8) and H(9), while H(3) was tentatively assigned to the resonance at δ6.68. The absence of the low field singlet (around δ8.0) customarily assigned to the H(11) was taken as a sign of the presence of one ether terminus at C(11). The high field signal at δ5.62 was assigned as H(2'). A pair of double doublets was observed in the aliphatic region (chemical shift unspecified) and were thought to represent H(6') and H(12'), which are bridgehead protons in the pavine portion. Addition of D_2O to the $CDCl_3$ resulted in a separation of the two *N*-methyl signals to δ2.51 and 2.54, with the methoxy groups being observed as four singlets at δ3.42, 3.61 (6H), 3.65, and 3.74. The aromatic protons were found at almost the same position, being observed at δ5.64, 6.39, 6.61, 6.68, and 6.78 (AB_q). The *O*-methyl ether of EP-10 was prepared, and the following chemical shift data was provided: *N*-methyl groups [δ2.54 and 2.60]; methoxy groups [δ3.41, 3.60 (6H), 3.64, 3.77, and 3.84); aromatic protons [5.63, 6.44, 6.59, 6.68, 6.79 (AB_q)]. The ^{13}C NMR spectrum (DEPT) demonstrated the presence of 7 methyl carbon atoms (δ41.1, 45.2, 58.0, 58.1, 58.6, 59.1, and 63.5), 5 methylene carbon atoms (δ25.9, 27.3, 30.2, 35.3, and 53.0), 8 methine carbon atoms δ51.6, 55.4, 64.0, 96.9, 111.4, 112.1, 113.3, and 121.9), and 18 quaternary carbon atoms (δ124.7, 125.6, 128.4, 130.3, 130.7, 132.2, 132.4, 132.7, 138.5, 144.6, 147.2, 147.4, 147.7, 149.6, 153.0, 154.2, 156.3, 157.3). Correlation spectroscopy (COSY) was also employed (data not provided) to provide supporting evidence for the pavine bridgehead protons H(5'), H(6'), H(11'), and H(12'), as well as the ABX system involving H(6a) and H(7) in the aporphine ring. The structure of EP-10 was thus assigned as **82**, but the authors were careful not to exclude the possibility of alternative structures, thereby finishing the paper with a precautionary note [35].

Preliminary biological testing of EP-10 demonstrated that the alkaloid possessed weak *in vitro* activity, as compared thalicarpine, in the lysis of Hela cells [35].



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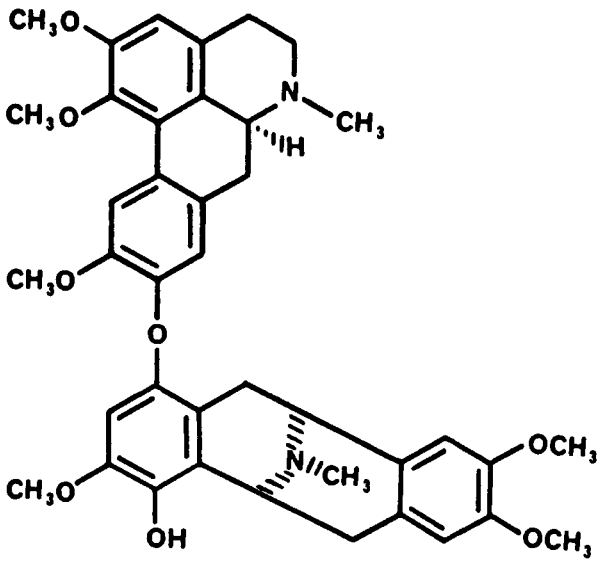

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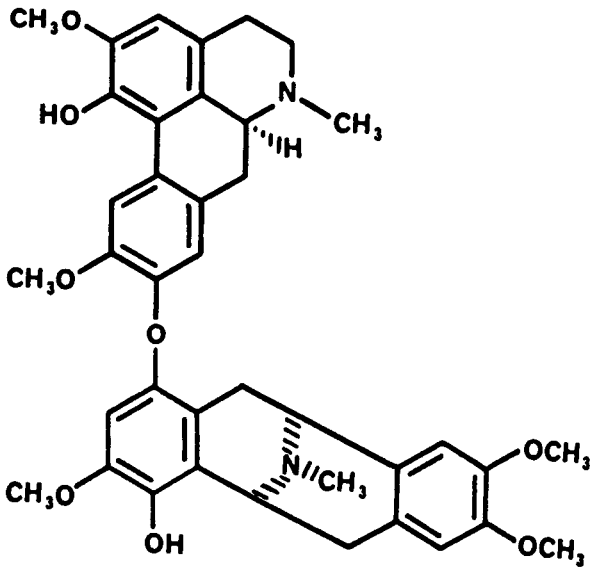
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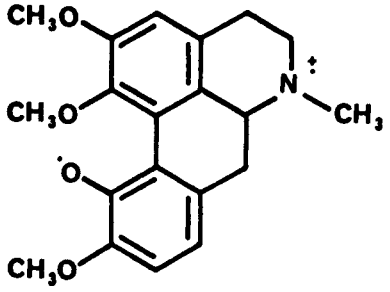
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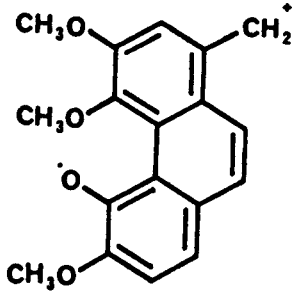
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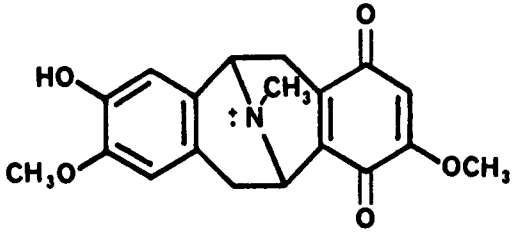
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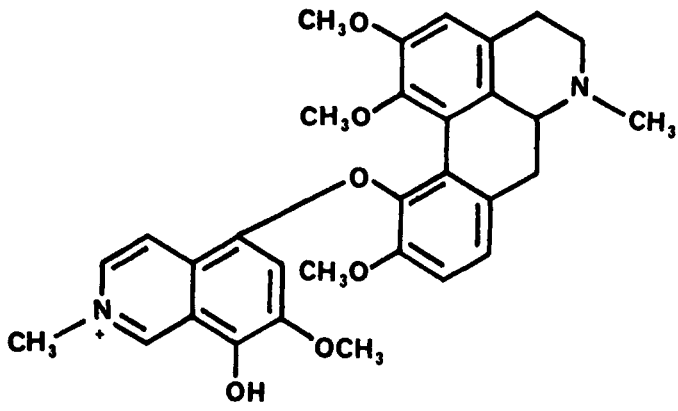

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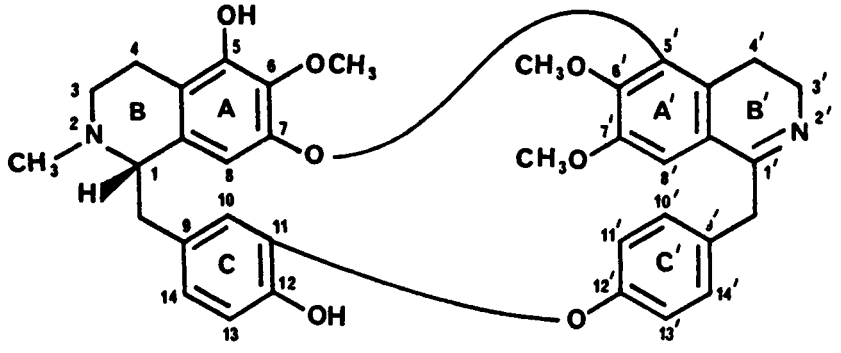
8.3. Bisbenzylisoquinolines

The numbering of the bisbenzylisoquinoline ring in the following alkaloids is according to accepted practice [1,100], and is illustrated in the structure of cultithalminine (**89**).

8.3.1. (+)-Cultithalminine. Tabular Review [100, Alkaloid No. 285], ^{1}H NMR [81,100], EIMS [81,100].

Cultithalminine (**89**), $C_{36}H_{36}O_7N_2$ (608.2522), $[\alpha]_D$ +7° (c 0.17, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The alkaloid displayed a parent ion and base peak at m/z 608, with a strong M-1 peak at m/z 607 (94%), and was thus characteristic of the pattern observed for bisbenzylisoquinoline imines [100,136-138]. Other EIMS fragment ions include those found at m/z 593 (10%), 304 (9), 192 (20), 191 (10), 190 (18), and 164 (11). The ^{1}H NMR spectrum of cultithalminine was very similar to that of the imine thalmiculimine (**4**), but the former spectrum displayed only one methoxy absorption [δ3.91 (3H)] near δ3.9, while the latter spectrum showed two methoxy absorptions [δ3.92 (6H)]. This observation is consistent with the placement of a phenolic group, rather than a methoxy group, at either C(7') or C(12). Reduction of cultithalminine with $NaBH_4$/MeOH afforded (-)-2'-norcultithalmine (**90**), $[\alpha]_D$ -39° (c 0.07, MeOH), whose ^{1}H NMR spectrum was very similar to that of the known (-)-2'-northalmiculine (**91**), the latter obtained on similar reduction ($NaBH_4$/MeOH) of (+)-thalmiculimine (**4**). An examination of the EIMS of (-)-2'-norcultithalmine (**90**) was critical in

the decision to place a methoxy group at C(12) in cultithalminine. The parent ion in the EIMS of (-)-2'-norcultithalmine (**90**) was observed at m/z 610 (65%), with other significant fragment ions at m/z 609 (47%), 411 (15), 397 (100), 383 (26), 199 (72), 192 (31), 191 (39), 190 (44), and 176 (21). The ion that is the base peak at m/z 397 may be represented as **92**, and is well-known to derive from the upper portion of the dimer [100,136-138], so that both secondary amine **90** and secondary amine **91** have an identical substitution in that portion of the dimer. Hence, it was concluded that the placement of the phenolic group in question must be at C(12), with a methoxy group at C(7') in both **90** and **91**, and that cultithalminine may be assigned as **89**. The reported ^{1}H NMR chemical shift assignments for cultithalminine (**89**) are as follows: δ2.22 [s, *N*(2)Me], 5.18 [s, H(8)], 5.79 [d, 1H, J = 1.8 Hz, H(10)], 6.85 [d, 1H, J = 8.1 Hz, H(13)], 6.80 [1H, dd, J = 1.8, 8.1 Hz, H(14)]; 7.05 [s, H(8')], 7.40 [br d, 2H, J = 7.8 Hz, H(10') and H(14')], 7.00 [br d, 2H, J = 7.8 Hz, H(11') and H(13')]; 4.07 [s, C(6)OMe], 3.69 [s, C(6')OMe], and 3.91 [s, C(7')OMe]. In addition, the reported ^{1}H NMR chemical shifts for (-)-2'-norcultithalmine (**90**) are as noted: δ2.23 [s, *N*(2)Me], 5.46 [s, H(8)], 6.06 [d, 1H, J = 1.8 Hz, H(10)], 6.85 [br s, 2H, H(13) and H(14)]; 6.87 [s, H(8')], 7.41 [br d, 2H, J = 7.7 Hz, H(10') and H(14')], 7.04 [br d, 2H, J = 7.7 Hz, H(11') and H(13')]; 4.05 [s, C(6)OMe], 3.67 [s, C(6')OMe], and 3.94 [s, C(7')OMe].



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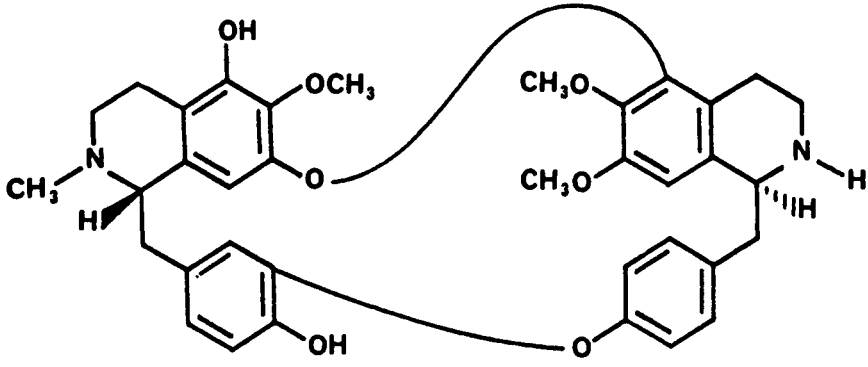

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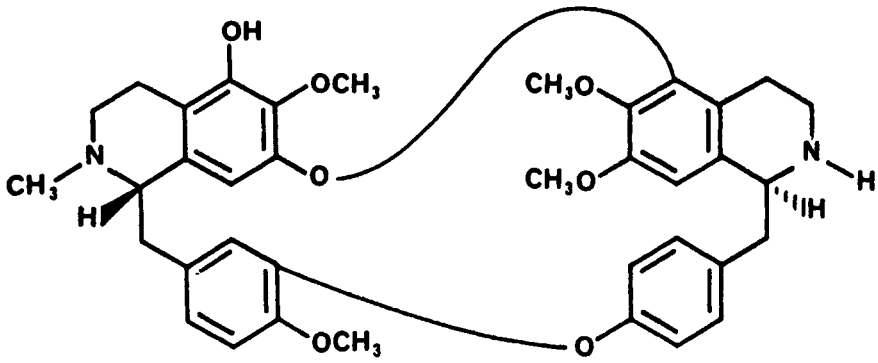

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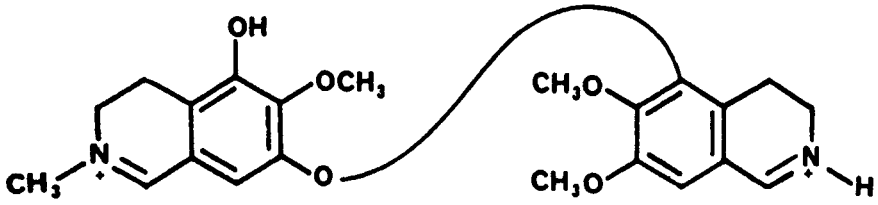


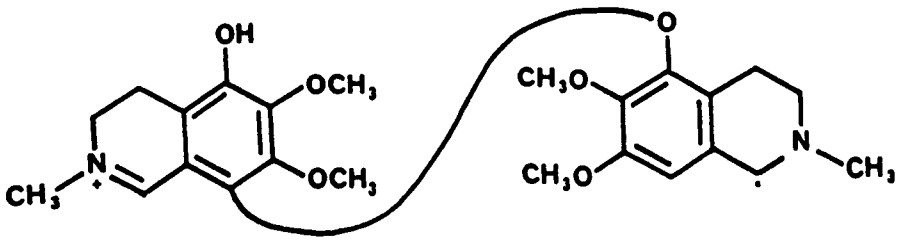
92

8.3.2. (-)-5-Hydroxythalidasine. Tabular Review [100, Alkaloid No. 311], UV [3,100], ^{1}H NMR [3,100], EIMS [3,100], CD [3,100].

5-Hydroxythalidasine (**6**), $C_{39}H_{44}O_8N_2$ (668.3098), $[\alpha]_D$ -51° (c 0.1, MeOH), was isolated as an amorphous solid along with eleven other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1986 [3,100]. The UV spectrum was characteristic of a benzyltetrahydroisoquinoline-derived alkaloid and showed a single maximum at 282 nm (log ε 3.54), with a shoulder at 237 (4.20) [100,136-138]. The CD spectrum exhibited extrema at Δε (nm) 0 (300), -6.8 (282), 0 (269), 0 (255), +12.0 (242), with a negative tail below 230 nm. The EIMS showed a molecular ion at m/z 668, with other important fragment ions at m/z 667 (31), 666 (75), 651 (21), 441 (65), 427 (60), 411 (36), 221 (100), 206 (28), 204 (30), and 190 (23), and was similar to that of thalidasine (**32**), but with singly charged ions being some 16 amu greater than their counterpart ions in the EIMS of thalidasine (**32**)[84,100,136-138]. The ion found at m/z 441 is logically represented as **93**, and is 16 amu greater than its corresponding ion (m/z 425) in the EIMS of thalidasine (**32**), hence suggesting the placement of the extra

hydroxy group in the top (upper) portion of the dimer. The ^{1}H NMR spectrum of the new alkaloid was extremely similar to that of thalidasine (**32**) [84,100,136-138], except for the absence of the H(5) singlet at δ6.30 in the ^{1}H NMR spectrum of thalidasine. The position of the hydroxy group in the new alkaloid **6** was determined via a consideration of the results of a ^{1}H NMR nOeds investigation, in which it was observed that there were no reciprocating nOes between the C(4) or C(4') protons and any of the methoxy signals, except for one of the C(4') protons (δ2.32) and the C(7) methoxy (δ3.35). This behavior is characteristic of C(8) to C(5') bridging, and a analogous situation exists with (-)-thalidasine (**32**). Hence, it was concluded that the new alkaloid bears a phenolic hydroxy group at C(5), and the compound was named (-)-5-hydroxythalidasine (**6**). Reciprocal nOe enhancements between the following were observed: *N*(2)Me and H(1), H(1) and H(αa), H(8') and C(7')OMe, H(8') and H(1'), H(8') and H(α'a), H(1') and H(α'a), *N*(2')Me and H(1'), and C(7)OMe and H(4'a). In addition, other nOe were observed from H(1) to H(10), H(αb) to H(14), H(α'b) to H(14'), and C(7)OMe to C(6)OMe. A comparison of the CD curve of (-)-5-hydroxythalidasine (**6**) with that of thalidasine (**32**) showed a great similarity, thus directing the assignment of an identical absolute configuration (S,S) for (-)-5-hydroxythalidasine (**6**). The reported ^{1}H NMR chemical shifts for 5-hydroxythalidasine (**6**) are as follows: δ3.81 [m, H(1)], 2.25 [s, *N*(2)Me], 2.51 [m, H(4a)], 2.74 [m, H(4b)], 3.04 [m, 1H, H(a)], 2.66 [m, 1H, H(b)], 6.30 [br s, 1H, H(10)], 6.81 [br s, 2H, H(13) and H(14)]; δ3.88 [m, H(1')], 2.62 [s, *N*(2')Me], 2.13 [m, H(4'a)], 2.32 [m, H(4'b)], 6.46 [s, 1H, H(8')], 2.72 [m, 1H, H(a')], 3.22 [m, 1H, H(b')], 6.36 [dd, 1H, J = 2.1, 8.3 Hz, H(10')], 6.56 [dd, 1H, J = 2.5, 8.3 Hz, H(11')], 6.98 [dd, 1H, J = 2.5, 8.3 Hz, H(13')], 7.53 [dd, 1H, J = 2.1, 8.3 Hz, H(14')]; 3.79 [s, C(6)OMe], 3.35 [s, C(7)OMe, 3.52 [s, C(6')OMe, and 3.89 [s, C(7')OMe].

Finally, both the 5-methyl ether and the 5-acetylester of (-)-5-hydroxythalidasine were prepared, and their UV, CD, IR, and ^{1}H NMR spectra were recorded.

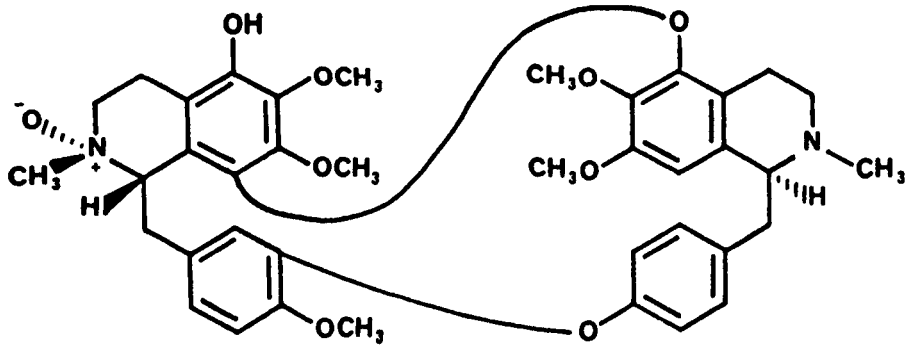


93

8.3.3. (-)-5-Hydroxythalidasine-2α-*N*-Oxide. Tabular Review [100, Alkaloid No. 312], ^{1}H NMR [81,100], EIMS [81,100].

5-Hydroxythalidasine-2α-*N*-Oxide (**94**), $C_{39}H_{44}O_9N_2$ (684.3046), $[\alpha]_D$ -11° (c 0.4, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from

an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The ^{1}H NMR spectrum was similar to that of the parent alkaloid, 5-hydroxythalidasine (**6**)[3], except for the presence of a low field NMe singlet at δ3.04 in the spectrum of the **94**, with the corresponding absence of the *N*(2)Me signal of 5-hydroxythalidasine (**6**)(δ2.25). The EIMS showed the parent ion at m/z 684 (0.4%), with other fragment ions at m/z 683 (0.6%), 682 (2), 668 (68)(M-16), 667 (27), 653 (23), 457 (2), 411 (86), 227 (58), 222 (57), 221 (100), 206 (22), 204 (34), 198 (35), 190 (35), and 176 (15). A nOe study of the alkaloid (+)-thalidasine-2α-*N*-oxide (**95**) had resulted in the assignment of the *N*-oxide function to the left portion of **95**, with a *syn*-relationship between the *N*(2)Me and H(1). In an analogous fashion, the same relationship was proposed to exist in 5-Hydroxythalidasine-2α-*N*-Oxide (**94**). Finally, the absence of the H(5) singlet that appears at δ6.39 in the spectrum of (+)-thalidasine-2α-*N*-oxide (**95**) directed the assignment of the phenolic hydroxy group in **94** to the C(5)-position. The ^{1}H NMR chemical shift assignments for **94** were made as follows: δ4.91 [m, H(1)], 3.04 [s, *N*(2)Me], 6.58 [d, 1H, J = 1.8 Hz, H(10)], 6.81 [d, 1H, J = 8.1 Hz, H(13)] and 6.69 [dd, 1H, J = 1.8, 8.1 Hz, H(14)]; δ3.89 [m, H(1')], 2.62 [s, *N*(2')Me], 6.48 [s, 1H, H(8')], 6.22 [dd, 1H, J = 2.1, 8.2 Hz, H(10')], 6.27 [dd, 1H, J = 2.1, 8.2 Hz, H(11')], 7.06 [dd, 1H, J = 2.1, 8.2 Hz, H(13')], 7.47 [dd, 1H, J = 2.1, 8.2 Hz, H(14')]; 3.77 [s, C(6)OMe], 3.89 [s, C(11)OMe], 3.42 [s, C(6')OMe, and 3.87 [s, C(7')OMe]. The ^{1}H NMR spectrum of 5-Hydroxythalidasine-2α-*N*-Oxide (**94**) was characteristic of either the 1*R*,1'*R* or the 1*S*,1'*S* configurations [3,84], but the sign of the specific rotation directed the assignment as 1*S*,1'*S* [139].



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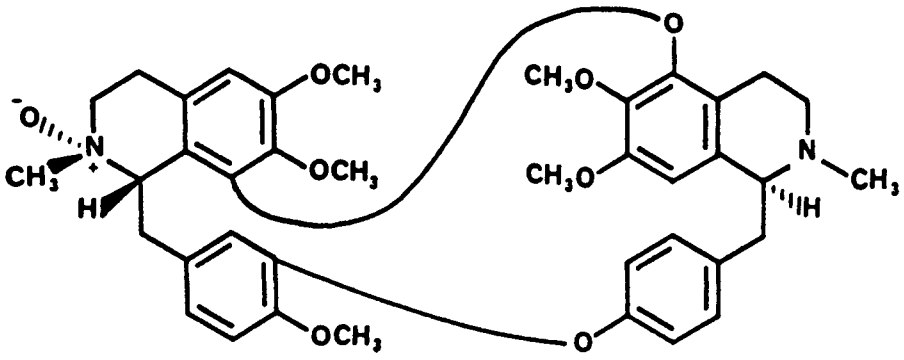
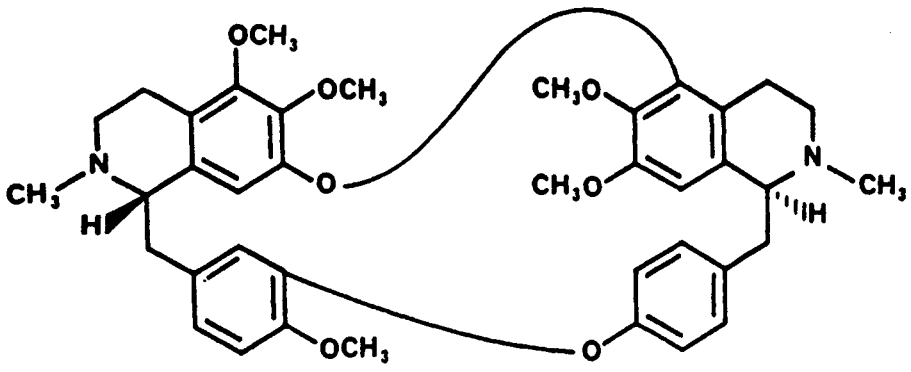


95

8.3.4. (-)-5-Hydroxythalmine. Tabular Review [100, Alkaloid No. 313], UV [3,100], ^{1}H NMR [3,100], EIMS [3,100], CD [3,100].

5-Hydroxythalmine (**2**), $C_{37}H_{40}O_7N_2$ (624.2836), $[\alpha]_D$ -68° (c 0.08, MeOH), was isolated as an amorphous solid along with eleven other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1986 [2,3,100]. The UV spectrum of the alkaloid exhibited maxima at 236 nm (sh) (log ε 4.43) and 281 (3.78), being typical of benzyltetrahydroisoquinoline-derived alkaloid. The CD spectrum (MeOH) showed extrema at Δε (nm) 0 (300), -3.5 (280), 0 (272), +1.6 (262), +1.1 (258), +12.1 (238), with a negative tail below 235 nm. The EIMS displayed the molecular ion at m/z 624 (50%), with other important fragment ions at m/z 623 (41%), 397 (100), 383 (37), 199 (57), 190 (57), and 176 (23). These fragment ions, particularly those at m/z 624 and 397, were consistent with a (-)-thalmiculine (**3**) analog in which one of the methoxy groups in the top portion of the molecule had been replaced with a phenolic hydroxy group. An inspection of the ^{1}H NMR spectrum revealed its close similarity to that of thalmiculine (**3**), but with the exception of a missing methoxy singlet close to δ3.60, suggesting the presence of a phenolic hydroxy group at C(6'). Methylation (CH_2N_2) of 5-hydroxythalmine afforded (-)-*O*-methylthalmiculine (**96**), thereby confirming the structure of 5-hydroxythalmine as **2** [3]. The complete ^{1}H NMR chemical shift assignments for (-)-5-hydroxythalmine are as follows: δ3.23 [m, H(1)], 2.19 [s, *N*(2)Me], 5.54 [s, H(8)], 6.11 [d, 1H, J = 2 Hz, H(10)], 6.80 [d, 1H, J = 8.2 Hz, H(13)] and 6.75 [dd, 1H, J = 2, 8.2 Hz, H(14)]; δ3.63 [m, H(1')], 2.66 [s, *N*(2')Me], 6.79 [s, H(8')], 7.35 [br s, 2H, H(10') + H(14')], 6.95 [br s, 2H, H(11') + H(13')]; 4.04 [s, C(6)OMe], 3.93 [s, 6H, C(12)OMe + C(7')OMe].

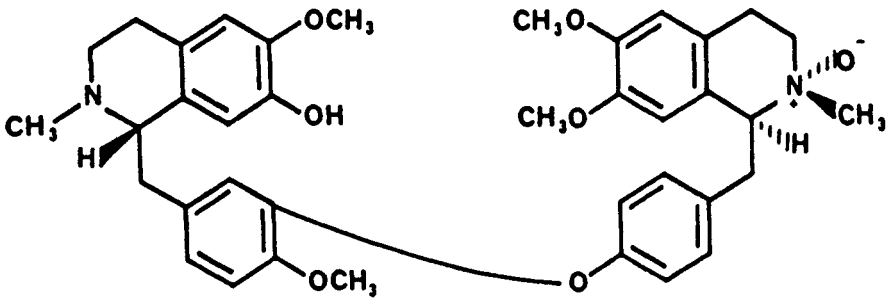


96

8.3.5. (+)-Neothalibrine-2'α-*N*-Oxide. Tabular Review [100, Alkaloid No. 325], ^{1}H NMR [81,100], EIMS [81,100].

Neothalibrine-2'α-*N*-Oxide (**97**), $C_{38}H_{44}O_7N_2$ (640.3149), $[\alpha]_D$ +74° (c 0.2, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The EIMS was characteristic of a singly bridged benzyltetrahydroisoquinoline dimer, in that no perceptible parent ion was observed, but the presence of two strong ionic species at m/z 206 (52%) and 192 (52) was apparent. These species represent the upper (head) *N*-methyltetrahydroisoquinoline portions of the molecule, the former containing two methoxy groups and the latter one methoxy group and one hydroxy group. The ^{1}H NMR spectrum of the alkaloid displayed signals that were representative of a singly-bridged dimer at the C(11)-C(12') positions, and strongly resembled that of neothalibrine (**98**). The notable exception was in the presence of a downfield signal at δ3.24 due to a *N*-methyl containing oxide. The elucidation of structure was completed via a nOe study, one salient feature of which demonstrated that no nOes were observed between H(1') (δ4.75) and the *N*-oxide methyl (δ3.24), thereby establishing the *anti*- relationship between these two groups. However, enhancement of H(8') was observed on irradiation of both H(1')(δ4.75) and C(7')OMe, while enhancement of H(14') was also observed on irradiation of H(1'). Reciprocal nOe enhancements between the following were observed: *N*(2)Me and H(1), H(1) and H(10), H(1) and H(8), H(1') and H(8'), H(5) and C(6)OMe, H(5') and C(6')OMe, C(7')OMe and H(8'). In addition, other nOe were observed from H(1') to H(10') and H(14'). The complete ^{1}H NMR chemical shift assignments for (+)-neothalibrine-2'α-*N*-Oxide (**97**) are as follows: δ3.67 [m, H(1)], 2.51 [s, *N*(2)Me], 6.48 [s, H(5)], 6.33 [s, H(8)], 6.51 [d, 1H, J = 8.3 Hz, H(10)], 6.88 [d, 1H, J = 8.3 Hz, H(13)] and 6.99 [dd, 1H, J = 1.8, 8.3 Hz, H(14)]; δ4.75 [m, H(1')], 3.24 [s,

N(2')Me], 6.63 [s, H(5')], 6.48 [s, H(8')], 7.25 [d, 2H, J = 8.5 Hz, H(10') + H(14')], 6.81 [d, 2H, J = 8.5 Hz, H(11') + H(13')]; 3.85 [s, C(6)OMe], 3.82 [s, 6H, C(12)OMe], 3.86 [s, C(6')OMe], and 3.62 [s, C(7')OMe].



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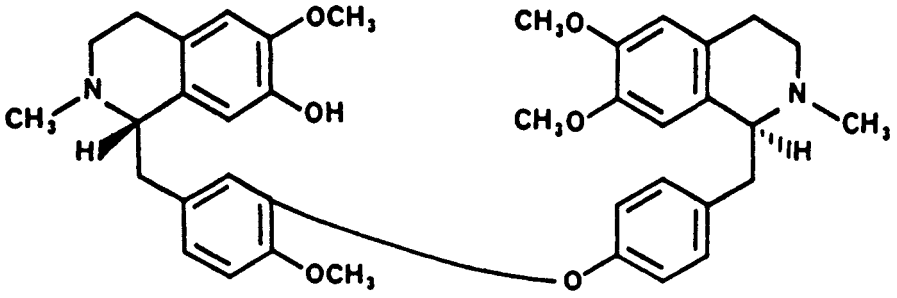
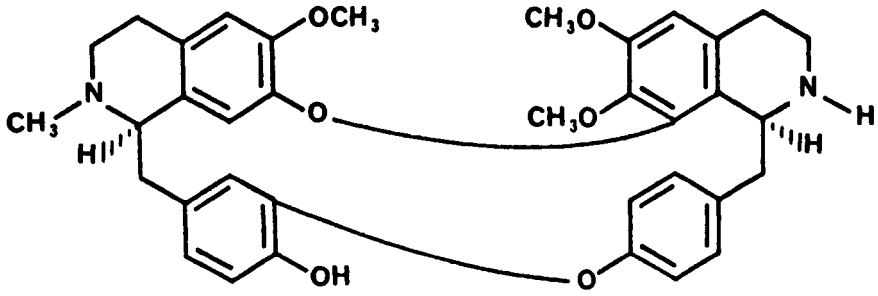


98

8.3.6. (+)-2'-Noroxyacanthine. Tabular Review [100, Alkaloid No. 338], ^{1}H NMR [81,100], EIMS [81,100].

(+)-2'-Noroxyacanthine (**99**), $C_{36}H_{38}O_6N_2$ (594.2730), $[\alpha]_D$ +125° (c 0.1, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The EIMS showed a parent ion at m/z 594 (67%), with other significant ions at m/z 593 (55), 382 (26), 381 (100), 367 (21), 192 (30), 191 (76), and 174 (27). The ^{1}H NMR spectrum revealed the presence of only one *N*-methyl group (δ2.57). These data were characteristic of a bisbenzyltetrahydroisoquinoline alkaloid of the oxyacanthine (**100**)-type containing three methoxy groups, one *N*-methyl group, and one secondary amine in the top portion of the molecule (supported by the presence of the EIMS fragment ions at m/z 382, 381, and 192 [100,136-138]), and one phenolic group in the

bottom portion of the molecule. A detailed inspection of the ^{1}H NMR spectrum showed that it was very similar to that of oxyacanthine (**100**), and the downfield shift of the H(1') multiplet from δ4.19 in oxyacanthine to δ4.56 in the new alkaloid demonstrated the presence of the secondary amine on the right-hand side of the alkaloid. Finally, the positive specific rotation of the alkaloid indicated a 1*R*, 1'*S* chirality, as in the parent (+)-oxyacanthine (**100**)[136]. The complete ^{1}H NMR chemical shift assignments for (+)-2'-noroxyacanthine (**99**) are as follows: δ3.62 [m, H(1)], 2.57 [s, *N*(2)Me], 6.35 [s, H(5)], 6.61 [s, H(8)], 5.42 [br s, 1H, H(10)], 6.78 [br s, 2H, H(13) + H(14)]; δ4.56 [m, H(1')], 6.34 [s, H(5')], 6.97 [m, 1H, C(10')], 6.30 [dd, 1H, J = 2.4, 8.4 Hz, H(11')], 6.92 [m, 1H, H(13')], 7.55 [dd, 1H, J = 2.4, 8.4 Hz, H(14')]; 3.66 [s, C(6)OMe], 3.80 [s, C(6')OMe], and 3.19 [s, C(7')OMe].



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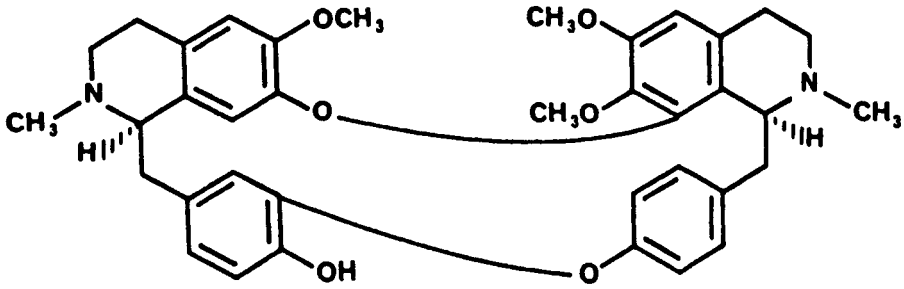


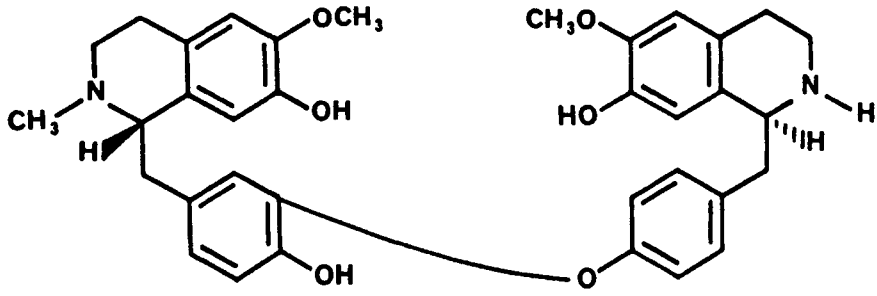
100

8.3.7. Northalibroline. Tabular Review [100, Alkaloid No. 341], UV [53,100], IR [53,100], ^{1}H NMR [53,100], EIMS [53,100].

Northalibroline (**101**), $C_{35}H_{38}O_6N_2$ (582.2730), $[\alpha]_D$ - none reported, was isolated from the phenolic alkaloid fraction of an extract (EtOH) of the roots and rhizomes of Turkish *T. minus* L. var. *minus* in 1988 [53,100]. The UV spectrum of the dimer displayed a single maximum at 282 nm, while the IR spectrum (KBr) showed bands at 3400, 2924, 1620, 1508, 1450, 1369, 1259, 1220, 1125, and 1021 cm^{-1}. The ^{1}H NMR spectrum ($CDCl_3$ + CD_3OD) indicated the presence of one *N*-methyl group (δ2.49) and two methoxy groups (δ3.81 and 3.85) as three singlets, as well as eleven aromatic protons. The EIMS (70 eV) failed to show a detectable parent ion but was characterized by fragment ions at m/z 192 (63%), 191 (14), 190 (90), 179 (12), 178 (100), and 177 (13). However, the EIMS (18 eV) did show a weak parent ion at m/z 582 (< 1%), and other meaningful fragment ions at m/z 404 (3%)(M-178), 192 (100), and 178 (66). This ^{1}H NMR and EIMS data (no detectable parent ion at 70 eV, plus the presence of two fragment ions of high relative intensity at m/z 192 and 178), was characteristic of a tail-to-tail linked benzyltetrahydroisoquinoline dimeric alkaloid containing two methoxy and two phenolic hydroxy groups in the top portion of the molecule and one phenolic group in the bottom portion of the molecule [100,136-138]. Furthermore, fragment ions at m/z 192 (**78**) and 178 (**102**), were indicative of the upper portions of the dimer containing one *N*-methyl group and one secondary amine function, respectively. Further support for the presence of a secondary amine came from the observation that the signal (dd, 1H) assigned as H(1') was found downfield at δ4.10 due to deshielding effects produced by the presence of the adjacent secondary amine [140]. Placement of the two methoxy groups at the C(7) and C(7') positions, as well as the two phenolic hydroxy groups at the C(8) and C(8'), positions was made from the observation that in bisbenzyltetrahydrosioquinoline alkaloids that are only tail-to-tail linked via an ether bridge, the H(8) signal is found more downfield than δ6.00 if there is a phenolic hydroxy group at C(7) [139]. Placement of the H(8) and H(8') protons at δ6.13 and 6.49, respectively, was affected by the observation that a downfield shift of approximately 0.35 ppm occurs for H(8') when this proton lies on the same side of the dimer as the imine function [139]. Finally, the presence of an EIMS fragment ion at m/z 404, as represented by ion **103**, was supportive of the placement of the remaining phenolic hydroxy group at C(12). A comparison of the spectral data for northalibroline (**101**) with those of northalibrine (**104**), an alkaloid isolated from *Thalictrum rochebrunianum* Franch. in 1976 [141], suggested that the former was the 7,12-*O*-demethyl-derivative of the latter, and the authors assigned the former the trivial name of northalibroline. (Note: Here it should be noted that the use of the prefix "nor-" traditionally means a *N*-demethylated analog, and the implication in this case is that there is a parent alkaloid named thalibroline that has two *N*-methyl groups and three phenolic hydroxy groups). In addition, the relative stereochemistry was assigned as 1*S*,1'*S*, in accord with accepted convention [121].

Finally, it was observed that the ^{1}H NMR values of northalibroline (**101**) were comparable to those of the diastereoisomeric 2-*N*-methyllindoldhamine, an alkaloid in which the

configurations of the chiral carbon atoms C(1) and C(1') are reversed [140]. The ^{1}H NMR chemical shift assignments of northalibroline (**101**) are as follows: δ3.67 [dd, H(1)], 2.49 [s, *N*(2)Me], 6.71 [s, H(5)], 6.13 [s, H(8)], 6.87 [s, 1H, J = 7.1 Hz, H(13)], 6.85 (s, 1H, J = 7.1 Hz, H(14)]; δ4.10 [dd, H(1')], 6.58 [s, H(5')], 6.49 [s, H(8')], 7.12 [d, 2H, J = 8.6 Hz, H(10') + H(14')], 6.82 (d, 2H, J = 8.6 Hz, H(11') + H(13')]; 3.81 [s, C(6)OMe or C(6')OMe], 3.85 [s, C(6')OMe or C(6)OMe]. (Note: Although a doublet proton, J = 7.2 Hz, was cited for H(5), this must be in error. Likely, this is H(10), but the coupling constant should then have been in the order of 2 Hz, for meta-coupling with H(14).



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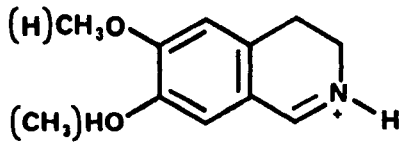

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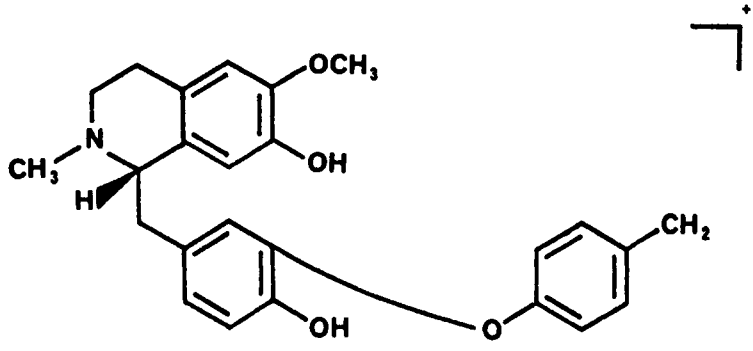

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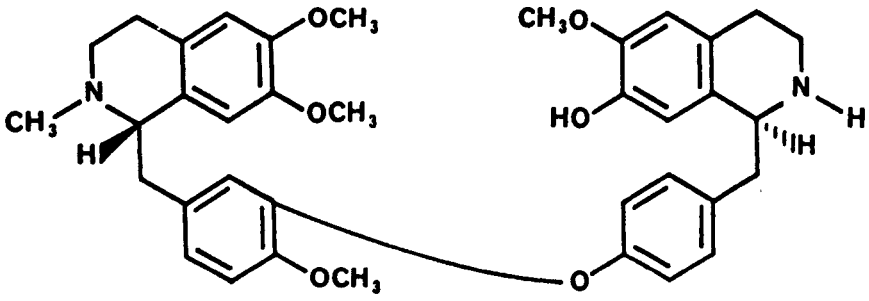
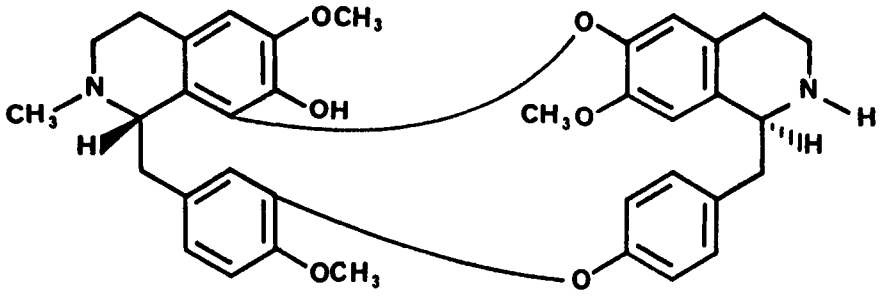


104

8.3.8. (+)-2'-Northaliphylline. Tabular Review [100, Alkaloid No. 342], UV [3,100], ^{1}H NMR [3,100], EIMS [3,100], CD [3,100].

2'-Northaliphylline (**105**), $C_{36}H_{38}O_6N_2$ (594.2730), $[\alpha]_D$ +197° (c 0.15, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The physicochemical properties and spectral data for this alkaloid had been described earlier [3], when reduction ($NaBH_4$) of (+)-thalsivasine (**7**) afforded (+)-2'-northalliphylline (**105**). The UV spectrum of **105** displayed maxima at 237 nm (sh) (log ε 4.16) and 285 (3.83), while the CD spectrum exhibited extrema at Δε (nm) 0 (300), +7 (285), 0 (270), -1 (250), with a positive tail below 220 nm. The ^{1}H NMR spectrum indicated the presence of only one *N*-methyl function (δ2.09), while the EIMS was characterized by a parent ion at m/z 594 (89%), with other important fragment ions at m/z 593 (84%), 592 (25), 367 (100), 353 (10), 208 (31), 192 (22), 191 (39), 190 (20), and 184 (84). These spectral data were characteristic of a head-to-head and tail-to-tail linked bisbenzylisoquinoline alkaloid containing two methoxy groups and one phenolic hydroxy group in the upper portion of the molecule, and one methoxy group in lower portion of the molecule [100,136-138]. Since the natural product was identical to the dihydro-derivative of the imino-alkaloid thalsivasine (**7**), and since the structure of the latter had been unequivocally established via conversion to (+)-thaliphylline (**106**), the identity of (+)-2'-northaliphylline (**105**) was established. The ^{1}H NMR chemical shift assignments for **105** are as follows: δ2.09 [s, *N*(2)Me], 6.55 [s, H(5)], 6.23 [d, 1H, J = 2.2 Hz, H(10)], 6.82 [d, 1H, J = 8.2 Hz, H(13)], 6.68 (dd, 1H, J = 2.2, 8.2 Hz, H(14)]; δ4.46 [m, H(1')], 6.11 [s, 2H, H(5') + H(8')], 6.69 [dd, 1H, J = 2.1, 8.5 Hz, H(10')], 6.94 [dd, 1H, J = 2.5, 8.5 Hz, H(11')], 7.08 [dd, 1H, J = 2.5, 8.1 Hz, H(13')], 7.26 [dd, 1H, J = 2.1, 8.1 Hz, H(14')]; 3.90 [s, 6H, C(6)OMe + C(12)OMe], 3.64 [s, C(6')OMe].



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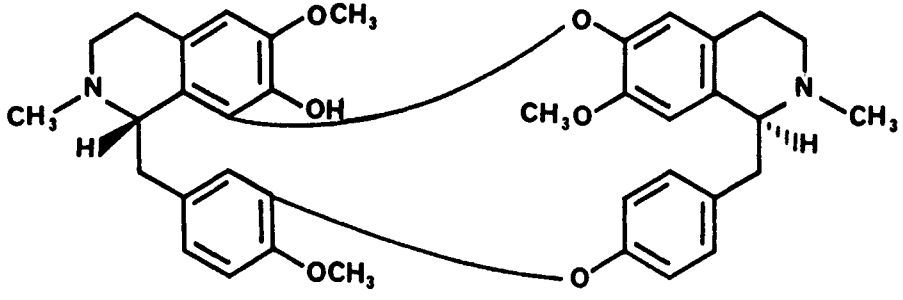
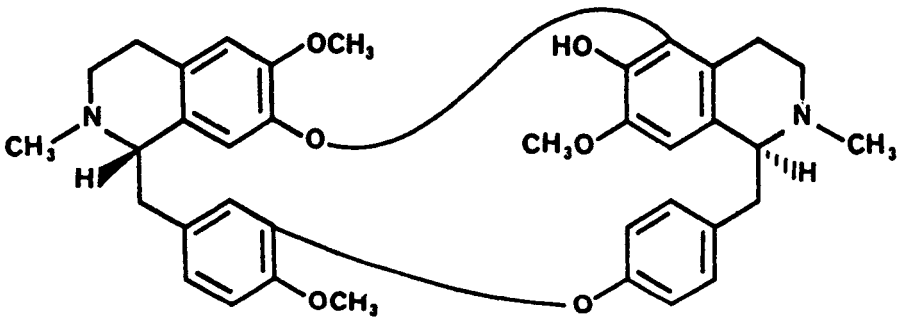


106

8.3.9. (-)-2-Northalmine. Tabular Review [100, Alkaloid No. 343], UV [84,100], ^{1}H NMR [84,100], EIMS [84,100], CD [84,100].

2-Northalmine (**1**), $C_{36}H_{38}O_6N_2$ (594.2730), $[\alpha]_D^{25}$ -31.8° (c 0.43, MeOH), was isolated as an amorphous solid from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1985 [84,100]. The UV spectrum of the alkaloid exhibited maxima at 234 nm (sh) (log ε 4.45) and 283 (3.97), while the CD spectrum exhibited extrema at Δε (nm) 0 (300), +7.6 (289), 0 (276), -2.1 (260), 0 (257), +6.3 (238) with a negative tail below 230 nm. The EIMS demonstrated a parent ion at m/z 594 (22%) and the base peak at m/z 367 (100%). These ions were 14 amu lower than corresponding ions in thalmine (**107**), an alkaloid that had been isolated along with (-)-*O*-methylthalmine (**108**), from similar fractions of the same extract. In addition, the ^{1}H NMR spectrum of the isolate was very similar to that of thalmine (**107**), the latter of which had undergone a complete nOe study, along with *O*-methylthalmine (**108**). It was readily apparent from the absence of the higher field *N*-methyl signal at approximately δ2.2 that the new alkaloid

was the 2-nor-derivative of thalmine (**107**), and this was confirmed by methylation ($CH_2O/NaBH_4$) of **1** to yield thalmine (**107**). A comparison of the CD curve of (-)-2-northalmine (**1**) with that of thalmine (**107**) showed a great similarity, and thus the assignment of an identical absolute configuration (**S,S**) for (-)-2-northalmine (**1**). The complete ^1H NMR chemical shift assignments, confirmed by nOes, are as follows: δ3.51 [m, H(1)], 6.62 [s, H(5)], 5.97 [s, H(8)], 5.93 [d, 1H, J = 1.9 Hz, H(10)], 6.80 [d, 1H, J = 8.0 Hz, H(13)], 6.68 (dd, 1H, J = 1.9, 8.0 Hz, H(14)]; δ3.62 [m, H(1')], 2.62 [s, *N*(2')Me], 6.77 [s, H(8')], 7.07 [br s, 1H, H(10')], 6.91 [br s, 2H, H(11') + H(13')], 7.63 [br s, 1H, H(14')]; 3.90 [s, C(6)OMe], 3.93 [s, C(12)OMe], and 3.94 [s, C(7')OMe]. An important nOe observation that was determined was the interconnection of H(1) with both H(8) and H(10). In addition, there was an interconnection between *N*(2')Me with H(1'), the latter of which was interrelated with H(8'), which was in turn interrelated with C(7')OMe.



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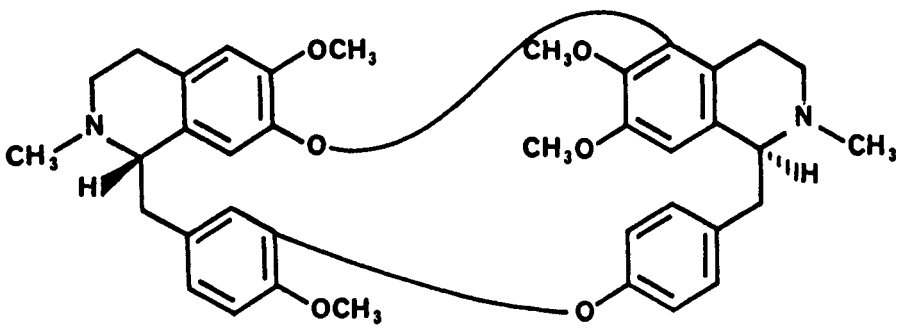


108

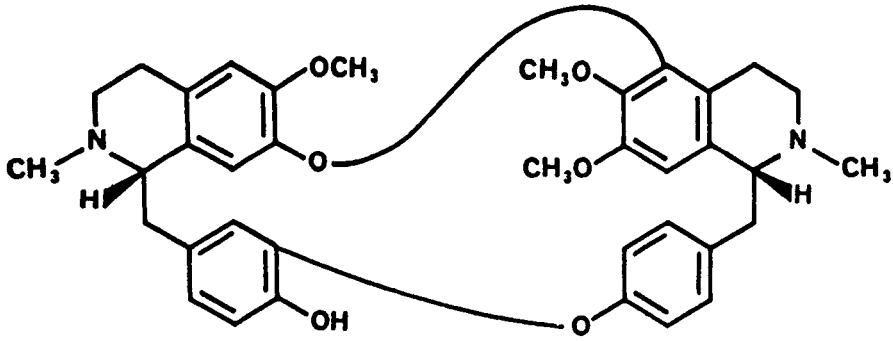
8.3.10. (+)-Thalidasine-2α-*N*-Oxide. Tabular Review [100, Alkaloid No. 377], ^{1}H NMR [81,100], EIMS [81,100].

Thalidasine-2α-*N*-oxide (**95**), $C_{39}H_{44}O_8N_2$ (668.3098), $[\alpha]_D$ +6° (c 0.15, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The ^{1}H NMR spectrum was similar to that of the parent alkaloid, thalidasine (**32**), while the EIMS showed the parent ion at m/z 668 (3%), with other fragment ions at m/z 667 (6%), 666 (12), 652 (70)(M-16), 637 (25), 425 (69), 411 (86), 213 (100), 206 (58), 204 (59), 190 (89), and 176 (18). A nOe study of the alkaloid (+)-thalidasine-2α-*N*-oxide (**95**) had resulted in the assignment of the *N*-oxide function to the left portion of **95**, with a *syn*-relationship between the *N*(2)Me and H(1). The ^{1}H NMR chemical shift assignments for the alkaloid were made as follows: δ4.88 [m, H(1)], 3.05 [s, *N*(2)Me], 6.39 [s, H(5)], 6.62 [d, 1H, J = 1.8 Hz, H(10)], 6.81 [d, 1H, J = 8.0 Hz, H(13)] and 6.68 [dd, 1H, J = 1.8, 8.0 Hz, H(14)]; δ3.89 [m, H(1')], 2.62 [s, *N*(2')Me], 6.44 [s, H(8')], 6.20 [dd, 1H, J = 2.0, 8.2 Hz, H(10')], 6.27 [dd, 1H, J = 2.0, 8.2 Hz, H(11')], 7.04 [dd, 1H, J = 2.0, 8.2 Hz, H(13')], 7.47 [dd, 1H, J = 2.0, 8.2 Hz, H(14')]; 3.76 [s, C(6)OMe], 3.20 [s, C(7)OMe], 3.90 [s, C(11)OMe], 3.41 [s, C(6')OMe, and 3.85 [s, C(7')OMe]. Finally, the ^{1}H NMR spectrum of thalidasine-2α-*N*-Oxide (**95**) was characteristic of either the 1*R*,1'*R* or the 1*S*,1'*S* configurations [3,84], but the sign of the specific rotation directed the assignment as 1*S*,1'*S* [139]. Reciprocal nOe enhancements between the following were observed: *N*(2)Me and H(1), H(1) and H(10), *N*(2')Me and H(1'), H(1') and H(8'), H(8') and C(7')OMe, H(5) and C(6)OMe, H(13) to C(12)OMe. In addition, other nOe were observed from *N*(2)Me to H(14), H(α'_b) to H(10'), and H(4) to H(5).

8.3.11. (+)-Thalifortine. UV [82], IR [82], ^{1}H NMR [82], ^{13}C NMR [82], EIMS [82], CD [82].

Thalifortine (**109**), $C_{37}H_{40}O_6N_2$ (608.2886), mp 143-145°, $[\alpha]_D^{25.5}$ +271.4° (c 0.37, MeOH), was isolated from an extract of the whole plant of *T. fortunei* S. Moore in 1990 [82]. The UV spectrum showed maxima at 215 nm (log ε 4.61) and 280 (3.91), while the CD spectrum exhibited extrema at $[\Theta]_{284.5}$ +56,300 and $[\Theta]_{212.5}$ +289,300. The IR spectrum (KBr) displayed bands at 3415, 2927, 2815, 1608, 1508, 1450, 1221, and 1169 cm^{-1}. The ^{1}H NMR spectrum indicated the presence of two *N*-methyl groups, three methoxy groups, and ten aromatic protons. The EIMS of thalifortine exhibited a parent ion at m/z 608 (93%), and other significant fragment ions at m/z 396 (67), 395 (71), 364 (19), 206 (17), 198 (100), 175 (53), 107 (20), and 91 (12). These spectral data were characteristic of a head-to-head and tail-to-tail linked bisbenzylisoquinoline alkaloid containing three methoxy groups in the upper portion of the molecule, and one hydroxy group in lower portion of the molecule [100,136-138]. The assignment of thalifortine as **109**, the (*S*,*R*) diastereoisomer of thalictine (*S*,*S*), was made by a consideration of the ^{1}H NMR chemical shift data (including nOe analysis), the ^{13}C NMR

spectrum, the EIMS fragmentation pattern, and the CD spectrum of thalifortine. The ^{1}H NMR chemical shift assignments for the alkaloid were made as follows: δ2.08 [s, *N*(2)Me], 6.57 [s, H(5)], 6.05 [s, H(8)], 6.31 [d, 1H, J = 1.7 Hz, H(10)], 6.73 [d, 1H, J = 8.9 Hz, H(13)] and 6.62 [dd, 1H, J = 1.7, 8.9 Hz, H(14)]; δ2.55 [s, *N*(2')Me], 5.98 [s, H(8')], 7.03 [dd, 1H, J = 2.6, 8.0 Hz, H(10')], 6.66 [dd, 1H, J = 2.6, 8.0 Hz, H(11')], 6.71 [dd, 1H, J = 2.6, 8.2 Hz, H(13')], 7.20 [dd, 1H, J = 2.6, 8.2 Hz, H(14')]; 3.86 [s, C(6)OMe], 3.77 [s, C(6')OMe, and 3.61 [s, C(7')OMe]. Reciprocal nOe enhancements between the following were observed and supported the assignment: H(1) and H(8), H(5) and C(6)OMe, *N*(2')Me and H(1'), and H(1') and H(8'). The ^{13}C NMR chemical shift assignments were as follows: 60.6 [C(1)], 41.7 [*N*(2)], 47.8 [C(3)], 26.5 [C(4)], 131.2 [C(4a)], 109.7 [C(5)], 141.0 [C(6)], 145.5 [C(7)], 111.5 [C(8)], 129.5 [C(8a)], 39.6 [C(α)], 133.1 [C(9)], 115.1 [C(10)]; 146.0 [C(11)], 152.1 [C(12)], 114.6 [C(13)], 124.0 [C(14)]; 63.4 [C(1')], 43.1 [*N*(2')], 42.8 [C(3')], 22.0 [C(4')], 131.4 [C(4'a)], 145.5 [C(5')], 145.2 [C(6')], 144.3 [C(7')], 113.8 [C(8')], 129.1 [C(8'a)], 36.9 [C(α')], 135.4 [C(9')], 124.2 [C(10')]; 119.7 [C(13')], 126.7 [C(14')];; 61.2 [C(6)OMe], 56.0 [C(6') or C(7')OMe], 56.1 [C(7') or C(6')OMe][82].

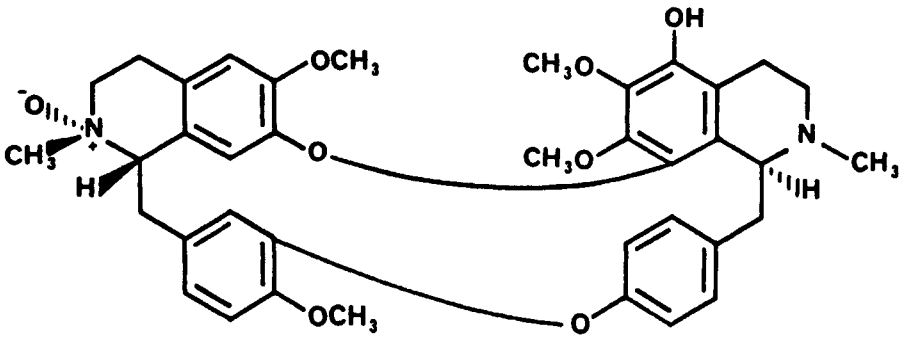


109

8.3.12. (-)-Thaligosine-2α-*N*-Oxide (Thalisopine-2α-*N*-Oxide). Tabular Review [100, Alkaloid No. 378], ^{1}H NMR [81,100], EIMS [81,100].

Thaligosine-2α-*N*-oxide (Thalisopine-2α-*N*-oxide)(**110**), $C_{38}H_{42}O_8N_2$ (654.2941), $[\alpha]_D$ -59° (c 0.13, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The EIMS of the alkaloid displayed a parent ion at m/z 654 (2%), with other important fragment ions at m/z 653 (8), 652 (18), 651 (21), 638 (47), 637 (39), 624 (13), 623 (21), 425 (5), 411 (100), 397 (79), 206 (57), 192 (54), 191 (15), 190 (21), and 176 (20) and was characteristic of head-to-head and tail-to-tail linked bisbenzylisoquinoline *N*-oxide [100,136-138]. The ^{1}H NMR spectrum

was found to be very similar to that of thalrugosaminine-2α-*N*-oxide (**111**), another alkaloid isolated from the same extract, and differed from **111** only by lacking one methoxy signal. A comparison of the chemical shift values of the H(1) and H(1') signals in the alkaloid *N*-oxide **110** with its parent base thaligosine (thalisopine) (**112**) revealed a strong downfield shift of the H(1) signal (δ3.50) in the parent base thaligosine (thalisopine) to δ4.81 in the alkaloid *N*-oxide, while the H(1') signal (δ4.23) of thaligosine (thalisopine) shifted only slightly downfield to δ4.42 in the *N*-oxide. This observation was consistent with the assignment of the *N*-oxide function to the left-hand portion of the molecule at *N*(2). Irradiation of the C(4') methylene protons of the *N*-oxide failed to induce a nOe on either a methoxy or an aromatic proton signal. Furthermore, irradiation of a methoxy signal in the *N*-oxide produced no enhancement of methylene protons. By contrast, a nOe was observed between the C(4') methylene protons and the C(5')OMe in the related thalrugosaminine-2α-*N*-oxide (**111**). These data furnished conclusive proof that the position of the phenolic hydroxy group in thaligosine-2α-*N*-oxide must be at C(5). A detailed nOe analysis was utilized to confirm the placement of the *N*-oxide at *N*(2), as well as to establish the relative configuration. The complete ^{1}H NMR chemical shift assignments for the alkaloid were determined as follows: δ4.81 [m, H(1)], 3.46 [s, *N*(2)Me], 6.51 [s, H(5)], 6.36 [s, H(8)], 6.57 [d, 1H, J = 1.8 Hz, H(10)], 6.98 [d, 1H, J = 8.2 Hz, H(13)] and 7.05 [dd, 1H, J = 1.8, 8.2 Hz, H(14)]; δ4.42 [m, H(1')], 2.61 [s, *N*(2')Me], 6.83 [br s, 2H, H(10') + H(11')], 7.11 [dd, 1H, J = 2.1, 8.1 Hz, H(13')], 7.50 [dd, 1H, J = 2.1, 8.1 Hz, H(14')]; 3.45 [s, C(6)OMe], 3.96 [s, C(12)OMe], 3.83 [s, C(6')OMe], 3.06 [s, C(7')OMe]. Reciprocal nOe enhancements between the following were observed: *N*(2)Me and H(1), H(8) and H(10), H(5) and C(6)OMe, H(13) to C(12)OMe. In addition, other nOes were observed from H(1) to H(8), *N*(2')Me to H(1'), and H(1') to H(14').



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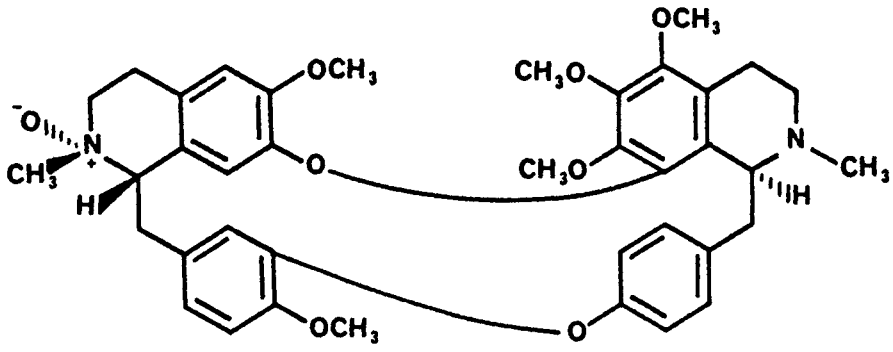

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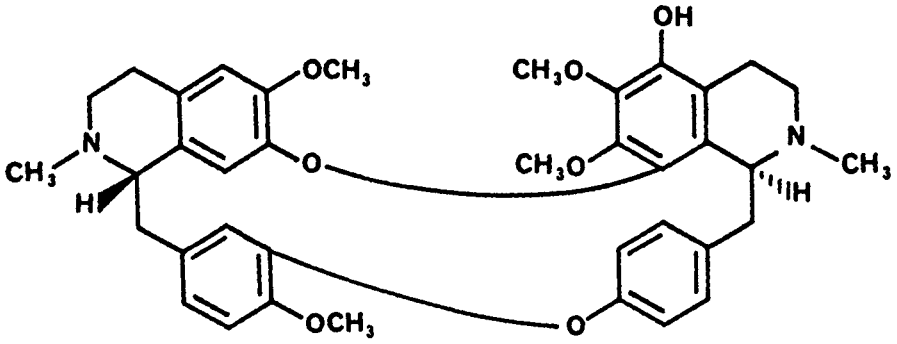
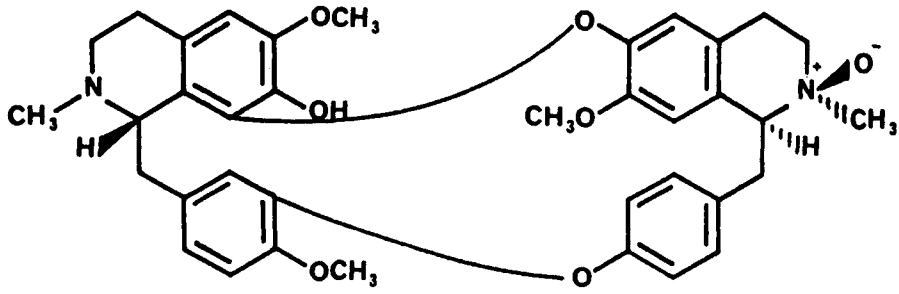


112

8.3.13. (+)-Thaliphylline-2'β-*N*-Oxide. Tabular Review [100, Alkaloid No. 379], ^{1}H NMR [81,100], EIMS [81,100].

Thaliphylline-2'β-*N*-oxide (**113**), $C_{37}H_{40}O_7N_2$ (624.2836), $[\alpha]_D$ +257° (c 0.7, MeOH), was isolated as an amorphous solid along with eight other benzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The EIMS showed the parent ion at m/z 624 (3%), with other significant fragment ions at m/z 623 (9), 622 (22), 608 (43), 607 (31), 594 (6), 381 (87), 367 (20), 192 (24), 191 (100), 190 (33), 176 (28), and 174 (39), and was characteristic of a head-to-head and tail-to-tail linked dimeric benzyltetrahydrosiquinoline alkaloid *N*-oxide [100,136-138]. The ^{1}H NMR spectrum of the alkaloid was quite like that of thaliphylline (**106**), but differed in the pronounced downfield shift of one of the *N*-methyl signals.

The chemical shift values of the *N*-methyl signals in thaliphylline were observed at δ2.07 [*N*(2')] and δ2.57 [*N*(2)], while those of the *N*-oxide were found at δ2.07 and δ3.26. It was obvious that the presence of the *N*-oxide was on the right-hand portion of the ring at *N*(2'). Further evidence was apparent from the downfield shift of the H(1') signal (δ3.95) in thaliphylline to δ4.63 in the *N*-oxide **113**. In addition, upfield shifts of both H(8') and H(10') in the alkaloid *N*-oxide were observed. A definitive assignment of the chemical shift values, as well as the *syn*- relationship between H(1') and *N*(2')Me was made via a detailed nOe study. The ^{1}H NMR chemical shift assignments for the alkaloid were determined as follows: δ3.20 [m, H(1)], 2.07 [s, *N*(2)Me], 6.58 [s, H(5)], 6.58 [d, 1H, J = 1.8 Hz, H(10)], 6.86 [d, 1H, J = 8.2 Hz, H(13)] and 6.77 [dd, 1H, J = 1.8, 8.2 Hz, H(14)]; δ4.63 [m, H(1')], 3.26 [s, *N*(2')Me], 6.21 [s, H(5')], 5.83 [s, H(8')], 6.26 [dd, 1H, J = 2.1, 8.0 Hz, H(10')], 6.64 [dd, 1H, J = 2.1, 8.0 Hz, H(11')], 7.22 [dd, 1H, J = 2.1, 8.0 Hz, H(13')], 7.33 [dd, 1H, J = 2.1, 8.0 Hz, H(14')]; 3.90 [s, C(6)OMe], 3.93 [s, C(12)OMe], and 3.60 [s, C(7')OMe]. Reciprocal nOe enhancements between the following were observed: *N*(2)Me and H(1), H(1) and H(10), H(1) and H(5'), H(10) and H(α_a), H(α_b) and H(14), H(1') and *N*(2')Me, H(1') and H(8'), H(8') and C(7')OMe, and H(5) and C(6)OMe. In addition, a nOe was observed from H(4) to H(5).

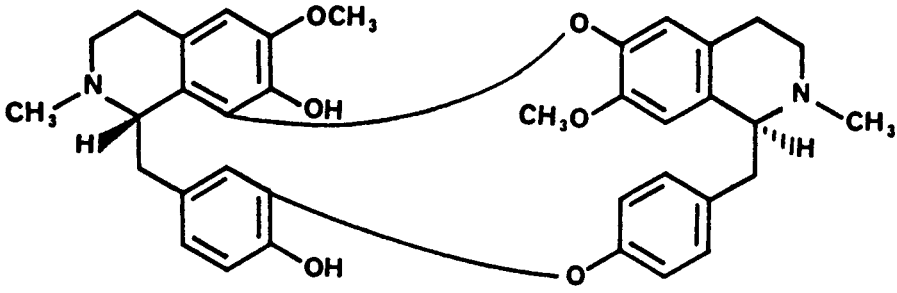


113

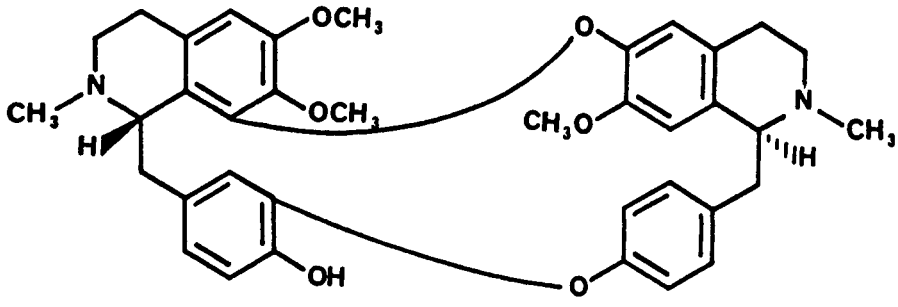
8.3.14. Thalivarmine. Tabular Review [100, Alkaloid No. 380], UV [77,100], ^{1}H NMR [77,100], EIMS [77,100].

Thalivarmine (**114**), $C_{36}H_{38}O_6N_2$ (594.2730), was isolated from an aqueous acetic acid extract of the above ground parts of *T. minus* var. *minus* L. of Turkish origin in 1985 [77,100]. The ^{1}H NMR spectrum of the alkaloid indicated the presence of two *N*-methyl groups and two methoxy groups, while the EIMS was characterized by the parent ion at m/z 594 (15%), with other significant fragment ions at m/z 593 (41%), 592 (23), 382 (23), 381 (87), 191.5 (20), 191 (100), 176 (18), 174 (24), and 168 (13). These spectral data were characteristic of a head-to-head

and tail-to-tail linked bisbenzyltetrahydroisoquinoline alkaloid containing two methoxy groups and one hydroxy group in the upper portion of the molecule and one phenolic hydroxy group in the lower portion of the molecule [100,136-138]. A comparison of the chemical shift values of thalivarmine (**114**) with those of thalicberine (**115**), *O*-methylthalicberine (**116**), and thaliphylline (**106**), alkaloids of established structure that had been isolated from the same fractions, showed a marked similarity. The major difference lay in the observation that the signals for the C(7) and C(12)-methoxy groups in these alkaloids at approximately δ3.76 and δ3.89, respectively, were missing in thalivarmine (**114**). Treatment of thalivarmine with an ethereal solution of CH_2N_2 afforded *O*-methylthalicberine (**116**), confirming the structure of thalivarmine as 7-*O*-demethylthalicberine (**114**). The ^1H NMR chemical shift assignments for the alkaloid were proposed as follows: δ2.09 [s, *N*(2)Me], 6.53 [s, H(5)], 6.27 [d, 1H, J = 1.8 Hz, H(10)], 6.80 [d, 1H, J = 8.0 Hz, H(13)] and 6.63 [dd, 1H, J = 1.8, 8.0 Hz, H(14)]; δ2.55 [s, *N*(2')Me], 6.11 [s, H(5')], 6.03 [s, H(8')], 6.68 [dd, 1H, J = 2.0, 8.0 Hz, H(10')], 6.74 [dd, 1H, J = 2.0, 8.0 Hz, H(11')], 7.02 [dd, 1H, J = 2.0, 8.0 Hz, H(13')], 7.22 [dd, 1H, J = 2.0, 8.0 Hz, H(14')]; 3.89 [s, C(6)OMe] and 3.63 [s, C(7')OMe]. It should be noted that the identification of the diazomethane methylation product of thalivarmine as *O*-methylthalicberine (**116**) was made solely by a TLC comparison with an authentic sample.



114



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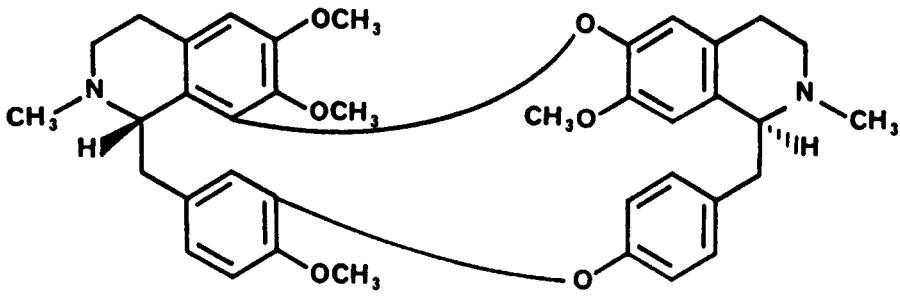

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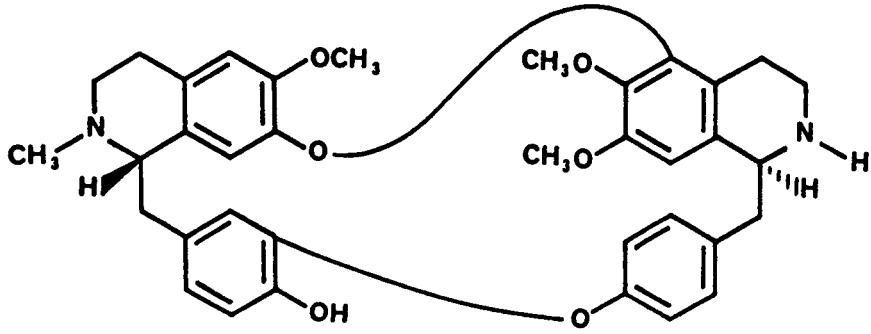
8.3.15. (+)-Thalmiculatimine. Tabular Review [100, Alkaloid No. 381], UV [3,100], ^{1}H NMR [3,100], EIMS [3,100], CD [3,100].

Thalmiculatimine (**5**), $C_{36}H_{36}O_6N_2$ (592.2573), $[\alpha]_D$ +7.5° (c 0.093, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The UV spectrum of the alkaloid showed a maximum at 280 nm (log ε 4.02), with a bathochromic shift in acidic media to 237 nm (sh)(log ε 4.38) and 285 (4.01). The CD spectrum exhibited extrema at Δε (nm) 0 (330), -6.5 (302), 0 (294), +7.5 (278), 0 (271), -10.5 (245), with a positive tail below 238 nm. The EIMS was characterized by an intense parent ion at m/z 592 (72%), a base peak at 591 (100)(M-1), and other significant fragment ions at m/z 397 (5), 296 (11), and 274 (3). The ^{1}H NMR spectrum revealed the presence of one *N*-methyl function at δ2.17, and three methoxy groups at δ3.76, 3.90, and 3.94. These spectral data were characteristic of an imine-containing, head-to-head and tail-to-tail linked bisbenzylisoquinoline alkaloid containing all three methoxy groups in the upper portion of the molecule [100,136-138]. Furthermore, the high field *N*-methyl group signal at δ2.17 was diagnostic of a *N*(2) function. Reduction of the imine group in the alkaloid with $NaBH_4$ afforded (-)-2'-northalictine (**117**) (a new compound) which upon *N*-methylation ($CH_2O/NaBH_4$) gave (-)-thalictine (**118**), the last being an alkaloid also isolated from the same extract. The complete ^{1}H NMR chemical shift assignments for thalmiculatimine (**5**) are as follows: δ3.09 [m, H(1)], 2.17 [s, *N*(2)Me], 6.64 [s, H(5)], 5.58 [s, H(8)], 5.81 [d, 1H, J = 2 Hz,H(10)], 6.79 [d, 1H, J = 8.2 Hz, H(13)] and 6.73 [dd, 1H, J = 2, 8.2 Hz, H(14)]; δ7.02 [s, H(8')], 7.40 [d, 2H, J_{app} = 8 Hz, H(10') and H(14')], 7.02 [d, 2H, J_{app} = 8 Hz, H(11') and H(13')]; 3.94 [s, C(6)OMe], 3.76 [s, C(6')OMe], and 3.90 [s, C(7')OMe]. Notable in the ^{1}H NMR spectrum of thalmiculatimine (**5**) are two features: first, the upfield position of the H((8) and H(10) protons at δ5.58 and 5.81, respectively, in comparison to the corresponding protons

in *N*-methyldihydrothalmiculatimine (thalictine)(**118**) which are found at δ5.89 and 6.05, respectively; and second, the well-defined pair of doublets at δ7.02 and 7.40 that represent the H(11') + H(13') protons and the H(10') and H(14') protons, respectively.

The following is a summary of the data reported for the new alkaloid (-)-2'-northalictine (dihydrothalmiculatimine)(**117**): $[\alpha]_D$ -54° (c 0.1, MeOH); UV - λ_{max} 237 nm (sh)(log ε 4.33) and 284 (3.90); CD - Δε (nm) 0 (300), +2.4 (291), 0 (288), -3.3 (281), 0 (268), 0 (258), +14.5 (240), with a negative tail below 230 nm; EIMS - M^+ m/z 594 (52%), 593 (77), 592 (78), 591 (100), 395 (17), 381 (47), 365 (24), and 191 (38); ^{1}H NMR - δ2.19 [s, *N*(2)Me], 6.63 [s, H(5)], 5.85 [s, H(8)], 6.04 [d, 1H, J = 2 Hz, H(10)], 6.84 [d, 1H, J = 8.2 Hz, H(13)] and 6.74 [dd, 1H, J = 2, 8.2 Hz, H(14)]; δ6.84 [s, H(8')], 7.37 [d, 2H, J_{app} = 8 Hz, H(10') and H(14')], 6.95 [d, 2H, J_{app} = 8 Hz, H(11') and H(13')]; 3.91 [s, C(6)OMe or C(7')OMe], 3.73 [s, C(6')OMe], and 3.92 [s, C(7')OMe or C(6)OMe].



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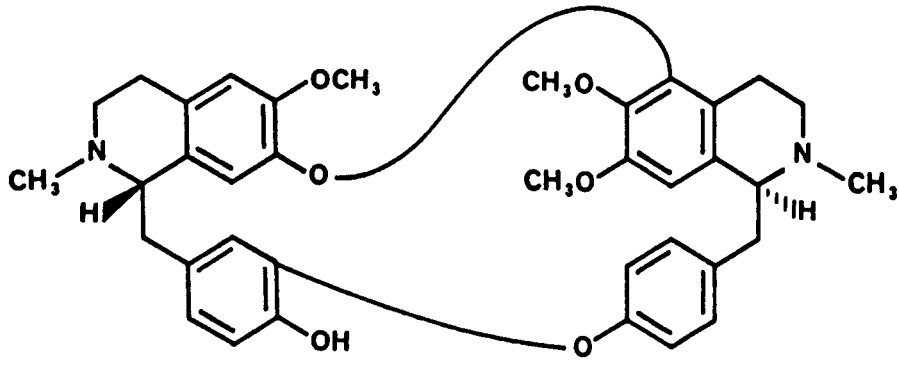

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8.3.16. (-)-Thalmiculimine. Tabular Review [100, Alkaloid No. 382], UV [3,100], ^{1}H NMR [3,100], EIMS [3,100], CD [3,100].

Thalmiculimine (**4**), $C_{37}H_{38}O_7N_2$ (622.2679), $[\alpha]_D$ -5° (c 0.09, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The UV spectrum was characterized by a maximum at 274 nm (log ε 3.97), with a bathochromic shift in acidic media to 237 nm (sh)(log ε 4.40) and 284 (3.93), while the CD spectrum exhibited extrema at Δε (nm) 0 (330), -5.4 (302), 0 (289), +7.4 (278), 0 (269), -12 (245), with a positive tail below 235 nm. Like thalmiculatimine (**5**), thalmiculimine (**4**) was also characterized by an EIMS with a very intense parent ion [m/z 622 (86%)], and with the base peak being the M-1 ion [m/z 621 (100%)]. Other fragment ions of significance include those at m/z 607 (44%), 591 (16), 561 (18), 311 (18), and 288 (10). The ^{1}H NMR spectrum of thalmiculimine was very similar to that of thalmiculatimine and was highlighted by the presence of one high field *N*-methyl group at δ2.16 (characteristic of the *N*(2) group in the thalmine-series) and four methoxy groups. These data were characteristic of an iminobisbenzylisoquinoline alkaloid of the thalmine (**107**) series [100,136-138]. Reduction of the alkaloid with $NaBH_4$ afforded (-)-2'-northalmiculine (**91**)(a new alkaloid), which upon *N*-methylation ($CH_2O/NaBH_4$) gave (-)-thalmiculine (**3**). The ^{1}H NMR chemical shift values for thalmiculimine (**4**) were reported as follows: δ3.04 [m, H(1)], 2.16 [s, *N*(2)Me], 5.17 [s, H(8)], 5.84 [d, 1H, J = 2 Hz,H(10)], 6.81 [d, 1H, J = 8.2 Hz, H(13)] and 6.75 [dd, 1H, J = 2, 8.2 Hz, H(14)]; δ7.06 [s, H(8')], 7.38 [d, 2H, J_{app} = 8 Hz, H(10') and H(14')], 7.04 [d, 2H, J_{app} = 8 Hz, H(11') and H(13')]; 4.07 [s, C(6)OMe], 3.92 (s, C(12)OMe], 3.69 [s, C(6')OMe], and 3.92 [s, C(7')OMe].

The following is a summary of the data reported for the new alkaloid (-)-2'-northalmiculine (dihydrothalmiculimine)(**91**): $[\alpha]_D$ -44° (c 0.1, MeOH); UV - λ_{max} 236 nm (sh)(log ε 4.33) and 281 (3.63); CD - Δε (nm) 0 (300), -7 (281), 0 (268), 0 (258), 0 (255), +23.5 (238), with a negative tail below 225 nm; EIMS - M^+ m/z 624 (72%), 623 (100), 397 (57), 383 (49), 199 (71), and 176 (64); ^{1}H NMR - δ3.30 (m, H(1)], 2.26 [s, *N*(2)Me], 5.46 [s, H(8)], 6.12 [d, 1H, J = 2 Hz, H(10)], 6.85 [d, 1H, J = 8.2 Hz, H(13)] and 6.81 [dd, 1H, J = 2, 8.2 Hz, H(14)]; δ6.90 [s, H(8')], 7.35 [d, 2H, J_{app} = 8 Hz, H(10') and H(14')], 6.99 [d, 2H, J_{app} = 8 Hz, H(11') and H(13')]; 4.05 [s, C(6)OMe], 3.95 [s, C(12)OMe], 3.69 [s, C(6')OMe], and 3.93 [s, C(7')OMe].

8.3.17. (-)-Thalmiculine. Tabular Review [100, Alkaloid No. 383], UV [3,100], ^{1}H NMR [3,100], EIMS [3,100], CD [3,100].

Thalmiculine (**3**), $C_{38}H_{42}O_7N_2$ (638.2992), $[\alpha]_D$ -35° (c 2.2, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The UV spectrum displayed maxima

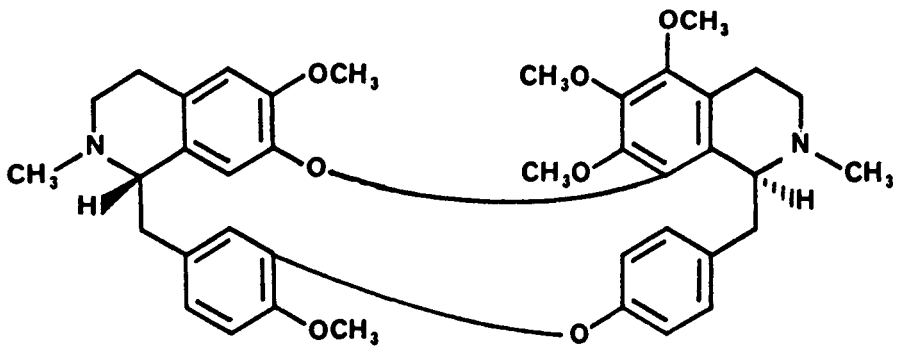
at 235 nm (sh)(log ε 4.28) and 281 (3.58), while the CD spectrum exhibited extrema at Δε (nm) 0 (300), -3.2 (280), 0.8 (sh)(268), 0 (260), +11.2 (238), with a negative tail below 225 nm. The EIMS showed the parent ion and base peak at m/z 638 (100%), with other significant fragment ions at m/z 637 (62%), 411 (67), 397 (41), 206 (73), and 183 (38), and was characteristic of a head-to-head and tail-to-tail linked bisbenzytetrahydroisoquinoline alkaloid containing two *N*-methyl groups, three methoxy groups, and one phenolic hydroxy group in the top portion of the molecule, and one methoxy group in the bottom portion of the molecule [100,136-138]. The ^{1}H NMR spectrum displayed the *N*-methyl resonances as two singlets at δ2.18 and 2.67, this being suggestive of an alkaloids of the thalmine (**107**) series. In addition, the usually well defined Ring C' aromatic protons were found as a poorly defined hump at δ7.30 [H(10') and H(14')] and a broad singlet at δ6.95 [H(11') and H(13')], both of which were sharpened by heating to 60°. Finally, the absence of a one-proton singlet at approximately δ6.63 was indicative of substitution at the C(5) position, and this was taken as preliminary evidence for the placement of a phenolic hydroxy group at that position. Methylation (CH_2N_2) of (-)-thalmiculine (**3**) gave (-)-*O*-methylthalmiculine (**96**), and a thorough nOeds study of this compound resulted in a complete assignment of the ^{1}H NMR chemical shift assignments. Several salient observations were made concerning the certain ^{1}H NMR chemical shifts of the structurally-related alkaloids (-)-thalmiculine (**3**), (-)-*O*-methylthalmiculine (**96**), and (-)-*O*-methylthalmine (**108**). First, while the position of the H(8) proton in (-)-*O*-methylthalmine is at δ5.88. this same proton is shifted upfield in the 5-oxygenated derivatives (-)-*O*-methylthalmiculine (**96**) and (-)-thalmiculine (**3**) to δ5.45 and 5.70, respectively. Hence, an upfield shift of about 0.2 ppm is characteristic of the 5-methoxylated derivative, while that of approximately 0.4 ppm is characteristic of the C(5) phenol. Second, the chemical shift associated with the C(6)OMe group is likewise indicative of C(5) substitution, as this signal is observed at δ3.92 in (-)-*O*-methylthalmine (**108**), but at δ4.00 in (-)-*O*-methylthalmiculine (**96**) and at δ4.05 in (-)-thalmiculine (**3**). The ^{1}H NMR chemical shift values for thalmiculine (**3**) were reported as follows: δ3.22 [m, H(1)], 2.18 [s, *N*(2)Me], 5.45 [s, H(8)], 6.09 [d, 1H, J = 2 Hz,H(10)], 6.80 [d, 1H, J = 8.2 Hz, H(13)] and 6.75 [dd, 1H, J = 2, 8.2 Hz, H(14)]; δ3.64 (m, H(1')], 2.67 [s, *N*(2')Me], 6.81 [s, H(8')], 7.30 [2H, H(10') and H(14')], 6.95 [br s, 2H, H(11') and H(13')]; 4.05 [s, C(6)OMe], 3.93 (s, C(12)OMe], 3.63 [s, C(6')OMe], and 3.90 [s, C(7')OMe].

The following is a summary of the data reported for the (-)-*O*-methylthalmiculine (**96**): $[\alpha]_D$ -38° (c 0.2, MeOH); EIMS - M^+ m/z 652 (91%), 651 (12), 638 (18), 622 (19), 426 (82), 412 (79), 410 (31), 213.5 (100), 206.5 (16), 204 (41), and 190.5 (73); ^{1}H-NMR - δ3.27 [m, H(1)], 2.18 [s, *N*(2)Me], 5.70 [s, H(8)], 6.12 [d, 1H, J = 2 Hz,H(10)], 6.79 [d, 1H, J = 8.2 Hz, H(13)] and 6.76 [dd, 1H, J = 2, 8.2 Hz, H(14)]; δ3.63 (m, H(1')], 2.66 [s, *N*(2')Me], 6.79 [s, H(8')], 7.37 [2H, H(10') and H(14')], 6.93 [br s, 2H, H(11') and H(13')]; 3.93 [s, 6H, C(5)OMe and C(12)OMe], 4.00 [s, C(6)OMe], 3.65 [s, C(6')OMe], and 3.88 [s, C(7')OMe]. Reciprocal nOe enhancements between the following were observed: *N*(2)Me and H(1), H(1) and H(8), H(8') and C(7')OMe, H(8') and H(α'a), H(4a) and C(5)OMe, and H(13) and C(12)OMe. In

addition, other observed nOes include H(αa) to H(10), H(αa) to H(8), H(αb) to H(14), H(11',13') to H(10), and H(1') to *N*(2')Me.

8.3.18. (-)-Thalrugosaminine-2α-*N*-Oxide. Tabular Review [100, Alkaloid No. 384], ^{1}H NMR [81,100], EIMS [81,100].

Thalrugosaminine-2α-*N*-oxide (**111**), $C_{39}H_{44}O_8N_2$ (668.3098), $[\alpha]_D$ -33° (c 0.2, MeOH), was isolated as an amorphous solid along with eight other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of *T. cultratum* Wall. in 1987 [81,100]. The EIMS of the alkaloid displayed a parent ion at m/z 668 (6%), with other important fragment ions at m/z 667 (10), 666 (22), 652 (82), 651 (60), 637 (35), 608 (18), 607 (19), 441 (1), 425 (63), 412 (26), 411 (92), 397 (15), 213 (100), 212 (13), 206 (51), and 174 (42) and was characteristic of head-to-head and tail-to-tail linked bisbenzylisoquinoline *N*-oxide [100,136-138]. The ^{1}H NMR spectrum was found to be very similar to that of thaligosine-2α-*N*-oxide (**110**), another alkaloid isolated from the same extract, and differed only by the presence of one additional methoxy signal. A comparison of the chemical shift values of the H(1) and H(1') signals in the alkaloid *N*-oxide **111** with its parent base thalrugosaminine (**119**) demonstrated a strong downfield shift of the H(1) signal (δ3.49) in the parent base thalrugosaminine to δ4.83 in the alkaloid *N*-oxide, while the H(1') signal (δ4.47) of the *N*-oxide was shifted only slightly downfield from that of the parent base (δ4.24). This observation was consistent with the assignment of the *N*-oxide function to the left-hand portion of the molecule at *N*(2). A detailed nOe analysis was utilized to confirm the placement of the *N*-oxide at *N*(2), as well as to establish the relative configuration. The complete ^{1}H NMR chemical shift assignments for the alkaloid were determined as follows: δ4.83 [m, H(1)], 3.45 [s, *N*(2)Me], 6.50 [s, H(5)], 6.37 [s, H(8)], 6.59 [br s, 1H, H(10)], 6.99 [br s, 2H, H(13) + H(14)]; δ4.47 [m, H(1')], 2.58 [s, *N*(2')Me], 6.82 [br s, 2H, H(10') + H(11')], 7.16 [dd, 1H, J = 2.2, 8.1 Hz, H(13')], 7.51 [dd, 1H, J = 2.2, 8.1 Hz, H(14')]; 3.45 [s, C(6)OMe], 3.96 [s, C(12)OMe], 3.81 [s, C(5')OMe], 3.89 [s, C(6')OMe], 3.05 [s, C(7')OMe]. Reciprocal nOe enhancements between the following were observed: *N*(2)Me and H(1), H(8) and H(10), H(1') and *N*(2')Me, H(5) and C(6)OMe, H(13) to C(12)OMe. In addition, other nOes were observed from H(1) to H(14), H(8) to H(α_a), H(1') to H(14'), H(1') to H(α'_b), H(14') to H(α'_a), and C(5')OMe to H(4').



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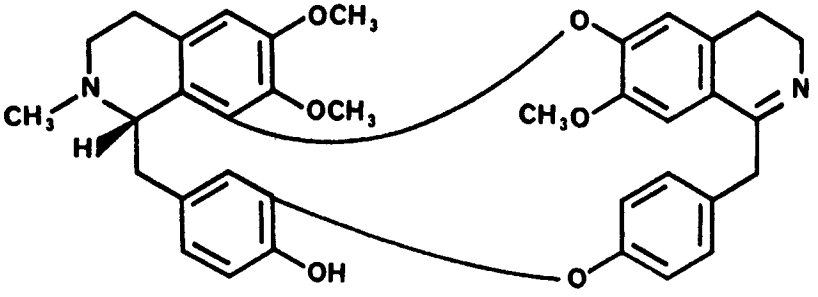
8.3.19. (+)-Thalsivasine. Tabular Review [100, Alkaloid No. 385], UV [77,3,100], ^{1}H NMR [3,77,100], EIMS [3,77,100], CD [3,100].

Thalsivasine (7), $C_{36}H_{36}O_6N_2$ (592.2573), $[\alpha]_D$ +196° (c 0.2, MeOH), was isolated from an aqueous acetic acid extract of the above ground parts of *T. minus* var. *minus* L. of Turkish origin in 1985 [77,100]. It was shortly thereafter isolated as an amorphous solid along with eleven other bisbenzylisoquinoline alkaloids from an extract (EtOH) of the whole plant of the Pakistani *T. cultratum* Wall. in 1986 [2,3,100]. The alkaloid displayed UV maxima at 234 (sh) (log ε 4.39), 281 (4.05), and 313 (3.78), with a bathochromic shift in acidic media to 239 nm (log ε 4.36), 285 (3.99), 308 (3.69), and 354 (3.82) [3]. The EIMS was characterized by a parent ion at m/z 592 (39%), a pronounced M-1 peak at 591 amu that was also the base peak, and other important fragment ions at 590 (50), 576 (15), 561 (12), 560 (26), 368 (17), 236 (14), 204 (8), and 183 (7)[77]. These spectral data were characteristic of an imine-containing bisbenzylisoquinoline-derived alkaloid containing two ether bridges, one in the upper portion and one in the lower portion of the molecule [100,136-138]. Among the unusual features of the ^{1}H NMR spectrum was the presence of an upfield three-proton singlet at δ1.96 which was characteristic of the *N*(2) methyl group in the thalmethine (**120**) series of alkaloids [100,136-138]. Since both thalmethine (**120**) and *O*-methylthalmethine (**121**) had been isolated from fractions that contained thalsivasine (**7**), and since thalsivasine possessed spectral properties that were characteristic of this group of compounds, thalsivasine was methylated with an ethereal solution of CH_2N_2 to afford *O*-methylthalmethine (**121**), thereby confirming the skeletal framework and positions of oxygenation in thalsivasine (**7**)[77]. This identification was made solely via a TLC comparison with an authentic sample. The assignment of structure of thalsivasine as 7-*O*-demethyl-*O*-methylthalmethine (**7**)[77] was made via a consideration of the ^{1}H NMR spectrum which failed to show the presence of the C(7) methoxy signal (at approximately δ3.83) that is characteristic in the thalmethine-series of alkaloids [100,136-138]. The structure of thalsivasine

as **7** was later confirmed [3] by reduction of the alkaloid to (+)-2'-northaliphylline (**105**), whose *N*-methylation product was the known alkaloid (+)-thaliphylline (**106**), that was also present in the same extract [3]. The CD spectrum of thalsivasine (**7**) exhibited extrema at Δε (nm) 0 (320), +14 (280), 0 (271), -9.6 (265), -11.0 (255), -14.4 (245), with a negative tail below 225 nm. The ^{1}H NMR chemical shift assignments for the alkaloid were proposed as follows: δ1.96 [s, *N*(2)Me], 6.56 [s, H(5)], 5.80 [d, 1H, J = 1.8 Hz, H(10)], 6.79 [d, 1H, J = 8 Hz, H(13)] and 6.63 [dd, 1H, J = 1.8, 8 Hz, H(14)]; δ6.67 [s, H(5')], 6.28 [s, H(8')], 6.78 [dd, 1H, J = 2, 8.2 Hz, H(10')], 6.81 [dd, 1H, J = 2, 8.2 Hz, H(11')], 7.16 [dd, 1H, J = 2, 8.2 Hz, H(13')], 7.48 [dd, 1H, J = 2, 8.2 Hz, H(14')]; 3.93 [s, C(6)OMe], 3.92 [s, C(12)OMe], and 3.71 [s, C(7')OMe].



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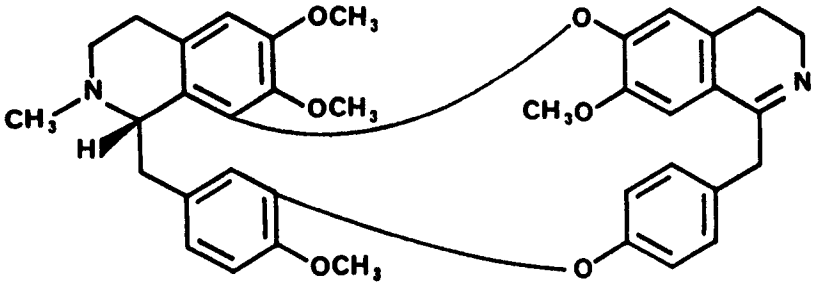

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8.3.20. Enzymatic Control of Stereochemistry Among the *Thalictrum* Bisbenzylisoquinoline Alkaloids

Approximately ten years ago, Guinaudeau et al. proposed four rules that appeared to govern the formation of bisbenzylisoquinoline alkaloids in *Thalictrum* species [1,121]. In order

to interpret these rules correctly, these alkaloids must be drawn uniformly with their two lower aromatic rings (tail portions) in a position such that the phenolic hydroxyl or methoxyl group in ring C at C(12) would be the lower left ring, which then fixed the termini of the diphenyl ether bridge at C(11) and C(12'). These proposed rules were as follows:

1. The dimers were members of any of seven different structural subgroups:

   Subgroup A: C(11) -> C(12') diphenyl ether bridge
   Subgroup B: C(8) -> C(7') + C(11) -> C(12') diphenyl ether bridges
   Subgroup C: C(7) -> C(8') + C(11) -> C(12') diphenyl ether bridges
   Subgroup D: C(8) -> C(6') + C(11) -> C(12') diphenyl ether bridges
   Subgroup E: C(8) -> C(5') + C(11) -> C(12') diphenyl ether bridges
   Subgroup F: C(7) -> C(5') + C(11) -> C(12') diphenyl ether bridges
   Subgroup G: C(5) -> C(8') + C(11) -> C(12') diphenyl ether bridges

2. When a benzylisoquinoline moiety was oxygenated at C(5) or C(5'), it had the (*S*) configuration

3. The right-hand benzylisoquinoline moiety incorporated the (*S*) configuration at C(1')

4. The left-hand benzylisoquinoline moiety incorporated the (*S*) configuration at C(1), except in Subgroups A, B, and C, where it may be (*R*)

It was also proposed that when an imine function (either as a single C=N bond or as part of an isoquinoline system) was present in these dimers, the imine was found on the right side of the dimer, whereas the left side was of the (*S*) configuration.

In a period of approximately ten years following the proposal of these rules, 19 new bisbenzylisoquinoline alkaloids were isolated from *Thalictrum* genera. The following is summary of the "concurrence" of these alkaloids to the proposed rules of Guinaudeau et al. as described above:

1. The new dimers were found to belong to five of the seven possible Subgroups, with Subgroup F having the most (7):

Subgroup A(2):	Neothalibrine-2'α-*N*-Oxide (**97**)[81,100], Northalibroline (**101**)[53,100]
Subgroup B(0):	None

Subgroup C(3):	2'-Noroxyacanthine(**99**)[81,100],Thaligosine-2α-*N*-Oxide (Thalisopine-2α-*N*-Oxide)(**110**)[81,100], Thalrugosaminine-2α-*N*-Oxide (**111**)[81,100]
Subgroup D(4):	2'-Northaliphylline (**105**)[81,100], Thaliphylline-2'β-*N*-Oxide (**113**)[81,100], Thalivarmine (**114**)[77,100], Thalsivasine (**7**)[77,100]
Subgroup E(3):	5-Hydroxythalidasine (**6**)[3,100], 5-Hydroxythalidasine-2α-*N*-Oxide (**94**)[81,100], Thalidasine-2α-*N*-Oxide (**95**)[81,100]
Subgroup F(7):	Cultithalminine (**89**)[81,100], 5-Hydroxythalmine (**2**)[2,3,100],2-Northalmine(**1**)[84,100],Thalifortine (**109**)[82], Thalmiculatimine (**5**)[81,100], Thalmiculimine (**4**)[81,100], Thalmiculine (**3**)[81,100]
Subgroup G(0):	None

2. Eight of the 19 new alkaloids were oxygenated at C(5) or C(5') with all eight of these alkaloids possessing the (*S*) configuration in that half of the dimer bearing the C(5) or C(5') hydroxylation. These eight alkaloids are: cultithalminine (**89**) [C(5)], 5-hydroxythalidasine (**6**) [C(5)], 5-hydroxythalidasine-2α-*N*-oxide [C(5)] (**94**), 5-hydroxythalmine (**2**) [C(5)], thaligosine-2α-*N*-oxide (thalisopine-2α-*N*-oxide)(**110**) [C(5')], thalmiculimine (**4**) [C(5)], thalmiculine (**3**) [C(5)], and thalrugosaminine-2α-*N*-oxide (**111**) [C(5')].

3. Fourteen of the 19 new alkaloids incorporated the (*S*) configuration at C(1'), while four [cultithalminine (**89**), thalmiculatimine (**5**), thalmiculimine (**4**), and thalsivasine (**7**)] of the remaining five alkaloids were imines at C(1'). The only exception to the rule was thalifortine (**109**), which was proposed to have the *S,R* configuration at C(1)/C(1') [82]. However, there was no CD spectrum provided in this paper, nor was the alkaloid subjected to Na/liquid NH_3 fission (in order to split the compound into its optically active monomeric halves, each of which could be assigned the (*S*) or (*R*) configuration based solely on their respective specific rotation). Hence, the proposed configuration of thalifortine was likely equivocal.

4. Eighteen of the 19 new alkaloids were characterized by having the (*S*) configuration at C(1). Only 2'-noroxyacanthine (**99**) [81,100] was the exception, and this alkaloid is a member of Subgroup C, one of the three subgroups in which the (*R*) configuration at C(1) is "allowed" by the rule.

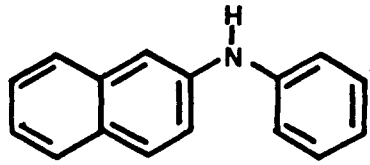
It is safe to conclude that the earlier rules proposed by Guinaudeau et al. have stood the test of time over the last decade. It will be interesting to look back after another decade, when we are in the 21st century, and follow the course of these interesting correlations.

9. ALKALOIDS OF UNUSUAL/UNEXPECTED STRUCTURE

9.1. *N*-Phenyl-2-Naphthylamine.

UV [82], IR [82], ^{1}H NMR [82], EIMS [82].

N-Phenyl-2-naphthylamine (**122**), $C_{16}H_{13}N$ (219.1048), mp 107.5-108.5°C, was isolated from an extract of the roots of *T. ichengense* Lecoy et Oliv. in 1989 [39]. The UV spectrum (EtOH) was characterized by maxima at 220 nm (log ε 4.59), 273 (4.38), and 310 (4.32), while the IR spectrum (KBr) displayed maxima at 3380, 1620, 1595 and 1490 cm^{-1}. The EIMS displayed a parent ion at m/z 219 (100%), with other reported ions being observed at m/z 127 (55), 93 (9), and 77 (4). The ^{1}H NMR spectrum displayed a broad one-proton singlet at δ5.13 (NH) and a complex cluster of aromatic protons at δ6.79-7.79 (m, 12H). To my knowledge, this is the first reported isolation of this unusual compound from nature.



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10. ANALYSIS OF THALICTRUM ALKALOIDS

10.1. Thin-Layer Chromatographic Densitometry

Samples of different species of *Thalictrum* were analyzed by TLC densitometry. This involved extraction of the pulverized sample (commonly a root) with methanol, followed by spotting on a silica gel TLC plate. The plate was first developed with C_6H_6-Me_2CO-NH_4OH (3:3:0.2), with the alkaloids protopine, cryptopine, and hernandezine being scanned at 280 nm. The plate was then developed with C_6H_6-$CHCl_3$-EtOAc-MeOH (2:7:1:3), and the alkaloids isotetrandrine, thalidezine, isothalidezine, berbamine, oxyacanthine, allocryptopine, jatrorrhizine,

and coptisine determined at 280 nm. Finally, the plate was developed with $CHCl_3$-EtOAc-Et_2NH-MeOH-NH_4OH (2:8:0.5:2:0.1), and berberine and palmatine were scanned at 280 nm. The authors report that the method was simple, rapid, and accurate [142].

10.2. Mass Spectrometry

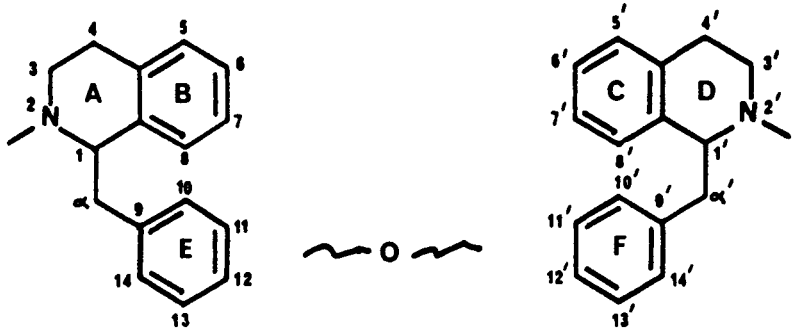
A study of the fast atom bombardment mass spectra and low-energy collision activation tandem mass spectra of a series of monoquaternary and bisquaternary bisbenzylisoquinoline *Thalictrum* alkaloids was undertaken [143]. It was determined that the relative molecular mass of the free base alkaloid and its bisquaternary salt can be obtained from FAB data, but that the monoquaternary ammonium salt derivatives produce only a $(M\text{-}X)^+$ ion, thus precluding the determination of the relative molecular mass. For example, the bisquaternary iodide ammonium salts produced ions $(M\text{-}I)^+$, $(M\text{-}I\text{-}HI)^+$, and $(M\text{-}I\text{-}CH_3I)^+$. The relative molecular mass of the counter anion may be determined with facility, thereby affording the relative molecular mass of the alkaloid. However, with a monoquaternary ammonium salt such as a monochloride, only a $(M\text{-}X)^+$ ion is produced, and there is insufficient mass spectral data to determine its counter anion from positive-ion FAB. Negative-ion FAB was unable to be employed to determine absolutely the counter anion of the alkaloid, since chloride anions occur as common background anions.

The single ether-linked alkaloids (Type A - tail-to-tail linkage, with Ring E linked to Ring F])(**123**) undergo fragmentation principally via benzylic and ether cleavages, and thus the total number of aromatic substituents (hydroxy, methoxy, methylenedioxy groups) may be readily determined for rings A-B, C-D, E and F. This fragmentation was studied using *N*-desmethylthalistyline (bistertiary), thalistyline methochloride (monotertiary-monoquaternary), and thalistyline dimethiodide (bisquaternary). In addition, the aporphine-benzylisoquinoline dimeric alkaloid thalicarpine and its dimethiodide salt were also studied, with parallel results.

The doubly ether-linked alkaloids (Type B - head-to-head and tail-to-tail linkage, with Ring B linked to Ring C and Ring E linked to Ring F)(**124**) undergo facile and prominent fragmentation via double benzylic cleavages, thus allowing the determination of the total number of aromatic substituents in the upper half of the molecule (Rings A-B + Rings C-D). The following alkaloids were studied, and found to support these findings: homoaromoline and homoaromoline dimethiodide; thalrugosine and thalrugosine dimethiodide; thalrugosidine and thalrugosidine dimethiodide; and thalidasine.

Finally, an alkaloid of undetermined structure was studied to demonstrate the utility of these methods. The FABMS of the alkaloid was first determined, followed by derivitization of the alkaloid to its bismethiodide (MeI/Me_2CO - room temperature), and subsequent determination of the FABMS of the derivative. The unknown alkaloid exhibited fragmentation characteristic of a type A aporphine-benzylisoquinoline alkaloid with one methoxy and one hydroxy group in

Ring B, two methoxy groups in Ring E, and either three methoxy groups or one methylenedioxy group + one hydroxy group + one methoxy group in Rings C + F [143].



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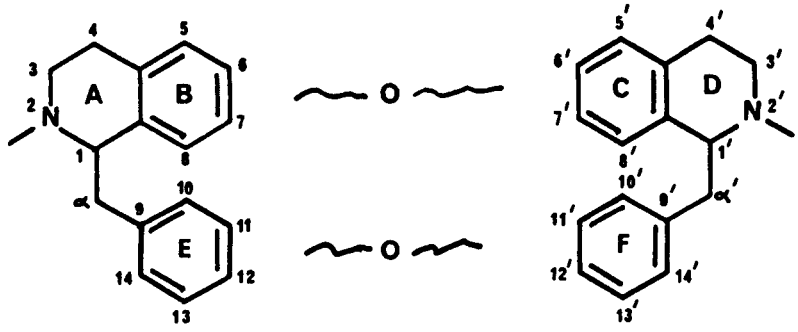

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10.3. X-Ray Crystallographic Diffraction Analysis

X-ray crystallographic diffraction analysis was performed on two alkaloids, thalactamine [65] and thalicsiline (73)[123].

10.4. Additional Spectral Data On Previously Reported Alkaloids

The ^{1}H NMR spectra were obtained for thalisopine (thaligosine) (112)[81] and thalrugosaminine (119)[81].

10.5. Glaucine, Thalicsimidine, and Oxopurpureine

The amount of glaucine, thalicsimidine, and oxopurpureine in an extract of the roots of the Chinese *Thalictrum microgynum* Lecoy. was determined by reverse-phase HPLC, as well as double wavelength TLC. The results (0.18%, 0.04%, and 0.09%, respectively) indicated a consistency in content that was substantiated by nearly the same values for each method [144].

10.6. Thalidasine

The decomposition kinetics of thalidasine in aqueous solution at pH 2.0-7.8 (salt concentration 0-0.132M) and different oxygen concentrations at 50-80° was studied via fluorescence spectrophotometry. Oxidation was found to significantly contribute to the overall decomposition, with the oxidation rate being mainly dependent upon the pH of the medium and the presence of oxygen. In the presence of excess oxygen, the reaction was found to follow pseudo first-order kinetics with respect to alkaloid concentration [145].

The construction and performance characteristics of a new variable-valency thalidasine-selective electrode, based on the tetraphenyl borate-thalidasine ion-pair complex as the electroactive material, was evaluated. The electrode response was fast and stable, with the slopes of calibration curves being reproducible. The slopes of electrode response and electrode potentials varied with the pH of the solution. The K_{a1} dissociation constant was 2.5×10^{-4} at 25°, while K_{a2} was 8.1×10^{-8} [146].

The encapsulation efficiency of a thalidasine liposome injection was studied using Sephadex G-50 column fluorimetry. The free alkaloid was separated from the liposome particles by the column, with satisfactory recovery of the liposome particles, as well as column reproducibility. The encapsulation efficiency of this particular liposome preparation (139-2) was approximately 93% due to the highly lipophilic property of the alkaloid. The authors concluded that the method was suitable for the quality control of encapsulation efficiency of this polyphase liposome injection [147].

In a separate study, the quantity of thalidasine in a polyphase-liposome injection (139-2) was determined by dilution of the injection with EtOH-0.01N HCl (4:1), followed by spectrofluorometric determination at 235 nm (for excitation) and at 318 nm (for emission). The recovery was > 96% and the coefficient of variation was < 3%. The polyphase-liposome encapsulation rate was also determined by this method after separation via Sephadex column chromatography [148].

The pharmacokinetics of thalidasine in a polyphase liposome preparation and in aqueous solution were determined by HPLC in mice following intravenous injection. The blood drug concentration curve fit a two-compartment open model, with the distribution and elimination half-lives being 3.52 and 23.58 minutes, respectively, for the liposome preparation, and 1.293 and 11.12 minutes, respectively, for the aqueous solution [149].

11. BIOSYNTHESIS

Systematic studies conducted with isotopically labelled precursors and intermediates were used to study the biosynthetic pathway leading to various aporphine-benzylisoquinoline dimeric alkaloids in *Thalictrum minus* L. A high rate of incorporation of isotopically labelled reticuline into thalicarpine was noted, and interpreted to mean that reticuline is a precursor of thalicarpine, and is active in the biosynthesis of both the aporphine and benzylisoquinoline portions of thalicarpine. The presence of isoboldine and its incorporation into the aporphine portion of the dimer was noted, with the particular substitution pattern found in reticuline and isoboldine occurring at the phenethylamine stage of the biosynthesis. Both adiantifoline and thalmelatidine are produced at a later biosynthetic stage, and are formed through the introduction of oxygen-bearing substitutents at the aporphine-benzylisoquinoline stage. The following biosynthetic scheme was derived to explain these results: dopamine -> 3-methoxy-4-hydroxyphenethylamine -> reticuline -> isoboldine -> thalmelatine -> thalicarpine -> adiantifoline and thalmelatidine [150].

Administration of triply ^{13}C-labelled (*S*)-reticuline to a stable variant non-alkaloid producing cell culture line of *Thalictrum tuberosum* in cell culture demonstrated a very high incorporation of label into protoberberine alkaloids that were subsequently produced. Since this cell line does not produce reticuline, because the cell line lacks or has insufficient quantities of three methyltransferases that lead to the formation of reticuline, cell cultures that contain this cell line are appropriate for the study of biosynthetic studies. ^{13}C NMR spectroscopy and CIMS were utilized to follow the time course of the metabolism, and demonstrated the rapid formation of scoulerine as a primary reaction product, followed by further tetrahydroprotoberberines and dehydroprotoberberines. The apparently reversible formation of dehydroscoulerine in significant amounts was interpreted as evidence for the role of this compound as an alkaloidal storage product from which scoulerine may be regenerated via enzymic reduction. Scoulerine, dehydroscoulerine, columbamine, and (*S*)-reticuline were detected by ^{13}C NMR in the crude extracts, while berberine was detected by HPLC. The biosynthetic pathway of these protoberberines in this variant cell culture were summarized as follows [151]: (*S*)-reticuline -> scoulerine -> tetrahydrocolumbamine (hypothetical) -> columbamine -> dehydroscoulerine -> candadine -> berberine.

12. CELL CULTURE

There has been an upsurge in the study of alkaloid production in cell suspension cultures in several *Thalictrum* species, notably *T. minus* and *T. rugosum*. These studies, as well as others conducted in the last decade are discussed in the following paragraphs.

12.1. *Thalictrum dipterocarpum*

A cell suspension culture of *T. dipterocarpum* produced berberine (0.4 g/L) as the major alkaloid. The production of berberine was markedly stimulated by NAA in combination with 6-benzylaminopurine. Berberine was released into the culture medium during the logarithmic growth phase, but afterwards the alkaloid that was produced accumulated in the cells [152].

A group of heavy-metal complexing peptides, designated as phytochelatins, were isolated from about thirteen different plant cell suspension cultures, including *Thalictrum dipterocarpum*. The structure of these phytochelatins was established as $(\gamma\text{-glutamic acid-cysteine})_n$-glycine (where n = 3-7), with these peptides appearing upon induction of plant cells with heavy metal ions. Although Cd^{+2} was principally utilized in this study, other inducing ions included Cu^{+2}, Pb^{+2}, Hg^{+2}, and Zn^{+2}. Cell extracts exposed to heavy metal ions did not produce any phytochelatins within the limits of detection. It has been proposed that these peptides may be viewed as linear polymers of the γ-glutamic acid-cysteine portion of glutathione, and perhaps may have been formed from glutathione. The presence of repetitive gamma γ-glutamic acid bonds precludes the possibility that phytochelatins are primary gene products. Most importantly, phytochelatins appear to be the most structurally simple natural products reported to date that are capable of the detoxification and homeostasis of heavy metals via metal-thiolate formation [153].

12.2. *Thalictrum flavum*

A cell suspension culture of *T. flavum* produced berberine (0.3 g/L) as the major alkaloid. The production of berberine was suppressed by NAA in combination with 6-benzylaminopurine. Berberine and columbamine accumulated in the cells of these cultures without being released into the medium [152].

12.3. *Thalictrum minus*

A new bioreactor, consisting of a liquid-gas two-phase system, was devised for utilization with berberine-secreting *T. minus* cells immobilized in calcium alginate beads. The cells were alternately soaked in medium, and then exposed to air, and the maximum yield of berberine production was 875 mg/L. The berberine productivity of immobilized cells was as high as that of freely suspended cells under such conditions of batch culture. In addition, the rate of production of berberine by the immobilized cells remained constant at a high value (50 mg/L/day) for 60 days of semicontinuous culture, achieved by the renewal of medium at 10 day intervals [154].

It was determined that the specific rate of consumption of oxygen by berberine-producing cells of a cell suspension culture of *T. minus* was twice as high as that of non-berberine

producing cells. In addition, the yield of the alkaloid increased with increases in the volumetric oxygen transfer coefficient. An estimate of the optimum conditions of oxygen supply in the suspension cultures and immobilized cells was made according to a known theoretical model assuming oxygen uptake by the cells to be a zero-order reaction, and was in good agreement with the experimental data. The oxygen supply to the immobilized cells was not improved via the reduction of the radius of the beads [155].

The transfer of cultured cells of *T. minus* from culture flasks to a bubble column bioreactor resulted in the production of a small amount of berberine, and with a change of color of these cells to a dark brown, even in the presence of sufficient oxygen. This was attributed to the removal of carbon dioxide from the culture medium via bubbling air, and was reproducible in flask cultures artificially deprived of carbon dioxide. Cell browning was also induced via the exogenous administration of ethylene, suggesting that carbon dioxide likely acts antagonistically against the endogenously generated ethylene. Addition of 2% carbon dioxide to the air of the bioreactor prevented the physiological damage produced by forced aeration [156].

The production of berberine in cell suspension cultures of *T. minus* was regulated by ethylene via the activation of berberine biosynthesis from (*S*)-reticuline. However, ethylene promoted the formation of bound polyphenolics that are associated with cell browning when added in the late stage of culture [157].

The addition of the cytokinin 6-benzylaminopurine (BAP) to growing cell suspension cultures of *T. minus* cells (medium containing 2,4-D) early in the growth cycle induced the production of berberine. The precursor L-tyrosine was rapidly converted into berberine in the presence of BAP, with the alkaloid being released into the medium. However, in the absence of BAP, large amounts of the intermediates tyramine and dopamine accumulated in the non-berberine-producing cells in the same medium. The authors concluded that BAP activates enzymic reactions prior to the formation of the amines in the biosynthesis of berberine [158].

12.4. *Thalictrum minus* var. *hypoleucum*

The addition of 6-benzyladenine (5-10 μM) to cell suspension cultures (Linsmaier and Skoog medium) of *T. minus* var. *hypoleucum* resulted in the production of large amounts of berberine (400-800 mg/L). The alkaloid was continuously released from the cells into the liquid medium, with an excess of the compound crystallizing as its nitrate salt in the medium. Culture of the cells in a modified Linsmaier and Skoog medium containing 20mM KNO_3 and 40mM NH_4Cl in place of 20.6 mM NH_4NO_3 as the nitrogen source, resulted in the crystallization of mainly berberine chloride instead of berberine nitrate. Thalifendine and magnoflorine were produced in small amounts by the culture, and were isolated and identified [159].

The production of berberine was achieved from cells of *T. minus* var. *hypoleucum* in gas phases via culturing the cells with spraying liquid media and supplying oxygen or air. The berberine that is produced is separated from the culture media [160].

The production of berberine in cell suspension cultures of *T. minus* var. *hypoleucum* was greatly enhanced via the administration of spermidine. However, the utilization of other polyamines including cadaverine, putrescine, and spermine were not effective. Experimental results indicate that spermidine induces an increase in the production of ethylene, the latter of which is definitively associated with the biosynthesis of berberine [161].

12.5. *Thalictrum rugosum*

The utilization of a carbohydrate fraction isolated from yeast extract by precipitation with ethanol was used as an elicitor in the induction of berberine biosynthesis (up to four-fold) in cultured cells of *T. rugosum*. These cells were optimally induced in the late exponential or early stationary growth phases, with the response of the cell cultures to elicitor treatment being dependent upon the amount of carbohydrate per unit of biomass and on the physiological state of the cells. Berberine has been classified as a prohibitin, a preinfectional metabolite with antimicrobial activity, and the presence of the alkaloid in the roots of *Cooperia pedunculata*, *Mahonia trifoliata*, and *Sanguinaria canadensis* protects these plants from invasion by the fungus *Phymatotrichum omnivorum*. In conclusion, the carboyhydrate fraction isolated from yeast extract appeared to be effective in the induction of phytoalexin production as well as the enhancement of prohibitin production in plant cell suspension cultures [162].

Treatment of cell suspension cultures of *T. rugosum* with a yeast glucan elicitor induced the production of tyrosine decarboxylase in the late exponential and early stationary growth phases of the cells. Tyrosine decarboxylase has been suggested as the key enzyme between primary and secondary metabolism in the biosynthesis of norlaudanosoline-derived alkaloids, and a good correlation between induced tyrosine decarboxylase activity and berberine biosynthesis has been established [163].

Transferral of growing cells of *T. rugosum* from cell culture media to a fresh medium lacking phosphate caused the release of protoberberine alkaloids into the second medium. Furthermore, cell cultures that have released their alkaloids into the medium continued to grow when the alkaloid-containing medium was replaced by fresh growth medium [164].

Better cell growth and a prevention in the rapid decrease in cell mass after substrate depletion in cell suspension cultures of *T. rugosum* was provided by light. In addition, continuous illumination enhanced the production of alkaloid in cell culture. Finally, light was found to significantly suppress the secretion of the major alkaloid berberine into the culture [23].

The cultivation of a cell suspension of *T. rugosum* utilizing bubble-free aeration was accomplished via a novel membrane-stirrer reactor. Advantages of this bubble-free aeration process included the absence of any foaming or flotation, and the gentle mixing of the suspension via the tumbling movement of a coil or basket of membranes. Suspensions of cell cultures were grown under these conditions in a 21 liter reactor to a high cell densities of 550 g fresh mass or 50 g of dry mass. Maintenance of a pO_2 of 30% up to 400 g/liter was achieved without air

bubble formation at the gas-exchange membranes. The growth rates and alkaloid production of shake-flask and fermentor-grown cells were similar, provided that the physiological states of the initial suspensions were comparable [165].

The addition of etephon to a cell suspension of a culture of *T. rugosum* decreased the fresh/dry weight ratio, and increased the yield of berberine by 31% (dry weight). The release of berberine into the culture increased by 8-9% on the addition of etephon, conceivably via an increase in plasmalemma permeabilization [24].

T. rugosum cell suspensions that produce berberine in a growth-associated manner were examined in two-stage cultures. The utilization of growth regulators, particularly IAA in place of 2,4-D was examined at various growth phases. Although enhancement was clear when IAA was used separately, combination of 2,4-D with IAA produced a suppression of growth, and the change of medium from 2,4-D to IAA was detrimental to the formation of products. A decreased production of berberine was noted in this two-stage culture, and was possibly due to the loss of conditional factors associated with medium replacement [25].

Cells of *T. rugosum* were immobilized in calcium alginate, where they remained viable and produced berberine both in shake flasks and in an airlift bioreactor. Most of the berberine that was produced was stored within the cells, and the formation of the alkaloid was growth associative. The rapid hydrolysis of sucrose, and the preference of glucose over fructose was observed during the growth stage of the cells. Berberine production was increased and the dissolution of the alginate beads were decreased in phosphate-deficient media. With respect to growth and the production of berberine, the behavior of the immobilized cells that were grown in the airlift reactor was comparable to those grown in shake-flasks. The rate of cellular growth and the production of berberine was higher in an airlift reactor operation than in a packed-column reactor due to the better transfer of oxygen [31].

Cultured cells of *T. rugosum* were immobilized via a glass fiber substratum and cultured in shake flasks. Under these conditions, the immobilized cells exhibited both a decrease in growth and a decrease in protoberberine alkaloid production in comparison to freely suspended cells. The decreased production rate of alkaloids was a function of the slower growth rate of the cells, as alkaloid production is known to be growth associated in this species. Maximum biomass and protoberberine alkaloid levels were maintained for longer than 14 days in immobilized culture. However, fresh weight, dry weight, and total alkaloid content decreased in suspension cultures following the linear growth phase. The total inoculum biomass of the cultured cells was introduced as a suspension, and was spontaneously immobilized within 8 hours. The growing biomass in the bioreactor was completely retained by the glass fiber substratum during the 28-day culture period, and the increase in specific production of protoberberine alkaloids was initially similar in both the bioreactor- and the shake-flask-cultivated immobilized cells [166].

The production of berberine in cell suspension cultures of *T. rugosum* was stimulated by the addition of $CuSO_4$, with a maximum enhancement in production of the alkaloid effected by adding the salt at final concentrations of 200-500 μM. The addition of cupric sulfate solution

also permeabilized or lysed cells, with the resulting release of a large amount of berberine into the culture medium [26].

An airlift bioreactor operation was studied for the growth-associated production of the secondary metabolite berberine from *T. rugosum*. A cell scraper used in this particular bioreactor was employed to overcome the problem of large aggregates of high density cells. In addition, the berberine content was increased twofold via the utilization of a mixture of CO_2 and ethylene as gas-stripping agents. It was evident that continuous gas sparging was detrimental to the production of berberine without supplementation with other gases [27].

Depletion of phosphate concentration in cell suspension cultures of *T. rugosum* enhanced the specific productivity of berberine with a significant release of the alkaloid into the culture. Extracellular berberine was found to account for 19% of the total berberine in culture without phosphate, but was only 2-5% of the total berberine in culture with even small amounts of phosphate being present. In addition, precursor feeding was not effective in enhancing the production of alkaloid, nor did exogenous berberine affect cell growth or the production of alkaloid. The culture cells take up large amounts of berberine, for when 500 mg of berberine/L was added at the beginning of the experiment, 81% was found in the cells [28].

The enhancement of the production of berberine by cultures of *T. rugosum* was enhanced by several factors, with some of these factors enhancing both cell growth and alkaloid production, while others resulted in a decoupling of growth and product formation with significant enhancement in the level of specific product. Because it is well known that the production of berberine usually follows a growth-associated product formation kinetics, a process strategy was developed utilizing enhancing factors to optimize growth and alkaloid production. Various combinations of positive effectors produced a synergism, with resultant increase in the formation of berberine [29].

Suspended and alginate-entrapped XAD-7 polycarboxyl ester resin was used for the *in situ* separation of berberine that was produced by a plant cell culture of *T. rugosum*. The resin was capable of adsorbing the alkaloid, with the amount of adsorbed alkaloid being dependent on pH. The neutral salt form of the alkaloid was well adsorbed, with the adsorption isotherm displaying a Langmuir-type appearance. The *in situ* removal of berberine from the culture enhanced the production of secondary cell metabolites in cell culture. The most effective enhancement of alkaloid production was achieved when the resin was added at the exponential phase of cell growth of the culture. Chitosan was employed in the cell culture to permeabilize intracellular berberine, and the secretion of berberine was significantly accelerated via the addition of alginate-entrapped resin at the stationary phase of growth, allowing more than 70% of the berberine that was produced to be adsorbed to the alginate-entrapped resin [32].

13. PHARMACOLOGY

13.1. Extract Screening

A 50% ethanol extract (LD_{50} 681 mg/kg in mice) of the above-ground portions of Indian *T. cultratum* Wall. was screened in a wide battery of *in vivo* and *in vitro* tests, and found to possess a confirmed stimulant activity on the isolated rat uterus, as well as to inhibit the growth of human epidermoid nasopharyngeal carcinoma cells in culture [167]. A similar extract of Indian *T. javanicum* Bl. (whole plant)(LD_{50} >1000 mg/kg in mice) was also screened in these tests, but failed to demonstrate significant activity [168]; however, a 50% ethanol extract of Indian *T. reniforme* Wall. (whole plant)(LD_{50} >1000 mg/kg in mice) produced a stimulation of rat ileum that was not confirmed in repeat testing [168].

A methanol extract of the Rwandese medicinal plant *T. rhynochocarpum* Dillon et A. Rich was evaluated in a pharmacological screen. The entire plant has been used for the treatment of snake bite, and the methanolic extract of the root exhibited spasmolytic activity, as measured in the isolated guinea pig uterus. In addition, the methanolic extract of the leaves was shown to be toxic to mice, as measured by the effects produced by an intraperitoneal administration of the extract in an agar solution [169]. Similar methanolic extracts of the leaves, stems, and roots of the same plant were evaluated for antimicrobial activity against the Gram-positive organisms *Staphylococcus aureus* and *Bacillus subtilis*, the Gram-negative bacilli *Pseudomonas aeruginosa* and *Salmonella gallinarum*, the acid-fast bacillus *Mycobacterium smegmatis*, and the fungus *Candida albicans* [170]. Only the root extract showed an inhibition of the growth of any of the organisms, and that only being against one organism, *M. smegmatis* [170]. Finally, a 95% ethanol extract of the roots of the same Rwandese plant failed to demonstrate *in vitro* activity against the three other species of mycobacteria, *Mycobacterium avium*, *M. simiae*, and *M. tuberculosis* [171].

A methanol extract of the crude drug "Thalictri Herba" (unknown composition) was screened, along with forty-six other kinds of crude drugs, for choleretic effects. A significant positive correlation existed between doses and the choleretic effects of sodium dehydrocholate and 1-phenylpropanol [172]. As a result, high choleretic effects were found in seventeen of the forty-seven kinds of crude drugs, but "Thalictri Herba" was not among them [172]. In a separate work, a methanol extract of "Thalictri Herba" was examined, along with sixty-six other crude drug extracts, for its potential protective effects on experimental α-naphthylisothiocyanate-induced hepatic injury in rats. Although nineteen of the extracts were found to suppress the increase in the concentration of serum bilirubin produced by this induced injury, the extract of "Thalictri Herba" was not among them [173].

An ethanol extract of the roots of the Indian *Thalictrum foliolosum* DC. was found to inhibit the *in vitro* growth of several Gram-positive and Gram-negative bacteria. In particular, the extract demonstrated its greatest activity against *Staphylococcus aureus* and *Pseudomonas*

aeruginosa. The extract had no inhibitory effect on the usual superficial and systemic mycoses, including *Trichophyton mentagrophytes, Candida albicans, Aspergillus fumigatus*, and *Cryptococcus neoformans* [174].

13.2. Pharmacology of Individual Alkaloids

13.2.1. Allocryptopine

Allocryptopine was demonstrated to enhance ^{3}H-γ-aminobutyric acid (GABA) binding to rat brain synaptic membrane receptors. Using this phenomenon as an *in vitro* paradigm, allocryptopine and other protopine alkaloids may be suggested to have benzodiazepine-like activity [175].

13.2.2. Aromoline

Aromoline was screened for antiplasmodial, antiamoebic, and cytotoxic activities via the use of *in vitro* microtests. The alkaloid was characterized by the following: IC_{50} 1.36 μM against a multidrug-resistant strain of *Plasmodium falciparum* (K1)(standard - chloroquine phosphate, IC_{50} 0.20 μM); IC_{50} 5.05 μM against *Entamoeba histolytica* (standard - metronidazole, IC_{50} 1.87 μM); IC_{50} 105 μM against nasopharyngeal KB cell line (standard - podophyllotoxin, IC_{50} 0.008 μM)[176].

Aromoline was screened for *in vitro* antimalarial properties using a multi-drug resistant (K1) strain of *Plasmodium falciparum*. The screen detected the inhibition of incorporation of [^{3}H]-hypoxanthine into *P. falciparum*, and aromoline was characterized by IC 0.67 μg/ml (with 95% confidence intervals of 0.53 - 0.85). The standard antimalarial drug chloroquine diphosphate exhibits IC 0.14 μg/ml (with 95% confidence intervals of 0.12 - 0.16)[177].

Aromoline and ten other bisbenzylisoquinoline alkaloids were evaluated in the *in vitro* histamine release inhibition assay. This assay is one of the basic tests that is used in the evaluation of substances for antiallergic effects. The order of potency of inhibitory effect of those bisbenzylisoquinoline alkaloids that have been isolated from *Thalictrum* species was as follows: homoaromoline > aromoline > isotetrandrine > obaberine > tetrandrine. Berbamine and oxyacanthine were found to have no inhibitory effect [178].

The vasodilator effects of aromoline, berbamine, isotetrandrine, and (+/-)-*N*-methylcoclaurine was evaluated in isolated vessels from male Wistar rats. The rats were anesthetized with thiopental (i.p.), and the thoracic aorta was rapidly excised, with the fat with connective tissues being removed. Rings (about 2 mm in length) were cut from the vessel, and fixed in a bath solution. Prior to addition of the alkaloid sample, the arterial rings were

contracted with K^+ (80 mM). Vasodilator effects of the alkaloids were obtained via the addition of sample solutions directly to the bathing media in cumulative concentrations. The potency of the vasodilator effects of the alkaloids on the contracted thoracic aorta in order of decreasing effectiveness was as follows: aromoline (IC_{50} 0.8 μM) > berbamine (IC_{50} 2.4 μM) > isotetrandrine (IC_{50} 3.0 μM) > *N*-methylcoclaurine (IC_{50} 32 μM). Because aromoline was the most potent of the group, its vasodilator effects were studied *in vivo*. Vital-microscopic observations of microcirculation through a transparent round chamber installed in a rabbit ear showed that intravenous administration of aromoline (3 mg/kg) provided a remarkable dilator effect on the microcirculatory vessels [179].

The anti-inflammatory and antiallergic effects of aromoline were evaluated via the measurement of antigen-induced histamine release from leukocytes, and it was found that the alkaloid inhibited antigen-induced histamine release [180].

13.2.3. Berbamine

The intraperitoneal administration of berbamine into rats suppressed subcutaneous rat air pouch leukocyte infiltration that was induced by interleukin-1 (IL-1), tumor necrosis factor (TNF), and platelet-activating factor (PAF), at an ED_{50} 20-30 mg/kg/3 days. The utilization of berbamine may be of value in the therapy of chronic inflammatory diseases where IL-1, TNF, and PAF play in role in pathogenesis [177].

Berbamine was screened for antiplasmodial, antiamoebic, and cytotoxic activities via the use of *in vitro* microtests. The alkaloid was characterized by the following: IC_{50} 0.45 μM against a multidrug-resistant strain of *Plasmodium falciparum* (K1)(standard - chloroquine phosphate, IC_{50} 0.20 μM); IC_{50} 36.8 μM against *Entamoeba histolytica* (standard - metronidazole, IC_{50} 1.87 μM); IC_{50} 17.8 μM against nasopharyngeal KB cell line (standard - podophyllotoxin, IC_{50} 0.008 μM)[176].

Kinetic analysis of luminol-dependent chemiluminescence demonstrated that a solution of berbamine (20 μM) was found to inhibit the generation of various types of reactive oxygens by guinea-pig neutrophils. The results of these and other experiments suggest that berbamine inhibits the active oxygen generation via the stabilization of plasma membrane and the inhibition of phospholipid-dependent protein kinase (PKC) and NADPH oxidase activation [178].

The scavenging effect of berbamine on active oxygen radicals was studied via a spin-trapping technique and a chemiluminescence (CL) method in phorbol myristate acetate (PMA) stimulated polymorphonuclear leukocytes (PMN) and in four-cell superoxide (O_2^+) or hydroxyl radical ($OH^·$) generating systems. The alkaloid (0.1-0.3 mM) effectively reduced active oxygen radicals in PMA-stimulated PMN, but had no obvious effect on oxygen consumption during the respiratory burst of PMN (as measured with spin probe oxymetry). In addition, berbamine (0.3 mM) inhibited the CL response of PMA-stimulated PMN, and quenched $O_2^·$ in the xanthine/xanthine oxidase and irradiation riboflavin systems, as well as $OH^·$ in the Fenton

reaction. The scavenging action of the alkaloid on O_2^+ was stronger than that of Vitamin E in the xanthine/xanthine oxidase system but the same as Vitamin E in the riboflavin system. Its action on OH^- was similar to that of Vitamin E [179].

Berbamine was noted to inhibit antigen- and compound 48/80-induced arachidonic acid liberation, but not diacylglycerol formation or histamine release. The alkaloid had no effect on A23187-induced arachidonic acid liberation, which was prevented by *p*-bromophenacyl bromide, a known phospholipase A_2 inhibitor, and also did not affect phospholipase A_2 activity in a cell-free system including an exogenous phospholipid substrate. Berbamine inhibited guanosine 5'-*O*-(3-thiotriphosphate)-induced arachidonic acid liberation in saponin-permeabilized mast cells, and by mastoparan or NaF plus $AlCl_3$ in intact cells. In addition, the alkaloid abolished the inhibitory effect of islet-activating protein on compound 48/80-induced arachidonic acid liberation. These data suggest that berbamine suppresses the receptor-mediated phospholipase A_2 activation through, at least in part, uncoupling of a GTP-binding protein from the enzyme, rather than by affecting the enzyme directly [180].

The interaction of the p-nitrobenzoyl ester of berbamine (compound E_6) and calmodulin (CaM)-dependent myosin light chain kinase (MLCK) was studied. The results indicated that the inhibition of MLCK activity was increased with increasing amounts of compound E_6 and was overcome completely by the addition of excessive CaM. The inhibition of MLCK activity by compound E_6 (K_i 0.95 μM) was concentration dependent, as shown by the observation that the stimulatory activity of MLCK induced by CaM was gradually inhibited by the increasing concentrations of compound E_6. Compound E_6 diminished the fluorescence intensity of dansyl-labeled CaM, with the intensity being gradually increased by the addition of different amounts of CaM. Compound E_6 had no effect on the activity of MLCK fragments produced by limited trypsinization, and was considered to be a novel and potent calmodulin antagonist [181].

Bisbenzylisoquinoline alkaloids block Ca^{+2} uptake through L-type Ca^{+2} channel and modulate binding of ligands to four distinct sites (dihydropyridine, benzothiazepine, aralkylamine, and (diphenylbutyl)piperidine) in the Ca^{+2} entry blocker receptor complex of the channel. All bisbenzylisoquinoline analogs tested, including berbamine, completely inhibit diltiazem binding, but many only partially inhibit D-600 and fluspirilene binding. For dihyrodpyridine binding, the compounds show either stimulation or inhibition or exhibit no effect. Data from this experiment suggest that berbamine and a variety of bisbenzylisoquinoline congeners act to block the L-type Ca^{+2} channel by binding to the benzothiazepine site on the channel. The novel patterns exhibited by berbamine and other bisbenzylisoquinoline alkaloid analogs in affecting allosteric coupling between the benzothiazepine site and the other receptors of the Ca^{+2} entry blocker receptor complex define a new class of Ca^{+2} entry blocker with binding properties distinct from diltiazem [182].

Berbamine was shown to have a similar but weaker effect than isotetrandrine or tetrandrine on overcoming resistance in the multidrug-resistant subline, Ch^R-24, derived from human KB carcinoma cells to various antineoplastic drugs. Berbamine partially overcomes the resistance of CH^R24 cells to daunomycin [183].

Treatment with berbamine was found to relax K^+-precontracted rat aortal rings, with an IC_{50} of about 20 μmol/liter. This relaxation was not inhibited by denudation of the endothelium or by premedication of the aortas with indomethacin, methylene blue or propranolol. In addition, the alkaloid inhibited contractions induced by norepinephrine, phenylephrine, and serotonin in a non-competitive manner, as well as competitively blocking calcium-induced contraction in a Ca^{+2}-free depolarizing PSS. The blockade of calcium entry appears to be the major mechanism of the dilatory effect of this alkaloid, but its interaction with α-adrenoceptors was not excluded [188].

The effect of berbamine on experimental allergic encephalitis (EAE) was investigated in Lewis rats. Amelioration of the acute-phase of EAE was only minimal, but the incidence of relapsing EAE was reduced by 65% for berbamine at a non-toxic treatment dosage of 60 mg/kg via gavage on alternate days. These results suggest that berbamine, and perhaps other bisbenzylisoquinoline alkaloids, may have a potential role in the therapy of progressive multiple sclerosis [189].

The anti-inflammatory and immunosuppressive properties of berbamine and tetrandrine were compared. These comparative *in vitro* studies demonstrated that tetrandrine had significantly greater suppressive effects on adherence, locomotion and ^3H-deoxyglucose uptake of neutrophils, as well as the mitogen-induced lymphocyte responses and mixed lymphocyte reactions. In addition, tetrandrine displayed anti-oxidant activity while berbamine did not. However, berbamine showed a significantly greater capacity for inhibition of NK cell cytotoxicity. These results indicated that tetrandrine was superior to berbamine in most aspects of anti-inflammatory and immunosuppressive activity [190].

A comparison of the effects of berbamine and tetrandrine in mice revealed that both alkaloids were equipotent in terms of enhancement of antibody responses and suppression of delayed-type hypersensitivity (DTH) responses to sheep erythrocyte cell antigens. Both alkaloids were equally active when administered to mice during the induction and expression phases of DTH. Neither alkaloid affected antibody response in brucellosis-infected mice, while both caused equipotent suppression of DTH. Berbamine, however, caused a significant suppression of spleen weight, while tetrandrine did not. Furthermore, berbamine produced a significantly greater enhancement of spleen colony counts of *Brucella abortus* than did tetrandrine. Bioactive dosage failed to induce toxic effects, as determined by short-term toxicology studies [191].

Berbamine and tetrandrine were investigated for their actions on guinea-pig airway microvascular leakage, an animal model of asthma and allergic inflammation. There was significant inhibition of microvascular leakage via tetrandrine with all four allergic mediators, but berbamine was only able to suppress microvascular leakage induced by platelet-activating factor and bradykinin, but not by leukotriene D4 or histamine [192].

Berbamine has been utilized in the Orient (China and Japan) in the therapy of leukopenic complications of cancer. Mice treated with non-toxic doses of the alkaloid displayed a significant enhancement of neutrophil and lymphocyte counts in their circulation, but a decrease in the numbers of bone marrow stem cells in a dose-dependent manner after two weeks. Similar results were observed in cyclophosphamide-treated mice. These results suggest that berbamine acts via

the stimulation of maturation and release of leukocyte progenitors, and may therefore have utility in leukopenic patients [193].

The potential therapeutic effect of berbamine (10, 25, and 50 μg/ml)(and thirteen other bisbenzylisoquinoline alkaloids) against the protozoan disease leishmaniasis was studied by biological assays on *in vitro* culture forms of three strains of *Leishmania*; *L. brasiliensis brasiliensis* (cutaneous and mucocutaneous leishmaniasis), *L. mexicana amazonensis* (cutaneous), and *L. donovani* (visceral leishmaniasis). Berbamine was found to inhibit the growth of all three types to differing degrees, but was not as active as daphnandrine, gyrocarpine, or obaberine [194].

The potential therapeutic effect of berbamine (10, 25, and 50 μg/ml) (and thirteen other bisbenzylisoquinoline alkaloids) against the protozoan disease American trypanosomiasis (Chagas' disease) was studied by biological assays on *in vitro* culture forms of three strains of *Trypanosoma cruzi* (Tulahuen, C8C11, and 1979 CL1). As in the case of *Leishmania*, berbamine was found to inhibit the growth of all three types to differing degrees, but was not as active as any daphnandrine, gyrocarpine, or obaberine [195].

Berbamine and tetrandrine were evaluated in terms of their production of inhibitory effects on the production of interleukin-1 and tumor necrosis factor (TNF_α) by monocytes and macrophages, and TNF_β production by lymphocytes. Tetrandrine was found to be 6-18 times more potent than berbamine in these tests. In addition, tetrandrine was found to significantly suppress phosphoinositide turnover while berbamine did not. Taken together, these findings suggest that tetrandrine may be superior to berbamine for chronic inflammatory diseases where inflammatory mediators and cytokines play a major role in pathogenesis (as in silicosis), while berbamine may be somewhat superior for the treatment of autoimmune disease where immunological mechanisms have a greater role in pathogenesis (as in experimental allergic encephalitis in rats). Comparative data regarding these two alkaloids may provide valuable insights into structure-activity relationships, and the design and development of synthetic analogues and congeners useful in the therapy of chronic inflammatory and autoimmune diseases [196].

Berbamine was found to inhibit arachidonic acid-induced inflammation in mice. Topical application of berbamine (2 μmol/mouse) markedly suppressed the tumor-promoting effect of 12-*O*-tetradecanoylphorbol-13-acetate (1 μg) in mouse skin initiated with 7,12-dimethylbenz[a]anthracene (50 μg), at a grade corresponding to that of another bisbenzylisoquinoline alkaloid, cepharanthine [197].

The effects of berbamine on energy metabolism in isolated rat alveolar type II cells (cells which play a critical role in the maintenance of normal lung function) were studied. Incubation of type II cells with berbamine produced a reduction in cellular ATP content, but there was no effect of the alkaloid on cellular oxygen consumption. Berbamine produced an increase in internal calcium levels of type II cells, and incubation of the cells with calcium ionophore, 4-bromo A-23187, led to increased amounts of cellular calcium and reductions in ATP levels, with no effect on oxygen consumption. Exposure of isolated lung mitochondria to calcium produced a concentration-dependent reduction in ATP synthesis with no effect on mitochondrial oxygen

consumption. Direct exposure of mitochondria to berbamine had no effect on ATP synthesis. These results were consistent with the belief that berbamine produced an increase in type II cell internal calcium levels which, in turn, led to reduced rates of mitochondrial ATP synthesis [198].

The inhibitory effects of berbamine and 150 natural products on the cytotoxic activity of polymorphonuclear leukocytes (PMN) was investigated. The research employed the effects of natural products on the PMN activation by the antitumor immunomodulator TAK (a linear β-1,3-D-glucan from *Alcaligenes faecalis* var. *myxogenes*) using a PMN cytotoxicity assay system. Berbamine was found to inhibit the activation of PMN activation by TAK, showing an ID_{50} 33 μg/ml. From this, and related studies, it was postulated that berbamine may impair the NADPH oxidase system in the plasma membrane of PMN [199].

Berbamine and ten other bisbenzylisoquinoline alkaloids were evaluated in the *in vitro* histamine release inhibition assay. This assay is one of the basic tests that is used in the evaluation of substances for antiallergic effects. The order of potency of inhibitory effect of those bisbenzylisoquinoline alkaloids that have been isolated from *Thalictrum* species was as follows: homoaromoline > aromoline > isotetrandrine > obaberine > tetrandrine. Berbamine and oxyacanthine were found to have no inhibitory effect [178].

The vasodilator effects of aromoline, berbamine, isotetrandrine, and (+/-)-*N*-methylcoclaurine was evaluated in isolated vessels from male Wistar rats. The rats were anesthetized with thiopental (i.p.), and the thoracic aorta was rapidly excised, with the fat with connective tissues being removed. Rings (about 2 mm in length) were cut from the vessel, and fixed in a bath solution. Prior to addition of the alkaloid sample, the arterial rings were contracted with K^+ (80 mM). Vasodilator effects of the alkaloids were obtained via the addition of sample solutions directly to the bathing media in cumulative concentrations. The potency of the vasodilator effects of the alkaloids on the contracted thoracic aorta in order of decreasing effectiveness was as follows: aromoline (IC_{50} 0.8 μM) > berbamine (IC_{50} 2.4 μM) > isotetrandrine (IC_{50} 3.0 μM) > *N*-methylcoclaurine (IC_{50} 32 μM) [179].

The effects of berbamine, oxyacanthine, and berberine on 5-lipoxygenase lipid peroxidation in phospholipid liposomes induced by 2,2'-azo-(bis-2-amidinopropane)(AAPH), deoxyribose degradation, and their reactivities against the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) were studied. None of the alkaloids showed any appreciable effects in the inhibition of 5-lipoxygenase. Reactivity against DPPH increased in the following order: berberine < oxyacanthine < berbamine. Pro-oxidant effects of the alkaloids were excluded, since deoxyribose degradation was not influenced as determined by the release of malondialdehyde [200].

Berbamine, berberine, and oxyacanthine were evaluated for antiproliferative activity against the growth of HaCaT cells (a rapidly multiplying human keratinocyte cell line). Proliferation of the keratinocytes was determined directly by counting the dispersed cells under a phase-contrast microscope after 48 hours of treatment. The antiproliferative activity of these alkaloids were as follows: berbamine (IC_{50} 11 μM) > oxyacanthine (IC_{50} 13 μM) > berberine (IC_{50} 30 μM). The standard was anthralin (IC_{50} 0.7 μM). These results indicate that the three

alkaloids tested were all antiproliferative agents when applied to HaCaT cells at micromolar concentrations [201].

The effects of berbamine of the isolated and perfused working heart of the guinea pig was studied. The alkaloid was observed to depress the function of the isolated working heart in a dose-dependent manner. The alkaloid (3 mol/l) decreased the left ventricular pressure, aortic pressure $-dP/dt_{max}$, aortic blood flow and coronary blood flow, and increased left ventricular end-diastolic pressure. At a concentration of 100 mol/l, ventricular asystole was produced, but there was no influence on atrial contraction. Berbamine was also observed to antagonize epinephrine-induced arrhythmias [202].

Berbamine was isolated from an extract of *Berberis poiretii* via bioassay-guided techniques using the ^{3}H-diltiazem receptor binding assay. The alkaloid was found to possess calcium channel blocking activities (IC_{80} 3 x 10^{-6} M) and to act as an antiperoxidant as measured by the erythrocyte lipid peroxidation test. These data may provide information about the antimyocardial ischemic and antiarrhythmic effects of the berbamine [203].

The effects of berbamine on KCl- and 5-HT-induced contraction of the basilar artery of pigs were studied *in vitro*. Berbamine was found to markedly relax KCl-induced contraction of the basilar artery (IC_{50} 4.63 μM), with this effect being antagonized by increasing the concentration of extracellular Ca^{+2}. The alkaloid inhibited 5-HT-induced contraction of this artery, displaying significant inhibition of sustained tonic contraction (IC_{50} 0.64 μM), but with the initial fast phasic contraction being relatively unaffected (IC_{50} 19.8 μM). The 5-HT-induced contraction of this artery was dependent on the concentration of extracellular Ca^{+2}, especially sustained tonic contraction. The results of Ca^{+2} withdrawal and replacement indicated that sustained tonic contraction was due to 5-HT-stimulated Ca^{+2} influx, while 5-HT-induced release of intracellular Ca^{+2} resulted in fast phasic contraction. The alkaloid (0.8 μM) produced a markedly inhibitory effect on 5-HT-induced Ca^{+2} influx. These effects were similar to those produced by nimodipine. These results suggest that berbamine has an antagonistic effect on the potential sensitive channels and the receptor operated channels [204].

The isolated perfused rat heart model was utilized to observe the protective effects of berbamine on myocardial ischemia/reperfusion injury. The rodent hearts were significantly injured by global ischemia (40 minutes), followed by reperfusion (20 minutes). Berbamine was observed to significantly improve heart function, prevent ventricular fibrillation, reduce creatine phosphokinase release, preserve NaK-ATPase activity, and reduce Na^+ gain and K^+ loss during ischemia and Ca^{+2} overload during perfusion. Oxygen-centered free radical signals became more intense with the use of low temperature ESR technique in hearts subjected to ischemia (40 minutes) followed by reperfusion (15 seconds). In the presence of berbamine, these free radical signals decreased. These results suggest that the alkaloid may alleviate myocardial ischemia/reperfusion injury. Reasons offered for this suggestion include the following: First, the effect might be due to preserved myocardial Na,K-ATPase activity and inhibition of sodium overload at the end of ischemia, which might further lead to attenuation of reperfusion-induced

calcium overload; and second, the reduction of oxygen free radical generation during reperfusion [205].

The antiinflammatory and antiallergic effects of berbamine were evaluated via the measurement of antigen-induced histamine release from leukocytes, and it was found that the alkaloid did not inhibit antigen-induced histamine release in a dose-dependent manner [180].

The effects of berbamine on the amplitude of contraction, automaticity, effective refractory period (ERP) and intensity duration of guinea-pig atria were studied. The alkaloid markedly inhibited the force of contraction of guinea-pig atria, and prolonged the ERP, attenuated epinephrine-induced automaticity, but had no influence on the intensity duration curve. Berbamine antagonized the actions of isoproterenol in a noncompetitive manner, and shifted the calcium dose-response curve to the right, acting as a noncompetitive antagonist. Finally, berbamine was also observed to shift the dose response curve of histamine to the right, also in a noncompetitive manner [206].

The plant sources, chemistry, pharmacology, and pharmacotherapeutics of berbamine were reviewed. A botanical survey revealed that there were thirty-two species of *Berberis* that contain berbamine in China that were utilized. It was observed that berbamine possessed significant leukogenic effects in rats and dogs that had been pretreated with cyclophosphamide, and clinical observations demonstrated a therapeutic effect in 405 leukopenic patients. The utility of berbamine as a leukogenic drug, and as an adjuvant in cancer chemotherapy and radiotherapy was discussed [334].

Berbamine was evaluated in BALB/c mice infected with *Leishmania amazonensis* (IFLA/BR/67/PH8 or MHOM/GF/84/CAY-H-142) or *L. venezuelensis* (VE/74/PM-H3). The treatments were initiated one day after parasitic infection at an alkaloid concentration of 100 mg/kg/day for fourteen days, and the reference compound (meglumine antimonate) at 200 mg/kg/day. Berbamine was found to be less potent than meglumine antimonate against *L. amazonensis*, and the alkaloid did not show significant activity against *L. venezuelensis* [334].

13.2.4. Berberine

Berberine sulfate was found to have no effect on the growth of uropathogenic *E. coli* or on the synthesis of major outer membrane proteins, but it did block adhesion of the organisms to erythrocytes and to epithelial cells. The decreased adhesion was accompanied by a reduction in the synthesis of fimbrial subunits and in the expression of assembled fimbriae, suggesting that the anti-infective activity of the alkaloid in *E. coli*-induced UTI may be mediated by the selective suppression of the synthesis and assembly of fimbriae by these organisms [207].

Berberine sulfate was reported to be bacteriostatic for streptococci, with sub-minimum inhibitory concentrations of the alkaloid blocking adherence of streptococci to host cells, immobilized fibronectin, and hexadecane. Two distinct mechanisms were advanced to explain the interference of berberine sulfate with the adherence of group A streptococci: first, by

releasing the adhesin lipoteichoic acid from the streptococcal cell surface; and second, by directly preventing or dissolving lipoteichoic acid-fibronectin complexes [208].

Berberine and 8-cyanodihydroberberine were found to exhibit significant activity (> 50% suppression of lesion size) against the lesions of *Leishmania braziliensis panamensis* in golden hamsters. Tetrahydroberberine (canadine) was more potent but less toxic than berberine against *Leishmania donovani*, but not as potent as meglumine antimonate, a standard drug utilized in the therapy of leishmaniasis [209].

Berberine was determined to inhibit rabbit lens aldose reductase (IC_{50} 5.2 x 10^{-5}M) and to exhibit a competitive inhibition as determined by Lineweaver-Burk plot [210].

The effects of berberine on contractions of the longitudinal muscle of the guinea-pig isolated ileum were studied, and found to be dose dependent. Lower concentrations of the alkaloid (=/< 5 x 10^{-1}M) induced a parallel rightward shift of the dose-response curve of acetylcholine, suggesting that the alkaloid is a competitive antagonist of the actions of acetylcholine at the receptors. However, at a higher concentration (1 x 10^{-4}M), the alkaloid produced a rightward shift of the dose-response curve of acetylcholine with a reduction of maximum response, thus indicating that the interactions of the two drugs changed from competitive to noncompetitive antagonism. Berberine was observed to dose-dependently antagonize the effects of the specific muscarinic receptor agonist muscarine, furnishing evidence that the site of action of the alkaloid is at the muscarinic receptors. The alkaloid had no effect on the contractions of the ileum preparation elicited by histamine, and concentration-dependently reduced electrically induced cholinergic contractions. In addition, berberine had no effects on KCl-elicited contractions (postreceptor sites). The action of the alkaloid was reversible and dependent on the duration of incubation with the preparation, with the longer tissue incubation time corresponding to a slower recovery. These results support the hypothesis that berberine blocks muscarinic receptors, and might thereby offer a partial explanation of the efficacy of the alkaloid in the reduction of intestinal motility and the treatment of diarrhea [211].

Berberine chloride exhibited reverse transcriptase inhibitory properties against both HIV-1RT (p66/p51)(Human immunodeficiency virus type 1 reverse transcriptase)(IC_{50} 60.9 μg/ml [163.8 μM]) and HIV-2RT (p68/p55)(Human immunodeficiency virus type 2 reverse transcriptase)(IC_{50} 57.8 μg/ml [155.5 μM]). The alkaloid is thought to inhibit various RNA and DNA polymerizing enzymes via interaction with nucleic acid template-primers [212].

The interaction of berberine chloride with poly(A) and tRNA has been studied by various spectroscopic techniques. In comparison with DNA or tRNA, berberine has a very high effective binding capacity to poly(A), as determined by binding parameters derived from spectrophotometric and spectrofluorimetric measurements via Scatchard analysis. Circular dichroism studies demonstrate that binding of berberine to poly(A) produces a significant change in the CD spectrum of poly(A) itself, as exhibited by both a decrease of positive and negative bands and the appearance of a conservative type of extrinsic CD spectrum in the 300-400 nm wavelength region, while not causing any significant alteration to the A form structure of tRNA. It was concluded that berberine interacts more strongly with poly(A) than DNA or tRNA [213].

The interaction of berberine chloride with calf thymus DNA was studied by spectrophotometry in buffers of various salt concentrations and temperatures. The salt and temperature dependence of the binding constants were used to estimate thermodynamic parameters involved in the complex formation of berberine with DNA. The binding process was observed to be exothermic over the entire range, with the values of enthalpy and entropy change being strongly dependent on the salt concentration. The negative enthalpy and positive entropy changes compensate one another to produce a relatively small Gibbs free energy change [214].

The methanol extract of the bark of *Phellodendron amurense* was shown to possess strong antifeedant activity against the paper/wood termite pest *Reticulitermes speratus*. Partitioning and chromatography of the extract afforded four active antifeedant compounds, one of which was berberine [215].

In order to study the structure-activity relationships of phenolics as regards xanthine oxidase inhibition, berberine and eleven other naturally occurring phenolics were tested. The IC_{50} of berberine chloride was 170.74 μM, while that of quercetin (the most potent compound evaluated) was 7.23 μM [216].

Berberine acted as a feeding deterrent toward the larvae of *Spodoptera littoralis* when incorporated into an artificial diet at concentrations corresponding to its approximate natural concentrations as found in plants. Under ecological conditions, therefore, berberine may have some kind of protective function against generalist herbivores, such as *S. littoralis*, if more palatable food is available to the insects [217].

The electropharmacological effects of berberine on canine cardiac Purkinje fibers and ventricular muscle and atrial muscle, as well as rabbit atrial muscle were studied via conventional microelectrode techniques to obtain intracellular recordings of transmembrane electrical potentials. The results suggest that berberine exerts Class III antiarrhythmic and proarrhythmic actions in canine cardiac muscle *in vitro* [218].

Four hundred adults presenting with acute watery diarrhea were entered into a randomized, placebo controlled, double blind clinical trial of berberine, tetracycline, and tetracycline + berberine to study the antisecretory and vibriostatic effects of the alkaloid. Of 185 patients with cholera, those given tetracycline or tetracycline + berberine had considerably reduced volume and frequency of diarrheal stools, duration of diarrhea, and volumes of required intravenous and oral rehydration fluid. Berberine did not produce an antisecretory effect, but analysis by factorial design equations showed a reduction in diarrheal stools by one liter and a reduction in cyclic AMP concentrations in stools by 77% in the groups given berberine. Many fewer patients given tetracycline or tetracycline + berberine excreted vibrios in their stools after 24 hours in comparison with those given berberine alone. Neither tetracycline nor berberine had any benefit over placebo in 215 patients with noncholera diarrhea [219].

A pluripotent human teratocarcinoma cell clone, NT2/D1, which was derived from the Tera-2 cell line, was induced to differentiate into cells with neuronal cell morphology by treatment with berberine. As early as one day after a 24 hour treatment of cells with berberine at a non-toxic dose of 0.1 mg/ml in culture medium, the cells began to show morphologic

changes, including the development of terminally differentiated neuronal cells with long, interconnecting network-like cellular structures. This process is much faster as compared with that induced by treatment with retinoic acid which took at least several days to develop. In view of the recent discovery of four novel *ras*-like genes expressed in undifferentiated NT2/D1 cells, berberine-induced differentiation may shed light on the differential expression of these genes of *ras* superfamily [220].

Forty-eight derivatives of berberine-type alkaloids were examined for their inhibition activity against the induction of mouse ear edema via application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Berberine chloride (ED_{50} 6.5 μmol/ear; 36% inhibition) and berberine sulfate (ED_{50} 1.2 μmol/ear; 53% inhibition) displayed inhibitory effects at a grade corresponding to quercetin, caffeine, and cepharanthine. Berberine derivatives had stronger inhibitory activity than palmatine derivatives. Since berberine and some of its derivatives are present in Chinese traditional drugs, which are used in combined Kampo prescriptions, it is important to determine if such alkaloids possess carcinogenic inhibiting properties [221].

The interaction of berberine chloride with natural and synthetic DNAs of differing base composition and sequences was followed by various spectroscopic and viscometric studies. The binding of the alkaloid was characterized by hypochromism and bathochromism in the absorption bands, enhancement of fluorescence intensity, stabilization against thermal denaturation, perturbations in the circular dichroism spectrum, increase in the contour length of sonicated rod-like DNA, and induction of unwinding-rewinding process of covalently superhelical DNA, depending on the base composition and sequences of base pairs. Binding parameters determined from absorbance and fluorescence titration by Scatchard analysis indicated a very high specificity of the alkaloid to AT-rich DNAs and alternate polymer. Fluorescence quantum yield was maximum for the complexes with AT-rich DNAs and alternate AT polymer. These results suggest that berberine chloride exhibits considerable specificity towards alternating AT polymer and binds to AT-rich DNAs by a mechanism of classical intercalation [222].

Intraperitoneal administration of berberine at dosages of 0.5, 1, and 5 mg/kg/day to original-type anti-GBM-induced nephritic rats was effective in the the inhibition of urinary protein excretion, elevation of serum cholesterol, and creatinine contents, as well glomerular histopathological changes. Furthermore, oral administration of berberine (20 mg/kg/day) also inhibited urinary protein excretion throughout the experimental periods. The alkaloid inhibited platelet aggregation in both *in vitro* and *in vivo* assays, and inhibited the decline of renal blood flow. Although berberine inhibited an increase in thromboxane B_2 formation, the alkaloid increased the formation of 6-ketoprostaglandin $F_{1\alpha}$ in platelets and isolated glomeruli. These results suggested that the antinephritic effect of berberine may be partly due to antiplatelet action and improved renal hemodynamics, via the alteration of prostanoid synthesis [223].

Berberine chloride was tested for *in vitro* activity against the epimastigotes of the protozoan parasite *Trypanosoma cruzi* (Costa Rican strain). This organism is the causative agent of Chagas' Disease. The alkaloid was found to inhibit the growth of the epimastigotes at concentrations of both 5 μg/ml and 50 μg/ml [224].

The effect of berberine chloride on the contraction of guinea pig aortic strips induced by various stimuli was studied. The alkaloid (25-200 μM) noncompetitively inhibited the response of the strips to both histamine and norepinephrine, but did not decrease the high K^+-elicited contraction. The alkaloid reduced both the rate and the relative contribution to developed tension of the initial, rapid phase to norepinephrine-induced contraction, whereas the slow, later component was less affected. Berberine inhibited the response of aortic strips incubated in Ca^{+2} to norepinephrine, but did not reduce caffeine-induced contraction. The alkaloid inhibited phospholipase C-activated contractile response, and in cultured arterial smooth muscle cells, did not significantly decrease the production of inositol phosphates activated by Arg^8-vasopressin. This pattern of activity of the alkaloid is not easily reconciled with an involvement of the contractile machinery and suggests that the drug has no effect on the voltage-operated calcium channels. Although an antagonism at the receptors or an increase of cyclic AMP or cyclic GMP was not completely excluded, the authors suggested that at least one component of the berberine inhibitory effect may be due to its action on some step of the chain of events linking receptors to contractile response [225].

Fluorescence data for the interaction with acetylcholinesterase (EC 3.1.1.7, ACHE) from the electric eel *Electrophorus electricus* with some quaternary protoberberine and benzophenanthridine alkaloids was obtained. Berberine and other related compounds were bound to the gamma γ-anionic site of ACHE via a comparison with known inhibitors of acetylcholinesterase, including tetramethylammonium and tacrine. Furthermore, during the interaction, two molecules of the ligand were bound to one molecule of the enzyme [226].

Aldehyde reductase I has been observed to be inhibited by a number of benzylisoquinoline-derived alkaloids. The sensitivity of aldehyde reductase I to test compounds was compared with that of alcohol dehydrogenase and/or aldehyde reductase II, and alcohol dehydrogenase was found to be more selective in binding the alkaloids. The kinetics of the inhibitory action of berberine and other results suggest that the binding site of aldehyde reductase I for alkaloids is relatively large, has a hydrophobic nature, and probably contains a group with a positive charge. In addition, the binding site is likely not identical with the active center of the enzyme [227].

Berberine chloride was evaluated for antimalarial activity against *Plasmodium falciparum in vitro* (two clones of human malaria; *Plasmodium falciparum* D-6 [Sierra Leone clone] and W-2 (Indochina clone) and *Plasmodium berghei in vivo* (mice). The alkaloid exhibited an antimalarial potency equivalent to that of quinine *in vitro*, but was inactive *in vivo*. The results were consistent with those of others who have found berberine to be a potent inhibitor *in vitro* of both nucleic acid and protein biosynthesis in *P. falciparum*, and have demonstrated a strong interaction of berberine with DNA. In addition, the lack of *in vivo* antimalarial activity in mice observed with berberine and other protoberberine alkaloids agrees with clinical reports that have claimed berberine to be inactive as an antimalarial drug [228].

Berberine chloride was evaluated in the human immunodeficiency virus reverse transcriptase assay and found to be moderately active (50 μg/ml < IC_{50} < 150 μg/ml)[229].

Berberine chloride was dissolved in culture medium and evaluated for its activity against two protozoa, *Plasmodium falciparum* (K1, multi-drug resistant strain) and *Entamoeba histolytica* (NIH 200). The alkaloid was toxic to KB cells (IC_{50} 7.3 μM), and was active against *P. falciparum* (IC_{50} 0.97 μM), but inactive against *E. histolytica* (IC_{50} 111 μM). In assessing relative *in vitro* cytotoxicity to antiplasmodial activity, berberine had the least favorable ratio (8), while jatrorrhizine had the most favorable ratio (> 106) of the alkaloids tested. The results were somewhat surprising in that berberine is used in some countries as an amoebicide. However, the results lend some support to the utilization of plants containing protoberberine alkaloids for the treatment of malaria [230].

Berberine chloride was evaluated for *in vitro* activity against *Candida albicans*, *Cryptococcus neoformans*, *Mycobacterium intracellularae*, *Trichophyton mentagrophytes*, and *Saccharomyces cerevasiae*, as well as representative Gram-positive and Gram-negative bacteria. The alkaloid was found to exhibit moderate to good antifungal activity, and the minimum inhibitory concentrations were determined as follows: *Candida albicans* (0.39 μg/ml); *Cryptococcus neoformans* (1.56 μg/ml); and *Mycobacterium intracellularae* (1.56 μg/ml). However, berberine chloride did not exhibit sufficient qualitative activity against bacteria to warrant the determination of minimum inhibitory concentration determinations [231].

Solutions of berberine sulfate were observed to inhibit the growth of various influenza viruses [232].

The cell-killing effect and its possible mechanism of berberine used alone or in combination with argon ion laser treatment on 9L rat glioma cells were studied. The survival fraction of 9L cells after single treatment of berberine was estimated via the LD_{50} which was 60 μg/ml. After the addition of low energy laser treatment, the LD_{50} markedly decreased to 10 μg/ml. The inhibitory effect of the alkaloid on the biosynthesis for DNA, RNA, and protein of 9L cells was enhanced via the argon laser. Furthermore, the 9L cells morphologically displayed lysis, encystation, and degeneration after treatment with berberine, with laser-treatment enhancing this cytocidal effect. These experiments validated the cytocidal effect of berberine combined with laser treatment on 9L rat glioma cell line, and introduced the possibility of the alkaloid as a photosensitive agent [233].

The interaction of berberine chloride with human platelet α_2 adrenoreceptor was investigated, and the alkaloid was found to competitively inhibit (IC_{50} 16.6 μM) the specific binding of [^{3}H]-yohimbine. The displacement curve was parallel to those of clonidine, epinephrine, and norepinephrine, with increasing concentrations of the alkaloid (from 0.1 μM to 10 μM) inhibiting [^{3}H]-yohimbine binding, and produced a shift of the saturation binding curve to the right without decreasing the maximum binding capacity. At concentrations of 0.1 μM to 0.1 mM, solutions of berberine chloride inhibited the platelet cyclic AMP accumulation that was induced by 10 μM prostaglandin E_1 in a dose-dependent manner, acting as an α_2 adrenoceptor agonist. In the presence of L-epinephrine, berberine blocked the inhibitory effect of the pressor, thus behaving as an α_2 adrenoceptor antagonist. These properties are similar to those of clonidine, suggesting that berberine is a partial agonist of platelet α_2 adrenoceptors [234].

The effects of berberine on guinea-pig ileum contractility were studied in both transmurally-stimulated and unstimulated preparations. Transdermal stimulation (80 V, 0.5 ms, 0.05 Hz) of the guinea-pig ileal segments produced a twitch response, with berberine (10^{-8} to 10^{-5} M) enhancing this response dose-dependently, as well as acetylcholine (3 x 10^{-9} and 10^{-8} M). Atropine (10^{-7} M) but not mecamylamine (10^{-5} M) abolished the response. Pretreatment with hemicholinium (3 x 10^{-5} M) antagonized the effect of berberine but failed to change that of acetylcholine. Berberine (10^{-5} M) also antagonized the α_2-adrenoceptor agonist xylazine (10^{-8} and 10^{-7} M)-induced inhibition of the twitch response to transmural stimulation. Berberine (10^{-5} M) also produced contractile responses in the unstimulated ileal preparations, with atropine (10^{-6}M) but not mecamylamine (10^{-4}M) abolishing the response. In addition, berberine (10^{-6} to 10^{-4} M) dose-dependently inhibited the cholinesterase activity of the guinea-pig blood. These results suggest that berberine increases ileal contractility via the following: first, increasing acetylcholine release from the postganglionic parasympathetic nerve terminal; second, increasing acetylcholine retention through an inhibition of cholinesterase activity; and, third, blocking α_2-adrenoceptors, possibly in the postganglionic parasympathetic nerve [235].

Berberine was evaluated for its effect on the expression of glucocorticoid receptors (GR) and its relation to cell cycle progression of human HepG2 hepatoma cells. Continuous exposure of HepG2 cells to various concentrations (1-50 μM) of berberine resulted in growth inhibition in a dose dependent manner. The viability of berberine-treated HepG2 cells was greater than 90% in all treatment groups. Flowcytometric analysis of berberine-treated HepG2 cells showed that the S phase fraction was significantly reduced. GR levels were higher in berberine-treated HepG2 cells than in vehicle (DMSO)-treated cells. Furthermore, the secretion of α-fetoprotein by HepG2 cells was inhibited by berberine. Finally, the berberine-induced cell growth arrest was partially reversible in HepG2 cells [236].

The antimicrobial effect of berberine chloride was evaluated on a large number of microorganisms. A marked inhibitory activity was observed on various *Candida* species, and a lesser effect on *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus cereus*, *Klebsiella pneumoniae*, and a diphtherioid [237].

The crude methanol extract of the roots of *Coptis teeta* from Burma was tested for growth inhibitory activity against *Giardia lamblia*, *Trichomonas vagnialis*, and *Entamoeba histolytica*. It was observed that the inhibitory effects of the extract were greater than that of berberine sulfate, one of the contained alkaloids of the extract. It was suggested that this greater inhibitory activity of the crude extract might have been due to the cumulative contributions of its contained protoberberine and other alkaloids, and that perhaps protoberberine alkaloids may be useful chemotherapeutic agents against the three parasites that were evaluated [238].

Berberine was submitted to an *in vitro* antimicrobial screen using eight Gram-positive and eight Gram-negative bacteria, as well as one yeast. The alkaloid was inactive (MIC > 50 mg/l) against virtually all of the organisms, save the acid-fast rod *Mycobacterium phlei* (MIC = 25 mg/l) [239].

The inhibitory effects of berberine and 150 natural products on the cytotoxic activity of polymorphonuclear leukocytes (PMN) was investigated. The research employed the effects of natural products on the PMN activation by the antitumor immunomodulator TAK (a linear β-1,3-D-glucan from *Alcaligenes faecalis* var. *myxogenes*) using a PMN cytotoxicity assay system. Berberine was found to fail to inhibit the activation of PMN activation by TAK, and was not cytotoxic to target cells [199].

Some protoberberine and structurally related alkaloids were tested for inhibitory activity on porcine pancreatic elastase (PPE) and human sputum elastase (HSE). Berberine chloride significantly inhibited the elastolytic activity of both enzymes, but tetrahydroberberine had no effect. It appears that the quaternary nitrogen atom of these alkaloids plays an important role in the inhibition of elastolytic activity. The amidolytic activity of the elastases was not affected by any of the test alkaloids [240].

Berberine chloride, as well as a series of twenty other pharmacologically active agents, was evaluated in a new microplate assay for cytotoxicity using the brine shrimp *Artemia salina*, and shown to furnish comparable results to those previously published in the test-tube method. The assay reliably detected all of the compounds toxic to KB cells in a series of twenty-one pharmacologically active agents, except for two which required metabolic activation in humans [241].

The effects of berbamine, oxyacanthine, and berberine on 5-lipoxygenase lipid peroxidation in phospholipid liposomes induced by 2,2'-azo-(bis-2-amidinopropane)(AAPH), deoxyribose degradation, and their reactivities against the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) were studied. None of the alkaloids showed any appreciable effects in the inhibition of 5-lipoxygenase. Reactivity against DPPH increased in the following order: berberine < oxyacanthine < berbamine. Pro-oxidant effects of the alkaloids were excluded, since deoxyribose degradation was not influenced as determined by the release of malondialdehyde [200].

Berberine, berbamine, and oxyacanthine were evaluated for antiproliferative activity against the growth of HaCaT cells (a rapidly multiplying human keratinocyte cell line). Proliferation of the keratinocytes was determined directly by counting the dispersed cells under a phase-contrast microscope after 48 hours of treatment. The antiproliferative activity of these alkaloids were as follows: berbamine (IC_{50} 11 μM) > oxyacanthine (IC_{50} 13 μM) > berberine (IC_{50} 30 μM). The standard was anthralin (IC_{50} 0.7 μM). These results indicate that the three alkaloids tested were all antiproliferative agents when applied to HaCaT cells at micromolar concentrations [201].

Four protoberberine alkaloids and one aporphine alkaloid were evaluated for lipoxygenase inhibition. Oxyberberine and columbamine were the most potent lipoxygenase inhibitors tested, with jatrorrhizine being intermediate, whereas berberine and magnoflorine exhibited only low potencies. A strong linear correlation between lipoxygenase inhibition and lipid antioxidant properties of these alkaloids was observed. These data suggest that the mechanism of

lipoxygenase inhibition by these alkaloids may be linked to the inhibition of lipid hydroperoxide substrate accumulation [242].

A series of berberine derivatives were prepared in order to attempt to find orally-active compounds for use in the therapy of human congestive heart failure and various arrhythmias. This research was stimulated by reports that berberine has been found to possess positive inotropic and negative chronotropic effects on the myocardium. However, since berberine is a quaternary compound, its absorption from the intestinal tract is limited. In the screening of inhibition of electrically-induced contraction of isolated guinea pig left atria and spontaneous beating of the guinea pig's right atria, the synthesized compounds were observed to inhibit spontaneous beating of the isolated guinea pig right atria. While berberine was relatively inactive (IC_{50} 204 μmol), the most active compound prepared in this study was 2,3-methylenedioxy-6,12-dimethoxytetrahydroberberine (IC_{50} 4.34 μmol)[243].

The effects of berberine on potassium channel subtypes were investigated via the use of patch-clamp whole cell recording techniques. The results suggested that berberine can prolong action potential duration (APD), decrease I_{K1} and outward I_{tail}, but had no effect on I_K. Furthermore, the alkaloid was shown to antagonized comakalim-induced inhibition of APD and the increase of K_{ATP}. Since berberine is known to be effective in lowering blood glucose levels as well as treating cardiac arrhythmias, these results indicate that berberine can inhibit voltage-dependent and ATP-sensitive potassium channels, with the mechanisms of action of the antiarrhythmic and antidiabetic effects of the alkaloid possibly being due to potassium channel blocking effects [244].

Berberine levels in plasma and in tissues were compared in normal mice after oral administration of berberine (100 mg/kg). The hypoglycemic effect of the alkaloid was also measured in these mice. Measurement of the alkaloid was via a simple and specific high performance TLC and fluor-densitometric method [245].

The cardiac effects of berberine were studied in isolated right and left atrial preparations from guinea pigs. The results suggested that berberine has a unique profile of action in isolated guinea pig atrial tissue, displaying both positive inotropic and negative chronotropic activity. Berberine enhanced both the force-velocity relationship and the duration of active state, thereby producing its positive inotropic effect. The mechanisms for these actions were postulated to include an alteration in trans-sarcolemmal flux of calcium, as well as an inhibition of intracellular calcium sequestration systems [246].

Small intravenous doses of berberine to dogs were noted to excite the myocardium, accelerate heart rate, and slightly increase coronary blood flow and cardiac contractility. Increasing doses were responsible for direct dilation of peripheral blood vessels, inhibition of the myocardium, and weakening cardiac contractility. Myocardial cells were observed to undergo serious damage, and were stopped in diastole [247].

The roots of *Allium cepa* were subjected to continuous treatment berberine sulfate solutions of differing concentrations. The root growth was completely inhibited at high concentrations (10^{-5} and 10^{-6} M), whereas a variable degree of growth inhibition was observed

at lower concentrations (10^{-7} to 10^{-9} M). Treatment of the roots with 10^{-9} M solutions, followed by irradiation with violet light (420 nm) resulted in complete growth inhibition, indicating the photodynamic activity of the alkaloid [248].

Intravenous administration of berberine (20 mg/kg) to anesthetized rats was observed to increase the cardiac index and stroke volume index. In the isolated guinea pig left atria, berberine (0.1 μmol/L) shifted the dose-response curve of ouabain to the left with an increase of the maximal effect. The alkaloid (10 μmol/L and 30 μmol/L) potentiated the maximal contractile force of ouabain and diminished the dose at which ouabain produced its peak effect. Berberine (30 μmol/L and 100 μmol/L) elevated the dose in which the toxicity of ouabain occurred. The investigators concluded that berberine might increase the cardiac output and widen the safety margin with ouabain-treatment [249].

Berberine was observed to reduce the amplitude of spontaneous miniature end plate potentials in the frog neuromuscular junction. At low concentrations, berberine diminished the frequency of miniature potentials, but increased it when the concentration exceeded a certain value [250].

Subcutaneous administration of berberine sulfate was observed to significantly decrease the acetic acid-induced writhing frequency in mice at a median effective dosage (ED_{50}) of 3.5 mg/kg. Berberine (8 mg/kg) also showed an analgesic effect in mice, as measured via the hot plate method. Oral or subcutaneous administration markedly inhibited the acetic acid-induced increased vascular permeability in rats. At intraperitoneal dosages of 4 and 8 mg/kg, the alkaloid produced obvious inhibition in the xylene-induced swelling of mouse ear. At subcutaneous dosages pf 20 and 40 mg/kg, the alkaloid inhibited the carrageenin-induced hind paw edema, with this inhibitory effect lasting longer than 7 hours. Both the anti-inflammatory and the analgesic actions of the alkaloid were dose dependent [251].

Berberine (0.50 mol/l) was found to dose-dependently inhibit collagen-, ADP-, and arachidonic acid-induced thromboxane A_2 release from platelets and to inhibit platelet aggregation. Intravenous administration of the alkaloid (25 mg/kg) resulted in a lowered plasma level of prostaglandin I_2 from 0.92 to 0.61 ng/m after one hour. These results suggest that berberine inhibits arachidonic acid metabolism, and that this effect may be an important factor in the antiaggregatory action of the alkaloid [252].

A soap for the therapy of gonorrhea was formulated to contain berberine as its active ingredient. The formulation was compounded via microgranulation processes, followed by heat-pressing to form a soap [253].

Intraperitoneal administration of berberine at a dosage of 0.1 mg/kg inhibited antiglomerular basement membrane nephritis in rats. The LD_{50} of the alkaloid was > 1 g/kg after oral administration. Tablets containing the alkaloid were formulated for oral administration [254].

Berberine was found to significantly inhibit hyperinsulinemia in noninsulin dependent diabetic rats, and to ameliorate abnormalities in glucose tolerance and lipid metabolism. The alkaloid also decreased lipid peroxide content and increased hepatic superoxide dismutase activity.

These results suggest that berberine possesses marked antioxidant activity, and thus may inhibit the formation of metabolic disorders that result from oxidative damage [255].

The administration of berberine (1-4 mg/kg, i.v.) to rabbits was observed to significantly and dose-dependently elevate the ventricular fibrillation threshold (VFT) induced by electrical stimulation. Neither reserpine nor vagotomy exerted any influence on the increase of VFT with berberine, which implies that the anti-arrhythmic action is most likely due to a direct activity on the myocardium, without involvement of the autonomic nervous system. The pronounced effect of the alkaloid on action potentials in the rabbit myocardium *in vivo* resulted in the reduction in the maximal velocity of depolarization (V_{max}). At higher dosage, berberine produced a prolongation in action potential duration at both 90 and 100% repolarization, in addition to a more potent decrease in V_{max}. These results may thus contribute to the effectiveness of berberine against myocardial arrhythmias [256].

The effects of a preparation containing berberine and the crude herb *Geranii Herba* on different diarrheal models in mice and the contractions of isolated guinea pig intestinal muscle were studied via comparison with a preparation containing creosote and loperamide. The results suggested that *Geranii Herba* exerted its antidiarrheal effect via the inhibition of intestinal movement, with the mechanism of action possibly differing from creosote and loperamide [257].

Further studies on the antidiarrheal effects of berberine and the crude herb *Geranii Herba* were undertaken, utilizing different diarrhea models of mice, as well as on the contractions of isolated guinea pig intestinal smooth muscle and rat intestinal peristalsis. The results were compared with those obtained after administration of atropine and papaverine. Berberine significantly inhibited both the castor oil- and $BaCl_2$-induced diarrheas at oral doses higher than 25 mg/kg, but did not inhibit pilocarpine- or serotonin-induced diarrhea even at 250 mg/kg. Berberine inhibited acetylcholine- or $BaCl_2$-induced contractions of the ileum and colon at a concentration of about 10^{-5} g/ml, and exhibited a noncompetitive inhibition of the contractile response induced by acetylcholine, whereas atropine displayed a competitive inhibition. Finally, berberine inhibited spontaneous intestinal peristalsis [258].

The effects of berberine and some other alkaloids and flavonoids on the lipogenesis in the sebaceous glands of the hamster ear (an excellent model for the human sebaceous gland) were studied. These alkaloids and flavonoids are major ingredients in Japanese-Chinese traditional herbal medicinals that are used in the therapy of acne vulgaris. Lipogenesis was assayed via determination of ^{14}C incorporation into sebaceous lipids extracted from the sebaceous glands that were preincubated with ^{14}C-acetate. Berberine (10^{-4} M) was shown to suppress the lipogenesis in the hamster sebaceous glands by 63% [259].

The administration of berberine (104 mg/kg, i.v.) was observed to markedly and dose-dependently reduce the incidence of ventricular arrhythmia after coronary artery occlusion in rats. At a dosage of 4 mg/kg (i.v.) the alkaloid also depressed the abnormal changes in hemorrheology, such as increased platelet and erythrocyte aggregation, as well as blood viscosity. At 30 μmol/l, berberine also prevented hypoxia from shortening action potential duration and ERP in the

isolated guinea pig myocardium. Berberine may produce its antagonistic effect on ventricular arrhythmia following myocardial ischemia via these electrophysiological mechanisms [260].

Berberine was observed to exhibit a hypoglycemic effect in normal mice, alloxan diabetic mice, and spontaneously diabetic KK mice. The alkaloid was also able to antagonize the elevation of serum glucose induced by intraperitoneal administration of glucose or epinephrine in normal mice. Administration of the alkaloid for 15 days resulted in an improvement of the glucose tolerance of KK mice. In addition, berberine was found to lower serum cholesterol in mice fed a high cholesterol diet and to inhibit *in vitro* platelet aggregation in rabbits [261].

^{3}H-Labelled berberine was found to bind specifically and reversibly to a microsomal fraction prepared from cultured cells of *Coptis japonica* capable of accumulating the alkaloid in the vacuole. The Scatchard plot of the data suggested the presence of a single [^{3}H]berberine-binding site with a dissociation constant of 1.67 μM for the alkaloid. The optimum pH for binding was between 9 and 10, with the addition of either coptisine or jatrorrhizine to the mixture significantly decreasing the [^{3}H]berberine binding, indicating that the binding is highly specific for protoberberine alkaloids. An excess amount of inorganic cation, as well as iodoacetate (a SH-blocking reagent) almost totally inhibited the binding. Fractionation of the microsomes via sucrose density-gradient centrifugation suggested that the binding site was not associated with the plasma membrane, endoplasmic reticulum or tonoplast, but with an unidentified membrane (density = 1.08-1.13 g/cm^3), that may be involved in berberine transport to the vacuole [335].

Berberine chloride was demonstrated to possess significant cytotoxic activity against cultured P-388 (murine lymphocytic leukemia) cells, in addition to three human cancer cell lines: BC1 (breast cancer) - ED_{50} 2.7 μM); HT-1080 (fibrosarcoma) - ED_{50} 2.4 μM); and KB (nasopharyngeal carcinoma) - ED_{50} 8.4 μM)[336].

13.2.5. Berberrubine

Forty-eight derivatives of berberine-type alkaloids were examined for their inhibition activity against the induction of mouse ear edema via application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Berberrubine chloride displayed inhibitory effects (ED_{50} 1.3 μmol/ear; 52% inhibition). Berberine derivatives had stronger inhibitory activity than palmatine derivatives. Since berberine and some of its derivatives are present in Chinese traditional drugs, which are used in combined Kampo prescriptions, it is important to determine if such alkaloids possess carcinogenic inhibiting properties [221].

Berberrubine was found to inhibit RAV-2 (reverse transcriptase)(IC_{50} 3 μg/ml). The alkaloid was formulated into tablets [262].

Berberrubine chloride was found to exhibit cytotoxic activity against cultured P-388 (murine lymphocytic leukemia) cells (1.7 μM)[336].

13.2.6. Columbamine

Columbamine iodide was evaluated in the human immunodeficiency virus reverse transcriptase assay and found to be moderately active (50 μg/ml < IC_{50} < 150 μg/ml)[229].

Columbamine chloride was dissolved in culture medium and evaluated for its activity against two protozoa, *Plasmodium falciparum* (K1, multi-drug resistant strain) and *Entamoeba histolytica* (NIH 200). The alkaloid was slightly toxic to KB cells (IC_{50} 78 μM), and was active against *P. falciparum* (IC_{50} 1.9 μM), but inactive against *E. histolytica* (IC_{50} 156 μM). In assessing relative *in vitro* cytotoxicity to antiplasmodial activity, berberine had the least favorable ratio (8), while jatrorrhizine had the most favorable ratio (> 106) of the alkaloids tested. Columbamine had an intermediate value (41). The results lend some support to the utilization of plants containing protoberberine alkaloids for the treatment of malaria [230].

Four protoberberine alkaloids and one aporphine alkaloid were evaluated for lipoxygenase inhibition. Oxyberberine and columbamine were the most potent lipoxygenase inhibitors tested, with jatrorrhizine being intermediate, whereas berberine and magnoflorine exhibited only low potencies. A strong linear correlation between lipoxygenase inhibition and lipid antioxidant properties of these alkaloids was observed. These data suggest that the mechanism of lipoxygenase inhibition by these alkaloids may be linked to the inhibition of lipid hydroperoxide substrate accumulation [242].

13.2.7. Coptisine

Coptisine chloride exhibited reverse transcriptase inhibitory properties against both HIV-1RT (p66/p51)(Human immunodeficiency virus type 1 reverse transcriptase)(IC_{50} 100.0 μg/ml [281.1 μM]) and HIV-2RT (p68/p55)(Human immunodeficiency virus type 2 reverse transcriptase)(IC_{50} 117.3 μg/ml [329.7 μM]). The alkaloid is thought to inhibit various RNA and DNA polymerizing enzymes via interaction with nucleic acid template-primers [212].

Intraperitoneal administration of coptisine at dosages of 0.5, 1, and 5 mg/kg/day to original-type anti-GBM-induced nephritic rats was shown to be effective in the inhibition of urinary protein excretion, elevation of serum cholesterol, and creatinine contents, as well glomerular histopathological changes. The alkaloid inhibited platelet aggregation in both *in vitro* and *in vivo* assays. These results suggest that the antinephritic effect of coptisine may be due partly to antiplatelet action and improved renal hemodynamics, via the alteration of prostanoid synthesis [223].

Coptisine chloride was evaluated in the human immunodeficiency virus reverse transcriptase assay and found to be moderately active (50 μg/ml < IC_{50} < 150 μg/ml)[229].

Some protoberberine and structurally related alkaloids were tested for inhibitory activity on porcine pancreatic elastase (PPE) and human sputum elastase (HSE). Coptisine chloride significantly inhibited the elastolytic activity of both enzymes, but tetrahydroberberine had no

effect. It appears that the quaternary nitrogen atom of these alkaloids plays an important role in the inhibition of elastolytic activity. The amidolytic activity of the elastases was not affected by any of the test alkaloids [240].

Intraperitoneal administration of coptisine at a dosage of 0.1 mg/kg inhibited antiglomerular basement membrane nephritis in rats. The LD_{50} of the alkaloid was > 1 g/kg after oral administration [254].

13.2.8. Corydine

Corydine was evaluated for its *in vitro* antimicrobial activity against a variety of microorganisms. The alkaloid was found to modestly inhibit the growth of *Bacillus subtilis* and *Mycobacterium phlei*. The oxoderivatives of aporphines were more active against Gram-positive bacteria than the reduced aporphines, but the former had a lower activity against yeast and no activity against Gram-negative bacteria [263].

13.2.9. Cryptopine

Cryptopine was demonstrated to enhance ^{3}H-γ-aminobutyric acid (GABA) binding to rat brain synaptic membrane receptors. Using this phenomenon as an *in vitro* paradigm, cryptopine and other protopine alkaloids may be suggested to have benzodiazepine-like activity [264].

13.2.10. 6a,7-Dehydromethoxyadiantifoline

6a,7-Dehydromethoxyadiantifoline was found to possess calcium channel blocking activity as measured by contraction of the guinea pig duodenum. The IC_{50} of the alkaloid was determined to be 2.53×10^{-6}M [71].

13.2.11. Dehydrothalifaberine

Dehydrothalifaberine, an alkaloid of the Chinese *Thalictrum faberi* Ulbr., was found to possess significant cytotoxicity as measured against the P-388 cell line [129].

13.2.12. Domesticine

Domesticine was determined to inhibit rabbit lens aldose reductase (IC_{50} 7.6 x 10^{-4}M) [210].

13.2.13. EP-10

Preliminary biological testing of the new aporphine-pavine alkaloid EP-10, isolated from extracts of the whole plant of the Taiwanese *Thalictrum fauriei* Hayata in 1986, demonstrated that the alkaloid possessed weak *in vitro* activity, as compared with thalicarpine, in the lysis of Hela cells [35].

13.2.14. Glaucine

The effects of glaucine were studied in mice and compared with haloperidol, phenobarbital, and morphine. The administration of glaucine was associated with an inhibition of the exploratory rearing activity, and elicited palpebral ptosis, catalepsy, hypothermia, and prolonged thiopental-induced anesthesia. In addition, the alkaloid reduced nociception, and exhibited anticonvulsive actions against harman and picrotoxin-induced convulsions, but not against those induced by bicuculline and pentetrazol. The alkaloid antagonized apomorphine- and methylphenidate-induced stereotyped gnawing, as well as apomorphine-induced climbing activity. The antinociceptive effect (writhing) was resistant to both naloxone and yohimbine, thus being in contrast to that of morphine. Opioid and adrenergic mechanisms were shown to be unequally involved in the antinociceptive effects of glaucine. In low doses, glaucine and haloperidol antagonized the antinociceptive effect of morphine, and thus these drugs may be considered as partial agonists or partial antagonists, respectively [330].

The scavenging and iron-reducing properties of a series of benzylisoquinoline alkaloids and synthetic benzylisoquinoline compounds was studied. Glaucine was found to act as a scavenger of the hydroxyl radical in the deoxyribose degradation by Fe^{+3}-EDTA + H_2O_2 [265].

Glaucine was shown to exhibit an inhibitory action on blood platelet aggregation induced by collagen, arachidonic acid, and ADP *in vitro* [266].

Glaucine was found to affect synaptic transmission in a fairly complicated manner. The alkaloid reduced the amplitude of spontaneous miniature end plate potentials in the frog neuromuscular junction. At low concentrations, glaucine diminished the frequency of miniature potentials, but increased it when the concentration exceeded a certain value. Glaucine provokes muscle contracture if present at a relatively high concentration [250].

Experiments were undertaken to study the α,β-adrenergic activity of glaucine as a preliminary step toward a study of the dopaminic activity in peripheral dopamine receptors. Experiments in isolated rat vas deferens demonstrated that glaucine failed to produce a direct response, but produced an increase of the maximum contractile effect of norepinephrine. These results suggest the absence of a direct action of glaucine on α-adrenoceptors. In contrast to apomorphine, the EC_{50} norepinephrine in the vas deferens was increased by glaucine. The potentiation of contractile effect of epinephrine and the fact that glaucine did not increase the EC_{50} of epinephrine in a dose-dependent manner argued against a competitive type of antagonism between these drugs, and suggested an action of glaucine at sites other than α-adrenergic receptors. Glaucine ((0.1, 1, and 10 μmol/l) did not produce any consistent modification of the contractile force of electrically driven rat atria. Glaucine (10 μmol/l)abolished the contractile effect of isoproterenol that produced a decrease in contractile force of rat atria. These results suggest that glaucine has a mechanism of action different from that of apomorphine. In conclusion, in contrast with apomorphine, it would appear that glaucine does not present a direct action on the α-adrenoceptors in isolated rat vas deferens [284].

13.2.15. Harmine

Harmine and other harmane-related compounds were observed to exhibit concentration-dependent inhibition of lipid peroxidation (measured as thiobarbiturate reactive products) in a hepatic microsomal preparation incubated with either enzymatic dependent (Fe^{+3}ADP/NADPH) or non-enzymatic (Fe^{+3}ADP/dihydroxyfumarate) oxygen radical producing systems. Alkaloids with hydroxyl substitution and a partially desaturated pyridyl ring were found to have the highest antioxidative potencies. Substitution of a hydroxyl group with a methoxyl group in the carboline ring system resulted in a decrease of greater than ten-fold in antioxidative activities. Of all the β-carbolines studied, harmine showed little if any antioxidative effects in the concentration range tested while harmalol was observed to have the highest antioxidant efficacy. These results suggest that β-carbolines may serve as endogenous antioxidants [333].

Histamine-*N*-methyltransferase (HNMT), the major enzyme responsible for the metabolism of histamine in rat brain, is strongly inhibited by tacrine (9-amino-1,2,3,4-tetrahydroacridine), although structural fragments of tacrine were less potent inhibitors of HNMT. In this study, a series of tacrine fragments were screened for inhibition of rat brain HNMT to elucidate the structural requirements for this class of HNMT inhibitors. It was found that harmine (IC_{50} 1.9 μM) and a number of other β-carbolines, inhibited HNMT with IC values in the range of 1-10 μM. The degree of saturation of the 3,4-position did not greatly affect the IC_{50}, nor did HNMT distinguish between the 7-hydroxy and 7-methoxy compounds [268].

Harmine was evaluated for its effects on both arachidonic acid metabolism and human platelet aggregation (platelet-activating factor) and found to be inactive [269].

The effects of harmine, harmaline, and other harmala alkaloids on the contractions induced in the vascular smooth muscle of rabbit aorta and intestinal smooth muscle of taenia isolated from guinea-pig caecum were examined. The order of inhibitory potency was 6-methoxyharman = harmine > harmaline = 2-methylharmine = harmane > 6-methoxyharmalan > harmalol = harmol for contractions induced by high K^{+1} in aorta and taenia and by carbachol in taenia. The order of inhibitory potency for contractions induced by noradrenaline in the aorta were 2-methylharmine > 6-methoxyharman > 6-methoxyharmalan = harmol = harmalol = harmane > harmine > harmaline. The results suggest that harmaline inhibits the contractile response of rabbit aorta and guinea-pig taenia via inhibition of different types of Ca^{+2} channel. The structure-activity relationship indicates that the potency and selectivity of the inhibitory effects on these channels are varied by modification of the structure within this alkaloid series [270].

The structure inhibitory activity relationships among numerous β-carboline alkaloids was studied. Harmine was found to inhibit the actions of cyclic adenosine monophosphate (cAMP) phosphodiesterase (IC_{50} 69.3 x 10^{-5} M). Among the di- and tri-substituted β-carbolines, the *O*-methylated β-carbolines and the *O*-acetylated β-carbolines had higher inhibitory activity than the corresponding hydroxy β-carbolines, while the dihydro- and tetrahydro-derivatives were not potent inhibitors [271].

The affinity of harmine and six related β-carbolines for serotonin, benzodiazepine, tryptamine, opiate and GABA receptors in rat brain was studied. These seven compounds showed great variability in their affinity spectra, and, on average, most of the alkaloids showed very low binding to GABA receptors (harmine - IC_{50} > 400 μM). Harmine was found to have a relatively high affinity to both μ- and δ-opiate receptors (μ-opiate receptor - IC_{50} 5.6 +/- 3.1 μM; δ-opiate - 13 +/- 8 μM). Harmine was intermediate in its binding capacity to 5-HT type 2 receptors (IC_{50} 58 +/- 6.8 μM), but showed a relatively high affinity for the tryptamine receptor (IC_{50} 0.10 +/- 0.02 μM) [272].

The potential cytotoxic activities of forty-six *Strychnos* alkaloids, including harmine, were tested on different cancer or normal cells cultured *in vitro*. At a concentration of 1-10 μg/ml, harmine showed modest activity against L 1210 cells derived from DB A/2 mouse ascites tumor and cultured HeLa cells derived from human carcinoma. The alkaloid displayed only slight activity against cultured flow 2002 cells derived from normal embryonic human lungs, and was inactive against cultured B16 melanoma cells derived from C57BL mouse melanoma [273].

Harmine and five other plant-derived simple β-carboline alkaloids and eight other plant derivatives were tested for *in vitro* activity against the epimastigotes of the protozoan parasite *Trypanosoma cruzi* (Costa Rican strain). This organism is the causative agent of Chagas' Disease. Harmine (as well as harmol, harmaline, and harman) at a concentration of 50μg/ml significantly reduced the population growth of epimastigotes to less than 50% of the control group within 96 hours. In addition, harmine was effective in the reduction of growth more than 90% at the 50 μg/ml level and showed significant activity at 5 μg/ml. Activity in this screen appears to be associated with the pyridyl ring 1,2 and 3,4 unsaturation, and increases with C(7) substitution [224].

Harmine, harmaline, and related derivatives were screened against a bank of microorganisms *in vitro* including eleven Gram-positive and twelve Gram-negative bacteria, and sixteen species of fungi (including eight dermatophytes, six filamentous fungi, and two species of *Candida*). Harmine (100-500 μg/ml) was found to have antibacterial activity against both Gram-positive and Gram-negative bacteria. In addition, the alkaloid was effective against all eight of the dermatophytes at a concentration of 100 μg/ml [274].

The behavior of some β-carboline derivatives, including harmine, as inhibitors of monoamine oxidase was studied in bovine retina. The di- and tetrahydro-β-carbolines behaved as reversible and competitive inhibitors. In contrast, harmine, and other fully unsaturated β-carbolines showed deviation from linearity at high substrate concentrations, and behaved as tight-binding inhibitors. Structure-activity relationships indicated that substitution of a methoxy group at C(7) position of the aromatic ring is determinant for this tight-binding behavior, with a substitution of a methoxy group at C(6) position greatly reducing this inhibition. Since β-carbolines have been reported to be endogenously formed, this suggests that they may have important physiological actions on monoamine oxidase activity *in vivo*. Finally, all the β-carbolines investigated in this study had low potencies as monoamine oxidase B inhibitors [275].

Harmine, a naturally-occurring photosensitizer, was examined for its photoactivity against the bacterial viruses T4 and M13. The kinetic curves of virus inactivation were used to calculate relative phototoxicities. Harmine exhibited activity against both viruses, but was considerably more active against M13 than T4. Harmine and other β-carbolines have been show to interact with nucleic acids in light-independent reactions, although this current study suggests that the photoactivity appears to affect single-stranded nucleic acids more. Harmine may thus conceivably affect viral proteins and other macromolecules [276].

13.2.16. Hernandezine

Hernandezine was observed to lack inhibitory activity (inactive) against both HIV-1RT (p66/p51)(Human immunodeficiency virus type 1 reverse transcriptase) and HIV-2RT (p68/p55)(Human immunodeficiency virus type 2 reverse transcriptase) [212].

Hernandezine was evaluated in the human immunodeficiency virus reverse transcriptase assay and found to be inactive (IC_{50} > 200 μg/ml)[229].

Hernandezine, which was isolated from *Thalictrum glandulosissimum*, was found to be effective for the treatment of mice bearing P388 leukemia, S180 ascites, and C26 colon cancer. Although the alkaloid inhibited the growth of mouse L1210 cells and human oral cancer KB cells *in vitro*, its inhibitory effect on normal hemopoietic progenitor cells (CFU-GM) in mice was relatively low. Preliminary results showed that hernandezine blocked cell-cycle transfer from G1 to S phase, and its cytocidal action might thus be cell cycle specific [277].

The total alkaloids of the Chinese *Thalictrum glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang, and the major alkaloid of the plant, hernandezine, were effectively employed

in the treatment of P388 leukemia, S180 ascites tumor, and C26 colon tumor in mice. Although hernandezine markedly inhibited the *in vitro* growth of mouse L1210 cells and human KB oral cancer cells, the alkaloid was characterized by only a low inhibition on normal hemopoietic progenitor cells (CFU-GM). Preliminary results demonstrated that hernandezine blocked cell-cycle transfer from the G_1 to the S phase, suggesting that its action may be cell cycle-specific [278]. It was also demonstrated that two other bisbenzylisoquinoline alkaloids isolated from the same plant, thalidezine and isothalidezine, possessed a similar inhibitory effect on the L1210 cells [277,278].

13.2.17. Homoaromoline

Homoaromoline was screened for antiplasmodial, antiamoebic, and cytotoxic activities via the use of *in vitro* microtests. The alkaloid was characterized by the following: IC_{50} 3.46 μM against a multidrug-resistant strain of *Plasmodium falciparum* (K1)(standard - chloroquine phosphate, IC_{50} 0.20 μM); IC_{50} 17.3 μM against *Entamoeba histolytica* (standard - metronidazole, IC_{50} >82.2 μM); IC_{50} 105 μM against nasopharyngeal KB cell line (standard - podophyllotoxin, IC_{50} 0.008 μM)[176].

The cytotoxic and antimalarial activities of homoaromoline were determined as follows: Cytotoxic activity [ED_{50} (μg/ml)]: P-388 (0.31), KB-3 (3.6), KB-V1 (15); Antimalarial activity [ED_{50} (ng/ml)]: *Plasmodium falciparum* Strain D-6 (232) and *P. falciparum* Strain W-2 (451)[279].

Homoaromoline was found to inhibit the growth of cultured *Plasmodium falciparum* strains D-6 and W-2, and to display nonselective cytotoxicity with a battery of cultured mammalian cells. These data were used to calculate a selectivity index (ED_{50} in cultured mammalian cells/ED_{50} in cultured *Plasmodium falciparum* strains). Agents that are useful antimalarial drugs, such as quinine and artemisinin, typically display selectivity indices that are > 1000. The ED_{50} (ng/ml) of homoaromoline against *Plasmodium falciparum* strain D-6 was 104.6, while that associated with strain W-2 was 288.3. The following is a summary of the cytotoxic activities (ED_{50}, μg/ml) of homoaromoline, along with the cytotoxicity/antimalarial activity ratio (selectivity index) with the first number in parentheses being the D-6 strain selectivity index and the second number being the W-2 strain selectivity index: BCA-1 [Human Breast Cancer] - 3.6 (34,13); HT-1080 [Human Fibrosarcoma] - 9.0 (86,31); LUC-1 [Human Lung Cancer] - 9.7 (93,34); MEL-2 [Human Melanoma] - 9.0 (86,31); COL-1 [Human Colon Cancer] - 10.0 (96,35); KB [Human Oral Epidermoid Carcinoma] - 6.3 (60,22); KB-V1 [Vinblastine-resistant KB] - 3.1 (30,11); P-388 [Murine Lymphoid Neoplasm] - 3.7 (35,13); A-431 [Human Epidermoid Carcinoma] - 8.9 (85,31); LNCaP [Hormone Dependent Human Prostate Cancer] - 3.4 (33,12); ZR-75-1 [Hormone Dependent Human Breast Cancer] - 2.0 (19,7). Based on the data that was generated and compared, homoaromoline and other bisbenzylisoquinoline alkaloids do not appear to be promising candidates for antimalarial drug development [280].

The inhibitory effects of homoaromoline and 150 natural products on the cytotoxic activity of polymorphonuclear leukocytes (PMN) was investigated. The research employed the effects of natural products on the PMN activation by the antitumor immunomodulator TAK (a linear β-1,3-D-glucan from *Alcaligenes faecalis* var. *myxogenes*) using a PMN cytotoxicity assay system. Homoaromoline was found to inhibit the activation of PMN activation by TAK, showing an ID_{50} 10 μg/ml. From this, and related studies, it was postulated that homoaromoline may impair the NADPH oxidase system in the plasma membrane of PMN [199].

Homoaromoline and ten other bisbenzylisoquinoline alkaloids were evaluated in the *in vitro* histamine release inhibition assay. This assay is one of the basic tests that is used in the evaluation of substances for antiallergic effects. The order of potency of inhibitory effect of those bisbenzylisoquinoline alkaloids that have been isolated from *Thalictrum* species was as follows: homoaromoline > aromoline > isotetrandrine > obaberine > tetrandrine. Berbamine and oxyacanthine were found to have no inhibitory effect [178].

7-*O*-Benzylhomoaromoline was prepared (NaH and benzyl chloride in DMF under Ar at room temperature overnight) and observed to have an IC_{50} 12.2 μg/ml against human colon cancer PRMI 4788 strain. By contrast, homoaromoline was characterized by IC_{50} > 64.0 μg/ml against the same cells. 7-*O*-Benzylhomoaromoline was subsequently formulated into tablets [281].

The anti-inflammatory and antiallergic effects of homoaromoline were evaluated via the measurement of antigen-induced histamine release from leukocytes, and it was found that the alkaloid inhibited antigen-induced histamine release [180].

13.2.18. Isoboldine

The antimicrobial activity of isoboldine against a series of bacteria and fungi was evaluated via *in vitro* screening. The alkaloid failed to demonstrate any inhibitory activity against the growth of the bacteria, but was strongly inhibitory to the growth of the superficial fungus *Trichophyton rubrum*. The alkaloid exhibited only weak inhibition of the growth of the superficial fungus *Microsporum gypseum* [282].

The *in vitro* antimicrobial activity of isoboldine was investigated by agar diffusion and agar dilution methods against various Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus faecalis*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Enterobacter aerogenes*, *Serratia marcescens*, *Serratia sonnei*), as well as the acid-fast organism *Mycobacterium phlei*, and the yeast *Candida albicans*. Isoboldine exhibited no significant activity against any of the test organisms [283].

Isoboldine was evaluated for its *in vitro* antimicrobial activity against a variety of microorganisms. The alkaloid was found to be inactive against Gram-positive and Gram-negative bacteria, as well as an acid-fast bacillus and a yeast. The oxoderivatives of aporphines were more

active against Gram-positive bacteria than the reduced aporphines, but the former had a lower activity against yeast and no activity against Gram-negative bacteria [263].

Isoboldine was determined to inhibit rabbit lens aldose reductase (IC_{50} 2.7 x 10^{-4}M) [210].

13.2.19. Isocorydine

Isocorydine was observed to significantly decrease the frequency of spontaneous contraction and muscle tension, but not the amplitude of contraction on the isolated isthmus of the oviduct of the rabbit. The tension and frequency of spontaneous contraction was suppressed by the alkaloid at concentrations of 3-300 μmol/l, but the amplitude of contraction was decreased only at 300 μmol/l. The alkaloid antagonized the norepinephrine-induced contraction of the oviduct, and the transport of ova through the oviduct was delayed by isocorydine [285].

(+)-Isocorydine was observed to antagonize the contraction of isolated guinea pig or rabbit biliary tracts induced by acetylcholine, histamine, angiotensin II, and K^+. The relaxant effect of the alkaloid on isolated gallbladder and the Sphincter of Oddi *in vitro* was similar to that observed with verapamil, thus suggesting that Ca^{+2} may be involved in the relaxant effect of isocorydine [286].

Standard microelectrode techniques were used to study the effects of isocorydine on potential characteristics of canine cardiac Purkinje fibers and ventricular myocardium *in vitro*. In the Purkinje fibers, the action potential durations APD_{50} and APD_{90} were prolonged at 3 μmol/l but shortened at 30 μmol/l by isocorydine. The action potential amplitude and maximal upstroke velocity were decreased at 100 μmol/l. In the ventricular myocardium, the action potential characteristics were changed by isocorydine at concentrations above 30 μmol/l. The APD_{50} was shortened, the APD_{90} was prolonged, and the maximal upstroke velocity was decreased at 30 μmol/l. The effective refractory period was prolonged by the alkaloid in Purkinje fibers and ventricular myocardium. These results indicated that the alkaloid may interfere with K^+, Na^+, and Ca^{+2} currents in myocardial cell membranes at different concentrations [287].

Isocorydine, a Chinese medicinal calcium antagonist, was found to decrease blood pressure and heart rate in anesthetized rabbits [288].

Intravenous administration of (+)-isocorydine hydrochloride to anesthetized rats at a dosage of 5-20 mg/kg significantly counteracted the arrhythmias induced by intravenously administered barium chloride (3 mg/kg) or aconitine (20 mg/kg), and decreased the incidence of ventricular fibrillation, as well as the mortality rate [332].

Intracellular microelectrodes were used to study the effects of isocorydine on the action potentials of the rabbit sinoatrial node and guinea pig ventricular muscle, in addition to the spontaneous electrical activity induced by barium ion in the ventricular muscle. Exposure of rabbit sinoatrial node cells to a 30 μM and 300 μM solutions of isocorydine resulted in a decrease in the APA, SP_0, and SP_4, and slowed the rate of spontaneous impulse initiation by the sinoatrial node. The duration of the action potential (APD_{50}, APD_{90}) and the effective refractory period

(ERP) were both prolonged via exposure to a 3 μM solution of the alkaloid. A 30 μM solution was observed to prolong the ERP, without any other significant effects on the other parameters of action potential. An obvious shortening of the duration of the action potential but not the ERP was observed on treatment with a 300 μM solution of isocorydine. In addition, this concentration also suppressed the spontaneous electrical activity induced by barium ion in the guinea pig ventricular muscle [331].

13.2.20. Isotetrandrine

Isotetrandrine was screened for antiplasmodial and antiamoebic activities via the use of *in vitro* microtests. The alkaloid was characterized by the following: IC_{50} 0.16 μM against a multidrug-resistant strain of *Plasmodium falciparum* (K1)(standard - chloroquine phosphate, IC_{50} 0.20 μM); IC_{50} 22.2 μM against *Entamoeba histolytica* (standard - metronidazole [176].

Kinetic analysis of luminol-dependent chemiluminescence demonstrated that a solution of isotetrandrine (20 μM) was found to inhibit the generation of various types of reactive oxygens by guinea-pig neutrophils. The results of these and other experiments suggest that isotetrandrine inhibits the active oxygen generation via the stabilization of plasma membrane and the inhibition of phospholipid-dependent protein kinase (PKC) and NADPH oxidase activation [182].

Isotetrandrine was noted to inhibit antigen- and compound 48/80-induced arachidonic acid liberation, but not diacylglycerol formation or histamine release. The alkaloid had no effect on A23187-induced arachidonic acid liberation, which was prevented by *p*-bromophenacyl bromide, a known phospholipase A_2 inhibitor, and also did not affect phospholipase A_2 activity in a cell-free system including an exogenous phospholipid substrate. Isotetrandrine inhibited guanosine 5'-*O*-(3-thiotriphosphate)-induced arachidonic acid liberation in saponin-permeabilized mast cells, and by mastoparan or NaF plus $AlCl_3$ in intact cells. In addition, the alkaloid abolished the inhibitory effect of islet-activating protein on compound 48/80-induced arachidonic acid liberation. These data suggest that isotetrandrine suppresses the receptor-mediated phospholipase A_2 activation through, at least in part, uncoupling of a GTP-binding protein from the enzyme, rather than by affecting the enzyme directly [184].

Bisbenzylisoquinoline alkaloids block Ca^{+2} uptake through L-type Ca^{+2} channel and modulate binding of ligands to four distinct sites (dihydropyridine, benzothiazepine, aralkylamine, and (diphenylbutyl)piperidine) in the Ca^{+2} entry blocker receptor complex of the channel. All bisbenzylisoquinoline analogs tested, including isotetrandrine, completely inhibit diltiazem binding, but many only partially inhibit D-600 and fluspirilene binding. For dihyrodpyridine binding, the compounds show either stimulation or inhibition, or exhibit no effect. Data from this experiment suggest that isotetrandrine and a variety of bisbenzylisoquinoline congeners act to block the L-type Ca^{+2} channel by binding to the benzothiazepine site on the channel. The novel patterns exhibited by isotetrandrine and other bisbenzylisoquinoline alkaloid analogs in affecting allosteric coupling between the benzothiazepine site and the other receptors of the Ca^{+2}

entry blocker receptor complex define a new class of Ca^{+2} entry blocker with binding properties distinct from diltiazem [186].

Isotetrandrine was shown to overcome resistance in the multidrug-resistant subline, Ch^{R}-24, derived from human KB carcinoma cells to vincristine, actinomycin D, and daunomycin, and partially overcame resistance to Adriamycin [187].

Isotetrandrine was found to inhibit the growth of cultured *Plasmodium falciparum* strains D-6 and W-2, and to display nonselective cytotoxicity with a battery of cultured mammalian cells. These data were used to calculate a selectivity index (ED_{50} in cultured mammalian cells/ED_{50} in cultured *Plasmodium falciparum* strains). Agents that are useful antimalarial drugs, such as quinine and artemisinin, typically display selectivity indices that are > 1000. The ED_{50} (ng/ml) of isotetrandrine against *Plasmodium falciparum* strain D-6 was 165.1, while that associated with strain W-2 was 54.6. The following is a summary of the cytotoxic activities (ED_{50}, μg/ml) of isotetrandrine, along with the cytotoxicity/antimalarial activity ratio (selectivity index) with the first number in parentheses being the D-6 strain selectivity index and the second number being the W-2 strain selectivity index: BCA-1 [Human Breast Cancer] - 5.4 (33,99); HT-1080 [Human Fibrosarcoma] - 11.0 (67,202); LUC-1 [Human Lung Cancer] - 10.0 (61,183); MEL-2 [Human Melanoma] - 19.5 (118,357); COL-1 [Human Colon Cancer] - 10.5 (64,192); KB [Human Oral Epidermoid Carcinoma] - 8.6 (37,40); KB-V1 [Vinblastine-resistant KB] - 1.5 (9,28); P-388 [Murine Lymphoid Neoplasm] - 5.6 (34,103); A-431 [Human Epidermoid Carcinoma] - 8.4 (51,154); LNCaP [Hormone Dependent Human Prostate Cancer] - 9.4 (57,172); ZR-75-1 [Hormone Dependent Human Breast Cancer] - 5.0 (30,92). Based on the data that was generated and compared, isotetrandrine and other bisbenzylisoquinoline alkaloids do not appear to be promising candidates for antimalarial drug development [280].

Isotetrandrine was screened for *in vitro* antimalarial properties using a multi-drug resistant (K1) strain of *Plasmodium falciparum*. The screen detected the inhibition of incorporation of [^{3}H]-hypoxanthine into *P. falciparum*, and isotetrandrine was characterized by IC 0.07 μg/ml. The standard antimalarial drug chloroquine diphosphate exhibits IC 0.14 μg/ml [289].

Isotetrandrine was found to inhibit arachidonic acid-induced inflammation in mice. Topical application of isotetrandrine (2 μmol/mouse) markedly suppressed the tumor-promoting effect of 12-*O*-tetradecanoylphorbol-13-acetate (1 μg) in mouse skin initiated with 7,12-dimethylbenz[a]anthracene (50 μg), at a grade corresponding to that of another bisbenzylisoquinoline alkaloid, cepharanthine [197].

The inhibitory effects of isotetrandrine and 150 natural products on the cytotoxic activity of polymorphonuclear leukocytes (PMN) was investigated. The research employed the effects of natural products on the PMN activation by the antitumor immunomodulator TAK (a linear β-1,3-D-glucan from *Alcaligenes faecalis* var. *myxogenes*) using a PMN cytotoxicity assay system. Isotetrandrine was found to inhibit the activation of PMN activation by TAK, showing an ID_{50} 38 μg/ml. From this, and related studies, it was postulated that isotetrandrine may impair the NADPH oxidase system in the plasma membrane of PMN [199].

Isotetrandrine and ten other bisbenzylisoquinoline alkaloids were evaluated in the *in vitro* histamine release inhibition assay. This assay is one of the basic tests that is used in the evaluation of substances for antiallergic effects. The order of potency of inhibitory effect of those bisbenzylisoquinoline alkaloids that have been isolated from *Thalictrum* species was as follows: homoaromoline > aromoline > isotetrandrine > obaberine > tetrandrine. Berbamine and oxyacanthine were found to have no inhibitory effect [178].

The vasodilator effects of aromoline, berbamine, isotetrandrine, and (+/-)-*N*-methylcoclaurine was evaluated in isolated vessels from male Wistar rats. The rats were anesthetized with thiopental (i.p.), and the thoracic aorta was rapidly excised, with the fat with connective tissues being removed. Rings (about 2 mm in length) were cut from the vessel, and fixed in a bath solution. Prior to addition of the alkaloid sample, the arterial rings were contracted with K^+ (80 mM). Vasodilator effects of the alkaloids were obtained via the addition of sample solutions directly to the bathing media in cumulative concentrations. The potency of the vasodilator effects of the alkaloids on the contracted thoracic aorta in order of decreasing effectiveness was as follows: aromoline (IC_{50} 0.8 μM) > berbamine (IC_{50} 2.4 μM) > isotetrandrine (IC_{50} 3.0 μM) > *N*-methylcoclaurine (IC_{50} 32 μM) [179].

The anti-inflammatory and antiallergic effects of isotetrandrine were evaluated via the measurement of antigen-induced histamine release from leukocytes, and it was found that the alkaloid suppressed antigen-induced histamine release, but this suppression was not dose-dependent [180].

Isotetrandrine was evaluated in BALB/c mice infected with *Leishmania amazonensis* (IFLA/BR/67/PH8 or MHOM/GF/84/CAY-H-142) or *L. venezuelensis* (VE/74/PM-H3). The treatments were initiated one day after parasitic infection at an alkaloid concentration of 100 mg/kg/day for fourteen days, and the reference compound (meglumine antimonate) at 200 mg/kg/day. Isotetrandrine was observed to exhibit activity approximately equal to or greater than meglumine antimonate against *L. amazonensis* (PH8 or H-142), and showed significant activity against *L. venezuelensis*. Single local treatment on the footpad of mice that had been inoculated with *L. amazonensis* 2 weeks earlier revealed that isotetrandrine (200 mg/kg) was less active than meglumine antimonate (400 mg/kg) [334].

13.2.21. Isothalidezine

The total alkaloids of the Chinese *Thalictrum glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang, and the major alkaloid of the plant, hernandezine, were effectively employed in the treatment of P388 leukemia, S180 ascites tumor, and C26 colon tumor in mice. Although hernandezine markedly inhibited the *in vitro* growth of mouse L1210 cells and human KB oral cancer cells, the alkaloid was characterized by only a low inhibition on normal hemopoietic progenitor cells (CFU-GM). Preliminary results demonstrated that hernandezine blocked cell-cycle transfer from the G_1 to the S phase, suggesting that its action may be cell cycle-specific

[205]. It was also demonstrated that two other bisbenzylisoquinoline alkaloids isolated from the same plant, thalidezine and isothalidezine, possessed a similar inhibitory effect on the L1210 cells [278]. Isothalidezine, which was isolated from *Thalictrum glandulosissimum*, was found to markedly inhibit the growth of mouse L1210 cells *in vitro*, but its inhibitory effect on normal hemopoietic progenitor cells (CFU-GM) in mice was relatively low. Preliminary results showed that isothalidezine blocked cell-cycle transfer from G1 to S phase, and its cytocidal action might thus be cell cycle specific [277].

13.2.22. Jatrorrhizine

Jatrorrhizine chloride exhibited reverse transcriptase inhibitory properties against both HIV-1RT (p66/p51)(Human immunodeficiency virus type 1 reverse transcriptase)(IC_{50} 42.4 μg/ml [113.4 μM]) and HIV-2RT (p68/p55)(Human immunodeficiency virus type 2 reverse transcriptase)(IC_{50} 39.8 μg/ml [106.5 μM]). The alkaloid is thought to inhibit various RNA and DNA polymerizing enzymes via interaction with nucleic acid template-primers [212].

Jatrorrhizine chloride was evaluated for antimalarial activity against *Plasmodium falciparum in vitro* (two clones of human malaria; *Plasmodium falciparum* D-6 [Sierra Leone clone] and W-2 (Indochina clone) and *Plasmodium berghei in vivo* (mice). The alkaloid exhibited an antimalarial potency equivalent to that of quinine *in vitro*, but was inactive *in vivo* [228].

Jatrorrhizine chloride was evaluated in the human immunodeficiency virus reverse transcriptase assay and found to be moderately active (50 μg/ml < IC_{50} < 150 μg/ml)[229].

Jatrorrhizine chloride was dissolved in culture medium and evaluated for its activity against two protozoa, *Plasmodium falciparum* (K1, multi-drug resistant strain) and *Entamoeba histolytica* (NIH 200). The alkaloid was not toxic to KB cells (IC_{50} > 334 μM), and was active against *P. falciparum* (IC_{50} 3.1 μM), but inactive against *E. histolytica* (IC_{50} 83 μM). In assessing relative *in vitro* cytotoxicity to antiplasmodial activity, berberine had the least favorable ratio (8), while jatrorrhizine had the most favorable ratio (> 106) of the alkaloids tested. The results lend some support to the utilization of plants containing protoberberine alkaloids for the treatment of malaria [230].

Jatrorrhizine was found to possess anti-inflammatory activity as measured in the cobra venom factor-induced (CVF) rat paw edema. CVF edema was used to examine jatrorrhizine and other substances in order to detect novel compounds, since it has been shown that joint cyclooxygenase and lipoxygenase inhibitors, as well as immunoreactive drugs, exhibit more pronounced inhibitory effects on CVF in comparison to carrageenin-induced edema. CVF edema is dependent on activation of the complement system which plays an important role in acute and chronic inflammatory reactions, mediating the activity of immune complexes. This test system represents a functionally new type of acute inflammation via activation of the alternative complement pathway [290].

Jatrorrhizine was found to exhibit toxicity to P388 cells (ED_{50} 2.0 μg/ml) and to brine shrimp (LD_{50} 0.2 μg/ml), but was inactive in antibacterial assays against *Escherichia coli*, *Streptococcus aureus*, or *Bacillus subtilis*, at concentrations up to 100 μg/ml)[291].

Four protoberberine alkaloids and one aporphine alkaloid were evaluated for lipoxygenase inhibition. Oxyberberine and columbamine were the most potent lipoxygenase inhibitors tested, with jatrorrhizine being intermediate, whereas berberine and magnoflorine exhibited only low potencies. A strong linear correlation between lipoxygenase inhibition and lipid antioxidant properties of these alkaloids was observed. These data suggest that the mechanism of lipoxygenase inhibition by these alkaloids may be linked to the inhibition of lipid hydroperoxide substrate accumulation [242].

The antibacterial activity of jatrorrhizine against the Gram-negative bacilli *Pseudomonas aeruginosa* and *Proteus vulgaris* was determined, but unfortunately not described in this abstract [292].

13.2.23. Liriodenine

An investigation of the pharmacological activities of liriodenine demonstrated that the alkaloid was a selective muscarinic receptor antagonist in isolated trachea, ileum and cardiac tissues of guinea-pigs, being more potent in smooth muscle than in cardiac preparations. High concentrations (300 μM) of liriodenine elicited a blockage of voltage-dependent Ca^{+2} [293].

The antimuscarinic properties of liriodenine were compared with methoctramine (cardioselective M_2 antagonist) and 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP, smooth muscle selective M_3 antagonist) via radioligand binding tests, functional tests and measurements of second messenger generation in canine cultured tracheal smooth muscle cells. The antimuscarinic characteristics of liriodenine were found to be similar to those of 4-DAMP, and the alkaloid may act as a selective M_3 receptor antagonist in canine tracheal smooth muscle [294].

Liriodenine was found to be the mutagenic principle in a methanol extract of the root, root stalk, and stem of *Sinomenium acutum* (Menispermaceae). The alkaloid exhibited potent mutagenic activity towards *Salmonella typhimurium* strains TA98 and TA100 in the presence of liver homogenate (S9 mix)(10.9 and 90.7 revertants/nmol, respectively), and accounted for about 40% of the mutagenicity of the total methanol extract. The mutagenic potency of liriodenine is comparable to that of benzo[a]pyrene [295].

Liriodenine was found to be active in the brine shrimp lethality test (LC_{50} 2.03 μg/ml) but was inactive in the 9PS (a chemically-induced murine lymphocytic leukemia) and 9KB (human nasopharyngeal carcinoma) cytotoxicity tests [296].

Liriodenine was found to exhibit potent cytotoxicity against KB, A-549, HCT-8, P-388, and L-1210 cells with ED_{50} values of 1.00, 0.72, 0.70, 0.57, and 2.33 μg/ml, respectively [297].

Mechanism-based bioassays involving DNA repair or recombination deficient yeast mutants have been increasingly utilized in attempts to monitor the isolation of natural products

with selective anti-cancer activity. Liriodenine was found to exhibit selective toxicity against DNA repair and recombination deficient mutants of the yeast *Saccharomyces cerevisiae*. The following activities were recorded for the alkaloid against selective *S. cerevisiae* strains: IC_{12} 16.7 μg/ml (strain - rad 52); 15.0 mm zone of inhibition at 500 μg/ml (strain - rad 6); IC_{12} 500 μg/ml (strain - rad^+). The presence of a methylenedioxy group in oxoaporphine alkaloids tends to confer activity in these assays, and certain oxoaporphinoid alkaloids (notably those with methylenedioxy groups), may represent a novel class of DNA topoisomerase inhibitors [298].

The *in vitro* antimicrobial activity of liriodenine was investigated by agar diffusion and agar dilution methods against various Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus faecalis*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Enterobacter aerogenes*, *Serratia marcescens*, *Serratia sonnei*), as well as the acid-fast organism *Mycobacterium phlei*, and the yeast *Candida albicans*. Liriodenine showed some activity against the Gram-positive organisms, as well as against *Mycobacterium phlei*. There was no activity exhibited against the Gram-negative organisms, and only slight activity against the yeast *Candida albicans* [283].

Liriodenine was evaluated for its *in vitro* antimicrobial activity against a variety of microorganisms. The alkaloid was found to modestly inhibit the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Mycobacterium phlei*, and *Candida albicans*. The oxoderivatives of aporphines were more active against Gram-positive bacteria than the reduced aporphines, but the former had a lower activity against yeast, and no activity against Gram-negative bacteria [263].

A series of fifty-three isoquinoline alkaloids were tested for cytotoxicity against A-549 (human lung carcinoma), HCT-8 (human colon carcinoma), KB, P-388, and L-1210 cells *in vitro*. Liriodenine showed potent and wide spectrum activity against all five cell lines tested: A-549 (ED_{50} 0.72 μg/ml), HCT-8 (0.70), KB (1.00), P-388 (0.57), and L-121-0 (2.33)[299].

Liriodenine was observed to demonstrate potent cytotoxicity against KB, A-549, P-388, and L-1210 cells with ED_{50} values of 1.00, 0.72, 0.57, and 2.33 μg/ml, respectively [300].

The efficacy of liriodenine and liriodenine methiodide as potential anticandidal agents was determined in a mouse model of disseminated candidiasis. Varying doses of each drug were administered intraperitoneally or intravenously 7 hours postinfection to mice infected with a lethal dose of *Candida albicans* NIH B311. Reductions in the number of colony-forming units (CFU) recovered per milligram of kidney tissue were observed in drug-treated animals as compared with vehicle-treated controls. Intravenous administration of both liriodenine and its *N*-methyl analog (liriodenine methiodide) produced a significant reduction in the number of recovered CFU [301].

An ethanol extract of the above-ground parts of the Taiwanese *Thalictrum sessile* Hayata was found to exhibit significant cytotoxicity against in vitro tissue culture cells in human KB, A-549 lung carcinoma and HCT-8 colon tumor, as well as murine P-388 and L-1210 lymphocytic leukemia [62]. Bioassay-guided fractionation of this extract led to the isolation and identification of two cytotoxic aporphinoid alkaloids; the oxoaporphine liriodenine and the aporphine-benzylisoquinoline dimer (+)-thalifarazine. The following are the activities of liriodenine against

various tumor cell lines, with the LD_{50} values (μg/ml) expressed in parentheses: KB (1.00), A-549 (0.72), HCT-8 (0.70), P-388 (0.57), and L-1210 (2.33) [62].

The minimum inhibitory concentrations (MIC) of liriodenine versus the superficial dermatophytes *Trichophyton mentagrophytes* (ATCC 9972) and *Microsporum gypseum* (ATCC 14683) in a complex medium were 3.12 μg/ml and 100 μg/ml, respectively. In a defined medium against the same organisms, the values were 0.78 μg/ml and 3.12 μg/ml, respectively [337].

13.2.24. Magnoflorine

Magnoflorine chloride was observed to lack inhibitory activity (inactive) against both HIV-1RT (p66/p51)(Human immunodeficiency virus type 1 reverse transcriptase) and HIV-2RT (p68/p55)(Human immunodeficiency virus type 2 reverse transcriptase)[212].

Magnoflorine was observed to decrease arterial blood pressure in rabbits and to induce hypothermia in mice. In addition, the alkaloid induced contractions in the isolated pregnant rat uterus and stimulated the isolated guinea pig ileum. Experiments with standard agents in these models suggest magnoflorine acts on the muscarinic and serotonergic systems, either directly or indirectly [302].

Magnoflorine chloride was evaluated in the human immunodeficiency virus reverse transcriptase assay and found to be inactive (IC_{50} > 200 μg/ml)[229].

The antimalarial activity [ED_{50} (ng/ml)] of magnoflorine was determined as follows: *Plasmodium falciparum* Strain D-6 (>10,000) and *P. falciparum* Strain W-2 (>10,000)[303].

Magnoflorine was observed to suppress the local graft-versus-host reactions when given to mice (5-20 mg/kg for 8 days, i.p.) from the day of spleen cell transfer to induce the reaction. The alkaloid suppressed picryl chloride-induced delayed type hypersensitivity (PC-DTH) when given to mice at 10 and 20 mg/kg for 5 days (i.p.) from the day of sensitization, but did not suppress it when given at the time of challenge. These results suggest that magnoflorine is able to suppress the induction phase but not the effector phase of the cellular immune response, and may have a role as a new type of immunosuppressor [304].

Four protoberberine alkaloids and one aporphine alkaloid were evaluated for lipoxygenase inhibition. Oxyberberine and columbamine were the most potent lipoxygenase inhibitors tested, with jatrorrhizine being intermediate, whereas berberine and magnoflorine exhibited only low potencies. A strong linear correlation between lipoxygenase inhibition and lipid antioxidant properties of these alkaloids was observed. These data suggest that the mechanism of lipoxygenase inhibition by these alkaloids may be linked to the inhibition of lipid hydroperoxide substrate accumulation [242].

13.2.25. Methoxyadiantifoline

Methoxyadiantifoline exhibited pulmonary antifibrotic properties, with substantial binding affinity for membrane lipids and alveolar macrophages, similar to those of tetrandrine. Experimental data indicated a strong relationship between the antifibrotic potential of the methoxyadiantifoline and its ability to bind alvelolar macrophages and to inhibit particle-induced activation of these macrophages. Methoxyadiantifoline should serve as a useful probe in the evaluation of the role of alveolar macrophages in pulmonary fibrosis [305].

Methoxyadiantifoline was observed to reverse multidrug resistance and to potentiate the effectiveness of a primary drug (antineoplastic, antimalarial, tuberculostatic) against a drug-resistant cell. This action is apparently via the reversal or inhibition of a glycoprotein pump that is present in a multidrug-resistant cell, such that the cell actually accepts a greater concentration of the drug than does a drug-resistant cell [306].

Methoxyadiantifoline was found to exhibit calcium blocker activity (IC_{50} 2.53 x 10^{-6} M) as determined by the *in vitro* assay for guinea pig duodenal contraction [71].

13.2.26. *N*-Methylcanadine

Forty-eight derivatives of berberine-type alkaloids were examined for their inhibition activity against the induction of mouse ear edema via application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). *N*-Methylcanadine chloride was found to possess inhibitory effects (6% inhibition), and berberine derivatives were found to have stronger inhibitory activity than palmatine derivatives. Since berberine and some of its derivatives are present in Chinese traditional drugs that are used in combined Kampo prescriptions, it is important to determine if such alkaloids possess carcinogenic inhibiting properties [221].

N-Methylcanadine was evaluated for antimalarial activity against *Plasmodium falciparum in vitro* (two clones of human malaria; *Plasmodium falciparum* D-6 [Sierra Leone clone] and W-2 (Indochina clone) and *Plasmodium berghei in vivo* (mice). It was found that the alkaloid was inactive in both *in vitro* and *in vivo* tests [228].

N-Methylcanadine chloride (canadine methochloride) was evaluated in the human immunodeficiency virus reverse transcriptase assay and found to be inactive (IC_{50} > 200 μg/ml)[229].

13.2.27. *N*-Methylcoclaurine

N-Methylcoclaurine, as well as eleven bisbenzylisoquinoline alkaloids were evaluated in the *in vitro* histamine release inhibition assay. This assay is one of the basic tests that is used in the evaluation of substances for antiallergic effects. The order of potency of inhibitory effect

of those bisbenzylisoquinoline alkaloids that have been isolated from *Thalictrum* species was as follows: homoaromoline > aromoline > isotetrandrine > obaberine > tetrandrine. *N*-Methylcoclaurine was found to exhibit an inhibitory effect almost comparable to that of obaberine. Berbamine and oxyacanthine were found to have no inhibitory effect [178].

The vasodilator effects of aromoline, berbamine, isotetrandrine, and (+/-)-*N*-methylcoclaurine were evaluated in isolated vessels from male Wistar rats. The rats were anesthetized with thiopental (i.p.), and the thoracic aorta was rapidly excised, with the fat with connective tissues being removed. Rings (about 2 mm in length) were cut from the vessel, and fixed in a bath solution. Prior to addition of the alkaloid sample, the arterial rings were contracted with K^+ (80 mM). Vasodilator effects of the alkaloids were obtained via the addition of sample solutions directly to the bathing media in cumulative concentrations. The potency of the vasodilator effects of the alkaloids on the contracted thoracic aorta in order of decreasing effectiveness were as follows: aromoline (IC_{50} 0.8 μM) > berbamine (IC_{50} 2.4 μM) > isotetrandrine (IC_{50} 3.0 μM) > *N*-methylcoclaurine (IC_{50} 32 μM) [179].

13.2.28. Nantenine

Nantenine was determined to inhibit rabbit lens aldose reductase (IC_{50} 4.3 x 10^{-4}M) [210].

13.2.29. Obaberine

The potential therapeutic effect of obaberine (10, 25, and 50 μg/ml)(and thirteen other bisbenzylisoquinoline alkaloids) against the protozoan disease leishmaniasis was studied by biological assays on *in vitro* culture forms of three strains of *Leishmania*; *L. brasiliensis brasiliensis* (cutaneous and mucocutaneous leishmaniasis), *L. mexicana amazonensis* (cutaneous), and *L. donovani* (visceral leishmaniasis). Obaberine was found to inhibit the growth of all three types to differing degrees, and was considered among the top three alkaloids screened (daphnandrine and gyrocarpine being the other two) [194].

The potential therapeutic effect of obaberine (10, 25, and 50 μg/ml)(and thirteen other bisbenzylisoquinoline alkaloids) against the protozoan disease American trypanosomiasis (Chagas' disease) was studied by biological assays on *in vitro* culture forms of three strains of *Trypanosoma cruzi* (Tulahuen, C8C11,and 1979 CL1). As in the case of *Leishmania*, obaberine was found to inhibit the growth of all three types to differing degrees, and was considered among the top three alkaloids screened (daphnandrine and gyrocarpine being the other two) [195].

Obaberine was found to inhibit the growth of cultured *Plasmodium falciparum* strains D-6 and W-2, and to display nonselective cytotoxicity with a battery of cultured mammalian cells. These data were used to calculate a selectivity index (ED_{50} in cultured mammalian cells/ED_{50} in cultured *Plasmodium falciparum* strains). Agents that are useful antimalarial drugs, such as

quinine and artemisinin, typically display selectivity indices that are > 1000. The ED_{50} (ng/ml) of obaberine against *Plasmodium falciparum* strain D-6 was 231.0, while that associated with strain W-2 was 216.0. The following is a summary of the cytotoxic activities (ED_{50}, μg/ml) of obaberine, along with the cytotoxicity/antimalarial activity ratio (selectivity index) with the first number in parentheses being the D-6 strain selectivity index and the second number being the W-2 strain selectivity index: BCA-1 [Human Breast Cancer] - 4.6 (20,21); HT-1080 [Human Fibrosarcoma] - 10.4 (45,48); LUC-1 [Human Lung Cancer] - 9.7 (42,45); MEL-2 [Human Melanoma] - 20.0 (87,93); COL-1 [Human Colon Cancer] - 10.8 (47,50); KB [Human Oral Epidermoid Carcinoma] - 8.6 (37,40); KB-V1 [Vinblastine-resistant KB] - 2.6 (11,12); P-388 [Murine Lymphoid Neoplasm] - 4.1 (18,19); A-431 [Human Epidermoid Carcinoma] - 8.7 (38,40); LNCaP [Hormone Dependent Human Prostate Cancer] - 5.0 (22,23); ZR-75-1 [Hormone Dependent Human Breast Cancer] - 4.0 (17,19). Based on the data that was generated and compared, obaberine and other bisbenzylisoquinoline alkaloids do not appear to be promising candidates for antimalarial drug development [280].

The relaxant activity of obaberine was examined in isolated rat uterus and its inhibitory potency compared with that of tetrandrine. Obaberine was observed to relax KCl-depolarized rat uterus and to totally or partially inhibit oxytocin-induced rhythmic contractions. Obaberine inhibited sustained K^+-induced contraction with the same potency as tetrandrine when extracellular calcium is present, but the rhythmic contraction induced by oxytocin was diminished to a much lesser extent. This observation indicates that the relaxant mechanism of obaberine may be mainly related to Ca^{+2} influx, possibly through voltage-operated channels. In Ca^{+2}-free medium, obaberine relaxed oxytocin- and vanadate-induced uterine contractions, while tetrandrine did not. These results suggest that obaberine has an intracellular site of action, and may interact with a stereospecific receptor site within the cell [307].

Obaberine and ten other bisbenzylisoquinoline alkaloids were evaluated in the *in vitro* histamine release inhibition assay. This assay is one of the basic tests that is used in the evaluation of substances for antiallergic effects. The order of potency of the inhibitory effect of those bisbenzylisoquinoline alkaloids that have been isolated from *Thalictrum* species was as follows: homoaromoline > aromoline > isotetrandrine > obaberine > tetrandrine. Berbamine and oxyacanthine were found to have no inhibitory effect [178].

13.2.30. Obamegine

Obamegine was screened for antiplasmodial, antiamoebic, and cytotoxic activities via the use of *in vitro* microtests. The alkaloid was characterized by the following: IC_{50} 0.74 μM against a multidrug-resistant strain of *Plasmodium falciparum* (K1)(standard - chloroquine phosphate, IC_{50} 0.20 μM); IC_{50} 30.8 μM against *Entamoeba histolytica* (standard - metronidazole, IC_{50} 1.87 μM); IC_{50} 55.4 μM against nasopharyngeal KB cell line (standard - podophyllotoxin, IC_{50} 0.008 μM)[176].

Obamegine was screened for *in vitro* antimalarial properties using a multi-drug resistant (K1) strain of *Plasmodium falciparum*. The screen detected the inhibition of incorporation of [^{3}H]-hypoxanthine into *P. falciparum*, and obamegine was characterized by IC 0.51 μg/ml. The standard antimalarial drug chloroquine diphosphate exhibits IC 0.14 μg/ml [289].

13.2.31. Oxoglaucine

The efficacy of oxoglaucine methiodide as a potential anticandidal agent was determined in a mouse model of disseminated candidiasis. Varying doses were administered intraperitoneally or intravenously 7 hours postinfection to mice infected with a lethal dose of *Candida albicans* NIH B311. There was no significant response to treatment with oxoglaucine methiodide [301].

Mechanism-based bioassays involving DNA repair or recombination deficient yeast mutants have been increasingly utilized in attempts to monitor the isolation of natural products with selective anti-cancer activity. Oxoglaucine was evaluated, and found to exhibit selective toxicity against DNA repair and recombination deficient mutants of the yeast *Saccharomyces cerevisiae*. The following activities were recorded for the alkaloid against selective *S. cerevisiae* strains: IC_{12} >500 μg/ml (strain - rad 52); IC_{12} >500 μg/ml (strain - rad 52. top 1); IC_{12} >500 μg/ml (strain - rad 6); IC_{12} >500 μg/ml (strain - rad$^+$). The presence of a methylenedioxy group in oxoaporphine alkaloids tends to confer activity in these assays, and certain oxoaporphinoid alkaloids (notably those with methylenedioxy groups), may represent a novel class of DNA topoisomerase inhibitors. In this regard, oxoglaucine with its 1,2,9,10-tetramethoxy- substitution, was consistently inactive [298].

A series of fifty-three isoquinoline alkaloids were tested for cytotoxicity against A-549 (human lung carcinoma), HCT-8 (human colon carcinoma), KB, P-388, and L-1210 cells *in vitro*. Oxoglaucine was active against HCT-8 (ED_{50} 1.00 μg/ml) and KB (ED_{50} 2.00 μg/ml)[299].

13.2.32. Oxyacanthine

Oxyacanthine HCl was screened for antiplasmodial, antiamoebic, and cytotoxic activities via the use of *in vitro* microtests. The alkaloid was characterized by the following: IC_{50} 1.06 μM against a multidrug-resistant strain of *Plasmodium falciparum* (K1)(standard - chloroquine phosphate, IC_{50} 0.20 μM); IC_{50} 32.3 μM against *Entamoeba histolytica* (standard - metronidazole, IC_{50} >82.2 μM); IC_{50} 74.4 μM against nasopharyngeal KB cell line (standard - podophyllotoxin, IC_{50} 0.008 μM)[176].

Bisbenzylisoquinoline alkaloids block Ca^{+2} uptake through L-type Ca^{+2} channel and modulate binding of ligands to four distinct sites (dihydropyridine, benzothiazepine, aralkylamine, and (diphenylbutyl)piperidine) in the Ca^{+2} entry blocker receptor complex of the channel. All bisbenzylisoquinoline analogs tested, including oxyacanthine, completely inhibit diltiazem binding,

but many only partially inhibit D-600 and fluspirilene binding. For dihyrodpyridine binding, the compounds show either stimulation or inhibition of exhibit no effect. Data from this experiment suggest that oxyacanthine and a variety of bisbenzylisoquinoline congeners act to block the L-type Ca^{+2} channel by binding to the benzothiazepine site on the channel. The novel patterns exhibited by oxyacanthine and other bisbenzylisoquinoline alkaloid analogs in affecting allosteric coupling between the benzothiazepine site and the other receptors of the Ca^{+2} entry blocker receptor complex define a new class of Ca^{+2} entry blocker with binding properties distinct from diltiazem [186].

Treatment with oxyacanthine was found to relax K^+-precontracted rat aortal rings, with an IC_{50} of about 20 μmol/liter. This relaxation was not inhibited by denudation of the endothelium or by premedication of the aortas with indomethacin, methylene blue or propranolol. In addition, the alkaloid inhibited contractions induced by norepinephrine, phenylephrine, and serotonin in a non-competitive manner, as well as competitively blocking calcium-induced contraction in a Ca^{+2}-free depolarizing PSS. Blockade of calcium entry appears to be the major mechanism of the dilatory effect of this alkaloid, but its interaction with α-adrenoceptors was not excluded [188].

Oxyacanthine was screened for antimalarial activity against *Plasmodium falciparum* K1 strain and found to be active with an IC_{50} = 0.31 μg/ml [308].

Oxyacanthine and ten other bisbenzylisoquinoline alkaloids were evaluated in the *in vitro* histamine release inhibition assay. This assay is one of the basic tests that is used in the evaluation of substances for antiallergic effects. The order of potency of inhibitory effect of those bisbenzylisoquinoline alkaloids that have been isolated from *Thalictrum* species was as follows: homoaromoline > aromoline > isotetrandrine > obaberine > tetrandrine. Berbamine and oxyacanthine were found to have no inhibitory effect [178].

The effects of berbamine, oxyacanthine, and berberine on 5-lipoxygenase lipid peroxidation in phospholipid liposomes induced by 2,2'-azo-(bis-2-amidinopropane)(AAPH), deoxyribose degradation, and their reactivities against the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) were studied. None of the alkaloids showed any appreciable effects in the inhibition of 5-lipoxygenase. Reactivity against DPPH increased in the following order: berberine < oxyacanthine < berbamine. Pro-oxidant effects of the alkaloids were excluded, since deoxyribose degradation was not influenced, as determined by the release of malondialdehyde [200].

Oxyacanthine, berbamine and berberine were evaluated for antiproliferative activity against the growth of HaCaT cells (a rapidly multiplying human keratinocyte cell line). Proliferation of the keratinocytes was determined directly by counting the dispersed cells under a phase-contrast microscope after 48 hours of treatment. The antiproliferative activity of these alkaloids were as follows: berbamine (IC_{50} 11 μM) > oxyacanthine (IC_{50} 13 μM) > berberine (IC_{50} 30 μM). The standard was anthralin (IC_{50} 0.7 μM). These results indicate that the three alkaloids tested were all antiproliferative agents when applied to HaCaT cells at micromolar concentrations [201].

13.2.33. Oxyberberine

Oxyberberine was evaluated for antimalarial activity against *Plasmodium falciparum in vitro* (two clones of human malaria; *Plasmodium falciparum* D-6 [Sierra Leone clone] and W-2 (Indochina clone) and *Plasmodium berghei in vivo* (mice). The alkaloid was found to be inactive in both *in vitro* and *in vivo* tests [228].

Four protoberberine alkaloids and one aporphine alkaloid were evaluated for lipoxygenase inhibition. Oxyberberine and columbamine were the most potent lipoxygenase inhibitors tested, with jatrorrhizine being intermediate, whereas berberine and magnoflorine exhibited only low potencies. A strong linear correlation between lipoxygenase inhibition and lipid antioxidant properties of these alkaloids was observed. These data suggest that the mechanism of lipoxygenase inhibition by these alkaloids may be linked to the inhibition of lipid hydroperoxide substrate accumulation [242].

13.2.34. Palmatine

The methanol extract of the bark of *Phellodendron amurense* was shown to possess strong antifeedant activity against the paper/wood termite pest *Reticulitermes speratus*. Partitioning and chromatography of the extract afforded four active antifeedant compounds, one of which was palmatine [215].

Forty-eight derivatives of berberine-type alkaloids were examined for their inhibition activity against the induction of mouse ear edema via application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Palmatine chloride was found to possess inhibitory effects (29% inhibition). Berberine derivatives had stronger inhibitory activity than palmatine derivatives. Since berberine and some of its derivatives are present in Chinese traditional drugs, which are used in combined Kampo prescriptions, it is important to determine if such alkaloids possess carcinogenic inhibiting properties [221].

The sedative effect of palmatine on locomotor activity and concentration of monoamine in rats was studied via behavioral and biochemical methods. Palmatine was shown to enhance the hypomotility induced by α-methyl-p-tyrosine, reserpine, and 5-hydroxytryptophan, but reduced the hypermotility produced by L-dopa plus benserazide and p-chlorophenylalanine. In addition, the alkaloid significantly decreased the concentration of dopamine and homovanillic acid in the cortex and the concentration of serotonin in the brain stem, but increased the concentration of 5-HT in the cortex and 5-hydroxyindole acetic acid in the brain stem. These results suggest that the sedative action associated with palmatine may be related to the decrease in catecholamine concentration in the cortex and serotonin in the brain stem, and the increase in the concentration of 5-HT in the cortex [309].

Palmatine chloride was evaluated for antimalarial activity against *Plasmodium falciparum in vitro* (two clones of human malaria; *Plasmodium falciparum* D-6 [Sierra Leone clone] and W-2

(Indochina clone) and *Plasmodium berghei in vivo* (mice). The alkaloid exhibited an antimalarial potency equivalent to that of quinine *in vitro*, but was inactive *in vivo* [228].

Administration of palmatine hydroxide orally to dogs at a dosage of 30 mg/kg/day for 60 days produced a consistent impairment of primary and secondary spermatocytes and elongated spermatids (Stages IV-VIII). Primary and secondary spermatocytes were reduced by 60 and 68%, respectively, and the elongated spermatids were decreased by 58%. The number of spermatogonia and Sertoli cells remained unaltered. The production of immature and mature Leydig cells decreased by 66% and 27%, respectively, while protein, sialic acid and glycogen content, as well as acid phosphatase activity of testes and epididymides were lowered to varying extents. Testicular cholesterol was elevated significantly, and the weights to the testes and epididymides were significantly reduced. The antispermatogenic action of the alkaloid may be mediated via disturbances in Leydig cell function [310].

Palmatine was submitted to an *in vitro* antimicrobial screen using eight Gram-positive and eight Gram-negative bacteria, as well as one yeast. The alkaloid was inactive (MIC > 50 mg/l) against virtually all of the organisms, save the acid-fast rod *Mycobacterium phlei* (MIC = 25 mg/l) [239].

A series of fifty-three isoquinoline alkaloids were tested for cytotoxicity against A-549 (human lung carcinoma), HCT-8 (human colon carcinoma), KB, P-388, and L-1210 cells *in vitro*. Palmatine iodide was active only against KB (ED_{50} 4.00 μg/ml)[299].

The mechanism of the antiarrhythmic of palmatine was studied in the guinea pig. Palmatine (50 μmol/l) was observed to markedly prolong the functional refractory period and decrease the epinephrine-induced automaticity in isolated guinea pig papillary muscles. However, it exerted no effect on the excitability and contractility of the muscles. At 10 μmol/l, the alkaloid prolonged both action potential duration $_{20}$ (APD_{20}) and action potential duration$_{90}$ (APD_{90}) of the guinea pig papillary muscles. The V_{max} was decreased at a concentration of 100 μmol/l, but the action potential amplitude and resting potential were unaltered. These results indicated that the antiarrhythmic actions of palmatine may be due to the ability of the alkaloid to decrease automaticity and to prolong the functional refractory period and action potential duration of the myocardium [311].

Palmatine was determined to inhibit rabbit lens aldose reductase (IC_{50} 3.8 x 10^{-5}M) [210].

13.2.35. Protopine

Protopine was demonstrated to enhance ^{3}H-γ-aminobutyric acid (GABA) binding to rat brain synaptic membrane receptors. Using this phenomenon as an *in vitro* paradigm, protopine and other protopine alkaloids may be suggested to have benzodiazepine-like activity [264].

The mode of action of protopine on rabbit platelet aggregation was investigated in the metabolic system of adenosine 3',5'-cyclic monophosphate (cyclic AMP) *in vitro* experimental models. The inhibitory activity of the alkaloid on adenosine 5'-diphosphate induced platelet

aggregation was increased in the presence of prostaglandin I_2 or papaverine in platelets. Protopine elevated content of the basal cyclic AMP accumulation in platelets and enhanced activity of crude adenylate cyclase prepared from platelets, but was ineffective on cyclic AMP phosphodiesterase. Protopine has an inhibitory activity on platelet aggregation, activates adenylate cyclase, and increases cyclic AMP content in platelets, in addition to other inhibitory actions in the metabolic system of [312].

The mode of action of protopine on blood platelet aggregation was investigated in the metabolic system of arachidonic acid and in liberation of platelet activating factor using *in vitro* experimental models. The alkaloid inhibited the release of arachidonic acid and of platelet activating factor from platelet membrane phospholipids. Protopine also inhibited the conversion of prostaglandin G_2 to thromboxane A_2, as well as carboxyheptyl imidazole, a thromboxane synthetase inhibitor. These experimental results indicate that protopine functions both as a phospholipase inhibitor and a thromboxane synthetase inhibitor, and suggest that the alkaloid may be useful as an antiplatelet drug in the therapy of thrombosis [313].

It has been observed that aldehyde reductase I has been inhibited by a number of benzylisoquinoline-derived alkaloids. The sensitivity of aldehyde reductase I to test compounds, including protopine, was compared with that of alcohol dehydrogenase and/or aldehyde reductase II, and alcohol dehydrogenase was shown to be more selective in binding the alkaloids. The kinetics of the inhibitory action of berberine, as well as other results suggest that the binding site of aldehyde reductase I for alkaloids is relatively large, has a hydrophobic nature, and probably contains a group with a positive charge. In addition, the binding site is likely not identical with the active center of the enzyme [227].

Protopine was observed to inhibit norepinephrine-induced tonic contraction in rat thoracic aorta in a concentration-dependent manner (25-100 μg/ml). Norepinephrine-induced phasic contraction was inhibited by only a high concentration (100 μg/ml). The alkaloid caused relaxation at the plateau of norepinephrine-induced tonic contraction. Neither indomethacin (20 μM) nor methylene blue (50 μM) antagonized this relaxing effect, and it still persisted in denuded rat aorta in the presence of nifedipine (2-100 μM). The alkaloid also inhibited high potassium (60 mM)-induced, calcium-dependent (0.03-3 mM) contraction of the rat aorta in a concentration-dependent manner. Neither cAMP nor cGMP level was changed by protopine, nor were the norepinephrine-induced formation of inositol monophosphate or the caffeine-induced contraction. $^{45}Ca^{+2}$ influx induced by either norepinephrine or K^+ was inhibited by protopine in a concentration-dependent fashion. Protopine relaxed rat thoracic aorta mainly via the suppression of Ca^{+2} influx through both voltage- and receptor-operated calcium channels [314].

The anticholinergic and antihistaminic properties of protopine and some derivatives were studied on the isolated guinea-pig ileum. Cumulative dose-response curves for acetylcholine were displaced to the right by protopine in a parallel, and were thus characteristic for competitive antagonism. The effect was qualitatively comparable to atropine, although higher concentrations of protopine ($> 10^{-5}$M) decreased the maximal contractions of acetylcholine, this effect indicating some noncompetitive antagonism. Atropine was estimated to be about 660 times more potent

than protopine as an anticholinergic agent. Incubation studies using histamine as the agonist indicated that protopine does not have true antihistaminic properties. Protopine was concluded to be only a weak anticholinergic alkaloid [315].

Protopine was shown to be an inhibitor of phosphoinositide breakdown. Thirty minutes after intraperitoneal administration of the alkaloid (up to 200 mg/kg) to mice, tail bleeding was observed to be markedly prolonged in a dose-dependent manner. However, administration of protopine did not prevent acute thromboembolic death in these animals. The antihemostatic effect of protopine and other Chinese herbal medicinals may be explained by their *in vitro* antiplatelet and vasorelaxing actions [316].

Protopine was observed to inhibit the aggregation and ATP release of rabbit platelets induced by ADP, arachidonic acid, PAG, collagen and ionophore A23187. Although the platelet aggregation caused by thrombin was not inhibited by protopine (100 μg/ml), the release reaction was partially suppressed. Protopine also inhibited the platelet aggregation caused by ADP, arachidonic acid, PAF, and collagen in rabbit platelet-rich plasma. The thromboxane B_2 formation of washed platelets caused by arachidonic acid, collagen, ionophore A23187 and thrombin was suppressed by protopine. The alkaloid also inhibited the intracellular calcium increase caused by arachidonic acid in quin-2/AM loaded rabbit platelets. In the presence of indomethacin, the intracellular calcium increase caused by collagen and PAF was completely suppressed by protopine, and the intracellular calcium increase caused by thrombin was partially inhibited. The phosphoinositides breakdown caused by collagen and PAF was inhibited by the alkaloid, but that by thrombin was not affected significantly. Protopine did not cause the elevation of cyclic AMP level of platelets. The antiplatelet effect of protopine was due to inhibition of thromboxane formation and phosphoinositides breakdown, which then was followed by a decrease in intracellular calcium [317].

Protopine was shown to exhibit an inhibitory action on blood platelet aggregation induced by collagen, arachidonic acid, and ADP *in vitro*. In addition, protopine demonstrated equal or superior inhibitory effects when compared with that of the standard drugs acetylsalicylic acid or dipyridamole [266].

The effects of protopine on blood platelet aggregation were investigated in *in vivo*, *ex vivo*, and *in vitro* models. Protopine (0.25 mM/kg, p.o.) displayed stronger inhibitory effects on endotoxin-induced disseminated intravascular coagulation (DIC) in rats and on pulmonary thromboembolism in mice compared with that of acetylsalicylic acid and dipyridamole (1.0 mM, kg, p.o., respectively), and also inhibited collagen- and ADP-induced rat *ex vivo* blood platelet aggregation. The alkaloid was noted to intensify the inhibitory effect of PGI_2 on platelet aggregation, but there was no effect on PGI_2 generation from rat arterial tissue. Administration of acetylsalicylic acid (0.1 mM/kg, p.o.) strongly inhibited the PGI_2 generation. These results suggest that protopine should serve as an anti-thrombotic agent without inhibitory effects on PGI_2 generation, as observed with acetylsalicylic acid [318].

Protopine was found to dose-dependently inhibit *in vitro* rabbit platelet aggregation induced by ADP, arachidonic acid (AA), collagen, or aggregoserpentin of *Trimeresurus*

mucrosquamatus venom (TMVA). The IC_{50} values were 25.3, 30.5, 46.9, and 33.4 μmol/l, respectively. Protopine (10, 20 mg/kg, i.v.) was found to inhibit the platelet aggregation produced by these inducers, with the effects (maximal at 5 minutes) lasting one hour. Via the use of fluorphotometry and RIA procedures, it was observed that protopine suppressed the release of 5-HT from platelets during collagen-, AA-, or TMVA-induced aggregation *in vitro*. Protopine did not block the formation of thromboxane A_2 during AA-induced aggregation, nor did it increase the content of cAMP in rabbit platelets. The alkaloid did, however, increase the content of cGMP in rabbit platelets. The antiplatelet effect of protopine may be related to an increase in cGMP in rabbit platelets, and to the suppression of the release of the active substances from platelets [319].

Protopine (0.5 - 5.0 mcg/ml) exhibited smooth muscle relaxant activity on the isolated ileum of guinea pig, rabbit, and albino rat, and a marked relaxation of the intestine *in situ* (5 mg/kg, i.v.) of the anesthetized dog. It addition, the alkaloid demonstrated hydrocholeretic activity at the same dose in the anesthetized dog (with cystic duct ligated and common bile duct cannulated). Protopine (2000 μg/ml) also exhibited prominent antifungal activity as assessed against the spore germination of a number of plant pathogenic fungi [338].

13.2.36. Puntarenine

Puntarenine was evaluated for *in vitro* antimicrobial activity against *Candida albicans*, *Cryptococcus neoformans*, *Mycobacterium intracellularae*, *Trichophyton mentagrophytes*, and *Saccharomyces cerevasiae*, as well as representative Gram-positive and Gram-negative bacteria. The alkaloid showed marginal activity against the dermatophyte *Trichophyton mentagrophytes* (25 μg/ml) and the yeast *Saccharomyces cerevasiae* (50μg/ml), but was inactive against all other test organisms [231].

13.2.37. Reticuline

The antimicrobial activity of reticuline against a series of bacteria and fungi was evaluated via *in vitro* screening. The alkaloid failed to demonstrate any inhibitory activity against the growth of the bacteria, but was moderately inhibitory to the growth of the superficial fungus *Trichophyton rubrum*, and only weakly inhibitory to the growth of the superficial fungus *Microsporum gypseum* [282].

The antimalarial activity [ED_{50} (ng/ml)] of reticuline was determined as follows: *Plasmodium falciparum* Strain D-6 (4050) and *P. falciparum* Strain W-2 (4470)[303].

The *in vitro* antimicrobial activity of reticuline was investigated by agar diffusion and agar dilution methods against various Gram-postive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus faecalis*, *Bacillus subtilis*) and Gram-negative

bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Enterobacter aerogenes*, *Serratia marcescens*, *Serratia sonnei*), as well as the acid-fast organism *Mycobacterium phlei*, and the yeast *Candida albicans*. Reticuline exhibited no significant activity against any of the test organisms [283].

A study was undertaken in order to understand the effect of reticuline and coclaurine on conflict and learning behavior. The results were not available in the abstract [320].

13.2.38. Tetrahydroberberine (Canadine)

Canadine failed to exhibit reverse transcriptase inhibitory properties against both HIV-1RT (p66/p51)(Human immunodeficiency virus type 1 reverse transcriptase)(IC_{50} > 200 μg/ml) and HIV-2RT (p68/p55)(Human immunodeficiency virus type 2 reverse transcriptase)(IC_{50} > 200 μg/ml)[212].

Forty-eight derivatives of berberine-type alkaloids were examined for their inhibition activity against the induction of mouse ear edema via application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Canadine was observed to displayed inhibitory effects (43% inhibition). Since berberine and some of its derivatives are present in Chinese traditional drugs, which are used in combined Kampo prescriptions, it is important to determine if such alkaloids possess carcinogenic inhibiting properties [221].

The scavenging and iron-reducing properties of a series of benzylisoquinoline alkaloids and synthetic benzylisoquinoline compounds was studied. Candadine was found to increase deoxyribose degradation by Fe^{+3}-EDTA + H_2O_2 via a mechanism related to generation of superoxide anion [265].

Canadine was evaluated for antimalarial activity against *Plasmodium falciparum in vitro* (two clones of human malaria; *Plasmodium falciparum* D-6 [Sierra Leone clone] and W-2 (Indochina clone) and *Plasmodium berghei in vivo* (mice). The alkaloid was found to be inactive in both *in vitro*, and *in vivo* tests [228].

Canadine was evaluated in the human immunodeficiency virus reverse transcriptase assay and found to be inactive (IC_{50} > 200 μg/ml)[229].

(+/-)-Canadine hydrochloride was dissolved in culture medium and evaluated for its activity against two protozoa, *Plasmodium falciparum* (K1, multi-drug resistant strain) and *Entamoeba histolytica* (NIH 200). The alkaloid was not toxic to KB cells (IC_{50} 730 μM), nor was it active against *P. falciparum* (IC_{50} > 147 μM). In addition, it was inactive against *E. histolytica* (IC_{50} 126 μM) [230].

Some protoberberine and structurally related alkaloids were tested for inhibitory activity on porcine pancreatic elastase (PPE) and human sputum elastase (HSE). Berberine chloride significantly inhibited the elastolytic activity of both enzymes, but tetrahydroberberine had no effect. It appears that the quaternary nitrogen atom of these alkaloids plays an important role in

the inhibition of elastolytic activity. The amidolytic activity of the elastases was not affected by any of the test alkaloids [240].

Canadine was determined to inhibit rabbit lens aldose reductase (IC_{50} 4.9 x 10^{-4}M) [210].

13.2.39. Tetrahydrocolumbamine (Isocorypalmine)

Tetrahydrocolumbamine was shown to exhibit an inhibitory action on blood platelet aggregation induced by collagen, arachidonic acid, and ADP *in vitro* [266].

13.2.40. Tetrandrine

The intraperitoneal administration of tetrandrine into rats suppressed subcutaneous rat air pouch leukocyte infiltration that was induced by interleukin-1 (IL-1), tumor necrosis factor (TNF), and platelet-activating factor (PAF), at an ED_{50} 20-30 mg/kg/3 days. The utilization of tetrandrine may be of value in the therapy of chronic inflammatory diseases where IL-1, TNF, and PAF play in role in pathogenesis [181].

Tetrandrine was screened for antiplasmodial and antiamoebic activities via the use of *in vitro* microtests. The alkaloid was characterized by the following: IC_{50} 0.57 μM against a multidrug-resistant strain of *Plasmodium falciparum* (K1)(standard - chloroquine phosphate, IC_{50} 0.20 μM); IC_{50} 16.9 μM against *Entamoeba histolytica* (standard - metronidazole [176].

Kinetic analysis of luminol-dependent chemiluminescence demonstrated that a solution of tetrandrine (20 μM) was found to inhibit the generation of various types of reactive oxygens by guinea-pig neutrophils. The results of these and other experiments suggest that tetrandrine inhibits the active oxygen generation via the stabilization of plasma membrane and the inhibition of phospholipid-dependent protein kinase (PKC) and NADPH oxidase activation [182].

Bisbenzylisoquinoline alkaloids block Ca^{+2} uptake through L-type Ca^{+2} channel and modulate binding of ligands to four distinct sites (dihydropyridine, benzothiazepine, aralkylamine, and (diphenylbutyl)piperidine) in the Ca^{+2} entry blocker receptor complex of the channel. Tetrandrine blocks the L-type Ca^{+2} channel through interaction at the benzothiazepine (diltiazem) site. The novel patterns exhibited by tetrandrine and other bisbenzylisoquinoline alkaloid analogs in affecting allosteric coupling between the benzothiazepine site and the other receptors of the Ca^{+2} entry blocker receptor complex define a new class of Ca^{+2} entry blocker with binding properties distinct from diltiazem [186].

Tetrandrine was shown to overcome resistance in the multidrug-resistant subline, Ch^{R}-24, derived from human KB carcinoma cells to vincristine, actinomycin D, and daunomycin, and partially overcame resistance to Adriamycin [187].

The effect of tetrandrine on experimental allergic encephalitis (EAE) was investigated in Lewis rats. Amelioration of the acute-phase of EAE was only minimal, but the incidence of

relapsing EAE was reduced by 41% for berbamine at a non-toxic treatment dosage of 60 mg/kg via gavage on alternate days. These results suggest that tetrandrine, and perhaps other bisbenzylisoquinoline alkaloids, may have a potential role in the therapy of progressive multiple sclerosis [189].

The anti-inflammatory and immunosuppressive properties of berbamine and tetrandrine were compared. These comparative *in vitro* studies demonstrated that tetrandrine had significantly greater suppressive effects on adherence, locomotion and ^{3}H-deoxyglucose uptake of neutrophils, as well as the mitogen-induced lymphocyte responses and mixed lymphocyte reactions. In addition, tetrandrine displayed anti-oxidant activity while berbamine did not. However, berbamine showed a significantly greater capacity for inhibition of NK cell cytotoxicity. These results indicated that tetrandrine was superior to berbamine in most aspects of anti-inflammatory and immunosuppressive activity [190].

A comparison of the effects of berbamine and tetrandrine in mice revealed that both alkaloids were equipotent in terms of enhancement of antibody responses and suppression of delayed-type hypersensitivity (DTH) responses to sheep erythrocyte cell antigens. Both alkaloids were equally active when administered to mice during the induction and expression phases of DTH. Neither alkaloid affected antibody response in brucellosis-infected mice, while both caused equipotent suppression of DTH. Berbamine, however, caused a significant suppression of spleen weight, while tetrandrine did not. Furthermore, berbamine produced a significantly greater enhancement of spleen colony counts of *Brucella abortus* than did tetrandrine. Bioactive dosage failed to induce toxic effects, as determined by short-term toxicology studies [191].

Berbamine and tetrandrine were investigated for their actions on guinea-pig airway microvascular leakage, an animal model of asthma and allergic inflammation. There was significant inhibition of microvascular leakage via tetrandrine with all four allergic mediators, but berbamine was only able to suppress microvascular leakage induced by platelet-activating factor and bradykinin, but not by leukotriene D4 or histamine. These results indicate that tetrandrine possesses a broad-spectrum inhibitory activity on allergic mediators, and thereby may have value in the pharmacotherapy of diseases in which these mediators are involved in pathogenesis [192].

The cytotoxic and antimalarial activities of tetrandrine were determined as follows: Cytotoxic activity [ED_{50} (μg/ml)]: P-388 (0.40), KB-3 (2.1), KB-V1 (3.7); Antimalarial activity [ED_{50} (ng/ml)]: *Plasmodium falciparum* Strain D-6 (179) and *P. falciparum* Strain W-2 (160)[279].

The relaxant activity of obaberine was examined in rat isolated uterus and its inhibitory potency compared with that of tetrandrine. Obaberine was observed to relax KCl-depolarized rat uterus and totally or partially inhibit oxytocin-induced rhythmic contractions, and was similar to that produced by tetrandrine. Obaberine inhibits sustained K^{+}-induced contraction with the same potency as tetrandrine when extracellular calcium is present, but the rhythmic contraction induced by oxytocin was diminished to a much lesser extent. This observation indicates that the relaxant mechanism of obaberine may be mainly related to Ca^{+2} influx, possibly through voltage-operated channels. In Ca^{+2}-free medium, obaberine relaxed oxytocin- and vanadate-induced uterine

contractions, while tetrandrine did not. These results suggest that obaberine have an intracellular site of action, and may interact with a stereospecific receptor site at the intracellular level [307].

Tetrandrine and berbamine were evaluated in terms of their production of inhibitory effects on the production of interleukin-1 and tumor necrosis factor (TNF_{α}) by monocytes and macrophages, and TNF_{β} production by lymphocytes. Tetrandrine was found to be 6-18 times more potent than berbamine in these tests. In addition, tetrandrine was found to significantly suppress phosphoinositide turnover while berbamine did not. Taken together, these findings suggest that tetrandrine may be superior to berbamine for chronic inflammatory diseases where inflammatory mediators and cytokines play a major role in pathogenesis (as in silicosis), while berbamine may be somewhat superior for the treatment of autoimmune disease where immunological mechanisms have a greater role in pathogenesis (as in experimental allergic encephalitis in rats). Comparative data regarding these two alkaloids may provide valuable insights into structure-activity relationships, and the design and development of synthetic analogues and congeners useful in the therapy of chronic inflammatory and autoimmune diseases [196].

Tetrandrine was shown to be a potent inhibitor of particle-stimulated oxygen consumption, superoxide release, and hydrogen peroxide secretion by alveolar macrophages. The alkaloid also exhibited substantial binding affinity for membrane lipids and alveolar macrophages. The data indicated a strong relationship between the antifibrotic potential of tetrandrine and its ability to bind alvelolar macrophages and to inhibit particle-induced activation of these macrophages. Tetrandrine should serve as a useful probe in the evaluation of the role of alveolar macrophages in pulmonary fibrosis [305].

The effects of tetrandrine on energy metabolism in isolated rat alveolar type II cells (cells which play a critical role in the maintenance of normal lung function) were studied. Incubation of type II cells with tetrandrine produced a reduction in cellular ATP content, but there was no effect of the alkaloid on cellular oxygen consumption. Tetrandrine produced an increase in internal calcium levels of type II cells, and incubation of the cells with calcium ionophore, 4-bromo A-23187, led to increased amounts of cellular calcium and reductions in ATP levels, with no effect on oxygen consumption. Exposure of isolated lung mitochondria to calcium produced a concentration-dependent reduction in ATP synthesis with no effect on mitochondrial oxygen consumption. Direct exposure of mitochondria to tetrandrine had no effect on ATP synthesis. These results were consistent with the belief that tetrandrine produced an increase in type II cell internal calcium levels which, in turn, led to reduced rates of mitochondrial ATP synthesis [198].

Tetrandrine and ten other bisbenzylisoquinoline alkaloids were evaluated in the *in vitro* histamine release inhibition assay. This assay is one of the basic tests that is used in the evaluation of substances for antiallergic effects. The order of potency of inhibitory effect of those bisbenzylisoquinoline alkaloids that have been isolated from *Thalictrum* species was as follows: homoaromoline > aromoline > isotetrandrine > obaberine > tetrandrine. Berbamine and oxyacanthine were found to have no inhibitory effect [178].

At a dosage of 5 mg/kg (i.v.), tetrandrine was found to inhibit the pressor action of norepinephrine release induced by electrical stimulation of spinal cord T_{11}-L_2. However, tetrandrine (5 mg/kg, i.a.) did not obviously attenuate the hypertensive responses to norepinephrine (0.51-16.91 μg/kg, i.v.), indicating that the alkaloid did not affect α^1-adrenoceptor-mediated vasoconstriction. Tetrandrine (5 mg/kg, i.a.) was found to decrease the pressor responses to norepinephrine (0.05 and 0.17 μg/kg, i.v.) and markedly reduce the dose-dependent hypertensive responses to a selective α_2-adrenoceptor agonist ($B\text{-}HT_{920}$, i.v.), suggesting that the alkaloid reduced α_2-adrenoceptor-mediated vasoconstriction [321].

Tetrandrine was found to not only reverse multidrug resistance, but also to potential the effectiveness of a primary drug (antineoplastic, antimalarial, tuberculostatic) against a drug-resistant cell. This action is apparently via the reversal or inhibition of a glycoprotein pump that is present in a multidrug-resistant cell such that the cell actually accepts a greater concentration of the drug than does a drug-resistant cell. Treatment of athymic nude mice bearing doxorubicin-resistant uterine leiomyosarcoma cells with (S)-(+)-tetrandrine (3 mg over 4 days), followed by treatment two days later with doxorubicin (5.5 mg/kg) resulted in an approximate inhibition of tumor growth by 50%. In addition, tetrandrine was noted to potentiate the antimalarial action of chloroquine in both sensitive and multidrug-resistant *Plasmodium falciparum* [306].

Tetrandrine, a Chinese medicinal calcium antagonist, was found to decrease blood pressure and heart rate in anesthetized rabbits [288].

13.2.41. Thalicarpine

Thalicarpine was observed to lack inhibitory activity (inactive) against both HIV-1RT (p66/p51)(Human immunodeficiency virus type 1 reverse transcriptase) and HIV-2RT (p68/p55)(Human immunodeficiency virus type 2 reverse transcriptase) [212].

The cytotoxicity of thalicarpine (thaliblastine, TBL, NSC-68075) and/or cisplatin (DDP) in DDP-sensitive (O-342) and -resistant (O-342/DDP) rat ovarian tumor cell lines was comparatively determined via the utilization of the MTT assay. The ID_{50} of DDP was found to be 6.2 μM in O-342 cells and 23.4 μM in O-342/DDP cells, while, vice versa, the ID_{50} of thalicarpine was 39.3 μM in the sensitive line and 27.3 μM in the resistant line. In addition, simultaneous exposure of cells to DDP and thalicarpine showed a significant superiority over DDP alone in O-342 cells, as evaluated by variance analysis ($P < 0.001$). This enhancing effect of thalicarpine on DDP cytotoxicity was not observed in the resistant cells. These results suggest that thalicarpine could be a candidate for the treatment of DDP-resistant ovarian tumors [322].

Thalicarpine (thaliblastine, TBL, NSC-68075) was found to reverse multidrug resistance in doxorubicin (ADR)-resistant murine leukemic P388/R-84 cells by direct interaction with P-glycoprotein, thereby increasing cellular ADR retention [323].

The antiproliferative activity of the nonmyelotoxic antitumor agent thalicarpine (thaliblastine, TBL) on two human glioma cell lines was investigated. The antiproliferative

activity was strongly dependent on concentration and time of incubation, and the ID_{50} in T406 and GW27 glioma lines was found to be 5.1 μg/ml and 8.2 μg/ml (7.0 μM and 11.2 μM), respectively. These results suggest that further *in vitro* experiments on the mechanism of TBL should be undertaken and *in vivo* investigations begun on human glioma xenografts in nude mice to address the question of whether the alkaloid might be a candidate for the treatment of brain tumors in human patients [324].

13.2.42. Thalicminine

The effects of thalicminine on guinea-pig isolated ileum, trachea, aorta, and pulmonary artery, as well as blood pressure and heart rate of anesthetized guinea-pigs were studied. Solutions (1-300 μM) of the alkaloid were observed to produce relaxation of longitudinal ileal segments, with the relaxation produced by the 300 μM solution being followed by vigorous contraction. Although the alkaloid produced only mild relaxation of the trachea, solutions (0.3-300 μM) of thalicminine caused a concentration-dependent relaxation of epinephrine-precontracted aorta and main pulmonary artery. Propranolol (1 μM) failed to reduce the relaxation or to affect the EC_{50} of thalicminine on the ileum or the pulmonary artery, but quinacrine (10 μM) potentiated thalicmininine-induced relaxation and reduced the EC_{50} on the aorta and the pulmonary artery. These results suggested that the alkaloid was involved in the interference of the metabolism of arachidonic acid. Intravenous injections (0.37-1.1 mg/kg) of the alkaloid to anesthetized animals produced a transient reduction of both systolic and diastolic blood pressure, as well as heart rate, with both of these signs returning to normal values in about 10 minutes [325].

13.2.43. Thalicsiline

Thalicsiline (**73**), a new ajaconine-type dipterpene alkaloid isolated from extracts of the roots of *T. sessile* Hayata, was observed to possess anti-inflammatory activity as measured by a 42% reduction in carageenan-induced inflammation in the hindfoot of Sprague Dawley rats when the alkaloid was intraperitoneally administered at a dosage of 20 mg/kg x 2 [123,127].

13.2.44. Thalidasine

Thalidasine, which was isolated from extracts of the root of the Chinese *Thalictrum fargesii*, was found to possess calcium channel antagonistic properties, with actions on both voltage-dependent and receptor-dependent channels [86].

13.2.45. Thalidezine

The total alkaloids of the Chinese *Thalictrum glandulosissimum* (Finet et Gagnep.) W.T. Wang et S.H. Wang, and the major alkaloid of the plant, hernandezine, were effectively employed in the treatment of P388 leukemia, S180 ascites tumor, and C26 colon tumor in mice. Although hernandezine markedly inhibited the *in vitro* growth of mouse L1210 cells and human KB oral cancer cells, the alkaloid was characterized by only a low inhibition on normal hemopoietic progenitor cells (CFU-GM). Preliminary results demonstrated that hernandezine blocked cell-cycle transfer from the G_1 to the S phase, suggesting that its action may be cell cycle-specific [RCH]. It was also demonstrated that two other bisbenzylisoquinoline alkaloids isolated from the same plant, thalidezine and isothalidezine, possessed a similar inhibitory effect on the L1210 cells [277,278].

13.2.46. Thalifabatine

Thalifabatine, an alkaloid of the Chinese *Thalictrum faberi* Ulbr., was found to possess significant cytotoxicity as measured against the P-388 cell line [129].

13.2.47. Thalifaberidine

In 1994, Lin et al. evaluated the phenolic alkaloid fraction of an extract of the Chinese *Thalictrum faberi* Ulbr., as well as three of its contained phenolic alkaloids (thalifaberidine (76), thalifaberine (77), and thalifasine), for cytotoxic, antimalarial,and HIV-reverse transcriptase inhibitory activities. The phenolic alkaloid fraction and the three alkaloids were characterized by significant cytotoxic activities as measured (ED_{50} about 0.6-20 μg/ml against the following cell lines: BCA-1 (human breast cancer), HT-1080 (human fibrosarcoma), Lu-1 (human lung cancer), Col-2 (human colon cancer), KB (human oral epidermoid carcinoma), KB-V (vinblastine-resistant KB), P-388 (murine lymphoid leukemia), A431 (human epidermoid cancer), LNCaP (hormone-dependent human prostatic cancer), ZR-75-1 (hormone-dependent human breast cancer), and U373 (human glioblastoma). Among the three alkaloids, thalifasine demonstrated the strongest antimalarial activity, as measured against the chloroquine-sensitive (D-6)(ED_{50} = 238 ng/ml) and chloroquine-resistant (W-2)(ED_{50} = 49.3 ng/ml) clones of *Plasmodium falciparum*. Although the phenolic alkaloid fraction displayed weak activity (IC_{50} = 139 μg/ml) in the HIV-reverse transcriptase inhibition assay, the three alkaloids were inactive [129].

13.2.48. Thalifaberine

In 1994, Lin et al. evaluated the phenolic alkaloid fraction of an extract of the Chinese *Thalictrum faberi* Ulbr., as well as three of its contained phenolic alkaloids (thalifaberidine (**76**), thalifaberine (**77**), and thalifasine), for cytotoxic, antimalarial,and HIV-reverse transcriptase inhibitory activities. The phenolic alkaloid fraction and the three alkaloids were characterized by significant cytotoxic activities as measured (ED_{50} about 0.6-20 μg/ml against the following cell lines: BCA-1 (human breast cancer), HT-1080 (human fibrosarcoma), Lu-1 (human lung cancer), Col-2 (human colon cancer), KB (human oral epidermoid carcinona), KB-V (vinblastine-resistant KB), P-388 (murine lymphoid leukemia), A431 (human epidermoid cancer), LNCaP (hormone-dependent human prostatic cancer), ZR-75-1 (hormone-dependent human breast cancer), and U373 (human glioblastoma). Among the three alkaloids, thalifasine demonstrated the strongest antimalarial activity, as measured against the chloroquine-sensitive (D-6)(ED_{50} = 238 ng/ml) and chloroquine-resistant (W-2)(ED_{50} = 49.3 ng/ml) clones of *Plasmodium falciparum*. Although the phenolic alkaloid fraction displayed weak activity (IC_{50} = 139 μg/ml) in the HIV-reverse transcriptase inhibition assay, the three alkaloids were inactive [129].

13.2.49. Thalifabine

Thalifabine, an alkaloid of the Chinese *Thalictrum faberi* Ulbr., was found to possess significant cytotoxicity as measured against the P-388 cell line [129].

13.2.50. Thalifaboramine

Thalifaboramine (**79**), an alkaloid of the Chinese *Thalictrum faberi* Ulbr., was found to possess significant cytotoxicity as measured against the P-388 cell line [129,133].

13.2.51. Thalifalandine

In 1986, Lin et al. demonstrated the cytotoxicity of eight thalifaberine-type alkaloids that had been obtained from extracts of the Chinese *Thalictrum faberi* Ulbr. against the P388 cell line (murine lymphoid leukemias) [83]. In particular, thalifalandine (**80**) possessed significant cytotoxicity against P388 and L1210 leukemia cells having an IC_{50} of 0.7-1.8 μg/ml [83].

Thalifalandine was found to possess significant cytotoxicity as measured against the P-388 cell line [129].

13.2.52. Thalifaramine

Thalifaramine (**9**), an alkaloid of the Chinese *Thalictrum faberi* Ulbr., possessed significant cytotoxicity as measured against the P-388 cell line [129].

13.2.53. Thalifarapine

Thalifarapine (**81**), an alkaloid of the Chinese *Thalictrum faberi* Ulbr., was found to possess significant cytotoxicity as measured against the P-388 cell line [129].

13.2.54. Thalifarazine

Thalifarazine (**10**), an alkaloid of the Chinese *Thalictrum faberi* Ulbr., was found to possess significant cytotoxicity as measured against the P-388 cell line [129].

An ethanol extract of the above-ground parts of the Taiwanese *Thalictrum sessile* Hayata exhibited significant cytotoxicity against in vitro tissue culture cells in human KB, A-549 lung carcinoma and HCT-8 colon tumor, as well as murine P-388 and L-1210 lymphocytic leukemia [62]. Bioassay-guided fractionation of this extract led to the isolation and identification of two cytotoxic aporphinoid alkaloids; the oxoaporphine liriodenine and the aporphine-benzylisoquinoline dimer (+)-thalifarazine. The following are the activities of thalifarazine against various tumor cell lines, with the LD_{50} values (μg/ml) expressed in parentheses: KB (1.50), A-549 (6.53), HCT-8 (4.30), P-388 (3.75), and L-1210 (5.69) [62].

13.2.55. Thalifaricine

Thalifaricine (**8**), an alkaloid of the Chinese *Thalictrum faberi* Ulbr., possessed significant cytotoxicity as measured against the P-388 cell line [129].

13.2.56. Thalifaronine

Thalifaronine (**12**), an alkaloid of the Chinese *Thalictrum faberi* Ulbr., possessed significant cytotoxicity as measured against the P-388 cell line [129].

13.2.57. Thalifasine

In 1994, Lin et al. evaluated the phenolic alkaloid fraction of an extract of the Chinese *Thalictrum faberi* Ulbr., as well as three of its contained phenolic alkaloids (thalifaberidine (**76**), thalifaberine (**77**), and thalifasine), for cytotoxic, antimalarial,and HIV-reverse transcriptase inhibitory activities. The phenolic alkaloid fraction and the three alkaloids were characterized by significant cytotoxic activities as measured (ED_{50} about 0.6-20 μg/ml against the following cell lines: BCA-1 (human breast cancer), HT-1080 (human fibrosarcoma), Lu-1 (human lung cancer), Col-2 (human colon cancer), KB (human oral epidermoid carcinona), KB-V (vinblastine-resistant KB), P-388 (murine lymphoid leukemia), A431 (human epidermoid cancer), LNCaP (hormone-dependent human prostatic cancer), ZR-75-1 (hormone-dependent human breast cancer), and U373 (human glioblastoma). Among the three alkaloids, thalifasine demonstrated the strongest antimalarial activity, as measured against the chloroquine-sensitive (D-6)(ED_{50} = 238 ng/ml) and chloroquine-resistant (W-2)(ED_{50} = 49.3 ng/ml) clones of *Plasmodium falciparum*. Although the phenolic alkaloid fraction displayed weak activity (IC_{50} = 139 μg/ml) in the HIV-reverse transcriptase inhibition assay, the three alkaloids were inactive [129].

13.2.58. Thalifendine

Thalifendine chloride was dissolved in culture medium and evaluated for its activity against two protozoa, *Plasmodium falciparum* (K1, multi-drug resistant strain) and *Entamoeba histolytica* (NIH 200). The alkaloid was not toxic to KB cells (IC_{50} > 698 μM), and was active against *P. falciparum* (IC_{50} 7.9 μM), but inactive against *E. histolytica* (IC_{50} 115 μM). In assessing relative *in vitro* cytotoxicity to antiplasmodial activity, berberine had the least favorable ratio (8), while jatrorrhizine had the most favorable ratio (> 106) of the alkaloids tested. Thalifendine had an intermediate ratio ((> 88). The results lend some support to the utilization of plants containing protoberberine alkaloids for the treatment of malaria [230].

Thalifendine chloride was found to exhibit cytotoxic activity against cultured P-388 (murine lymphocytic leukemia) cells (0.84 μM)[336].

13.2.59. Thaliporphine

Thaliporphine was observed to inhibit the formation of nitrites/nitrates caused by lipopolysaccharide (LPS) in J774.2 macrophages. This inhibition was similar to that produced by cycloheximide, N^6-methyl-L-arginine and dexamethasone. The results of these and other studies in this report demonstrated that thaliporphine inhibited LPS induction of NO synthase expression, and that the mechanism of action of the alkaloid was via inhibition of LPS-stimulated interleukin-1β synthesis in macrophages. Pretreatment of anesthetized rats with thaliporphine

prior to LPS partially restored the fall in mean arterial pressure and the vascular hyporeactivity to norepinephrine 3 hours after LPS injection. Since thaliporphine selectively inhibits expression of inducible NO synthase, the alkaloid may be of potential in the therapy of endotoxemia [326].

Thaliporphine (0.1-100 μM), a potent vasoconstrictor, was observed to produce contractions in the isolated guinea-pig ileum in a concentration-dependent manner. These contractions were not affected by pretreatment of the ileum with tetrodotoxin, phentolamine, prazosin, propranolol, naloxone, atropine, diphenhydramine, methysergide, indomethacin or staurosporine. However, the contraction was markedly inhibited by nifedipine and verapamil, suggesting that thaliporphine produces intestinal smooth muscle contraction by a direct effect on muscle mediated by an increased Ca^{+2} influx through voltage-dependent Ca^{+2} channels [327].

The effects of thaliporphine on contractions and electrophysiological properties of cardiac tissues were examined in rat left atrial and right ventricular strips. The results indicated that thaliporphine is a weak Ca^{+2} channel agonist with strong Na^{+} and K^{+} channel blocking activities. The alkaloid possessed a positive inotropic effect that may be due to an increase in calcium entry mediated via partial activation of calcium channels or by inhibition of K^{+} efflux. Inhibition of K^{+} efflux would result in prolongation of APD_{50} and contribute to the negative chronotropic effect of the alkaloid [328].

13.2.60. Thalrugosine

The cytotoxic and antimalarial activities of thalrugosine were determined as follows: Cytotoxic activity [ED_{50} (μg/ml)]: P-388 (0.36), KB-3 (3.4), KB-V1 (11); Antimalarial activity [ED_{50} (ng/ml)]: *Plasmodium falciparum* Strain D-6 (65.1) and *P. falciparum* Strain W-2 (78.0)[279].

Thalrugosine was found to inhibit the growth of cultured *Plasmodium falciparum* strains D-6 and W-2, and to display nonselective cytotoxicity with a battery of cultured mammalian cells. These data were used to calculate a selectivity index (ED_{50} in cultured mammalian cells/ED_{50} in cultured *Plasmodium falciparum* strains). Agents that are useful antimalarial drugs, such as quinine and artemisinin, typically display selectivity indices that are > 1000. The ED_{50} (ng/ml) of thalrugosine against *Plasmodium falciparum* strain D-6 was 120.6, while that associated with strain W-2 was 229.7. The following is a summary of the cytotoxic activities (ED_{50}, μg/ml) of thalrugosine, along with the cytotoxicity/antimalarial activity ratio (selectivity index) with the first number in parentheses being the D-6 strain selectivity index and the second number being the W-2 strain selectivity index: BCA-1 [Human Breast Cancer] - 5.0 (42,22); HT-1080 [Human Fibrosarcoma] - 10.0 (83,44); LUC-1 [Human Lung Cancer] - 9.7 (80,42); MEL-2 [Human Melanoma] - 14.9 (124,65); COL-1 [Human Colon Cancer] - 11.1 (92,48); KB [Human Oral Epidermoid Carcinoma] - 6.5 (54,28); KB-V1 [Vinblastine-resistant KB] - 3.7 (31,16); P-388 [Murine Lymphoid Neoplasm] - 7.4 (61,32); A-431 [Human Epidermoid Carcinoma] - 4.9 (41,21); LNCaP [Hormone Dependent Human Prostate Cancer] - 5.9 (49,26); ZR-75-1 [Hormone

Dependent Human Breast Cancer] - 2.5 (21,11). Based on the data that was generated and compared, thalrugosine and other bisbenzylisoquinoline alkaloids do not appear to be promising candidates for antimalarial drug development [280].

14. AN INCLUSIVE COMPILATION OF THE BOTANICAL SOURCES OF THE *THALICTRUM* ALKALOIDS

Table 1

T. acteaefolium Sieb. & Zucc. [1]
- Tetrahydroberberine

T. acutifolium (Hand.-Mazz.) Boivin [61]
- Acutifolidine
- Oxyberberine
- Trilobinine

T. alpinum L. [1]
- Berberine
- Columbamine
- *N*-Desmethylthalrugosidine
- Hernandezine (Thalicsimine, Thaliximine)
- Isoboldine
- Jatrorrhizine
- Magnoflorine
- *N*-Methyl-6,7-dimethoxy-1,2-dihydroisoquinolin-1-one
- Neothalibrine
- Noroxyhydrastinine
- Oxyberberine (Berlambine)
- Palmatine
- Thalicarpine
- Thalidasine
- Thalifendine
- Thaliporphine (*O*-Methylisoboldine)
- Thalpindione
- Thalrugosaminine
- Thalrugosidine

T. amurense Maxim. [1]
- β-Allocryptopine
- Thalictricine (Thalictrisine)

Table 1. Continued

T. aquilegifolium L.
- Isoboldine (*N*-Methyllaurelliptine) [43]
- Isocorydine [1,43]
- Magnoflorine [43]
- *O*-Methylthalicberine [329]

T. baicalense Turcz.
- Baicalidine [1]
- Baicaline [1,34]
- Berberine [1,34]
- Glaucine [1,34]
- Magnoflorine [1]
- Oxobaicaline (7-Oxobaicaline) [111]
- Thalbaicalidine [111]
- Thalbaicaline [34,111]

T. buschianum [44]
- *O*-Methylthalicberine
- Talcamine (Likely thalmine)
- Thaliporphine (Thalicmidine)
- Thalmine

T. calabricum Spreng. [1]
- Magnoflorine

T. collinum Wallr.
- Berberine [37]
- Glaucine [37]
- Isoboldine (*N*-Methyllaurelliptine) [37,44]
- Magnoflorine [37]
- *O*-Methylthalicberine [37,44]
- Thalmine [37,44]

T. contortum L. [1]
- β-Allocryptopine

T. cultratum Wall.
- Adiantifoline [4]
- Aromoline [81]
- Berberine [2,20]
- Columbamine [20]
- Cultithalminine [81]

Table 1. Continued

5-Hydroxythalidasine [2,3]
5-Hydroxythalidasine-2α-*N*-oxide [81]
5-Hydroxythalmine [2,3]
Jatrorrhizine [20]
Magnoflorine [20]
O-Methylthalicberine [2,3]
O-Methylthalmine [84]
Neothalibrine [81]
Neothalibrine-2'α-*N*-oxide [81]
2'-Noroxyacanthine [81]
2-Northalidasine [2,84]
2'-Northaliphylline [81]
2-Northalmine [84]
Obaberine [81]
Oxyacanthine [81]
Palmatine [20]
Reticuline [2]
Revolutinone [2]
Thalflavidine [2]
Thalibulamine [2,4]
Thalicsimidine [2]
Thalictine [3]
Thalidasine [84]
Thalidasine-2α-*N*-oxide [81]
Thalidastine [20]
Thalifaberine [4]
Thalifaramine [2,4,129]
Thalifarapine (Thalifaroline) [4]
Thalifarazine [2,4,129]
Thalifaretine [2,4]
Thalifaricine [2,4,129]
Thalifaronine [2,4,129]
Thalifendine [20]
Thaliglucine [2]
Thalilutine [4]
Thaliphylline [2,3]
Thaliphylline-2'β-*N*-oxide [81]

Table 1. Continued

Thalirugine [81]
Thalisopavine [2]
Thalisopine (Thaligosine) [3,81]
Thalisopine-2α-*N*-oxide (Thaligosine-2α-*N*-oxide) [81]
Thalmelatidine [4]
Thalmiculatimine [2,3]
Thalmiculimine [2,3]
Thalmiculine [2,3]
Thalmine [2,3]
Thalmineline [4]
Thalrugosaminine [2,3]
Thalrugosaminine-2α-*N*-oxide [81]
Thalrugosidine [2,3]
Thalrugosinone [84]
Thalsivasine [3]

T. dasycarpum Fisch. and Lall. [1]
Argemonine
Bisnorargemonine
Corypalline
Dehydrothalicarpine
Laudanidine
Magnoflorine
Norargemonine
Thalicarpine
Thalidasine
Thalisopavine

T. dasycarpum Fisch. and Lall. var. *hypoglaucum* (Rydb.) Boivin [1]
Berberine
Magnoflorine

T. delavayi Franch.
Berberine [68]
Cryptopine [68]
Hernandezine [45,68]
Isocorydine [45]
Isothalidezine [68]
Leucoxylonine [45]
Magnoflorine [45]

Table 1. Continued

Ocoteine (Thalicmine) [45]
Pseudoprotopine [68]
Thalicthuberine [45]
Thalidezine [68]
Thalmirabine [68]

T. dioicum L. [1]
Berberine
Berberrubine
Corydine
Isocorypalmine
(Tetrahydrocolumbamine)
Magnoflorine
N-Methylcoclaurine
O-Methylcorypalline
N-Methyllaurotetanine
Pallidine
Pennsylvanine
Thalicarpine
Thalictrogamine
Thalictropine
Thalicminine
Thalidicine
Thalidine
Thalidoxine
Thalifendine
Thalmelatine

T. faberi Ulbr.
Berberine [1]
Dehydrohuangshanine [1]
Dehydrothalifaberine [1]
N-Desmethylthalidasine [1]
Faberidine [1]
Faberonine [1]
Huangshanine [1]
O-Methylthalibrine [1]
O-Methylthalicberine [1]
Pallidine [1]

Table 1. Continued

Thalfinine [1]
Thalidasine [1]
Thalifabatine [1]
Thalifaberidine [129]
Thalifaberine [1]
Thalfabine [1]
Thalifaboramine [133]
Thalifalandine [83]
Thalifarapine [1]
Thalifasine [1]
Thaliracebine [1]
Thalisopine (Thaligosine) [1]
Thalrugosidine [1]

T. fargesii Fr. ex Fin. et Gagnep.
Thalfoetidine [85,86]
Thalidasine [85,86]
Thaligosinine [86]
Thalisopidine [86]

T. fauriei Hayata
Corydine [35,15]
Dehydrodiscretine [1,35]
3-*O*-Demethyloconovine [15]
EP-10 [35]
Fauridine [15]
Faurine [15]
Fauripavine [15]
Faurithaline [15]
Isocorydine [15,35]
Isooconovine [15]
Magnoflorine [1,35]
3-Methoxyfaurithaline [15]
O-Methylfaurine [15]
Ocobotrine [15,35]
Oconovine [15,35]
Pallidine [15]
Thalifaurine [1,35]
Thalisopynine [15]

Table 1. Continued

T. fendleri Engelm. ex Gray [1]
- Berberine
- Glaucine
- Hernandezine
- Jatrorrhizine
- Magnoflorine
- *N*-Methylcorydaldine
- *N*-Methylthalidaldine
- Ocoteine
- Preocoteine
- Tetrahydrothalifendine
- Thaldimerine
- Thalicarpine
- Thalidastine
- Thalidezine
- Thalifendine
- Thalifendlerine
- Thaliporphine
- Veronamine

T. filamentosum Maxim. [1]
- Glaucine
- Thalicsimidine

T. flavum. L.
- Berberine (Thalsine) [1,38,69]
- Cryptopine [1]
- Glaucine [38]
- Hernandezine [38,69]
- Magnoflorine [1]
- *O*-Methylthalicberine [69]
- Thalflavidine [1]
- Thalflavine [1]
- Thalfoetidine [38,69]
- Thalicarpine [1]
- Thalicflavine [1]
- Thalicsimidine [38]
- Thalicsine (Thalixine) [1]

Table 1. Continued

Thalidasine [69]
Thaliglucine [38]

T. foetidum L.

Argemonine [49]
Argemonine-*N*-Oxide [64]
Berbamine [1]
Berberine [1,48,49,70,71]
Bisocobotrine [15]
Corunnine [1]
6a,7-Dehydromethoxyadiantifoline [71]
Fetidine [1]
Glaucine [1]
Harmine [1]
Hernandezine [64]
Isoboldine [1]
Isotetrandrine [1]
Magnoflorine [1,48,49]
Methoxyadiantifoline [71]
O-Methylthalicberine [64]
Ocobotrine [15]
Oxoglaucine [1]
Oxyberberine [71]
Protopine [64]
Reticuline [15]
Sinoacutine [15]
Thalactamine [64]
Thalfine [1]
Thalfinine [1]
Thalflavine [48]
Thalfoetidine [1]
Thalibrine [15]
Thalicarpine [1]
Thalictrogamine [49]
Thalidasine [15,49]
Thalidezine [64]
Thaligosinine [49]
Thalipine [49]

Table 1. Continued

Thaliporphine (Thalicmidine) [1]
Thalisopine (Thaligosine) [64]
Thalmelatine [49]
Thalrugosaminine [49]
Thalrugosidine [15]

T. foliolosum DC. [1]
Berberine
Columbamine
Dehydrodiscretamine
Jatrorrhizine
Magnoflorine (Thalictrine)
Noroxyhydrastinine
Oxyberberine (Berlambine)
Palmatine
Reticuline
Rugosinone
Tembetarine (*N*-Methylreticuline)
Thalicarpine
Thalidasine
Thalidastine
Thalifendine
Thalirugidine
Thalisopine (Thaligosine)
Thalrugosaminine
Thalrugosidine
N,O,O-Trimethylsparsiflorine
Xanthoplanine

T. fortunei [82]
Aromoline
N-Phenyl-2-Naphthylamine
Thalifortine

T. glandulosissimum (Finet et Gagnep.) W.T. Wang et S.H. Wang
Berberine [50]
Berberrubine [75]
Columbamine [50]
Coptisine [50]
Cryptopine [75,78]

Table 1. Continued

Groenlandicine [50]
Hernandezine [75,78]
Isothalidezine [78]
Izmirine [78]
Jatrorrhizine [50]
Magnoflorine [50]
O-Methylthalibrine [78]
8-Oxocoptisine [75]
Palmatine [50]
Protopine [75,78]
Puntarenine [75]
Thalidezine [75,78]
Thalifendine [50]

T. glaucum Desf. [1]
Berberine
Magnoflorine

T. hazarica R.R.S. [57]
N-Methyllaurotetanine
Thalicthuberine
Thalihazine

T. hernandezii Tausch [1]
Hernandezine

T. honanense W.T. Wang et S.H. Wang [72]
Adiantifoline
Berberine
Jatrorrhizine
Thalifendine
Thalmelatidine

T. ichengense Lecoy et Oliv.
Dehydroglaucine [39,40]
Dehydrothalicsimidine [40]
Glaucine [39,40]
N-Phenyl-2-Naphthylamine [39]
Thalicsimidine [39,40]
Thaliporphine (Thalicmidine) [39,40]

Table 1. Continued

T. isopyroides C.A.M.
- Cabudine [1]
- Cryptopine [1]
- Dehydroocoteine (Dehydrothalicmine) [1,17]
- Delporphine [17]
- Magnoflorine [1,17]
- Ocoteine (Thalicmine) [1,17]
- *N*-Methylcassythine [17]
- *N*-Methyl-6,7-dimethoxy-1,2-dihydroisoquinolin-1-one [1]
- *N*-Methyllaurotetanine [17]
- Preocoteine [17]
- Thalicminine [1,17]
- Thaligosinine [17]
- Thaliporphine (Thalicmidine) [17]
- Thalisopidine [1,17]
- Thalisopine [1]
- Thalisopynine (thalisopinine) [1]

T. javanicum Bl.
- Berberine [1,63]
- Columbamine [1]
- Demethyleneberberine [1]
- Jatrorrhizine [1]
- Magnoflorine [1]
- Oxyberberine [63]
- Palmatine [1]
- Rugosinone [63]
- Thalifendine [63]
- Thalisopine (Thaligosine) [63]
- Thalrugosaminine [63]

T. kuhistanicum Ovcz. [60]
- *O*-Methylthalicberine
- Thalicsimidine
- Thalmine

T. lankesteri Standl. [73]
- Berberine
- Columbamine
- Hernandezine

Table 1. Continued

Jatrorrhizine
Palmatine

T. longipedunculatum E. Nik.
Berberine [1,41]
Columbamine [41]
Glaucine [41]
Magnoflorine [1,41]
O-Methylthalicberine [41]
Ocoteine (Thalicmine) [41]
Thalfoetidine [1,41]
Thalicberine [41]
Thalicsine (Thalixine) [1]
Thalidasine [1,41]
Thaliglucinone [41]

T. longistylum DC.
Berberine [1]
Berberlongine [15]
Berberstyline [15]
Columbamine [1]
5-*O*-Demethyllongine [15]
N-Desmethylthalistyline [1,15]
Hernandezine [15]
5'-Hydroxylongine [15]
5'-Hydroxythalidezine [15]
Isothalidezine [15]
Jatrorrhizine [1]
Longiberine [15]
Longine [1]
Magnoflorine [1]
O-Methyllongiberine [15]
O-Methylthalibrine [15]
N-Methylthalistyline (Thalistyline metho salt, Methothalistyline) [1]
N-Norhernandezine [15]
N-Northalidezine [15]
Oxyberberine (Berlambine) [1]
Palmatine [1]
Thalibrine [1,15]

Table 1. Continued

Thalidezine [15]
Thalifendine [1]
Thaliglucinone [1]
Thalistyline [1]
8-Trichloromethyldihydroberberine [1]

T. lucidum L. [1]
Aromoline (Thalicrine)
Berberine
Columbamine
Homoaromoline (Homothalicrine, *O*-Methylaromoline, Thalrugosamine)
Jatrorrhizine
Magnoflorine
O-Methylthalicberine
Obaberine
Obamegine
Oxyacanthine
Oxyberberine (Berlambine)
Palmatine
Thalicberine
Thalidasine
Thalifendine
Thaliglucinone
Thalrugosine
8-Trichloromethyldihydroberberine

T. minus L.
Adiantifoline [1]
Allocryptopine [42]
α-Allocryptopine [48]
β-Allocryptopine (Thalictrimine) [1]
Argemonine [1]
Aromoline (Thalicrine) [1]
Berberine [1,10,42,66]
Bursanine [1]
Corunnine [1]
O-Desmethyladiantifoline [1]
Eschscholtzidine [1]
Glaucine [1,42]

Table 1. Continued

Istanbulamine [1]
Iznikine [1]
Jatrorrhizine [1]
Magnoflorine [1]
N-Methylargemonine [1]
N-Methylcanadine [1,48]
O-Methylthalicberine (Thalmidine) [1,10,42,48]
O-Methylthalmethine [1,6,10,42]
Ocoteine (Thalicmine) [1]
Oxyacanthine [6]
Palmatine [48]
Preocoteine *N*-oxide [1]
Thalabadensine [1]
Thalactamine [1,10,65,66]
Thalflavidine [1]
Thalicarpine [1,6]
Thalicberine [1,10,42,48]
Thalicmidine *N*-oxide (Thaliporphine *N*-oxide) [1]
Thalicminine [1]
Thalicsimidine [1]
Thalictrine [1]
Thalidasine [1]
Thalifendine [1]
Thalflavine [42,66]
Thalfoetidine [42]
Thaliglucine [10]
Thalipine [1,6]
Thalipoline [1]
Thaliporphine (Thalicmidine) [1,10]
Thalmelatidine [1]
Thalmelatine [1,6]
Thalmethine [1,6,10,42]
Thalmine [1]
Thalmineline [1]

T. minus L. var. *adiantifolium* Hort.

Adiantifoline [1,36]
Berberine [1,36]

Table 1. Continued

Choline [36]
Columbamine [36]
Domesticine [36]
Glaucine [36]
Isoboldine (*N*-Methyllaurelliptine) [36]
Jatrorrhizine [36]
Magnoflorine [1,36]
O-Methylisoboldine [36]
O-Methylthalicberine [1]
Nantenine [36]
Noroxyhydrastinine [1]
Palmatine [36]
Thalifendine [1,36]
Thalifoline [1]
Thaliglucine [36]
Thaliglucinone [1,36]

T. minus L. var. *elatum* (Jacq.) Stoj. et Stefanov (*T. minus* L. ssp. *majus*)(*T. minus* var. *majus* (Crantz) Crepin)

Adiantifoline [1,51,59]
Berberine [1,51,52]
Dehydrothalicarpine [1]
Dehydrothalmelatine [1]
O-Demethyladiantifoline [1,58,59]
Eschscholtzidine [59]
Hernandaline [1]
4-Hydroxyeschscholtzidine [59]
Isonorargemonine [59]
Jatrorrhizine [52]
Magnoflorine [51,52]
O-Methylthalicberine [51]
O-Methylthalmethine [51]
Noroxyhydrastinine [1,59]
Obaberine [51]
Oxyacanthine [51]
1-Oxo-6,7-dimethoxy-*N*-methyltetrahydroisoquinoline [1]
Thaliadine [1,58,59]
Thalicarpine [1]

Table 1. Continued

Thalicberine [51]
Thalicthuberine [1,58,59]
Thalifendine [52]
Thalisopine (Thaligosine) [51]
Thalmelatidine [1,51,58,59]
Thalmelatine [1]
Thalmeline [1,59]
Thalmineline [1]
Thalphenine [52]

T. minus L. var. *hypoleucum* [80]
O-Methylthalicberine
Thalmelatidine

T. minus L. var. *microphyllum* Boiss. [1]
Adiantifoline
Aromoline (Thalicrine)
Berberine
Bursanine
Homoaromoline (Homothalicrine, *O*-Methylaromoline, Thalrugosamine)
Istanbulamine
Iznikine
Jatrorrhizine
Magnoflorine
O-Methylthalicberine
N(2')-Noradiantifoline
Obaberine
Obamegine
Palmatine
Takatonine
Thalactamine
Thaliadanine
Thalicberine
Thaliglucinone
Thaligrisine
Thaliphylline
Thalirugine
Thalisopine (Thaligosine)
Thalmelatidine

Table 1. Continued

Thalmicrinone
Thalrugosine
8-Trichloromethyldihydroberberine
Uskudaramine

T. minus L. var. *minus*
Adiantifoline [53]
Berberine [1,53]
Magnoflorine [1,53]
N-Methylcanadine [77]
O-Methylthalicberine [77]
O-Methylthalmethine [77]
Northalibroline [53]
Thalicberine [77]
Thaliglucinone [53]
Thaliphylline [77]
Thalivarmine [77]
Thalmelatidine [53,77]
Thalmethine [77]
Thalmineline [53]
Thalsivasine [77]

T. minus L. race B [1]
Adiantifoline
Berberine
Columbamine
Jatrorrhizine
Magnoflorine
N-Methylcorydaldine
O-Methylthalibrine
Obaberine
Oxyberberine (Berlambine)
Palmatine
Reticuline
Thalfine
Thalfinine
Thaliadanine
Thaliadine
Thalidasine

Table 1. Continued

Thalifendine
Thaliglucinone
Thalirabine
Thaliracebine
Thalistine
Thalmirabine
Thalphenine
Thalrugosaminine
Thalrugosine

T. minus L. race C [14]
Adiantifoline
6a,7-Dehydroadiantifoline
6a,7-Dehydrothaliadine
Delporphine
7'-Dihydrodehydrothaliadine
7'-Dihydrooxothaliadine
7'-Dihydrothaliadine
Isoboldine
Laudanidine
N-Methylcorydaldine
O-Methylthalibrine
O-Methylthalicberine
Obaberine
6-Noradiantifoline
Oxothaliadine
Squarosine
Thalfine
Thalfinine
Thalicarpine
Thaliracebine
Thalisopynine
Thalistine
Thalmelatidine
Thalmineline
Thalmirabine
Thalmirine
Thalrugosine

Table 1. Continued

T. pedunculatum Edgew.
- Berbamine [1]
- Berberine [1]
- Isocorydine [46]
- *N*-Methyldanguyelline [46]
- Noroconovine [46]
- Oconovine [46]
- Pronuciferine [46]
- Reticuline [46]
- Thalicsimidine [46]

T. petaloideum L. [1]
- Magnoflorine

T. podocarpum Humb. [1]
- Berberine
- Columbamine
- *N*-Desmethylthalidezine
- *N*-Desmethylthalistyline
- Hernandezine
- Isothalidezine
- Jatrorrhizine
- Magnoflorine
- *N*-Methylthalistyline (Methothalistyline, Thalistyline metho salt)
- Oxyberberine (Berlambine)
- Palmatine
- Thalidezine
- Thalifendine
- Thaliglucinone
- Thalistyline
- 8-Trichloromethyldihydroberberine

T. polygamum Muhl. [1]
- Berberine
- Berberrubine
- Bisnorthalphenine
- Deoxythalidastine
- Magnoflorine
- *N*-Methylnantenine
- *N*-Methylpalaudinium

Table 1. Continued

N-Methylthaliglucine
N-Methylthaliglucinone
Pennsylpavine
Pennsylpavoline
Pennsylvanamine
Pennsylvanine
Thalicarpine
Thalictrogamine
Thalictropine
Thalifendine
Thaliglucinone
Thalphenine
Thalphenine methine
Thalipine
Thalrugosine (Thaligine)

T. purpurascens DC [74]
Berberine

T. revolutum D.C. [1]
β-Allocryptopine
Argemonine
Berberine
Bisnorthalphenine
Choline chloride
Columbamine
N-Desmethylthalphenine
Deoxythalidastine
Eschscholtzidine
Isonorargemonine
Jatrorrhizine
Laudanidine
Magnoflorine
N-Methylargemonine
N-Methylarmepavine
N-Methylcoclaurine
N-Methyl-6,7-Dimethoxyisoquinolinium
N-Methyleschscholtzidine
N-Methyllaurotetanine

Table 1. Continued

O-Methylthalicberine
O-Methylthalmethine
Neothalibrine
N(2')-Northalicarpine
Palmatine
Pennsylvanine
Platycerine
Reticuline
Revolutinone
Revolutopine
Thalflavidine
Thalicarpine
Thalictrogamine
Thalidasine
Thalifendine
Thaliglucinone
Thalilutidine
Thalilutine
Thalipine
Thalirevoline
Thalirevolutine
Thalmelatine
Thalphenine
Thalrugosaminine
T. rochebrunianum Franc. and Sav. [1]
Berberine
Dihydrothalictrinine
Hernandezine
Jatrorrhizine
Magnoflorine
O-Methylthalibrunimine
N'-Norhernandezine
Northalibrine
N'-Northalibrunine
Oxothalibrunimine
Thalibrine
Thalibrunimine

Table 1. Continued

Thalibrunine
Thalictrinine
Thaliglucinone
Thalsimine

T. rugosum Ait. (*T. glaucum* Desf.) [1]
Aromoline (Thalicrine)
Berberine
Columbamine
Corypalline
Deoxythalidastine
Homoaromoline (Homothalicrine, *O*-Methylaromoline, Thalrugosamine)
Jatrorrhizine
Magnoflorine
6,7-Methylenedioxy-1,2-dihydroisoquinolin-1-one
Neothalibrine
Noroxyhydrastinine
Obaberine
Obamegine
Oxyberberine (Berlambine)
Protothalipine
Protopine
Rugosine
Rugosinone
Thalicthuberine
Thalidasine
Thalifendine
Thaliglucine
Thaliglucinone
Thaligosidine
Thaligosine
Thaligosinine
Thalirugidine
Thalirugine
Thaliruginine
Thalphenine
Thalrugosaminine
Thalrugosidine

Table 1. Continued

Thalrugosine
Thalrugosinone
Thalsimine

T. sachalinense Lecoyer. [1]
Berberine
Glaucine
Magnoflorine
N-Methylnantenine
Thalrugosine

T. sessile Hayata
Berberine [54]
Liriodenine [62]
Magnoflorine [54]
Spiradine A [54,79]
Spirasine I [54]
Spirasine II [54]
Spirasine III [54]
Spiredine [54]
Thalicessine [79]
Thalicsiline [54,123]
Thalifarazine [54,62]

T. simplex L.
β-Allocryptopine [4]
Argemonine [67]
2-Demethylthalimonine [67]
9-Demethylthalimonine [67]
Hernandezine [1]
Leucoxylonine [107]
Leucoxylonine *N*-oxide [107]
Magnoflorine [1]
N'-Norhernandezine (Thalisamine) [1]
Northalicthuberine [119]
Ocoteine (thalicmine) [1]
Thalfoetidine [1]
Thalicminine [1]
Thalicsimidine [1]
Thalicsine (thalixine) [1]

Table 1. Continued

Thalictrisine [1]
Thalidezine [1]
Thalimonine [117]
Thalimonine *N*-oxide A [107]
Thalimonine *N*-oxide B [107]
Thalsimidine [1]
Thalsimine [1]

T. squarrosum Steph. ex Willd.
Magnoflorine [1]
Thalidasine [88,89]

T. strictum Ledeb. [1]
Argemonine
Berberine
Magnoflorine
O-Methylcassyfiline (Northalicmine, *O*-Methylcassythine, Hexahydrothalicminine)
2,3-Methylenedioxy-4,8,9-trimethoxypavinane
Ocoteine (Thalicmine)
Preocoteine
Thalicminine
Thalicsimidine
Thalicthuberine

T. sultanabadense Stapf.
Berberine [55]
Hernandezine [1,55]
Hernandezine *N*-oxide [1]
Magnoflorine [55]
O-Methylthalmine [87]
Thalabadensine (Thalbadensine) [1,55]
Thalictine [55,87]
Thalidezine [1]
Thalifoline [55]

T. thunbergii DC. (*T. minus* L. var. *hypoleucum*) [1]
Aromoline (Thalicrine)
Berberine
Columbamine
Deoxythalidastine
Homoaromoline (Homothalicrine, *O*-Methylaromoline, Thalrugosamine)

Table 1. Continued

	Jatrorrhizine
	Magnoflorine
	O-Methylthalicberine
	Palmatine
	Takatonine
	Thalicberine
	Thalicthuberine
	Thalictine
	Thalidastine
	Thalifendine
T. tuberiferum Maxim.	
	Berberine [1]
	Magnoflorine [56]
T. uchiyamai Nakai	
	Corypalline [1]
	Deoxythalidastine [76]
	Jatrorrhizine [76]
	Protothalipine [76]
	Thalifendine [76]
T. urbaini Hayata [1]	
	Isocorydine
	Oconovine

15. AN INCLUSIVE COMPILATION OF ALKALOIDS THAT HAVE BEEN ISOLATED FROM *THALICTRUM* SPECIES BY ALKALOID TYPE

Table 2

Monomeric Alkaloids

Aporphines

Acutifolidine
Baicalidine
Baicaline
Bisnorthalphenine
Corydine
Delporphine
N-Demethylthalphenine
Domesticine
Glaucine (*O*-Methylthalicmidine)
Isoboldine (*N*-Methyllaurelliptine)
Isocorydine
Isooconovine
Leucoxylonine
Leucoxylonine *N*-Oxide
Magnoflorine (Thalictrine)
O-Methylcassyfiline (Northalicmine, *O*-Methylcassythine, Hexahydrothalicminine)
N-Methylcassythine
N-Methyldanguyelline
O-Methylisoboldine
N-Methyllaurotetanine
N-Methylnantenine
Nantenine
Noroconovine
Oconovine
Ocoteine (Thalicmine, *N,O*-Dimethylcassyfiline)
Preocoteine
Preocoteine *N*-Oxide
Thalbaicalidine
Thalbaicaline
Thaliadine (3-Methoxyhernandaline)
Thalicmidine *N*-Oxide (Thaliporphine *N*-Oxide)
Thalicsimidine (*O*-Methylpreocoteine, Purpureine)

Table 2. Continued

Thaliporphine (Thalicmidine, *O*-Methylisoboldine)
Thalisopynine (Thalisopinine)
Thalphenine
Trilobinine
N,O,O-Trimethylsparsiflorine
Xanthoplanine

Dehydroaporphines

Cabudine
Dehydroglaucine
Dehydroocoteine (Dehydrothalicmine)
Dehydrothalicsimidine

Proaporphines

Pronuciferine

Oxoaporphines

Corunnine
Liriodenine
Oxobaicaline
Oxoglaucine (*O*-Methylatheroline)
Thalicminine

Benzylisoquinolines

Laudanidine
N-Methylarmepavine
N-Methylcoclaurine
N-Methylpalaudinium
Reticuline
Takatonine
Tembetarine (*N*-Methylreticuline)
Thalifendlerine
Thalmeline
Veronamine

Oxobenzylisoquinolines

Rugosinone
Thalmicrinone

Isopavines

Thalidicine
Thalidine
Thalisopavine

Table 2. Continued

Isoquinolines

Corypalline

O-Methylcorypalline

N-Methyl-6,7-Dimethoxyisosquinolinium

Isoquinolones

N-Methylcorydaldine

N-Methyl-6,7-Dimethoxy-1,2-Dihydroisoquinolin-1-one

6,7-Methylenedioxy-1,2-Dihydroisoquinoline-1-one

N-Methylthalidaldine

Noroxyhydrastinine

Thalactamine

Thalflavine

Thalifoline

Morphinans

Ocobotrine

Pallidine

Sinoacutine

Pavines

Argemonine

Argemonine-N-Oxide

Bisnorargemonine

2-Demethylthalimonine

9-Demethylthalimonine

Eschscholtzidine

4-Hydroxyeschscholtzidine

Isonorargemonine

N-Methylargemonine

N-Methyleschscholtzidine

Norargemonine

Platycerine

2,3-Methylenedioxy-4,8,9-Trimethoxypavinane

Thalimonine

Thalimonine *N*-Oxide A

Thalimonine *N*-Oxide B

Phenanthrenes

N-Methylthaliglucine (Thaliglucine Metho Salt)

N-Methylthaliglucinone (Thaliglucinone Metho Salt)

Table 2. Continued

Northalicthuberine
Thalflavidine
Thalicsine (Thalixine)
Thalicthuberine
Thaliglucine
Thaliglucinone
Thalihazine

Protoberberines

Berberine
Berberrubine
Columbamine
Coptisine
Dehydrodiscretamine
Dehydrodiscretine
Demethyleneberberine
Deoxythalidastine
Groenlandicine
Jatrorrhizine
8-Oxocoptisine
Oxyberberine (Berlambine)
Palmatine
Thalidastine
Thalifaurine
Thalifendine
8-Trichloromethyldihydroberberine (Berberine-Chloroform)

Tetrahydroprotoberberines

N-Methyltetrahydroberberine (*N*-Methylcanadine)
Tetrahydroberberine (Canadine)
Tetrahydrocolumbamine (Isocorypalmine)
Tetrahydrothalifendine

Isohomoprotoberberines

Puntarenine

Protopines

Allocryptopine (Thalictrimine, β-Allocryptopine)
α-Allocryptopine
Cryptopine (Thalisopyrine)
Izmirine

Table 2. Continued

- Protopine
- Protothalipine
- Pseudoprotopine
- Thalictricine (Thalictrisine)

Diterpenes

- Spiradine A
- Spirasine I
- Spirasine II
- Spirasine III
- Spiredine
- Thalicessine
- Thalicsiline

β-Carbolines

- Harmine

Betaines

- Choline

Miscellaneous

- *N*-Phenyl-2-Naphthylamine

Dimeric Alkaloids

Aporphine-Benzylisoquinolines

- Adiantifoline
- Bursanine
- *O*-Desmethyladiantifoline
- Faberidine
- Faberonine
- Fetidine (Foetidine)
- Huangshanine
- Istanbulamine
- Iznikine
- Methoxyadiantifoline
- *N*(2')-Noradiantifoline
- *N*(2')-Northalicarpine
- Pennsylvanamine
- Pennsylvanine
- Revolutopine
- Thaliadanine
- Thalibulamine

Table 2. Continued

Thalicarpine
Thalictrogamine
Thalictropine
Thalidoxine
Thalifabatine
Thalifaberidine
Thalifaberine
Thalifabine
Thalifaboramine
Thalifalandine
Thalifaramine
Thalifarapine (Thalifaroline)
Thalifarazine
Thalifaretine
Thalifaricine
Thalifaronine
Thalifasine
Thalilutidine
Thalilutine
Thalipine
Thalirevoline
Thalirevolutine (*O*-Methylfetidine)
Thalmelatidine
Thalmelatine
Thalmineline
Uskudaramine

Dehydroaporphine-Benzylisoquinolines

Dehydrohuangshanine
6a,7-Dehydromethoxyadiantifoline
Dehydrothalifaberine
Dehydrothalicarpine

Aporphine-Pavines

EP-10
Pennsylpavine
Pennsylpavoline

Table 2. Continued

Bisbenzylisoquinolines

Aromoline (Thalicrine)
Berbamine
Cultithalminine
N-Desmethylthalidasine
N-Desmethylthalidezine
N-Desmethylthalistyline
N-Desmethylthalrugosidine
Dihydrothalictrinine
Hernandezine
Hernandezine *N*-Oxide
Homoaromoline (Homothalicrine, *O*-Methylaromoline, Thalrugosamine)
5-Hydroxythalidasine
5-Hydroxythalidasine-2α-*N*-Oxide
5-Hydroxythalmine
Isotetrandrine
Isothalidezine
O-Methylthalibrine
O-Methylthalibrunimine
O-Methylthalicberine (Thalmidine)
N-Methylthalistyline (Methothalistyline, Thalistyline Metho Salt)
O-Methylthalmethine
O-Methylthalmine
Neothalibrine
Neothalibrine-2'α-*N*-Oxide
N'-Norhernandezine (Thalisamine)
2'-Noroxyacanthine
Northalibrine
Northalibroline
N'-Northalibrunine (*N*(2')-Northalibrunine, 2'-Northalibrunine)
2-Northalidasine
N-Northalidezine
2'-Northaliphylline
2-Northalmine
Obaberine
Obamegine
Oxothalibrunimine

Table 2. Continued

Oxyacanthine
Talcamine (Likely Thalmine)
Thalabadensine (Thalbadensine)
Thalfine (Thalphine)
Thalfinine (Thalphinine)
Thalfoetidine
Thalibrine
Thalibrunimine
Thalicberine
Thalictine
Thalictrinine
Thalidasine
Thalidasine-2α-*N*-Oxide
Thalidezine
Thalifortine
Thaligosidine
Thaligosine-2α-*N*-Oxide (Thalisopine-2α-*N*-Oxide)
Thaligosinine
Thaligrisine
Thaliphylline
Thaliphylline-2'β-*N*-Oxide
Thalirabine
Thaliracebine
Thalirugidine
Thalirugine
Thaliruginine
Thalisopidine
Thalisopine (Thaligosine)
Thalistine
Thalistyline
Thalivarmine
Thalmethine
Thalmiculatimine
Thalmiculimine
Thalmiculine
Thalmine
Thalmirabine

Table 2. Continued

Thalpindione
Thalrugosaminine
Thalrugosaminine-2α-*N*-Oxide
Thalrugosidine
Thalrugosine (Thaligine, Isofangchinoline)
Thalrugosinone
Thalsimidine (Thalcimidine)
Thalsimine
Thalsivasine

Secobisbenzylisoquinolines

Revolutinone

16. AN INCLUSIVE COMPILATION OF THE CALCULATED MOLECULAR WEIGHTS OF THE *THALICTRUM* ALKALOIDS

Table 3

104.1075 $C_5H_{14}ON$
Choline

189.0426 $C_{10}H_7O_3N$
6,7-Methylenedioxy-1,2-dihydroisoquinolin-1-one

191.0582 $C_{10}H_9O_3N$
Noroxyhydrastinine

193.1103 $C_{11}H_{15}O_2N$
Corypalline

204.1024 $C_{12}H_{14}0_2N$
N-Methyl-6,7-dimethoxyisoquinolinium

207.0895 $C_{11}H_{13}O_3N$
Thalifoline

207.1259 $C_{12}H_{17}O_2N$
O-Methylcorypalline

Table 3. Continued

212.0950 $C_{13}H_{12}ON_2$
Harmine
219.0895 $C_{12}H_{13}O_3N$
N-Methyl-6,7-dimethoxy-1,2-dihydroisoquinolin-1-one
219.1048 $C_{16}H_{13}N$
N-Phenyl-2-Naphthylamine
221.1052 $C_{12}H_{15}O_3N$
N-Methylcorydaldine
233.0688 $C_{12}H_{15}O_3N$
Thalmirine
235.0845 $C_{12}H_{13}O_4N$
Thalflavine
249.1001 $C_{13}H_{15}O_4N$
Thalactamine
251.1158 $C_{13}H_{17}O_4N$
N-Methylthalidaldine
275.0582 $C_{17}H_9O_3N$
Liriodenine
299.1521 $C_{18}H_{21}O_3N$
N-Methylcoclaurine
311.1521 $C_{19}H_{21}O_3N$
Pronuciferine
311.1885 $C_{20}H_{25}O_2N$
Spiradine A
320.0923 $C_{19}H_{14}O_4N$
Coptisine
Deoxythalidastine
322.1079 $C_{19}H_{16}O_4N$
Berberrubine
Groenlandicine
Thalifaurine
Thalifendine
323.1158 $C_{19}H_{17}O_4N$
Bisnorthalphenine
324.1236 $C_{19}H_{18}O_4N$
Dehydrodiscretamine
Demethyleneberberine

Table 3. Continued

325.1314 $C_{19}H_{19}O_4N$
Domesticine
Tetrahydrothalifendine
325.1677 $C_{20}H_{23}O_3N$
N,O,O-Trimethylsparsiflorine
327.1471 $C_{19}H_{21}O_4N$
Bisnorargemonine
Isoboldine (*N*-Methyllaurelliptine)
Pallidine
Thalidicine
Thalidine
328.1913 $C_{20}H_{26}O_3N$
N-Methylarmepavine
329.1627 $C_{19}H_{23}O_4N$
Ocobotrine
Reticuline
Thalmeline
335.0794 $C_{19}H_{13}O_5N$
8-Oxocoptisine
336.1236 $C_{20}H_{18}O_4N$
Berberine
337.1314 $C_{20}H_{19}O_4N$
Cabudine
N-Demethylthalphenine
338.1028 $C_{19}H_{16}O_5N$
Thalidastine
338.1392 $C_{20}H_{20}O_4N$
Columbamine
Dehydrodiscretine
Jatrorrhizine
339.1471 $C_{20}H_{21}O_4N$
Eschscholtzidine
Nantenine
Northalicthuberine
Tetrahydroberberine (Canadine)
340.1547 $C_{20}H_{22}O_4N$
N-Methylpalaudinium

Table 3. Continued

341.1627	$C_{20}H_{23}O_4N$
	Corydine
	Isocorydine
	Isonorargemonine
	O-Methylisoboldine
	N-Methyllaurotetanine
	Norargemonine
	Platycerine
	Tetrahydrocolumbamine (Isocorypalmine)
	Thaliporphine (Thalicmidine)
	Thalisopavine
342.1705	$C_{20}H_{24}O_4N$
	Magnoflorine (Thalictrine)
	Trilobinine
343.1783	$C_{20}H_{25}O_4N$
	Laudanidine
	Thalifendlerine
344.1862	$C_{20}H_{26}O_4N$
	Tembetarine
351.1107	$C_{20}H_{17}O_5N$
	Corunnine
	Oxoglaucine
	Oxyberberine
351.1471	$C_{21}H_{21}O_4N$
	Thaliglucine
352.1549	$C_{21}H_{22}O_4N$
	Palmatine
	Thalphenine
353.0899	$C_{19}H_{15}O_6N$
	Rugosinone
353.1263	$C_{20}H_{19}O_5N$
	Protopine
	Pseudoprotopine
	Thalmicrinone
353.1627	$C_{21}H_{23}O_4N$
	Dehydroglaucine
	Thalicthuberine

Table 3. Continued

353.1991 $C_{21}H_{23}O_4N$
Spiredine
354.1705 $C_{21}H_{24}O_4N$
N-Methyleschescholtzidine
N-Methylnantenine
N-Methyltetrahydroberberine (*N*-Methylcanadine)
Takatonine
355.1420 $C_{20}H_{21}O_5N$
Baicaline
2-Demethylthalimonine
9-Demethylthalimonine
4-Hydroxyeschscholtzidine
Izmirine
O-Methylcassyfiline (Northalicmine, *O*-Methylcassythine, Hexahydrothalicminine)
N-Methylcassythine
Thalictricine (Thalictrisine)
355.1783 $C_{21}H_{25}O_4N$
Argemonine
Glaucine (*O*-Methylthalicmidine)
355.2147 $C_{22}H_{29}O_3N$
Spirasine I
Spirasine II
356.1861 $C_{21}H_{26}O_4N$
Xanthoplanine
357.1576 $C_{20}H_{23}O_5N$
Delporphine
3-*O*-Demethyloconovine
N-Methyldanguyelline
Noroconovine
Thalbaicaline
Thalicmidine *N*-oxide (Thaliporphine *N*-oxide)
365.0899 $C_{20}H_{15}O_6N$
Oxobaicaline (7-Oxobaicaline)
Thalicminine
365.1263 $C_{21}H_{19}O_5N$
Thaliglucinone
Thalicsine (thalixine)

Table 3. Continued

366.1705 $C_{22}H_{24}O_4N$

N-Methylthaliglucine (Thaliglucine metho salt)

367.1420 $C_{21}H_{21}O_5N$

Dehydrothalicmine (Dehydroocoteine)

369.1576 $C_{21}H_{23}O_5N$

Allocryptopine (β-Allocryptopine, Thalictrimine)

α-Allocryptopine

Baicalidine

Cryptopine (Thalisopyrine)

2,3-Methylenedioxy-4,8,9-trimethoxypavinane

Ocoteine (Thalicmine, *N,O*-Dimethylcassyfiline)

Thalimonine

369.1940 $C_{22}H_{27}O_4N$

Spirasine III

Thalicessine

370.2018 $C_{22}H_{28}O_4N$

N-Methylargemonine

371.1733 $C_{21}H_{25}O_5N$

Acutifolidine

Argemonine-*N*-Oxide

Oconovine

Preocoteine

Protothalipine

Thalbaicalidine

Thalisopynine (Thalisopinine)

380.1498 $C_{22}H_{22}O_5N$

N-Methylthaliglucinone (Thaliglucinone metho salt)

381.1212 $C_{21}H_{19}O_6N$

Puntarenine

383.1733 $C_{22}H_{25}O_5N$

Dehydrothalicsimidine

Thalihazine

385.1524 $C_{21}H_{23}O_6N$

Thalimonine *N*-Oxide A

Thalimonine *N*-Oxide B

385.1889 $C_{22}H_{27}O_5N$

Thalicsimidine (*O*-Methylpreocoteine, Purpureine)

Table 3. Continued

387.1682 $C_{21}H_{25}O_6N$
Preocoteine *N*-Oxide
395.1369 $C_{22}H_{21}O_6N$
Thalflavidine
399.1681 $C_{22}H_{25}O_6N$
Leucoxylonine
415.1631 $C_{22}H_{25}O_7N$
Leucoxylonine *N*-Oxide
417.2515 $C_{24}H_{35}O_5N$
Thalicsiline
454.4826 $C_{21}H_{18}O_4NCl_3$
8-Trichloromethyldihydroberberine
461.2202 $C_{28}H_{31}O_5N$
O-Methylfaurine
489.2363 $C_{26}H_{35}O_8N$
Veronamine
507.2257 $C_{29}H_{33}O_7N$
Faurine
531.1530 $C_{29}H_{25}O_9N$
Oxothaliadine
533.1686 $C_{29}H_{27}O_9N$
Dihydrooxothaliadine
533.2050 $C_{30}H_{31}O_8N$
6a,7-Dehydrothaliadine
535.2207 $C_{30}H_{33}O_8N$
7'-Dihydrodehydrothaliadine
Thaliadine (3-Methoxyhernandaline)
537.2363 $C_{30}H_{35}O_8N$
7'-Dihydrothaliadine
582.2730 $C_{35}H_{38}O_6N_2$
Northalibroline
592.2573 $C_{36}H_{36}O_6N_2$
Thalmethine
Thalmiculatimine
Thalsivasine

Table 3. Continued

594.2730	$C_{36}H_{38}O_6N_2$
	Aromoline (Thalicrine)
	2'-Noroxyacanthine
	2'-Northaliphylline
	2-Northalmine
	Obamegine
	Thalabadensine (Thalbadensine)
	Thalivarmine
606.2730	$C_{37}H_{38}O_6N_2$
	O-Methylthalmethine
608.2522	$C_{36}H_{36}O_7N_2$
	Cultithalminine
608.2886	$C_{37}H_{40}O_6N_2$
	Berbamine
	Homoaromoline (Homothalicrine, *O*-Methylaromoline, Thalrugosamine)
	Oxyacanthine
	Thalicberine
	Thalictine
	Thalifortine
	Thaliphylline
	Thalmine
	Thalrugosine (Thaligine, Isofangchinoline)
610.3043	$C_{37}H_{42}O_6N_2$
	Northalibrine
	Thaligrisine
622.2679	$C_{37}H_{38}O_7N_2$
	Thalmiculimine
	Thalsimidine (Thalcimidine)
622.3043	$C_{38}H_{42}O_6N_2$
	Isotetrandrine
	O-Methylthalicberine (Thalmidine)
	Obaberine (*O*-Methyloxyacanthine)
624.2836	$C_{37}H_{40}O_7N_2$
	N-Desmethylthalidezine
	N-Desmethylthalrugosidine
	5-Hydroxythalmine
	Thaligosidine

Table 3. Continued

		Thaliphylline-2'β-*N*-Oxide
		Thalisopidine
624.3199	$C_{38}H_{44}O_6N_2$	
		Neothalibrine
		Thalibrine
636.2836	$C_{38}H_{40}O_7N_2$	
		Longiberine
		Thalsimine
638.2992	$C_{38}H_{42}O_7N_2$	
		N-Desmethylthalidasine
		Isothalidezine
		N-2-Norhernandezine
		N'-Norhernandezine (Thalisamine)
		2-Northalidasine
		Thalfoetidine
		Thalidezine
		Thaligosinine
		Thalisopine (Thaligosine)
		Thalmiculine
		Thalrugosidine
638.3356	$C_{39}H_{46}O_6N_2$	
		O-Methylthalibrine
640.3149	$C_{38}H_{44}O_7N_2$	
		Neothalibrine-2'α-*N*-Oxide
		Thalirugine
648.2472	$C_{38}H_{36}O_8N_2$	
		Thalfine (Thalphine)
650.2992	$C_{39}H_{42}O_7N_2$	
		O-Methyllongiberine
652.2421	$C_{37}H_{36}O_9N_2$	
		Thalpindione
652.2785	$C_{38}H_{40}O_8N_2$	
		Revolutinone
		Thalibrunimine
652.3149	$C_{39}H_{44}O_7N_2$	
		Hernandezine
		Thalidasine

Table 3. Continued

Thalifaramine
Thalifaboramine
Thaliracebine
Thalrugosaminine

654.2941 $C_{38}H_{42}O_8N_2$
5'-Hydroxythalidezine
N'-Northalibrunine (*N*-2'-Northalibrunine, 2'-Northalibrunine)
Thaligosine-2α-*N*-Oxide (Thalisopine-2α-*N*-Oxide)

654.3305 $C_{39}H_{46}O_7N_2$
5-*O*-Demethylongine
Thaliruginine

656.3098 $C_{38}H_{44}O_8N_2$
Bisocobotrine

664.2421 $C_{38}H_{36}O_9N_2$
Thalictrinine

666.2577 $C_{38}H_{38}O_9N_2$
Dihydrothalictrinine
Oxothalibrunimine
Thalrugosinone

666.2941 $C_{39}H_{42}O_8N_2$
EP-10
Fauripavine
O-Methylthalibrunimine
Pennsylpavoline
Squarosine
Thalfinine (Thalphinine)

666.3293 $C_{40}H_{46}O_7N_2$
Berberlongine
Thalifaronine

668.3097 $C_{39}H_{44}O_8N_2$
Faurithaline
Hernandezine *N*-oxide
5-Hydroxythalidasine
Istanbulamine
Pennsylvanamine
Revolutopine
Thalibrunine

Table 3. Continued

Thalictrogamine
Thalidasine-2α-*N*-Oxide
Thalifaberidine
Thalifaricine
Thalipine
Thalistine
Thalmirabine
Thalrugosaminine-2α-*N*-Oxide
Uskudaramine

668.3462 $C_{40}H_{48}O_7N_2$
Longine

670.3254 $C_{39}H_{46}O_8N_2$
Thalirugidine

680.3097 $C_{40}H_{44}O_8N_2$
Berberstyline
Pennsylpavine

682.3254 $C_{40}H_{46}O_8N_2$
N-Desmethylthalistyline
Faberidine
Fauridine
Fetidine
N(2')-Northalicarpine
Pennsylvanine
Thalibulamine
Thalictropine
Thalidoxine
Thalifalandine
Thalifarapine (Thalifaroline)
Thalifarazine
Thalifaretine
Thalilutidine
Thalirevoline
Thalmelatine

683.3332 $C_{40}H_{47}O_8N_2$
Thalirabine

684.3046 $C_{39}H_{44}O_9N_2$
5-Hydroxythalidasine-2α-*N*-Oxide

Table 3. Continued

684.3411 $C_{40}H_{48}O_8N_2$
5'-Hydroxylongine
694.3254 $C_{41}H_{46}O_8N_2$
Dehydrothalifaberine
Dehydrothalicarpine
696.3410 $C_{41}H_{48}O_8N_2$
Thalicarpine
Thalifaberine
Thalirevolutine
697.3489 $C_{41}H_{49}O_8N_2$
Thalistyline
698.3200 $C_{40}H_{46}O_9N_2$
Bursanine
Iznikine
3-Methoxyfaurithaline
Thalifasine
710.3191 $C_{41}H_{46}O_9N_2$
Thalifabine
712.3360 $C_{41}H_{48}O_9N_2$
O-Desmethyladiantifoline
Faberonine
N(2')-Noradiantifoline
6-Noradiantifoline
Thaliadanine
Thalifabatine
Thalilutine
712.3724 $C_{42}H_{52}O_8N_2$
N-Methylthalistyline (Methothalistyline, Thalistyline Metho Salt)
724.3360 $C_{42}H_{48}O_9N_2$
6a,7-Dehydroadiantifoline
Dehydrohuangshanine
726.3516 $C_{42}H_{50}O_9N_2$
Adiantifoline
Huangshanine
740.3309 $C_{42}H_{48}O_{10}N_2$
Thalmelatidine

Table 3. Continued

742.3465	$C_{42}H_{50}O_{10}N_2$
Thalmineline	
754.3465	$C_{43}H_{50}O_{10}N_2$
6a,7-Dehydromethoxyadiantifoline	
756.3622	$C_{43}H_{52}O_{10}N_2$
Methoxyadiantifoline	

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Chapter Two

Taxine

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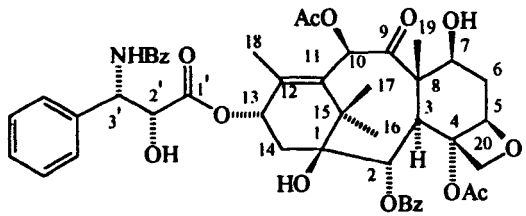
CONTENTS

1.1. INTRODUCTION

Over the past few years, no plant has been investigated more thoroughly than the yew tree [1]. Indeed, the commercialization of one of its constituents as an anticancer drug (paclitaxel = Taxol® [2-4]) has prompted a flurry of research activity on plants from the genus *Taxus*, resulting in the isolation of hundreds of new metabolites [1]. Curiously, little attention was given to taxine, the oldest and more typical of the yew constituents. Taxine is a collective name, referring to mixtures of diterpene alkaloids obtainable from yew biomass or extracts through an acid/base partition scheme. Paclitaxel-type alkaloids, where the amino-acidic nitrogen is part of an amide bond, lack basic properties and are not among the constituents of taxine. The most recent review on taxine dates back to 1968 [5], at a time when the structure of none of its genuine constituents had been elucidated. Several recent surveys on taxoids (*taxane diterpenoids*) are instead available [1,6,7]. To avoid duplication of information, only topics specifically related to alkaloidal taxoids will be addressed. The reader is referred to the above mentioned references for a discussion of the general features of taxoids.



Paclitaxel (= Taxol®)

1.2 HISTORY

Taxine is responsible for the poisonous properties of the yew and its long and legendary history. The toxicity of this tree is well documented in historical and fictional literature, and has fascinated poets and writers [8]. The yew is one of the few poisons known to the ancients which can be identified with certainty today. Thus, Julius Caesar in his *De Bello Gallico* wrote of the suicide of the defeated Gaulish chieftain Catuvolcus, who '*taxo, cuius magna in Gallia Germaniaque copia est, exanimavit*' (killed himself with the yew, which is abundant in Gaul and Germany) [9]. Homer [10] and Virgil [11] mentioned the yew tree and its poisonous properties, as did Shakespeare [12] and, in more recent times, the novelist Agatha Christie [13] and the

poet T. S. Eliot [14]. Hebenon (hebona in Quartos), the venom used to kill Hamlet's father and possibly the most famous of all literary poisons, might also have been a yew extract [15].

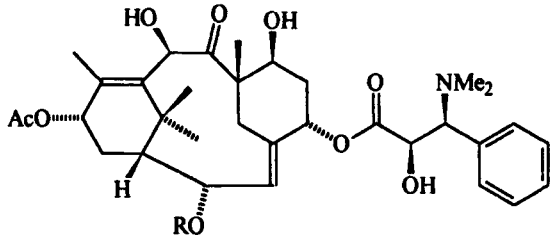
Scientific investigations on the poisonous constituents of the yew date from the early 19th century. The needles were mentioned in several pharmacopoeias as a foxglove substitute, but the yew was better known as a livestock poison. The first chemical investigation of the yew tree seems to have been carried out by Piero Peretti, a professor at Rome University, in 1828 [16]. He isolated a bitter substance from the needles, but failed to recognize its basic properties. The early work was characterized by confusion rather than rigor, and culminated in the isolation of an alkaloid mixture, named taxine, from the needles. This was reported by Lucas in 1856 [17], and the circumstances of the isolation are worth mentioning.

Lucas was a pharmacist in Arnstadt, a town in Thuringia known to music lovers for the years J.S. Bach spent there. One day Lucas read a report by a French veterinarian (Dujardin) on several cases of yew poisoning in domestic animals (horses, pigs, cows, donkeys). This reminded him of a case that had occurred in his own town two decades earlier, when a flock of sheep had been kept in a fenced yard landscaped with a few large yew trees. The following day, five or six sheep were found dead, and a veterinarian was called out. Poisoning from heavy metals was suspected, and Lucas was asked to analyze the stomach of the dead animals. He could not find any evidence for the presence of heavy metals, and suggested that the animals might have ingested a poisonous plant. Indeed, an inspection of the yard where the sheep had been kept revealed that the yews had been stripped of leaves as high as the animals could reach, and Lucas was aware of the poisonous properties of the tree. He did not try to isolate the poisonous principle of yew that time, but only years later, when the report by Dujardin reminded him of his previous experience. By then, the isolation of alkaloids had become a hot topic, and many toxic compounds of this type had been isolated (strychnine, nicotine, coniine). After a laborious procedure, Lucas obtained an amorphous powder giving amorphous salts, which he named taxine. The yield was not high (*ca* 3 g from 1.5 Kg of dried needles), but 'not optimized' (*vermute Ich, daß bei recht sorgfältiger Behandlung und Bearbeitung größerer Messen die Ausbeute reichlicher ausfallen wird*).

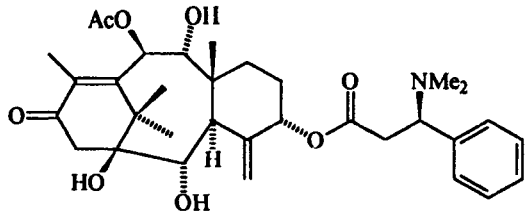
The poisonous properties of the alkaloidal material obtained from the yew tree were first investigated by Borchers in 1876 [18], but its structural characterization was exceedingly slow, despite intensive work by numerous research groups. The first clue came only in 1923, when Winterstein, the chemist who coined the classical definition of an alkaloid, showed that taxine is the ester of a polyalkol esterified with acetic acid and (*L*)-β-dimethylamino-β-phenylpropionic acid [19]. This β-aminoacid was then named after Winterstein. The early studies on taxine were

hampered by the amorphous properties of this material and its salts, and by the lack of a purity criterion. The melting point (82° → 124° C) and rotatory power (+ 35° → + 96°) reported for taxine from the European yew varied over a wide range, and the early studies could not dispel the obvious suspicion that the material was a mixture of products.

It was only in 1956, a hundred years after the isolation of taxine, that Graf in Würzburg showed by electrophoresis that taxine is actually a mixture of several compounds [20]. By column chromatography on buffered silica gel he obtained three constituents in a pure state: taxine A (1%), taxine B (30%) and taxine C (traces), the alphabetical designation presumably referring to their order of isolation [20].

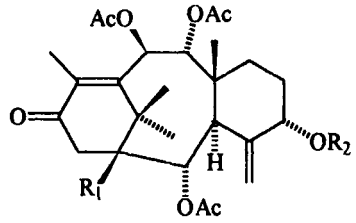


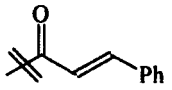
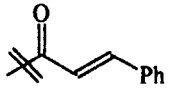
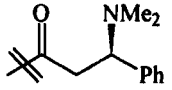
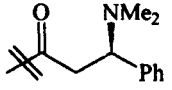
Taxine A (R= Ac); Taxine C (R=H)



Taxine B

A different approach was pursued by Lythgoe in England and by Takahashi and Kondo in Japan [5]. They discovered that chemical modification of taxine through acetylation and Hoffmann degradation can afford pure compounds, and the constitutions and stereochemistries of two of these degradation products (triacetylcinnamoyl taxicin I and II) were reported by Lythgoe in 1967 [21]. The corresponding alkaloids were named taxine I and II, and were recently isolated from the seeds of the European yew [22].



	R_1	R_2
Triacetylcinnamoyltaxicin I	OH	
Triacetytlcinnamoyltaxicin II	H	
Taxine I	OH	
Taxine II	H	

The structures of the natural alkaloids isolated by Graf were not clear for a long time, nor were their relationship with taxine I and II. Thus the paradoxical statement by Lythgoe that "those components [of taxine] which have been isolated (taxines A, B and C) have not received structural elucidation, and those whose structures have been determined (taxine I and II) have not been isolated. [5]." Taxine I was presumably related to taxine B, the major constituent of taxine, but the exact number of the acetyl groups and their location remained elusive. The structure of taxine A was solved in 1982 [23], and this compound turned out to have a different terpenoid skeleton from taxine I and II. In 1991 the group in Gif finally reported the structure of taxine B [24], and the relationship between this alkaloid and taxine I became obvious.

In the early seventies, the discovery of the antitumor properties of paclitaxel [25] and the detection of large amounts of ecdysones [26] in yew tissues shifted the attention of the scientific community toward other constituents of the yew, and the number of papers devoted to taxine plummeted. However, interest was revived in the early nineties by the possibility of using taxine

as starting material for the semisynthesis of antitumor taxoids [27], and by the relevance of its detection in medicinal taxoids.

1.3 DISTRIBUTION AND ISOLATION TECHNIQUES

Taxane alkaloids have a very limited distribution within the plant kingdom, and occur only in two genera of the yew family (*Taxus* and *Austrotaxus*). The genus *Austrotaxus* is monotypic (*A. spicata* Compt), but confusion exists on the systematics of the genus *Taxus*, especially as regards the taxonomic status of certain Asian yews. Reference will be given here to the Krüssmann classification (Table 1) [28].

Table 1: The systematics of the genus *Taxus*

Trivial name	Krüssmann Calssification	Presence of Alkaloids
European yew	*Taxus baccata* L.	+
Himalayan yew	*Taxus wallichiana* Zucc.	+
Chinese yew	*Taxus celebica* (Warburg) Li	+
Japanese yew	*Taxus cuspidata* Sieb. et Zucc.	+
Pacific yew	*Taxus brevifolia* Nutt.	+
Canadian yew	*Taxus canadensis* Marsh.	? *
Florida yew	*Taxus floridana* Nutt.	?
Mexican yew	*Taxus globosa* Schlechtd.	?
	Taxus x media Rehd	+
	Taxus x hunnewelliana Rehd.	?

* The presence of alkaloids has been reported, but no constituent was identified (G. Masson, Rapport Office Recherches Ministère Sci Affaires Munic, *Ind Com Provice Québec*, 49 (1942). *Chem Abstr* 36:7238.

Not all species have been investigated for the presence of alkaloids, and some constituents of taxine are unstable in the plant material, being degraded during its drying and storing (see infra). In many investigations the state (dried/fresh) of the plant material was not specified, nor was the time lapse between the collection of the plant material and its analysis. Furthermore, some species were only analyzed for the presence of paclitaxel and paclitaxel-

equivalent compounds. It is thus likely that alkaloids in yew species have a larger distribution than described in Table 1, since they might have escaped detection for one of the above-mentioned reasons.

Few data are also available on the concentration of alkaloids in different plant parts. The red aril surrounding the seeds does not contain taxoids, and the alkaloids, at least in the European yew, seem to be concentrated mainly in the needles, where their concentration can be higher than 1% on dry weight basis [29]. The alkaloid contents varies greatly during the course of the year, and is higher in winter [29]. It was recognized early that taxine from different yews is different [5], but the complexity and instability of taxine has so far prevented its full characterization in species different from the European yew. Furthermore, no data on the concentration of taxine in the other species have been reported.

Taxine can be extracted from yew tissues using a conventional acid/base partition scheme. The following procedure has been used in the author's laboratory:

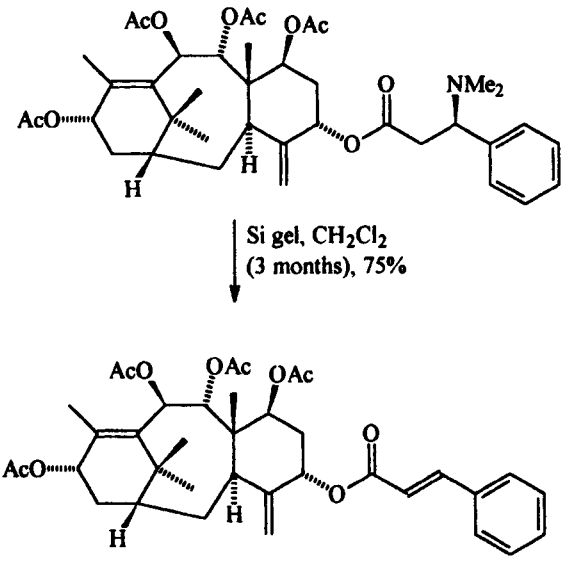
The fresh plant material (1Kg) is extracted with acetone (3 x 5 l) at room temperature; the pooled organic phases are concentrated to ca 150 ml and EtOAc (1l) and 2N HCl(300 ml) are added. The extraction with acid is repeated three times, and the pooled acid phases are extracted with EtOAc (2 x 200 ml) to remove polar phenolics (lignans, flavonoids). The aqueous phase is then neutralized (pH 7 -8) with conc. NH_3, and extracted with EtOAc (2 x 200 ml). The pooled organic phases are washed with brine, dried ($MgSO_4$) and evaporated to give taxine as a yellow amorphous powder (yield 2 - 4 g, depending on the plant source and the season).*

* In more basic medium, taxine A is partially isomerized and degraded [30]

Early separation methods, based on the selective partition of crude taxine between an organic solvent and water solutions of different pH values [31] have been abandoned, and column chromatography on silica gel using mixtures of $CHCl_3$-EtOH as eluant is today the method of choice. Further purification can be achieved on TLC or with HPLC. Unlike crude taxine, taxane alkaloids are often crystalline compounds, especially those of the taxine A-type.

Winterstein esters easily undergo β-elimination to *E*-cinnamic acid esters (*Scheme 1*). It is not clear to what extent this conversion is enzymatic or only chemical. The conversion can take place on storage [31] or in mild acidic medium. Prolonged times (months) are required with silica gel [32]. With compounds of the taxine B-type, the reaction can take place fairly easily on plant storage, though the extent of this reaction in the seeds is much less [30]. The degradation of Winterstein esters of the taxine B-type (**2a-d**) has a dramatic effect on the composition of taxine, since Winterstein esters of the 2'-deacetoxyaustrospicatine-type (**4f**) and

phenylisoserine esters (e,g, taxine A, **1a**) are more stable in the plant material. Thus, taxine obtained from fresh needles and from the stored plant material is different, owing to the degradation of alkaloids of the taxine B-type [30].



Scheme 1. Acidic degradation of 2'-deacetoxyaustrospicatine (**4f**) [32].

Winterstein esters corresponding to all taxoids isolated as cinnamates might well exist in yew tissues, but many of them have not yet been isolated, due to enzymatic and/or chemical degradation or to a very low concentration.

1.4 CLASSIFICATION AND BIOGENETIC CONSIDERATIONS

Taxane alkaloids are made up of a terpenoid core and a phenylpropanoid β-amino acid, joined by an ester bond. They can be classified according to the carbon-carbon connectivity of the terpenoid core and the type of the side-chain. Thus, the diterpenoid core can be of the taxane- (Tables 3-8), 11(15→1)abeotaxane-(Table 10), 2(3→20)abeotaxane-(Table 2) or 3-11-cyclotaxane (Table 9) type, whereas the side chain can be Winterstein's acid [*L*(*R*) β-dimethylamino-β-phenylpropionic acid] or *N,N*-dimethylphenylisoserine (2*R*, 3*S* (*threo*) α-hydroxy-β-dimethylamino-β-phenylpropionic acid). Further modification occurs in the

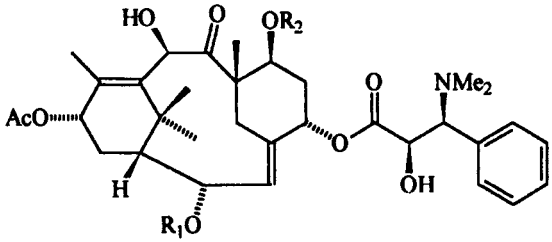
oxygenation/acylation pattern of the terpenoid core, and in the *N*-methylation or *O*-acetylation state of the side chain.

A few main subclasses can accommodate most alkaloids, and a system of nomenclature based on names defined in the literature can avoid the confusing use of very similar trivial names that plague the literature on taxoids. Thus, taxine B is a taxane alkaloid [24], whereas taxin B is a non-nitrogenous 2(3→20)abeotaxane [33].

The thirty-seven *Taxus* alkaloids isolated through July 1995 are reported in Tables 2--10, along with their botanical source [species, plant part (l = leaves; b = bark, r = roots)]. *A. spicata* Compt is the richest source of taxane alkaloids, in terms of chemical diversity, and 2'-deacetoxyaustrospicatine **(4f)** is the most widespread alkaloid (three *Taxus* species and *A. spicata*). The various taxane alkaloids can be divided into the following structural types:

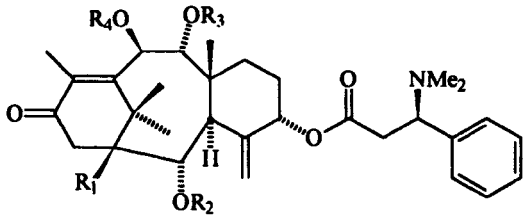
- *Taxine A-type* (Table 2, **1a-c**): the terpenoid core is of the 2(3→20)abeotaxane type, and the side chain is phenylisoserine. Modification occurs in the acetylation pattern of the terpenoid core (acetylation of the 7-hydroxyl, hydrolysis of the 2-acetate)
- *Taxine B-type* (Table 3, **2a-d**): the terpenoid core is normal (taxane) and bears a 13-oxo group, whereas the side chain is Winterstein's acid. Modification occurs by deoxygenation at C(1) and in the acetylation pattern of the terpenoid core.
- *Comptonine-type* (Table 4, **3a**): the terpenoid core is normal (taxane) and bears a 13-oxo group. The side chain however, is phenylisoserine rather than Winterstein's acid as in taxine B-type alkaloids. Comptonine **(3a)** and spicaledonine **(8a)** are the only alkaloids with a 13-oxo group where the aminoacyl moiety is not Winterstein's acid.
- *Austrospicatine-type* (Table 5, **4a-p**): the terpenoid core is normal (taxane) and bears a 13-acetoxyl group. The side chain can be Winterstein's acid or phenylisoserine. Modification occurs in the oxygenation [C(1), C(2), C(7)] and acetylation pattern of the terpenoid core and by acetylation, deoxygenation and demethylation of the side chain.
- *Austrotaxine-type* (Table 6, **5a-d**): the terpenoid core is normal (taxane) and C-14 is oxygenated.
- *Aminoacylnicotaxine-type* (Table 7, **6a,b**): the terpenoid core is normal (taxane), the 4(20) double bond is epoxidized and there is a 13-oxo group.
- *Spicataxine-type* (Table 8, **7a-e**): the terpenoid core is normal (taxane), the 4(20) double bond is epoxidized and there is an acetoxyl at C(13).
- *Spicaledonine-type* (Table 9, **8a,b**): these alkaloids are the 3,11-cycloderivatives of taxane alkaloids of the 13-oxo-type (taxine B and comptonine types).

Table2: Alkaloids of the Taxine A-type



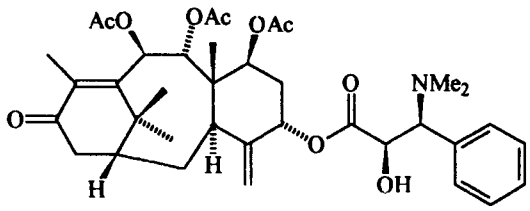
Compound	Trivial name	R_1	R_2	Source	Reference
1a	Taxine A	Ac	H	*T. baccata* (l)	23
1b	Taxine C	H	H	*T. baccata* (l)	45,46
1c	7-Acetyltaxine A	Ac	Ac	*T. baccata* (l)	46

Table 3: Alkaloids of the Taxine B- type



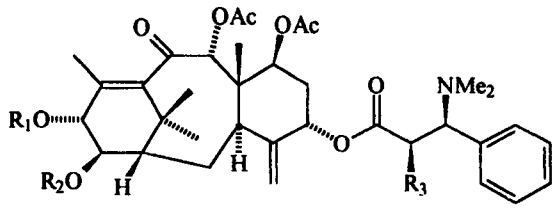
Compound	Trivial name	R_1	R_2	R_3	R_4	Source	Ref.
2a	Taxine B	OH	H	H	Ac	*T. baccata* (l)	24
2b	Isotaxine B	OH	H	Ac	H	*T. baccata* (l)	45
2c	Taxine I	OH	Ac	Ac	Ac	*T. baccata* (s)	22
2d	Taxine II	H	Ac	Ac	Ac	*T. baccata* (s) *T. cuspidata* (l)	22 47

Table 4: Alkaloids of the Comptonine-type



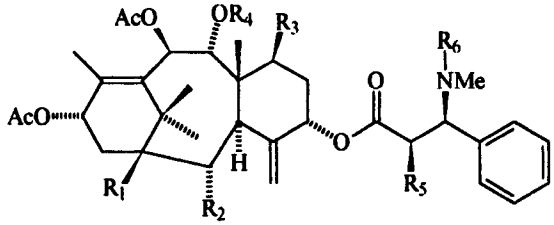
Compound	Trivial name	Source	Refrernce
3a	Comptonine	*A. spicata* (l)	32

Table 6: Alkaloids of the Austrotaxine-type.



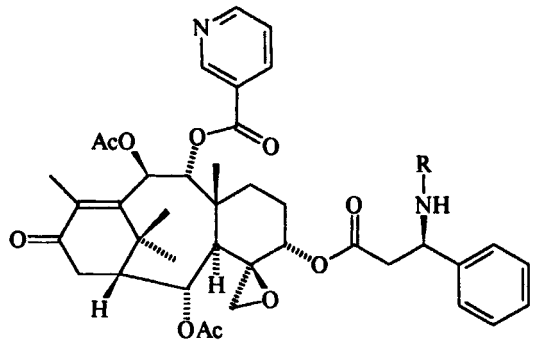
Compound	Trivial name	R_1	R_2	R_3	Source	Ref.
5a	Austrotaxine	Ac	Ac	OAc	*A. spicata* (l)	32
5b	2'-deacetylaustrotaxine	Ac	Ac	OH	*A. spicata* (l)	32
5c	2'-Deacetoxyaustrotaxine	Ac	Ac	H	*A. spicata* (l)	32
5d	Trideacetylaustrotaxine	H	H	OH	*A. spicata* (b)	51

Table 5: Alkaloids of the Austrospicatine-type



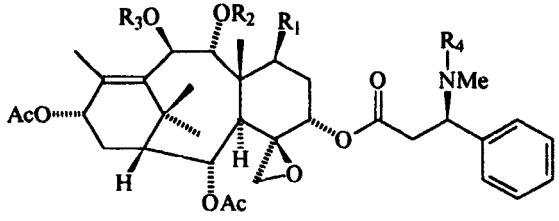
Compound	Trivial name	R_1	R_2	R_3	R_4	R_5	R_6	Source	Ref
4a	Austrospicatine	H	H	OAc	Ac	OAc	Me	*A. spicata* (l)	32
4b	2'-Deacetylaustro-spicatine	H	H	OAc	Ac	OH	Me	*A. spicata* (l)	32
4c	7-Deacetylaustro-spicatine	H	H	OH	Ac	OAc	Me	*A. spicata* (l)	32
4d	7,9-Bisdeacetyl-austrospicatine	H	H	OH	H	OAc	Me	*A. spicata* (l)	32
4e	2',7,9-Trisdeacc-tylaustrospicatine	H	H	OH	H	OH	Me	*A. spicata* (l)	32
4f	2'-Deacetoxy-austrospicatine	H	H	OAc	Ac	H	Me	*A. spicata* (l) *T. cuspidata* (l) *T.wallichiana*(b) *T. x media* (r)	32 49 30 30
4g	2α-Acetoxyaustro-spicatine	H	OAc	OAc	Ac	OAc	Me	*A. spicata* (l)	32
4h	2α-Acetoxy-2'-de-acetylaustrospicati-ne	H	OAc	OAc	Ac	OH	Me	*A. spicata* (l)	32
4i	2α-Acetoxy-2'-de-acetoxyaustrospica-tine	H	OH	OAc	Ac	H	Me	*A. spicata* (l)	32
4l	1, 2α-Dihydroxy-7,2'-deacetoxy-9-deacetylaustrospi-catine	OH	OH	H	H	H	Me	*T. baccata* (l)	48
4m	2α–Hydroxy-7,2'-deacctoxy-9-dea-cetylaustrospica-tine	H	OH	H	H	H	Me	*T. baccata* (l)	48
4n	Nor-2α-hydroxy-7,2'-deacetoxy-9-deacetylaustrospi-catine	H	OH	H	H	H	H	*T. baccata* (l)	48
4o	7,2'-Bisdeacetoxy-austrospicatine	H	H	H	Ac	H	Me	*T. wallichiana*(b)	50
4p	1-Hydroxy,2α-acetoxy-2'-deace-tylaustrospicatine	OH	OAc	Oac	Ac	OH	Me	*T. baccata* (l)	46

Table 7: Alkaloids of the Aminoacylnicotaxine-type



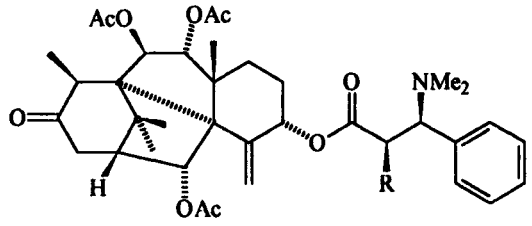
Compound	Trivial name	R	Source	Referernce
6a	Aminoacylnicotaxine	Me	*A. spicata* (b)	51
6b	Noraminoacylnicotaxine	H	*A. spicata* (b)	51

Table 8: Alkaloids of the Spicataxine-type



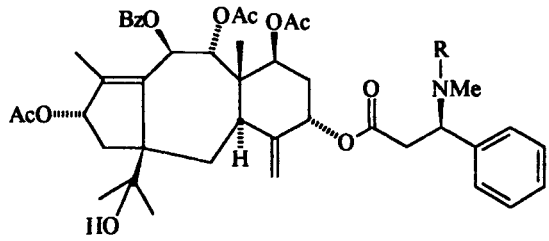
Compoud	Trivial name	R1	R2	R3	R4	Source	Ref.
7a	Spicataxine	H	H	Ac	Me	*A. spicata* (b)	51
7b	9-Acetyl,10-deacetylspicataxine	H	Ac	H	Me	*A. spicata* (b)	51
7c	7-Acetoxy-9-acetylspicataxine	OAc	Ac	Ac	Me	*T. x media* (r)	52
7d	Nicaustrine	H	Nic*	Ac	Me	*A. spicata* (b)	51
7e	Nornicaustrine	H	Nic*	Ac	H	*A. spicata* (b)	51

Table 9: Alkaloids of the Spicaledonine-type



Compound	Trivial name	R	Source	Refrernce
8a	Spicaledonine	OH	*A. spicata* (b)	51
8b	Taxuspine H	H	*T. cuspidata* (l)	53

Table 10: Alkaloids of the Aminoacylbrevifoliol-type



Compound	Trivial name	R	Source	Refrernce
9a	13-Acetyl-5-[(3*R*)-3-(*N*, *N*-dimethyl-amino)-3-phenylpropionyl]brevifoliol	Me	*T. brevifolia* (l)	54
9b	13-Acetyl-5-[(3*R*)-3-(*N*-methyla-mino)-3-phenylpropionyl]brevifoliol	H	*T. brevifolia* (l)	54

- *Aminoacylbrevifoliol-type* (Table 10, **9a,b**): the terpenoid core is the rearranged 11(15→1)abeo type. The other features are similar to those of alkaloids of the austrospicatine-type.

The derivation of taxanes from geranylgeranyl diphosphate, first postulated by Lythgoe [34], has been confirmed by feeding experiments [35]. However, the exact details of the biosynthesis are not known. From the biogenetic point of view, taxane alkaloids can be divided into two classes: those derived from a 4(20),7-verticilladiene (taxine A type) and those derived from a 3,7-verticilladiene (all the other structural types) (Scheme 2) [36, 37]. Taxanes and 2(3→20)abeotaxanes are thus biogenetically distinct, and taxanes are the precursors of compounds of the 11(15→1)abeo- and 3,11-cyclo series (Scheme 2). Taxanes of the 1-hydroxy type are easily converted into their A,B-contracted derivatives [38]. However, the acylation and oxygenation pattern of 1-hydroxytaxanes and 11(15→1)abeotaxanes is different [39], and the chemical cyclization generally gives compounds of the Δ^{15}-type, which have never been isolated from yew extracts. Similarly, although taxanes of the 13-oxo-$\Delta^{4(20)}$-type can be converted into 3,11-cyclotaxanes by UV-light [40], reductive deamination to phenylpropionates is also observed under these conditions [41] (see **1.6.2.**), but phenylpropionate esters of taxoids are unknown as natural products.

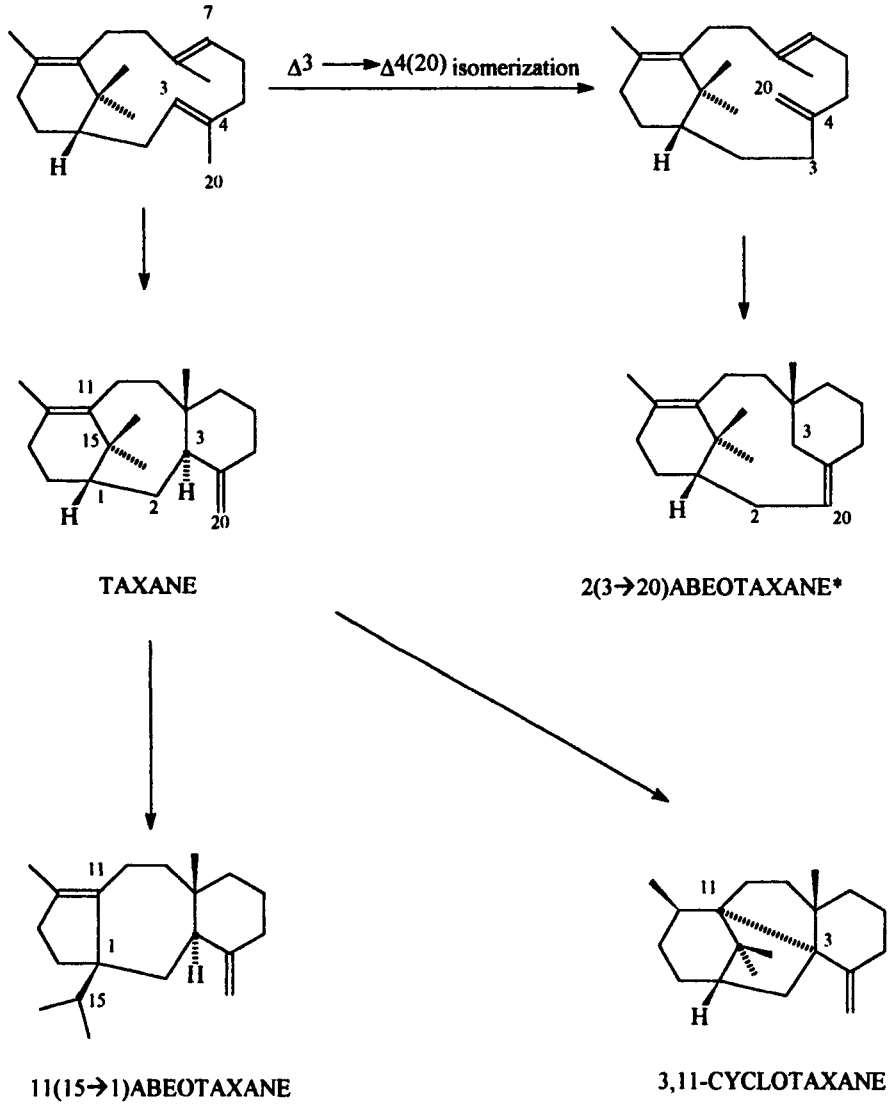
A relationship seems to exist between the functionalization pattern of the terpenoid core and the type of side-chain. Thus, alkaloids of the 13-oxo type are generally esterified with Winterstein's acid, and those of the 2(3→20) abeo-type with phenylisoserine, whereas *N*-demethylation is observed mainly in alkaloids of the 13-acetoxyl type.

The biogenesis of Winterstein's acid and phenylisoserine has been investigated in detail [42-43]. These β-aminoacids are derived from phenylalanine, probably through a diotopic reaction involving the migration of the amino group from the α- to the β-carbon of the amino acid [44]. Cinnamic acid was not incorporated in the yew β-amino acids [44].

1.5 STRUCTURAL DETERMINATION

The structure elucidation of taxine defied the efforts of generations of natural products chemists. Taxine A was the first native alkaloid to be structurally elucidated in 1982 [23], through a combination of spectroscopic techniques (^{1}H-and ^{13}C-NMR and X-ray spectroscopy). Taxine A is a minor (*ca* 1%) component of taxine, and is one of the structurally more

challenging of all taxoids, having a rearranged skeleton and being highly functionalized. Its high crystallinity undoubtedly helped its purification and structure elucidation.



Scheme 2: Biogenetic relationship between the skeleta of Taxus *alkaloids*

*: Numbering refers to the structural formula and not to the biogenesis.

Taxoids are a structurally homogeneous class of secondary metabolites and only a few skeletal types have been reported. It is thus relatively easy to infer the gross structure of the

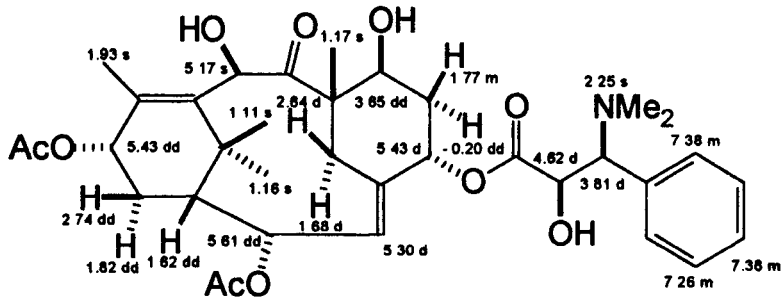
molecule from a routine 1D-NMR analysis, and the most challenging part is the determination of the acylation pattern. Several spectroscopic features of taxoids are very unusual, and their rationalization provides unique challenges to spectroscopists.

1.5.1 NMR Spectroscopy

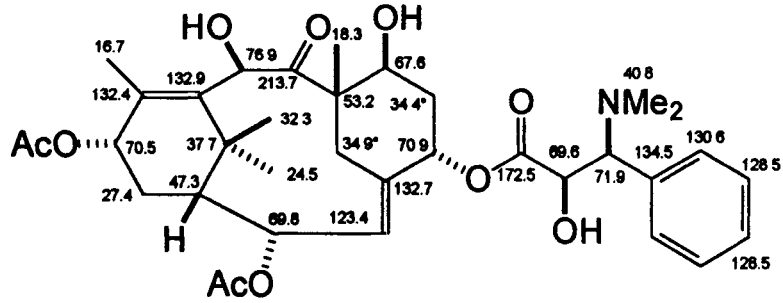
The ^{1}H NMR spectra of the most significative taxane alkaloids are reported in Tables 11-14. The general spectroscopic features of the resonances of the terpenoid core have been discussed elsewhere [55], and attention will be given only to the effect of the basic side-chain on the terpenoid core signals. The most relevant feature of the ^{1}H NMR spectra of several types of alkaloids is the upfield chemical shift of H(6α) or H(14α). These protons are oriented toward the concave α-face of the terpenoid core, and are subjected to anisotropic diamagnetic shielding from the phenyl ring of the aminoacidic side chain. The effects are especially marked in esters of phenylisoserine. Thus, in taxine A **(1a)**, H(6α) resonates upfield of TMS at δ -0.20 [46], and in 2'-deacetylaustrospicatine **(4b)** at δ 0.47 [32]. The removal of the side-chain moves these signals downfield, to δ 1.78 [37] and 1.95 [46] respectively. In Winterstein's esters, the effect on H(6α) is less marked, but still detectable [δ H(6α) 1.25 in 2'-deacetoxyaustrospicatine **(4f)**] [32], and H(14α) is the main proton affected (δ 0.96 in **4f**) [32]. In phenylisoserine esters, the diamagnetic shielding depends on the side chain conformation, and requires a close proximity between H-6α and the side chain phenyl. Assuming the usual Z-'hydrogen eclipsed' conformation around the C-O bond of secondary esters [56], this can take place only when the side chain has a conformation of the type A (*Scheme 3*), with H(2') and H(3') antiperiplanar. A conformation of this type has been found in the X-ray analysis of taxine A [23], and is in accordance with the J values observed in $CDCl_3$ ($J_{2',3'}$ *ca* 10 Hz).

All factors changing this arrangement are expected to affect the chemical shift of H(6α). Thus, acetylation of the 2'-hydroxyl of taxine A moves the signal of H(6α) downfield (from δ -0.20 to + 1.80), and causes a decrease of $J_{2',3'}$ (from 10.0 Hz to 4.7 Hz) [46]. Comparison of the ^{1}H NMR spectra of 2'-deacetylaustrospicatine **(4b)** and austrospicatine **(4a)** shows a similar trend (δ H(6α) = 0.47 and 1.68; $J_{2',3'}$ = 9 Hz and 4 Hz, respectively) [32]. Similar changes are observed when the spectra are taken in solvents more polar than $CDCl_3$. Thus, in taxine A H(6α) resonates at -0.20 in $CDCl_3$, but at δ + 0.90 in DMSO- d_6, where $J_{2',3'}$ drops from 10.0 to 6.6 Hz [46].

Table 11: NMR data of taxine A (1a) ($CDCl_3$)[23,50]

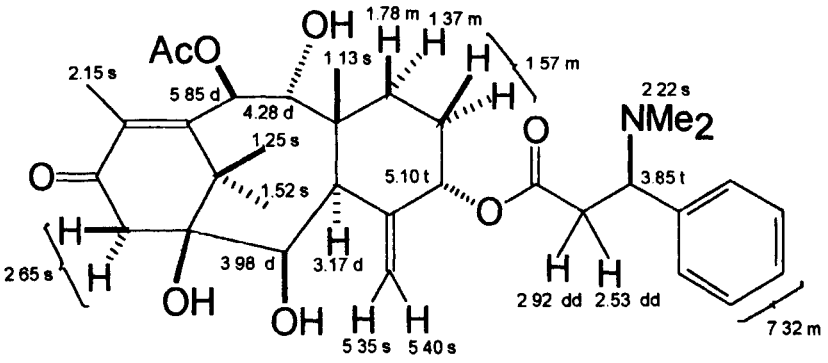


Ac: 2.29, 1.98 (s).
J(Hz): 1,2=2.5; 2,20= 10.0; 3α,3β=15.5; 5,6β=6.4; 6α,6β=15.0; 7,6α=3.6; 7,6β=13.6; 13,14α=3.1; 13,14β=11.0; 14α,14β=16.5; 14β,1=7.5; 2',3'=10.0.

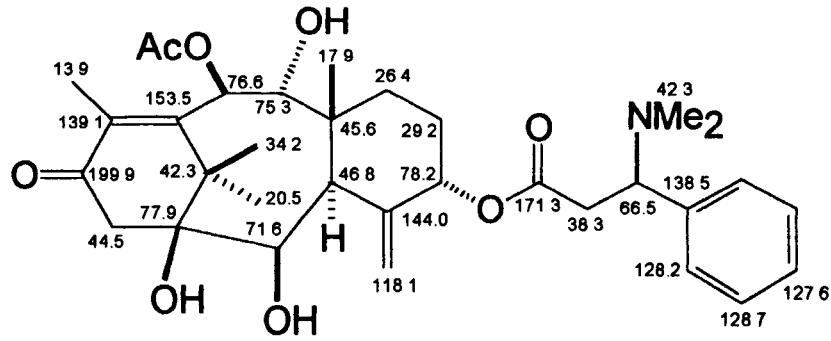


Ac: 170.7, 169.9 (s); 21.4, 21.2 (q).

Table 12: NMR data of taxine B (2a) ($CDCl_3$)[24]

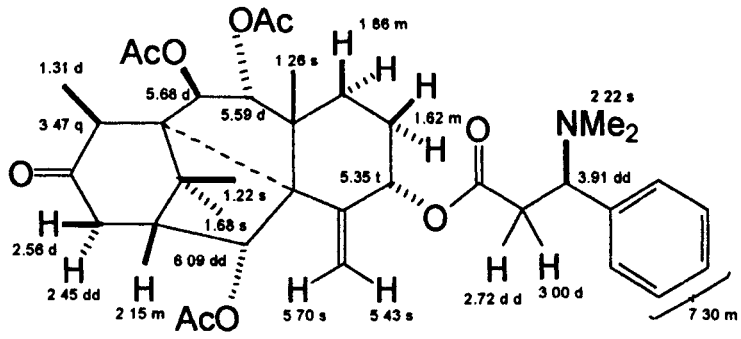


Ac: 2.22 (s)
J(Hz): 2,3=7.0; 5,6α= 5,6β=2.5; 9,10=9.5; 2'α,3 = 7.0; 2'β,3 = 7.0; 2'α,2'β = 16.0,.

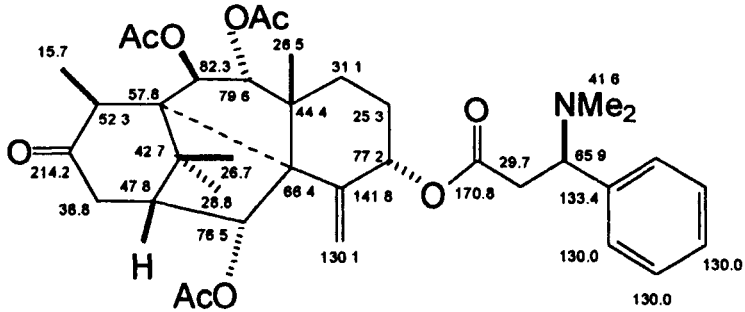


Ac:170.2 (s), 21.2 (q).

Table 13: NMR data of taxuspine H (8b) ($CDDCl_3$)[53]

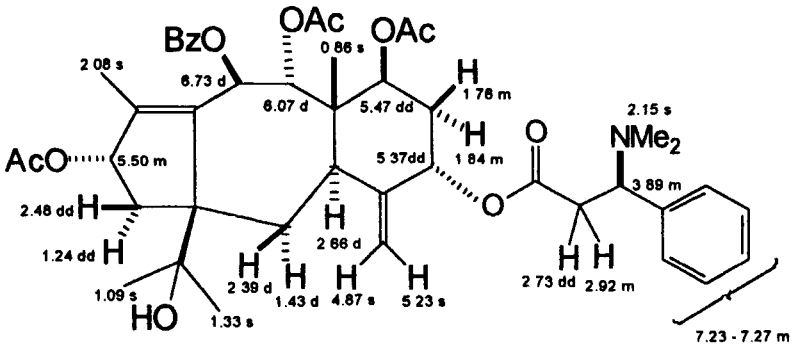


Ac: 2.07, 2.06, 2.04 (s).
J(Hz): 1,2 =5.3; 5,6α=5,6β=4.0; 9,10=9.5; 12,18=7.2; 14α,14β=20.5; 2a,2b=15.0; 2a,3'=6.0; 2b,3'=8.0.

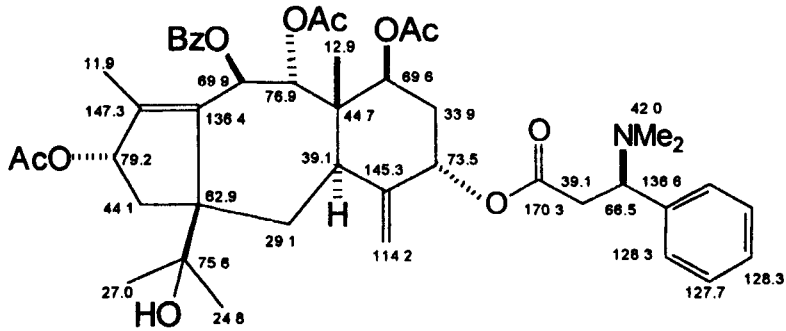


Ac: 169.9, 169.5, 168.8 (s), 21.4, 21.1, 20.9 (q).

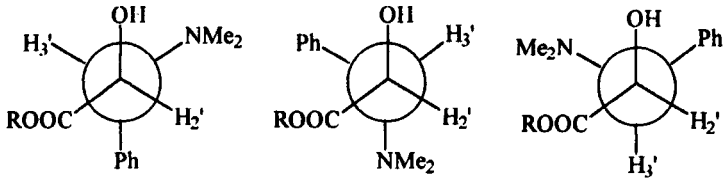
Table 14: NMR data of 9a ($CDCl_3$)[54]



Ac: 2.06, 1.87, 1.74 (s): Bz: 7.87 (d, J=7.7 Hz), 7.43 (t, J=7.7 Hz), 7.56 (t, J=7.6 Hz).
J(Hz): 2α,2β=14.1; 2β,3=9.1; 5,6α =1.8; 5,6β=4.0; 7,6α=5.2; 7,6β=11.3; 9,10=10.5; 13,14α=8.6; 13,14β=7.3; 14α,14β=13.9; 2'a,3=8.2; 2'a,2'b=13.9.



Ac:170.9, 169.9, 169.6 (s); 21.4, 20.7, 20.7 (q); Bz: 166.1 (s), 129.1 (s, *i*), 129.5 (d, *o*), 128.8 (d, *m*), 133.4 (d, *p*).



Scheme 3: Staggered conformations of N,N-dimethylphenylisoserine derivatives around the C(2')-C(3') bond.

These results can be explained by postulating that the amino-acidic side chain of phenylisoserine-type alkaloids is essentially ananchomeric (conformationally locked) in $CDCl_3$ and apolar solvents, presumably as a result of a strong intramolecular H-bonding between the vicinal hydroxyl and the dimethylamino group and a smaller number of gauche interactions between the substituents Acetylation of the 2'-hydroxyl suppresses the intramolecular hydrogen bonding within the side chain, and in polar solvents intermolecular hydrogen bonding with the solvent competes with intramolecular bonding. Acetylation also decreases the steric requirements of the oxygen at C(2'). As a result, the contribution of conformations **B** and **C**, with H-2' and H-3' synclinal and the phenyl away from the taxoid core, becomes more important or even predominant (2'-acetylderivatives) (*Scheme 3*).

Solvent-related conformational changes in the side chain of paclitaxel have been reported [57]. However, in this compound the 'extended' conformation, corresponding to **C** in *Scheme 3*, prevails in apolar solvents, and the 'globular' conformation, corresponding to **A** in *Scheme 3*, in polar solvents. These differences are presumably related to the very good hydrogen acceptor properties of the basic dimethylamino group of phenylisoserine esters like taxine A and 2'-deacetylaustrospicatine. In paclitaxel-type compounds, the aminoacidic nitrogen is acylated, and can only act as a weak hydrogen bonding donor.

1.5.2 X-Ray Diffraction

Two taxane alkaloids have been investigated by diffraction techniques: taxine A (**1a**) [23] and 2'deacetoxyaustrospicatine (**4f**) [49]. The results gave important information on the orientation of the side chain relatively to the terpenoid core. In both cases the aromatic ring is close to the terpenoid core, but in **4f** the phenyl and the carbonyl are antiperiplanar, whereas in taxine A they are synclinal (cf. C and A in Scheme 3). As a result, the protons closer to the phenyl ring are H(6α) in taxine A and H(14α) in **4f**. This is in accordance with the results of the ^{1}H-NMR analysis [46]. In **4f**, the conformation with C-1' and the phenyl antiperiplanar is stabilized by hydrophobic interactions with the methyl of the 13-acetate [49]. The X-ray features of the terpenoid core of **4f** and taxine A show linear strain at several carbon-carbon bonds, as generally observed in taxoids [55].

1.5.3 Other techniques and the assignment of the absolute configuration

Mass spectrometry is generally useful only to obtain information on the molecular weight and to identify the type and functional groups bound to the terpenoid core. Soft ionization techniques must be employed (FAB, DCI, TSP). The spectra of Winterstein esters are characterized by a prominent peak at *m/z* 210, and those of the *N,N*-dimethylphenylisoserine esters at 226, corresponding to the aminoacidic side chain [58].

The absolute configuration of 2'-deacetoxyaustrospicatine (**4f**) was assigned on the basis of a strong positive Cotton effect at 218 nm [49]. This was assigned to the π-π* transition of the exocyclic methylene at C-4. However, a previous detailed analysis of the CD spectra of several related taxanes led to the assignment of this Cotton effect to the π-π* transition of the endocyclic double bond [59]. It was also observed that the $\Delta^{4(20)}$-chromophore was responsible for a weak negative Cotton effect of no practical use, being overshadowed by the strong positive effect of the Δ^{11}-chromophore.

The absolute configuration of the terpenoid core of taxine I was deduced by the X-ray analysis of the bromoderivative of a transformation product of its corresponding cinnamate (taxinine) [60]. The *L* (*R*) absolute configuration of the dextrorotatory Winterstein acid obtained from the degradation of crude taxine was established by comparison with the rotatory power of the semisynthetic enantiomers prepared from *D*- and *L* β-phenyl-β-alanine [61].

1.6 REACTIONS

1.6.1. Elimination of dimethylamine

Winterstein esters are easily turned into *E*-cinnamates by the action of acids (H^+, silica gel) [32]. For preparative purposes the reaction has been carried out using the Hoffmann elimination of the corresponding methylammonium hydroxides [27] or the Cope elimination of the corresponding *N*-oxides [51]. The latter procedure is more convenient, since, if the reaction is carried out in THF, elimination of dimethylhydroxylamine takes place spontaneously at room temperature. If only a moderate excess of peracid is employed, the exocyclic double bond at C-4 is not affected [30]. The transformation of Winterstein esters into cinnamates can be very useful for separation purposes. Thus, whereas taxine B **(2a)** and isotaxine B **(2b)** are difficult to separate by HPLC, their corresponding cinnamates can be separated by column chromatography (!) [62].

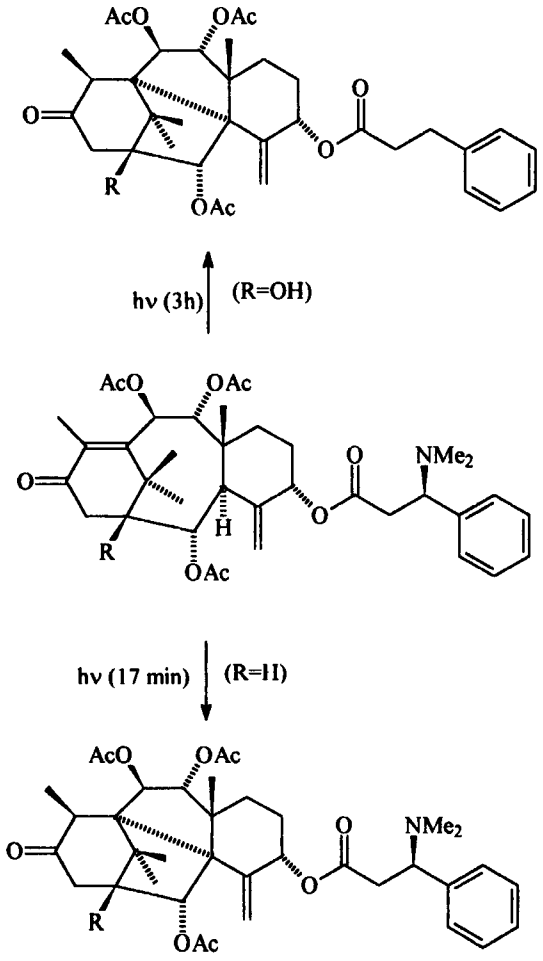
1.6.2. Photochemical Reactions

Prolonged (*ca* 3 h) irradiation of Winterstein esters leds to the formation of the corresponding phenylpropionates [41] (*Scheme 4*) The reaction is typical of Winterstein's acid derivatives and does not involve antenna-like effects from chromophores on the terpenoid moiety. Similar results were obtained in fact with simple aliphatic esters of this β-aminoacid [41]. Under these conditions (irradiation for several hours), alkaloids of the 13-oxo,$\Delta^{4(20),11}$-taxadiene-type (taxine B and comptonine-type) undergo photocyclization to 3,11-cyclotaxanes [40]. The photocyclization of this type of alkaloids could be accomplished without reductive deamination using shorter (17 min) irradiation times [53].

1.7 PHARMACOLOGY

The most common form of yew poisoning is the accidental swallowing of seeds by children, who are attracted to the colorful and sweet-tasting aril surrounding the seeds [63]. Unless the seeds are broken or chewed, only very mild or no toxic signs at all are observed,

since the seed coat is resistant to the gastric juices [63]. The ingestion of yew needles is a classical way of committing suicide, and most cases of yew poisoning reported in the forensic literature refer to the intentional consumption of plant material by psychiatric patients or by prison inmates [64].



Scheme 4: Photochemical reactions of alkaloids of the taxine B-type.

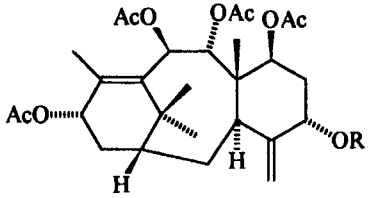
Taxus poisoning is rare, but nevertheless important because of the widespread availability of the plant material and its general fatal outcome [64, 65]. The diagnosis is usually

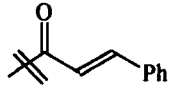
botanical (examination of the stomach contents) more than clinical, and on autopsy the anatomical findings are non- specific [64]. The exact fatal dose is not known, but 50-100 fresh needles are considered lethal for a human adult [66]. No clinical treatment for the management of yew poisoning is known. Cardiac pacing, digoxin-specific FAB antibody fragments, high doses of lidocaine and the conventional therapy of arrhythmias were tried, but the general utility of these measurements and their rationale have not been established [64]. The yew tree is also a notorious threat for livestock, and animal poisoning is not uncommon. Spectacular cases in terms of public interest have been reported, like the death of seven precious Brahman-type bulls from a rodeo at the Ohio State Fairground in April 1975 [67].

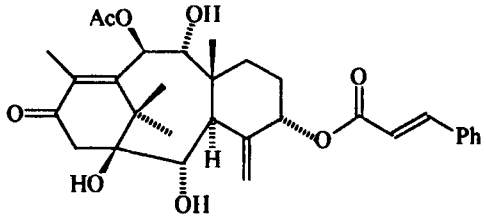
Taxine is a powerful heart poison, and has traditionally been considered responsible for the toxic properties of the yew [65], although recent work suggests a non-negligible contribution also from certain degradation products of the alkaloid mixture (see infra). Most investigations were carried out using the crude alkaloid mixture from the European yew [65]. The infusion of taxine in different animal species causes multiple rhythm disturbances, including severe bradycardia, atrio-ventricular blocks, enlargement of the QRS complex, ventricular tachycardia and/or fibrillation, complicated by respiratory and neuromuscular disorders [65]. These cardiac alterations are similar to those reported for yew poisoning [65]. The mechanism of action of taxine has not been elucidated at the cellular and molecular level. Preliminary experiments showed that taxine inhibits sodium and calcium currents in isolated ventricular guinea-pigs cells [68], a finding consistent with the observation that taxine reduces the maximum rate of depolarization of the action potential in simple *Xenopus* nerve fibres [69].

Only two studies have been done on the cardiac activity of pure alkaloids. One study compared the activity of taxine A (**1a**) and taxine B (**2a**) in different animal species [70]. Only taxine B was toxic, causing the typical symptoms of yew poisoning, whereas the cardiac activity of taxine A was not significant. The other study [71] investigated the effect of four alkaloids and related compounds on two cardiac preparations, the isolated, self-paced heart and the isolated papillary muscle stimulated at constant rate. In this way electrical and mechanical effects could be investigated independently from variations in oxygen supply and beating frequency. In the isolated heart, the Winterstein esters **2a** (taxine B), **4f** (2'-deacetoxyaustrospicatine) and **4m** (2α-hydroxy-7-deacetoxy-9-deacetylaustrospicatine) induced electrical and mechanical effects similar to those reported in *in vivo* experiments with crude taxine (atrio-ventricular blocks, widening of the QRS complex, ventricular tachycardia, fibrillation), but had no effect on the coronary flow. These effects are similar to those of class I antiarrhythmic drugs, and taxine B was the most active compound. The phenylisoserine ester taxine A (**1a**) had only minimal effect

on the electrical and mechanical activity of the isolated heart. Non-alkaloidal taxoids related to **2a** and **4f** were also investigated. 2-Deacetoxydecinnamoyltaxinine J, corresponding to the terpenoid core of **4f**, showed no activity, nor did Winterstein's acid [70], highlighting the importance of both the terpenoid core and the aminoacidic moiety for cardiac activity. On the other hand, the cinnamates corresponding to the Winterstein esters **2a** and **4f** (10-acetylcinnamoyltaxicin I and 2-deacetoxytaxinine J, respectively) could induce significant changes in cardiac performance on the isolated heart (rise in diastolic pressure, arrhythmia, transient increase followed by profound reduction of the left ventricular systolic pressure), most probably due to a reduction of the coronary flow, since no effect on the papillary muscle could be observed.



	R
2-Deacetoxydecinnamoyltaxinine J	H
2-Deacetoxytaxinine J	



10-Acetylcinnamoyltaxicin I

These data suggest that the powerful toxicity of yew alkaloids might be due to the synergic activity of Winterstein esters, which have direct cardiotoxicity, and their corresponding cinnamates, which cause an ischemic state through a reduction of the coronary flow. Winterstein esters and their corresponding cinnamates co-occur in yew tissues [62], where Winterstein

esters prevail. However, a non negligible conversion of these compounds to cinnamates might take place in the acidic stomach medium. The variety of targets and mechanisms involved in the cardiac activity of taxine makes it difficult to develop a specific antidote.

2'-Deacetoxyaustrospicatine **(4f)** and taxinine II **(2d)** are the only yew alkaloids which have been tested for an activity different from cardiac toxicity [47]. Both compounds showed a certain cytotoxicity *in vitro* on L1210 murine leukemia cells, significantly lower, however, than that of paclitaxel. On the other hand, 2'-deacetoxyaustrospicatine had a powerful activity, comparable to that of verapamil, as an inhibitor of P-glycoprotein-mediated transport, thus acting as a multidrug resistance (MDR)-reversing agent [47].

1.8 CONCLUSIONS

Taxine is a mixture of diterpenoid alkaloids present in all parts of the yew tree except the red aril surrounding the seed. The composition of taxine depends on the botanical source (yew species and variety) and the plant part. Taxine is unstable in the plant material and part of the degradation is due to the elimination of dimethylamine from Winterstein's acid-type alkaloids. Diversity in yew alkaloids derives from the presence of different terpenoid cores (taxane, 3,11-cyclotaxane, 11(15→1)abeotaxane, 2(3→20)abeotaxane) and side chains (Winterstein acid, phenylisoserine and demethylated or acetylated forms of these β-aminoacids). Further modification occurs in the oxygenation pattern of the terpenoid core. Winterstein esters and their corresponding cinnamates are powerful heart poisons and, owing to the widespread availability of the yew tree, *Taxus* poisoning is relevant in human and veterinarian toxicology. Furthermore, the presence of alkaloid impurities in paclitaxel might be clinically relevant, since this compound already shows a certain cardiotoxicity [72]. Our knowledge on *Taxus* alkaloids would greatly benefit from the development of analytical methods for the detection and characterization of taxine in plant tissues. Future research on *Taxus* alkaloids is expected to shed light on the complex post-harvest transformations and the marked seasonal and intraspecific variation of their production, as well as to establish the mechanism of their cardiac toxicity at the molecular level. The therapeutic potential of subclinical doses of paclitaxel to prevent restenosys after angioplasty [73] is expected to spur investigations on the cardiac activity of taxoids. These studies might eventually lead to a better understanding of the activity of taxine at the molecular level.

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The Alkaloids of South American Menispermaceae

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CONTENTS

1. INTRODUCTION

Menispermaceae (A. L. Jussieu), the moonseed family, is widely distributed throughout the tropical world. There are about seventy genera identified of which about seventeen are present in the New World. These genera belong to five subfamilies: Anomospermeae, Cocculeae, Hyperbaeneae, Tinosporeae and Triclisieae. The number of species identified is approximately 142. South American Menispermaceae species are woody-vines, shrubs, or small trees. Proper identification of most of these menispermaceous plants is difficult because flowers and fruits are seldom available, and if so, have not been collected at the same time. The flowers of some genera, for example, *Telitoxicum* spp., are very small and delicate. As a result, they break away and drop to the ground separately from the stem when the bush ropes are cut down. Sometimes the collector has to rely on his ability to distinguish the menispermaceous bush-ropes from other lianas by the striking color of fresh cut stems. For example, in *Abuta obovata*, *Abuta rufescens*, and *Anomospermum reticulatum* the slash is yellow, while in *Anomospermum chloranthum*, it is lavender [1].

Diels's monograph was the first comprehensive monograph on Menispermaceae [2]. The ethnobotany of South American Menispermaceae is well described by Krukoff, Barneby and Moldenke who identified some species [1, 3-5]. A few were re-identified even though in some cases staminate inflorescences, flowers, fruits, and seeds were not available [1, 3-5]. Sometimes species are renamed that were found to be the same as another species identified earlier. For example, *Abuta concolor* has been reclassified under *Abuta grandifolia* by Krukoff and Moldenke [3]. By 1982, it was evident to Krukoff that tropical lowland forests of Venezuela and of Amazonian Bolivia, Ecuador, Peru and Colombia are very rich in Menispermaceae. He expected new species, particularly in the genera *Abuta*, *Sciadotenia*, and *Anomospermum* [5].

The subfamilies and the genera of the New World Menispermaceae are given in *Table I*. It is worthwhile to note that *Synandropus* and *Ungulipetalum* are still unknown in fruit and may not be generically distinct from *Odontocarya* and *Sciadotenia*, respectively [6].

Table I: Subfamilies and genera of New World Menispermaceae

ANOMOSPERMEAE	COCCULEAE	HYPERBAENEAE	TINOSPOREAE	TRICLISIEAE
1. *Abuta*	7. *Cissampelos*	9. *Hyperbaenea*	10. *Borismene*	14. *Chondrodendron*
2. *Anomospermum*	8. *Cocculus*		11. *Disciphania*	15. *Curarea*
3. *Caryomene*			12. *Odontocarya*	16. *Sciadotenia*
4. *Elephantomene* = *Cionomene*			13. Synandropus	17. *Ungulipetalum*
5. *Orthomene*				
6. Telitoxicum				

The major contribution of South American Menispermaceae to medicine is the curare activity of several species. Native Indians from Brazil, Bolivia, Colombia, Ecuador, French Guiana, Peru and Surinam utilized this principle to make arrow poisons for hunting small animals such as monkeys [7]. The curare activity will be discussed later in the section on pharmacology. Two of the recent reviews dealing with this Amazonian family are "The Healing Forest" by Schultes and Raffauf [8], (which covers 1516 species: 145 families and 594 genera) and "Amazonian Ethnobotanical Dictionary" by Duke and Vasquez [9] (which covers only some 20% of the flora of the area). Both reviews provide a good account of the medicinal and toxic plants used by the natives of the northwest Amazon. Local uses and colloquial names are also given for the flora described; however, neither gives the structures of the compounds present. The aim of this review is to cover all of the species identified from South American Menispermaceae, the compounds isolated from them and their pharmacological activities.

2. DISTRIBUTION OF SPECIES

The species (number in parenthesis) of Menispermaceae identified from South America are in *Table II*. Botanical nomenclature is given in reference to genus name except for more recent reassignment of species which are given in reference to species name.

Table II: Species of South American Menispermaceae

ANOMOSPERMEAE

1. ***Abuta* (27)** [1] is the genus with the most rapidly increasing number of species.

1. *aristeguietae*
2. *antioquiana*
3. *barbata*
4. *brevifolia*
5. *bullata*
6. *candollei*
7. *chocoënsis* [5]
8. *colombiana*
9. *convexa* [4]
10. *fluminum* [10]
11. *grandifolia*
12. *grisebachii*
13. *imene*
14. *longa*
15. *mycetandra* [4]
16. *negroensis*
17. *obovata*
18. *pahni* [4]
19. *panurensis*
20. *racemosa*
21. *rufescens* = *splendida* [11]
22. *sandwithiana*
23. *seemanni*
24. *selloana*
25. *solimoesensis*
26. *vaupesensis*
27. *velutina*

Table II: Species of South American Menispermaceae (Continued...)

ANOMOSPERMEAE (Continued...)

2. *Anomospermum* (8) [4]

1. *andersonii* [12]
2. *bolivianum*
3. *chloranthum*
4. *grandifolium*
5. *matogrossense*
6. *solimoesanum*
7. *steyermarkii*
8. *reticulatum*

3. *Caryomene* (5) [4]

1. *foveolata*
2. *glaucescens*
3. *grandifolia* [12]
4. *olivascens*
5. *prumnoides*

4. *Elephantomene* (1) [13] = ***Cionomene* (1)** [12,14]

eburnea *javariensis*

5. *Orthomene* (4) [4]

1. *hirsuta*
2. *prancei*
3. schomburgkii
4. *verruculosa*

6. *Telitoxicum* (8) [3]

1. *duckei*
2. *glaziovii*
3. *inopinatum*
4. *krukovii*
5. *minutiflorum*
6. *negroense* [10]
7. *peruvianum*
8. *rodriguesii* [15]

COCCULEAE

7. *Cissampelos* (10) [16]

1. *andromorpha*
2. *fasciculata*
3. *glaberrima*
4. *grandifolia*
5. *laxiflora*
6. *ovalifolia*
7. *pareira*
8. *sympodialis*
9. *tropaeolifolia*
10. *verticillata*

8. *Cocculus* (0) [6]

No native species of Cocculus have been found in South America, but *C. laurifolius* DC was formerly cultivated at Lima, Peru, and mistakenly identified as an *Abuta*.

HYPERBAENEAE

9. *Hyperbaena* (3) [17]

1. *domingensis*
2. *hassleri*
3. *oblongifolia*

Table II: Species of South American Menispermaceae (Continued...)

TINOSPOREAE

10. *Borismene* (1) [2]
japurensis

11. *Disciphania* (19) [18]

1. *cubijensis*
2. *calocarpa*
3. *convolvulacea*
4. *cryptobotrya*
5. *dioscoreoides* [19]
6. *ernstii*
7. *heterophylla* [20]
8. *inversa*
9. *juliflora*
10. *killipii*
11. *lobata*
12. *modesta*
13. *peltata*
14. *remota*
15. *sagittaria*
16. *sarcostephana*
17. *smithii*
18. *tessmannii*
19. *tricaudata*[19]

12. Odon*tocarya* (29) [18]

1. *acuparata*
2. *arifolia*
3. *asarifolia*
4. *deminuta*
5. *dielsiana*
6. *diplobotrya*
7. *duckei*
8. *echinus*
9. *emarginata*
10. *floribunda*
11. *hastata*
12. *klugii*
13. *krukoviana*
14. *macarenae*
15. *magnifolia*
16. *mallosperma*
17. *micrantha*
18. *miersiana*
19. *petiolaris*
20. *rusbyi*
21. *steyermarkii*
22. *syncretica*
23. *tamoides*
24. *tenacissima*
25. *tripetala*
26. *truncata*
27. *ulei*
28. *wullschlaegelii*
29. *zuliana*

13. *Synandropus* (1) [18]
membranaceus

TRICLISIEAE

14. *Chondrodendron* (3) [4]
(sometimes spelled *Chondodendron*)

1. *microphyllum*
2. *platiphyllum*
3. *tomentosum*

15. *Curarea* (4) [4]

1. *candicans*
2. *cuatrecasasii*
3. *tecunarum*
4. *toxicofera*

Table II: Species of South American Menispermaceae (Continued...)

TRICLISIEAE (Continued...)

16. *Sciadotenia* (18) [1]

1. *acutifolia*
2. *amazonica*
3. *brachypoda*
4. *campestris [21]*
5. *cayennensis*
6. *duckei*
7. *eichleriana*
8. *javariensis*
9. *mathiasiana*
10. *nitida [4]*
11. *pachnococca [4]*
12. *paraënsis*
13. *pubistaminea*
14. *ramiflora*
15. *sagotiana*
16. *solimoesana*
17. *sprucei*
18. *toxifera*

17. *Ungulipetalum* (1) [4]

1. *filipendulum*

3. OCCURRENCE OF ALKALOIDS

A list of all the alkaloids identified from the species of South American Menispermceae is given by genus in Table III [22]. All the species investigated to date and original reference are included in this list.

Table III: Occurrence of Alkaloids

Genus/Species Name	Alkaloid	References
I. *Abuta*		
1. *A. bullata*	Palmatine	23
	(+/-)Saulatine	23
	Trichloromethyl-8-dihydropalmatine	23
2. *A. concolor*	Grandirubrine	24
(*A. grandifolia*)*	Pareirubrine-A	24
3. *A. grandifolia*	Grandirubrine	25
	Palmatine	26
4. *A. grisebachii*	(-)-Grisabine	27
	(-)-Magnoline (Grisabutine)	27, 28
	(-)-Macolidine	28
	(-)-Macoline	28

Table III: Occurrence of Alkaloids (Continued...)

Genus/Species Name	Alkaloid	References
4. *A. grisebachii (Continued...)*	(-)-Peinamine	28
	(-)-*N*-methyl-7-*O*-demethylpeinamine	28
	(-)-7-*O*-Demethylpeinamine	28
5. *A. imene*	Imeluteine	29, 30
	Imenine	30, 31
	Imerubrine	30, 31
	Homomoschatoline	30
	Norrufescine	30
	Rufescine	29, 30
6. *A. pahni*	(+)- Coclaurine	32
	(-)- Daurisoline	32
	(-)-2'-*N*-Nordaurisoline	32
	(-)- Lindoldhamine	32
	(-)- *N, N'*-Dimethylindoldhamine	32
	(-)-2-*N*-Methyllindoldhamine	32
	(-)-2'-*N*-Methyllindoldhamine	32
	(+)-Stepharine	32
	Thalifoline	32
7. *A. panurensis*	(-)- Norpanurensine	33
	(-)- Panurensine	33
8. *A. rufescens/splendida*	(+)-Aromaline	34
	(+)-Homoaromaline	34
	Homomoschatoline	30
	Imeluteine	29, 30
	Imenine	30
	Imerubrine	30
	Lysicamine	34
	(-)-Krukovine	34
	Norrufescine	30
	Rufescine	29, 30
	Splendidine	35
9. A. sandwithiana	protoberberines	9

II. *Anomospermum*

None of the species of this genus have been completely investigated except that King has showed that tertiary and quaternary bases are present in the bark of *A. grandifolium* [36,1,7].

Table III: Occurrence of Alkaloids (Continued...)

Genus/Species Name	Alkaloid	References
III. *Caryomene*		
1. *C. olivascens*	(-) -Caryolivine	37
	(+)-Coclaurine	37, 38
	(-)-Coreximine	38
	(+)-1,2-Dehydro-2-norlimacusine	37
	(+)-2-Norlimacusine	37
	(-)-*N, N'*-Dimethyllindoldhamine	37
	(-)-10-Demethyldiscretine	38
	(-)-Discretine	38
	(-)-*N*-Formystepharine	37
	(-)-Govadine	38
	(-)-2-Norlimacine	37
	(-)-Pronuciferine	37
	(+)-Pseudopalmatine	37, 38
	(-)-Stepharine	37, 38
	(-)-Xilopinine	38
IV. *Chondrodendron*		
1. *C. limaciifolium* (*Curarea candicans*)**	(+)-Isochondrodendrine	39
2. *C. microphyllum*	(+)-Curine	40
	(+)-Isochondodendrine	40
3. *C. platiphyllum*	(-)-Chondrofoline	40, 41
	(-)-Curine	40, 41
	(+)-Isochondodendrine	40
4. *C. tomentosum*	(+)-Chondrocurine	36, 42, 43
	(+)-Chondocurarine	42
	(-)-Curine	36, 43, 44

Table III: Occurrence of Alkaloids (Continued...)

Genus/Species Name	Alkaloid	References
4. *C. tomentosum (Continued...)*	(-)- Cycleanine (*O,O*-dimethylisochondrodendrine)	36, 43
	(+)-Isochondrodendrine	36, 42, 43
	(-)-Norcycleanine	43
	N-Benzylphthalimide	43
	(+)-Tomentocurine	36, 43
	(-)-Tomentocurarine	42
	(+)-Tubocurarine	36, 42
	(-)-Tubocurarine	44
5. *C. toxicoferum* (*Curarea toxicofera*)***	(-)-Curine	45
	(+)-Isochondodendrine	45
	(-)-Toxicoferine	45
	(1:1 mixture of (-)-Curine and (-)-Tubocurine)	
V. *Cissampelos*		
1. *C. pareira*	(-)-Cissampareine	46
	Grandirubrine	47
	Isoimerubrine	47
	Norimeluteine	47
	Norrufescine	47
	Pareirubrine A	47
	Pareirubrine B	47
	Pareitropone	48
2. *C. ovalifolia*	(-)- Warifteine	49
	(-)- Cissampareine (Methylwarifteine)	49
	(-)- *O*-Methylcissampareine (Dimethylwarifteine) and corresponding dihydroderivatives	49
3. *C. sympodialis*	(+)- Milonine	50
	(-)- Warifteine	50

Table III: Occurrence of Alkaloids (Continued...)

Genus/Species Name	Alkaloid	References
VI. *Curarea*		
1. *C. candicans*	(+)-Candicusine	51
*(Chondrodendron limaciifolium)***	(+)-Curine	40
*(Chondrodendron candicans)***	(+)-Isochondrodendrine	40
	(-)-Limacine	51
	(+)-Limacusine	51
	(-)-Limacine-2'α-*N*-oxide	51
	(-)-Limacine-2β-*N*-oxide	51
	(-)-Limacine-2'β-*N*-oxide	51
	(-)-Krukovine	51
VII. *Sciadotenia*		
1. *S. eichleriana*	(+)-Coclaurine	52
	(-)-Grisabine	52
	(+)-2-Norlimacusine	52
	(+)-Stepharine	52
2. *S. cayennensis*	(+)-Actinodaphnine	52
	(+)-Launobine	52
3. *S. toxifera*	(+)-*O,O'*- Dimethylcurine	53
	Epi-norcycleanine	53
	(-)-Isochondodendrine	53
	(+)-Isochondodendrine	54
	(+)-Sciadenine	54-55
	(+)-Sciadoferine	54
	(+)-Sciadoline	54
VIII. *Telitoxicum*		
1. *T. glaziovii*	Imenine	56
	Homomoschatoline	57
	Lysicamine	57
	Ouregidione	56
	Splendidine	56

Table III: Occurrence of Alkaloids (Continued...)

Genus/Species Name	Alkaloid	References
1. *T. glaziovii (Continued...)*	Telazoline	57
	Teladiazoline	57
	Telitoxine	56
2. *T. krukovii*	7-Chloronorcephardione B	58
	N-Formyldehydroanonaine	58
	Norcepharadione B	58
	N-Formylnornuciferine	58
	N-Demethyl-*N*-Formyldehydro-nuciferine	58
	Telikovinone	58
3. *T. peruvianum*	Homomoschatoline	59
	Lysicamine	59, 60
	Norrufescine	60
	Peruvianine	60
	Subsessiline	60
	Telazoline	60
	Telitoxine	60
	Telitoxinone	59
	Telisatin A	59
	Telisatin B	59

* *Abuta concolor* (Poepp & Endl.) = *Abuta concolor* (Benth) = *Abuta grandifolia* (Mart.) [3].

** *Chondrodendron candicans* (Rich.) Sandw. = *Chondrodendron limaciifolium* (Diels) Mold. = *Curarea candicans* (Rich.) Barneby &Krukoff [4]

*** *Chondrodendron toxicoferum* (Wedd.)Kruk. & Mold. = *Curarea toxicofera* (Wedd.) Barneby & Krukoff [4]

In the seventies, the main thrust of investigation was the isolation of pharmacologically active alkaloids. Recent research has indicated that in some of these species, the weakly basic alkaloids are present in the neutral fractions [56, 59]. A number of species from different genera have given positive tests for alkaloids but have not been investigated completely. These are *Abuta velutina, Cissampelos fasciculata, Disciphania lobata, Sciadotenia amazonica, S. duckei, S. paraënsis, S. sagotiana, S. sprucei*, and *Telitoxicum rodriguesii* [61].

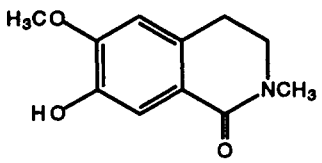
The genera from South American Menispermaceae which have given negative test results for alkaloids are *Odontocarya*, *Orthomene* and *Hyperbaena* [61]. The most collected and most wide spread plant, *O. schomburgkii*, gave an alkaloidal fraction [62] thus proving that the test results employed earlier are not completely reliable.

4. ALKALOIDS

The alkaloids of the tropical family, Menispermaceae have been studied throughout the world and reviewed by Tomita in 1952 [63] and Thornber in 1970 [64]. Most of the alkaloids are either 1-benzylisoquinoline or compounds theoretically derived from such precursors. The structures of alkaloids found to date in South American Menispermaceae are classified under general structure types.

4.1. Simple Isoquinolines and Benzyltetrahydroisoquinolines

Simple isoquinolines are not found in the Menispermaceae of South America. The isoquinolone thalifoline (**1**) was identified from *Abuta pahni* [32]. Benzyltetrahydroisoquinolines are the biogenetic precursors for aporphines, proaporphines, and oxoaporphines. Yet the only such compound isolated from South American Menispermaceae is (+) coclaurine (**2**) obtained from *Sciadotenia eichleriana* [52] and *Caryomene olivascens* [37-38]. The chemistry of simple isoquinolines [65], isoquinolones [66], and benzyltetrahydroisoquinolines [67] have been reviewed.



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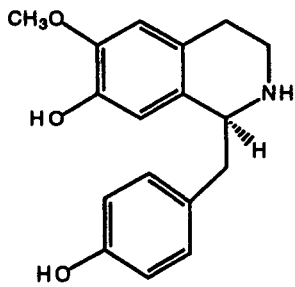

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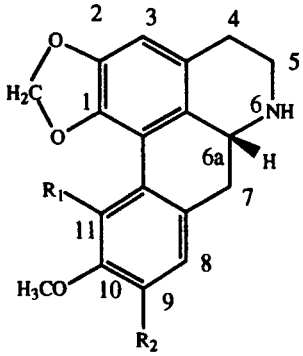
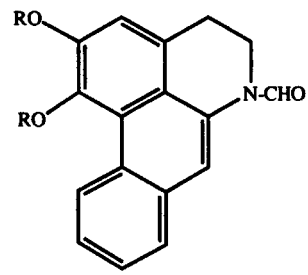
4.2. Aporphines

The number of aporphine alkaloids isolated from plants is increasing exponentially. Kametani and Honda covers the chemistry of these compounds in detail [68]. Comprehensive reviews of aporphinoids, their molecular formula, structural formula, and spectral data have been published [69]. Their presence in South American Menispermaceae is represented by (+)-

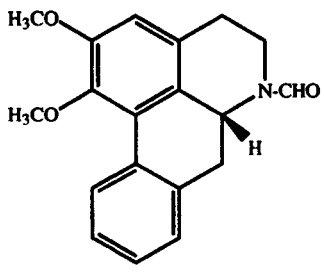
launobine (**3**) and (+)-actinodaphnine (**4**) from *Sciadotenia cayennensis* [52], and is also represented by *N*-formyldehydroanonaine (**5**), *N*-demethyl-*N*-formyldehydronuciferine (**6**), and *N*-formylnornuciferine (**7**) from *Telitoxicum krukovii* [58]. Recently, two isatins were isolated from *T. peruvianum* neutrals [59]. Aporphines and dehydroaporphines are thought to be the precursors of oxoaporphines [70,71]. These compounds can react with oxalic acid present in the plant to give isatins which can undergo oxidation to the corresponding oxoaporphines. Oxidation of telisatin A (**8**) with hydrogen peroxide in the presence of sodium hydroxide gave the oxoaporphine lysicamine (**10**) [72], while telisatin B (**9**) will give homomoschatoline (**11**).



3. R_1 = OH; R_2 =H
4. R_1 = H; R_2 = OH



5. R + R = CH_2
6. R = R = CH_3



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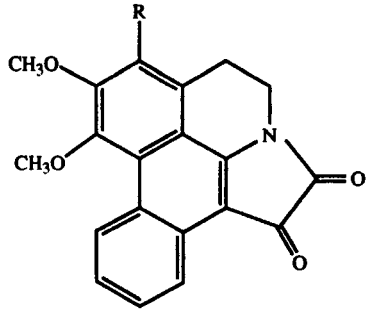
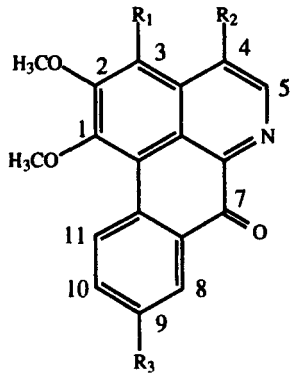


8. R = H
9. R = OCH_3

4.3. Oxoaporphines

Although the aporphine reviews [69] give about forty-eight oxoaporphines, only six were isolated from South American Menispermaceae: lysicamine (**10**) [35, 57, 60, 73], *O*-methylmoschatoline (homomoschatoline) (**11**) [30, 35, 57, 59], imenine (**12**) [31, 35, 56, 74], splendidine (**13**) [35, 56], subsessiline (**14**) [60, 75], and peruvianine (**15**) [60, 76]. These

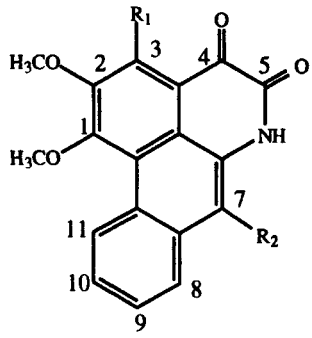
compounds are fully aromatic and highly colored with color ranging from yellow to orange. Imenine and splendidine are the only 4-oxygenated alkaloids of this type and were isolated from *Abuta imene*, *A. rufescens* (= *A. splendida*), and *T. glaziovii*. The structures of these alkaloids have been confirmed by synthesis. So far only some species of the genus *Abuta* and the three *Telitoxicum* species studied have given these colored compounds. Biogenetically, oxoaporphines are considered to be formed from aporphines [70,71].



10. $R_1 = R_2 = R_3 = H$
11. $R_2 = R_3 = H$; $R_1 = OCH_3$
12. $R_3 = H$; $R_1 = R_2 = OCH_3$
13. $R_1 = R_3 = H$; $R_2 = OCH_3$
14. $R_2 = H$; $R_1 = OCH_3$; $R_3 = OH$
15. $R_1 = R_2 = H$; $R_3 = OH$

4.4. 4,5-Dioxoaporphines

About eighteen 4,5-dioxoaporphines have been isolated from the plant kingdom [69]. The only three isolated from South American Menispermaceae are norcepharadione B (**16**) and 7-chloronorcepharadione B(**17**) from the neutral fraction of *Telitoxicum krukovii* [58] and ouregidione (**18**) from the neutral fraction of *T. glaziovii* [56]. But 7-chloronorcepharadione-B was considered to be an artifact [69]. Dehydro- and didehydroaporphines are the precursors of these reddish-orange compounds which fluoresce in dichloromethane solution [71].

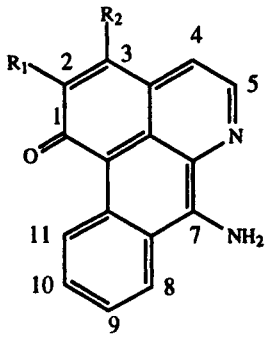


16. $R_1 = R_2 = H$
17. $R_1 = H$; $R_2 = Cl$
18. $R_1 = OCH_3$; $R_2 = H$

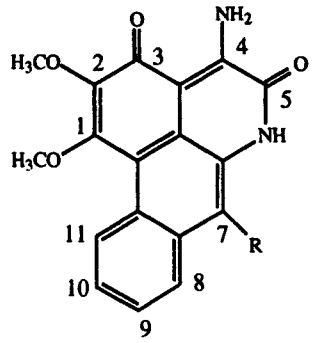
The *Telitoxicum* genus is closely related to some *Abuta* species such as *A. imene* and *A. rufescens* based on the constituents present which are mainly oxoaporphines and azafluoranthenes. It is possible that 4,5-dioxoaporphines may also be present in some of these *Abuta* species.

4.5. Miscellaneous Aporphinoids

A few alkaloids have been isolated with free amino groups on the aporphine nucleus. The isolation procedure employed ammonium hydroxide (1:1) to moisten the powdered plant material before extracting into ethyl acetate/95% ethanol (9:1). Currently, there is no strong evidence to prove whether these compounds are artifacts or not. Telazoline (**19**) from the nonphenolic fraction of *T. peruvianum* [60], telazoline and teladiazoline (**20**) from the neutral fraction of *T. glaziovii* [57], telitoxinone (**21**) from the neutral fraction of *T. peruvianum* [59], and telikovinone (**22**) from the neutral fraction of *T. krukovii* [58] were isolated.



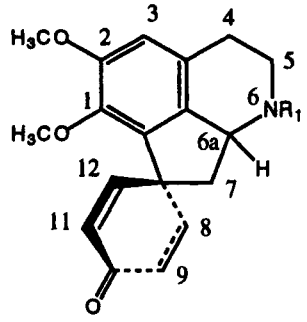
19. $R_1 = OCH_3$; $R_2 = H$
20. $R_1 = R_2 = OCH_3$



21. $R = OCH_3$
22. $R = H$

4.6. Proaporphines

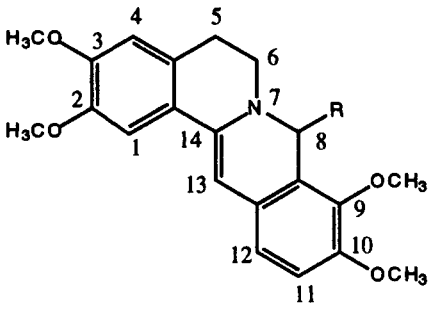
The genus *Caryomene* was split along with the genus *Orthomene* from *Anomospermum* by Krukoff and Barneby in 1971 [4]. The following proaporphines: (-)-stepharine (**23**); (-)-*N*-formylstepharine (**24**) and (-)-pronuciferine (**25**) were obtained from the stems or fruits of *Caryomene olivascens* [37, 38]. (+)-Stepharine (**26**) was isolated from *S. eichleriana* [52] and Abuta pahni [32]. For reviews of proaporphines, refer to Stuart and Cava [77] and Kyle and Cava [77].

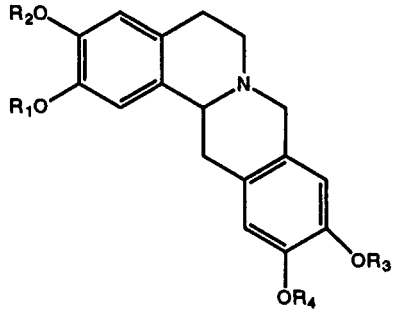


23. $R_1 = H$ (R)
24. $R_1 = CHO$ (R)
25. $R_1 = CH_3$ (R)
26. $R_1 = H$ (S)

4.7. Protoberberines and Tetrahydroprotoberberines

In the plant kingdom, one of the most widely distributed isoquinoline-type alkaloids is the protoberberines. Bhakuni and Jain, and Hanaoka give reviews dealing with the chemistry and the transformation reactions of these alkaloids [78]. Protoberberines, such as, (+)-pseudopalmatine (**27**) was isolated from *Caryomene olivascens* [38], and palmatine (**28**) and trichloromethyl-8-dihydropalmatine (**29**) have been identified from *Abuta bullata* [23]. Compound **29** may be an artifact. Even though palmatine (**28**) has been stated to have been isolated from *Abuta grandifolia*, the identity of the plant material cannot be verified [26]. Five 2,3,10,11-tetrasubstituted tetrahydroprotoberberines, (-)-coreximine (**30**), (-)-10-demethyldiscretine (**31**), (-)-discretine (**32**), (-)-govadine (**33**), (-)-xilopinine (**34**), were also found in the fruits of *Caryomene olivascens* [38].



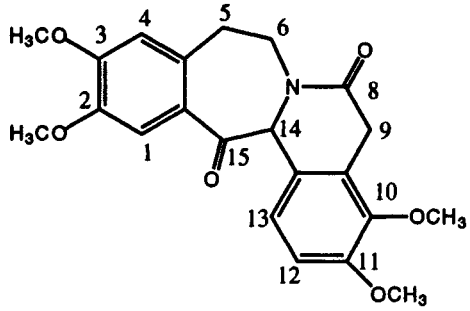


28. R = H; $\Delta^{7(8)}$ N^+ X^-
29. R = CCl_3

27. $R_1 = R_2 = R_3 = R_4 = OCH_3$, $\Delta^{7(8),\ 13}$;N^+ X^-
30. R_1 = H; $R_2 = R_3 = R_4 = OCH_3$
31. $R_1 = R_4 = OCH_3$; $R_2 = R_3$ = H
32. $R_1 = R_3 = R_4 = OCH_3$; R_2 = H
33. $R_2 = R_4 = OCH_3$; $R_1 = R_3$ = H
34. $R_1 = R_2 = R_3 = R_4 = OCH_3$

4.8. Isohomoprotoberberine

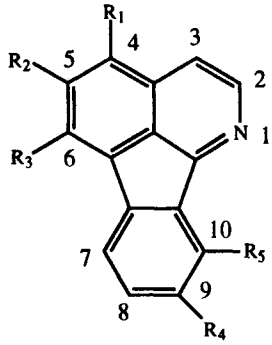
(+/-)Saulatine (**35**) is the only alkaloid of the isohomoprotoberberine type found in one South American Menispermaceae, *Abuta bullata*, collected from Guyana [23]. Homologation of protoberberine is thought to be the biogenetic pathway for the formation of this compound.



35

4.9. Azafluoranthenes

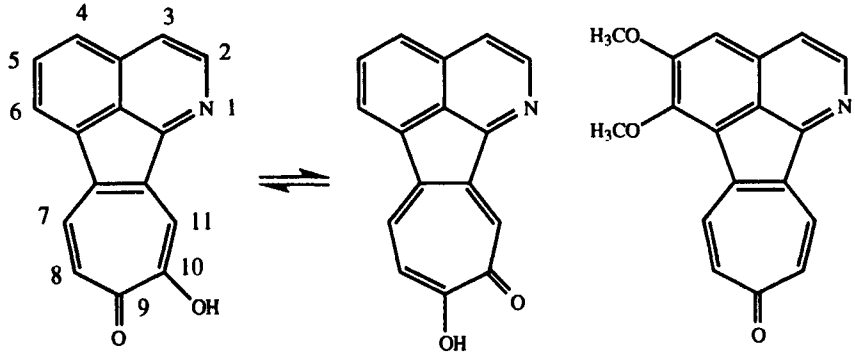
Indeno [1,2,3-i,j] isoquinoline (2-azafluoarnthene) was first reported in coal tar and its structure was confirmed by degradation to known compounds [79]. It is also a component of cigarette smoke [80] and is considered to be an air pollutant [81]. The natural products of this class were isolated for the first time from *Abuta rufescens* and *A. imene* [29]. Currently, there are six known azafluoranthene alkaloids, all isolated from Menispermaceae species: imeluteine (**36**) [29, 82], rufescine (**37**) [29, 82], norrufescine (**38**) [28, 83, 84], 5,6-dimethoxyindeno [1,2,3-i,j] isoquinoline (**39**) [85, 86], telitoxine (**40**) [56, 60, 87], and norimeluteine (**41**) [47]. Except norimeluteine, the azafluoranthene structures are confirmed by total syntheses. Of these, **39** is the only one isolated from *Triclisia gillettii* (Dewild) Staner [85], however it is not a South American Menispermaceae. Others were obtained from South American *Abuta* [29, 30], *Telitoxicum* [56, 60] and *Cissampelos* [47]. For a comprehensive review of azafluoranthene alkaloids including occurrence, structure, and syntheses refer to Buck [88].



36. $R_1 = R_2 = R_3 = R_4 = R_5 = OCH_3$
37. $R_1 = R_2 = R_3 = R_4 = OCH_3$; $R_5 = H$
38. $R_1 = R_2 = R_3 = OCH_3$; $R_4 = OH$; $R_5 = H$
39. $R_2 = R_3 = OCH_3$; $R_1 = R_4 = R_5 = H$
40. $R_2 = R_3 = OCH_3$; $R_4 = OH$; $R_1 = R_5 = H$
41. $R_1 = R_2 = R_3 = R_5 = OCH_3$; $R_4 = OH$

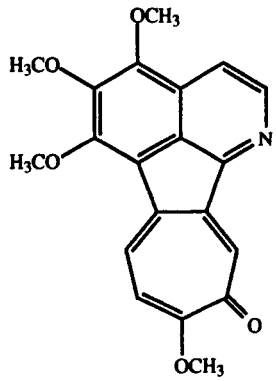
4.10. Tropoloisoquinolines

An isoquinoline system condensed to a tropolone is the skeleton for a tropoloisoquinoline alkaloid. The general structure of the five alkaloids of this type known so far are derivatives of the tautomers of 10-hydroxy-9H-azuleno[1,2,3-i,j]isoquinolin-9-one (**42**) and 9-hydroxy-10H-azuleno[1,2,3-i,j]isoquinoline-10-one (**43**) [88]. Recently, the first tropone-isquinoline alkaloid, pareitropone (**44**) has been isolated from the roots of the South American Menispermaceae, *Cissampelos pareira* [48].



42 **43** **44**

The first two known compounds imerubrine (**45**) [30] and grandirubrine (**46**) [25] were isolated in 1972 and 1980, respectively, from *A. imene* (*A. rufescens*) and *A. grandifolia*. The structure of imerubrine was determined by single crystal X-ray analysis [89]. Grandirubrine was the first tropoloisoquinoline alkaloid isolated with a free tropolone system, which on methylation gave imerubrine and isoimerubrine (**47**), thus proving the existence of the two tautomeric forms **42** and **43** [25]. Isoimerubrine along with the antileukemic pareirubrine A (**48**) and B (**49**) were isolated from *Cissampelos pareira* [47]. In ^{1}H-NMR spectra, pareirubrines A and B showed a closer resemblance to structure **42** than **43** by comparing the chemical shifts of H(7) and H(8) with those of imerubrine and isoimerubrine [47]. X-ray crystallography of pareirubrines showed that the molecules are almost completely planar and the conjugation extends throughout the molecules except for the substituents, the methoxyl groups. In the crystalline state, pareirubrines exist in only the tautomeric form **43** [47]. In other words, tropoloisoquinoline alkaloids with a free tropolone system are 10-hydroxy-9H-azuleno[1,2,3-i,j]isoquinolin-9-one (**42**) in solution and are 9-hydroxy-10H-azuleno[1,2,3-i,j]isoquinolin-10-one (**43**) in the solid state [47]. Brazilian *Abuta concolor (Abuta grandifolia)* also gave the antileukemic principles pareirubrine A and grandirubrine [24].



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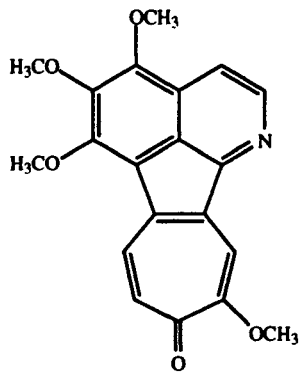

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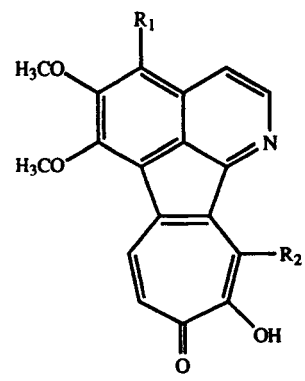
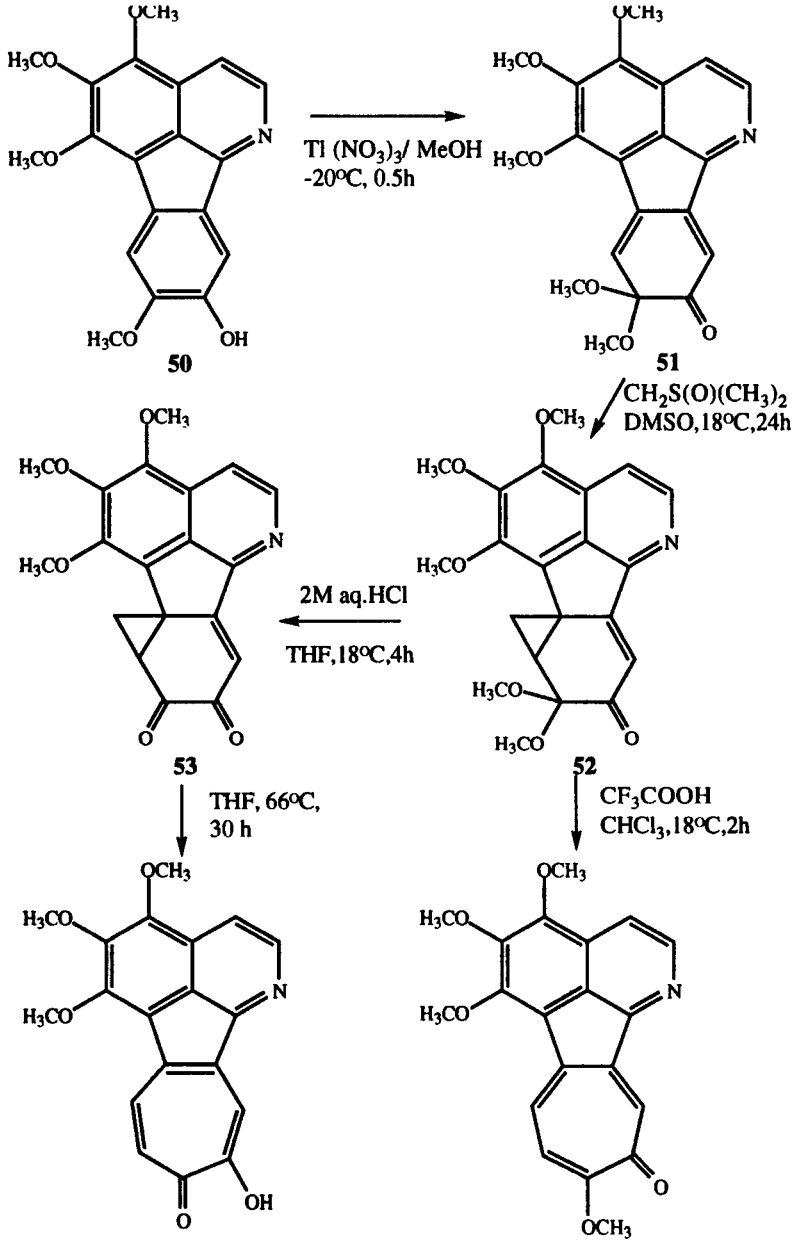


46. $R_1 = OCH_3$; $R_2 = H$
48. $R_1 = R_2 = OCH_3$
49. $R_1 = R_2 = H$

The first total synthesis of imerubrine was accomplished using azafluoranthene as an intermediate [90]. Treatment of the azafluoranthene (**50**) with thallium (III) nitrate in methanol at -20°C gave the dienone **51** in greater than 95% yield. Cyclopropanation of the dienone was accomplished using dimethylsulfoxonium methylide to give **52** in 38% yield. Reaction of compound **52** with trifluoroacetic acid in chloroform at room temperature gave imerubrine (**45**) (70%) [90]. Reaction of **52** with 2 M aqueous HCl in THF produced an orange product which was thought to be the diketone **53**. When the dienone was heated in refluxing THF for 30 h., an

87% yield of grandirubrine (**46**) was produced (Scheme 1). Another synthetic approach to these tropoloisoquinolines is reported by Boger and Takahashi [91].

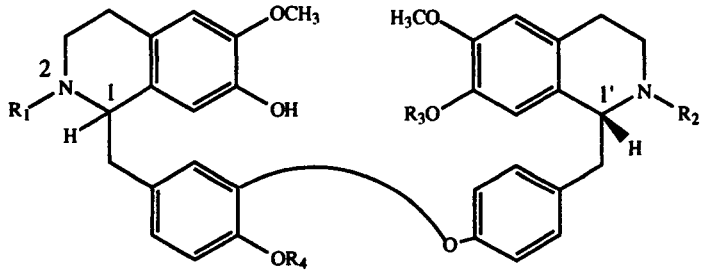


Scheme 1

4.11. Bisbenzylisoquinolines

Bisbenzylisoquinolines received importance based on their presence in the pharmacologically active arrow poisons of South American Indians. A recent review gives the chemistry, biogenesis and syntheses [92]. A comprehensive tabular review of over 400 of these phenylalanine-derived alkaloids was published by Guha [93] and Schiff [93].

There are approximately forty-five bisbenzylisoquinolines isolated from South American Menispermaceae. All the genera investigated so far (*Abuta, Sciadotenia, Caryomene, Curarea, Chondrodendron, Cissampelos*) have given these alkaloids except *Telitoxicum (Table III).* In the case of *Abuta,* a few species give these compounds while a few others give oxoaporphines, azafluoranthenes, or a combination of both (*A. splendida*). The structures of all the bisbenzylisoquinolines are given below based on their structural type and not based on the species from which the compound was isolated. For clarity, the names of the alkaloids are included with the structure instead of giving numerical numbers in the drawing.



(-)-Daurisoline: $R_1 = R_2 = R_3 = Me$; $R_4 = H$ (1R)

(-)-Grisabine: $R_1 = R_2 = R_4 = Me$; $R_3 = H$ (1S)

(-)-Lindoldhamine: $R_1 = R_2 = R_3 = R_4 = H$ (1R)

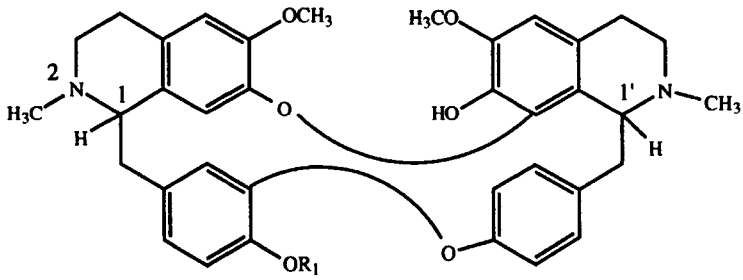
(-)-2-*N*-Methyllindoldhamine: $R_1 = Me$; $R_2 = R_3 = R_4 = H$ (1R)

(-)-2'-*N*-Methyllindoldhamine: $R_2 = Me$; $R_1 = R_3 = R_4 = H$ (1R)

(-)-*N,N'*-Dimethyllindoldhamine: $R_1 = R_2 = Me$; $R_3 = R_4 = H$ (1R)
(Guattegaumerine)

(-)-Magnoline (Grisabutine): $R_1 = R_2 = Me$; $R_3 = R_4 = H$ (1S)

(-)-2'-*N*-Nordaurisoline: $R_1 = R_3 = Me$; $R_2 = R_4 = H$ (1R)



(+)-Aromaline : R_1 = H (1R,1'S)

(+)-Candicusine: R_1 = H (1R,1'R)

(+)-1,2-Dehydro-2-norlimacusine: R_1 = Me; $\Delta^{1(2)}$ (1'R)

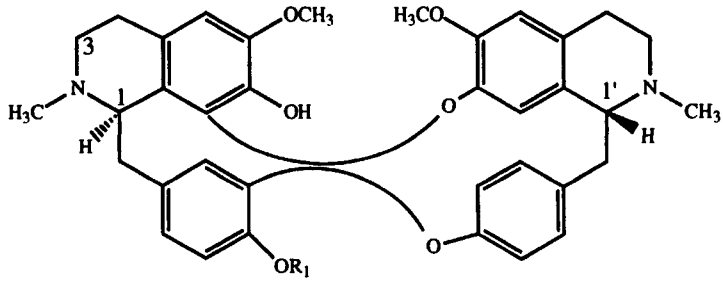
(+)-Homoaromaline : R_1 = Me (1R,1'S)

(+)-Limacusine : R_1 = Me (1R,1'R)

(-)-Macolidine: R_1 = H (1S,1'R)

(-)-Macoline: R_1 = H, $N^+MeH.X^-$,$N'^+Me_2.X^-$ (1S,1'R)

(+)-2-Norlimacusine: R_1 = Me; N(2)H, (1R,1'R)



(-)-Caryolivine: R_1 = Me, $\Delta^{1(2),3}$

(-)-Krukovine: R_1 = H

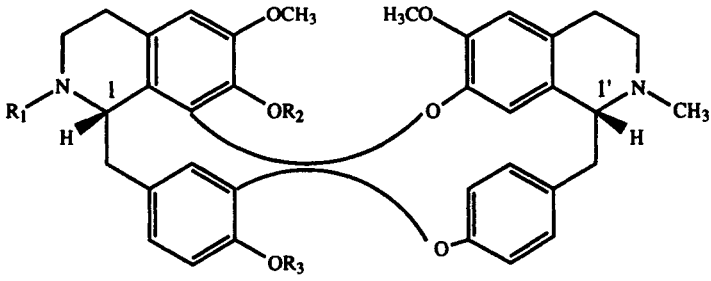
(-)-Limacine: R_1 = Me

(-)-Limacine 2'-α-*N*-oxide: R_1 = Me $N'^+—O^-$

(-)-Limacine 2-β-*N*-oxide: R_1 = Me $N^+—O^-$

(-)-Limacine 2'-β-*N*-oxide: R_1 = Me $N'^+— O^-$

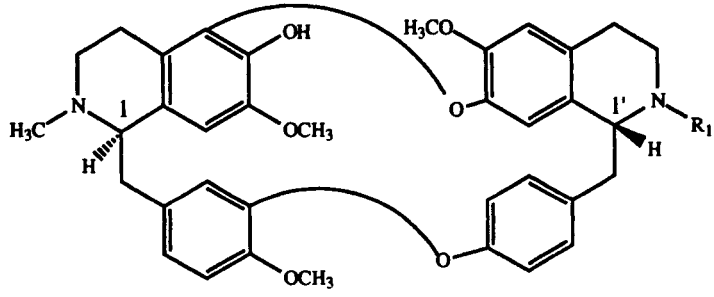
(-)-2-Norlimacine: R_1 = Me, N(2)-H



(-)-7-*O*-Demethylpeinamine: $R_1 = R_2 = R_3$=H

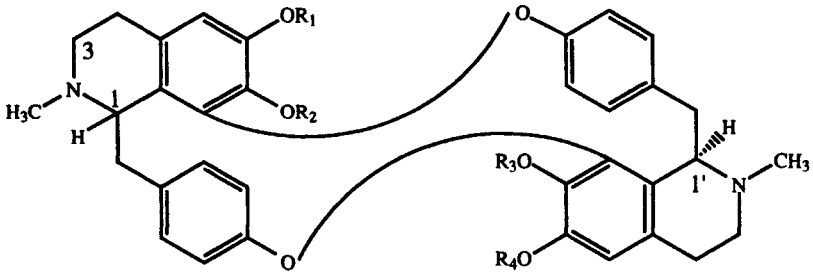
(-)-Peinamine: $R_1 = R_3$ = H; R_2 = Me

(-)-*N*-Methyl-7-*O*-demethylpeinamine: R_1 = Me; $R_2 = R_3$=H

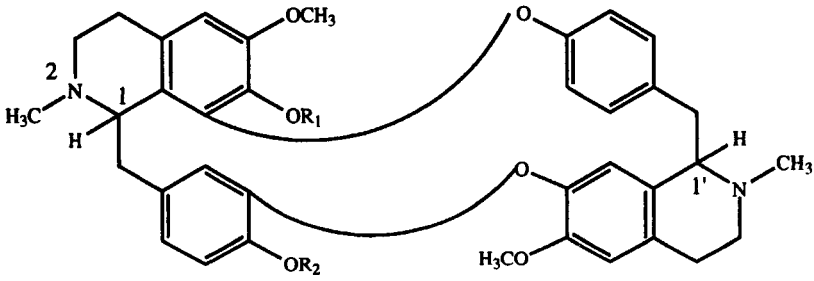


(-)-Norpanurensine: R_1 = H

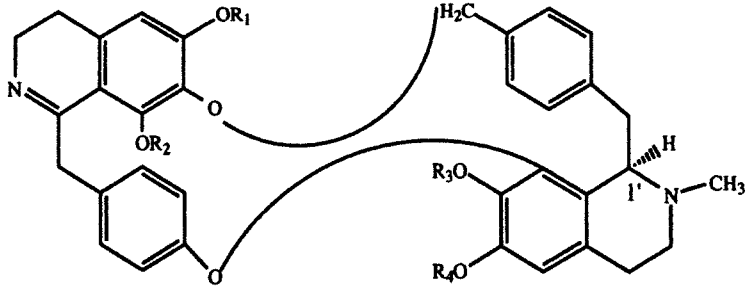
(-)-Panurensine: R_1 = Me



(-)-Cycleanine: $R_1 = R_2 = R_3 = R_4 = Me$ (1R)
(*O,O*-Dimethylisochondrodendrine)
(+)-Isochondodendrine: $R_1 = R_4 = Me$; $R_2 = R_3 = H$ (1R)
(-)-Norcycleanine: $R_1 = R_2 = R_4 = Me$; $R_3 = H$ (1R)
(+)-Sciadenine: $R_1 = R_3 = R_4 = Me$; $R_2 = H$, (1S)
(+)-Sciadoferine: $R_1 = R_3 = R_4 = Me$; $R_2 = H$, $\Delta^{1(2)}$
(+)-Sciadoline: $R_1 = R_3 = R_4 = Me$; $R_2 = H$, $\Delta^{1(2),3}$



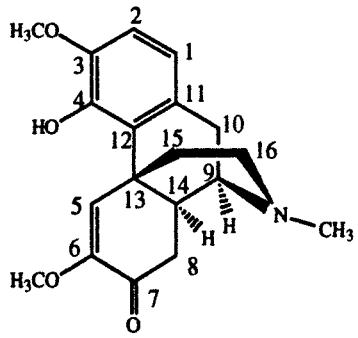
(+)-Chondrocurine [(+)-Tubocurine]: $R_1 = R_2 = H$ (1R, 1'S)
(+)-Chondrocurarine: $R_1 = R_2 = H$; $N^+Me_2.X^-$, $N'^+Me_2.X^-$ (1R,1'S)
(-)-Chondrofoline: $R_1 = Me$; $R_2 = H$ (1S,1'S)
(-)-Curine [(-)-Bebeerine]: $R_1 = R_2 = H$ (1R,1'R)
(+)-Curine [(+)-Bebeerine]: $R_1 = R_2 = H$ (1S,1'S)
(+)-*O,O'*-Dimethylcurine: $R_1 = R_2 = Me$ (1R,1'R)
(+)-Tubocurarine: $R_1 = R_2 = H$; $N^+Me_2.X^-$, $N'^+MeH.X^-$ (1R,1'S)
(-)-Tubocurarine: $R_1 = R_2 = H$; $N^+Me_2.X^-$, $N'^+MeH.X^-$ (1S,1'R)



(-)-Cissampareine (*O*-methylwarifteine): $R_1 = R_3 = R_4 = Me$; $R_2 = H$
(-)-*O*-Methylcissampareine: $R_1 = R_2 = R_3 = R_4 = Me$
(*O,O*-dimethylwarifteine)
(-)-Warifteine: $R_1 = Me$, $R_2 = H$; $R_3 = H$, $R_4 = Me$, or vice versa

4.12. Morphinandienone

The one and only morphinandienone, (+)-milonine (8,14-Dihydromorphinandienone) (**54**), was isolated from the dried leaves of *Cissampelos sympodialis*, a plant used in medicine in northeastern Brazil [50]. Blasko and Cordell reviewed morphinandienone, homomorphinandienone, and other related alkaloids with respect to isolation, structure, elucidation, spectroscopy, and synthesis [94].



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5. PHARMACOLOGY

Menispermaceae contains many medicinal and toxic plants. Some of the South American Menispermaceae were used by native Indians for their curare activity [7]. The western world was introduced to the muscle relaxants by the Spaniards, who encountered these arrow poisons in 1514 [7, 95]. The main South American Menispermaceae species used to prepare pot/tube curare are *Chondrodendron tomentosum*, and three of the four known curarea genus, *Curarea candicans, C. tecunarum and C. toxicofera* [7].

The active principle of tube-curare, (+) tubocurarine chloride, was first isolated in 1935 [96]. Its structure was revised based on nmr results that indicated both nitrogens were not quaternary, as previously thought, but that one is tertiary [97]. X-ray crystallographic analysis also confirmed the above conclusion [98].

Since its discovery, this compound is used as a muscle relaxant in surgical anesthesia [36]. The literature on pharmacology of tubocurarine is vast. An overview of the pharmacological activity of (+)-tubocurarine (chloride) has been reported by Schiff [93]. *Chondrodendron tomentosum* is the only known natural source of (+)-tubocurarine [95]. Pot curare was also investigated and found to comprise alkaloids for about half of its weight, mainly salts of bisbenzylisoquinolines [99]. The two species used in the production of the drug, pareira brava are *Chondrodendron platiphyllum* and *Chondrodendron microphyllum* [40], the first one being the major source of the drug [3].

Other ingredients found in the curare preparation of the natives belong to the genera *Abuta, Anomospermum, Cissampelos, Orthomene, Sciadotenia and Telitoxicum* [7, 8]. The neuromuscular blocking activity of the extracts of roots of *Cissampelos ovalifolia* was equivalent to that of *Chondrodendron tomentosum* [7]. Cissampareine, the cytotoxic bisbenzylisoquinoline alkaloid has been isolated from both *C. ovalifolia* and *C. pareira* [49, 46]. Caryomene is not included in the curare preparation of the Indians, but *Caryomene foveolata* (Barneby & Krukoff) from Brazil is said to be poisonous [4].

It is worthwhile to mention that curarizing arrow/dart poisons have been reported from Central Africa [7, 100], and Western Malaysia [101]. Even though other ingredients from different families are present, the curare activity may come from *Strychnos* (Loganiaceae), which is also a main ingredient from Amazon curare. More information on the wider use of curare plants from both Menispermaceae and Loganiaceae, their geographical distribution, botany, chemistry and pharmacology is given by Bisset [7]. Two other reviews of the Amazon plants have been published by Schultes and Raffauf [8] and by Duke and Vasquez [9]. The active principles are found in stem woods and roots. The genera and the species used as medicine by native Indians are given in Table IV.

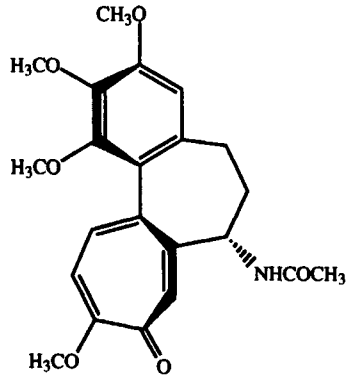
Table IV. The genera and the species of South American Menispermaceae used as medicine by native Indians.

Genera	*Species*				
ABUTA	*grandifolia* [8,9]	*grisebachii* [8,14]	*imene* [1,3,8,9]	*obovata* [1,8]	*pahni* [4,8]
	rufescens (*splendida*) [1,4,8,9]	*sandwithiana* [9,14]	*selloana* [8]	*solimosensis* [9]	*vaupesensis* [8]
ANOMOSPERMUM	*chloranthum* [8,9]	*grandifolium* [1]	*reticulatum* [8]		
CHONDRODENDRON	*microphyllum* [1]	*platiphyllum* [1,8]	*tomentosum* [1,8,9]		
CISSAMPELOS	*andromorpha* [8,9]	*fasciculata* [8]	*ovalifolia* [49]	*pareira* [8,9]	
CURAREA	*candicans* [3,4]	*tecunarum* [3,4,8,9]	*toxicofera* [3,4,8,9]		
ORTHOMENE	*schomburgkii* [8,9]				
SCIADOTENIA	*duckei* [8]	*toxifera* [1,8]			
ODONTOCARYA	*tripetala* [8]				
TELITOXICUM	*duckei* [8]	*minutiflorum* [3,8]	*peruvianum* [3,8]		

The National Cancer Institute under the Cancer Chemotherapy National Service Center (CCNSC) has screened plant and animal extracts for anticancer activity since 1955 [102]. A correlation of NCI screening data with several groups of medicinal and poisonous plants found increased activity in plants used as arrow and fish poisons and anthelmintics when compared with plants selected at random [103]. The highest correlation was found to be between poisonous or toxic plants and in vitro cell cytotoxicity. The total bases and neutrals or the ethanolic extracts of *Abuta, Sciadotenia, Telitoxicum, Caryomene, Cissampelos, Odontocarya, Borismene, Orthomene and Chondrodendron* were screened by NCI [104]. A few of the species tested were active but the active principles were not isolated, except the cytotoxic bisbenzylisoquinoline alkaloid, cissampareine, from *Cissampelos pareira* [46]. Although *A. panurensis* is not reported as an ingredient of curare, its alkaloid extract has shown significant activity in KB-nasopharynx cell system [33]. The alkaloids isolated are bisbenzyisoquinolines.

The antimicrobial activity of many of bisbenzyisoquinolines have been reported. Candicusine, cycleanine and aromaline were the most active compounds [105].

Even though the tropoloisoquinoline alkaloids imerubrine (**45**), from *Abuta imene* [30] and grandirubrine (**46**), from *Abuta grandifolia* [25], were isolated in 1972 and 1980, respectively, the amount of material available prevented their biological testing. Both of these alkaloids are biogenetically and structurally related to the potent antimitotic agent colchicine (**55**) and are compounds of potential therapeutic interest [106].



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Crude extracts of the plants from which imerubrine and grandirubrine have been isolated are the subject of patents as wound-healing agents and neoplasm inhibitors [107]. These compounds were also tested for antitubulin activity, and imerubrine was found to show weak inhibition of polymerization [91]. Along with grandirubrine (**46**) and isoimerubrine (**47**), the antileukemic pareirubrines A (**48**) and B (**49**), and a cytotoxic azafluoranthene, norimeluteine (**41**), were identified from the extract of South American *Cissampelos pareira* [47]. Pareirubrine A and grandirubrine were also isolated from *Abuta concolor (Abuta grandifolia)* and were found to be antileukemic [24]. Based on cytotoxic bioassay against P-388 leukemia cells, the authors showed that in the tropolo-isoquinoline system, the C(9) carbonyl group (isoimerubrine) shows stronger cytotoxicity than C(10) carbonyl group (imerubrine). This data does not agree with that of colchicine which is more potent than isocolchicine [106]. Pareitropone (**44**) with a tropone moiety showed more potent cytotoxicity against cultured P-388 cells than four of the five tropoloisoquinolines: imerubrine, grandirubrine, pareirubrines A and B [48]. Also azafluoranthenes are found to be less active than these tropoloisoquinolines.

The antimicrobial activity of aporphine alkaloids in general has been tested and oxoaporphines were the most active [108]. Some of these alkaloids are found to be toxic for mammalian cells in tissue cultures [109]. In another study of antimicrobial activity of fourteen benzylisoquinoline alkaloids, two oxoaporphines isolated from species of South American Menispermaceae, lysicamine (**10**) and *O*-methylmoschatoline (homomoschatoline) (**11**), had activity against cocci and gram positive bacilli, including *Mycobacterium phlei* [110].

Mutagenecity to Salmonella typhimurium TA 100 and TA 98 in the presence or absence of S9 mix was tested for forty-four isoquinoline alkaloids [111]. Liriodenine was the most active oxoaporphine while lysicamine was the least active. Based on the absence of mutagenicity of 1,2,10-trimethoxy-7-oxoaporphine, the authors concluded that substitution at position 10 of the 7-oxoaporphine system cancelled the mutagenicity.

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The Chemistry and Biological Activity of Calystegines and Related *Nor*tropane Alkaloids

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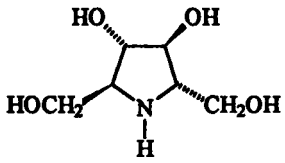
CONTENTS

1. INTRODUCTION

Polyhydroxy alkaloids have steadily increased in both numbers and biological significance since the discovery of the first members of the group in the late 1970s. Unlike most classes of alkaloids, which are characterized by a common structural feature, these are conceptually associated by the presence of a high degree of hydroxylation and an ability to inhibit glycosidases, a property which has profound effects upon biological systems as a consequence of disruption of the essential cellular function of glycoprotein processing [1,2].



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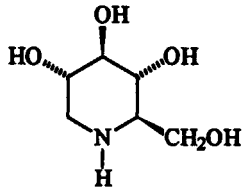

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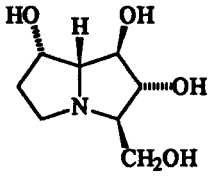

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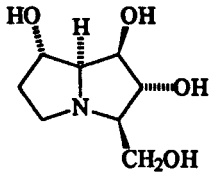

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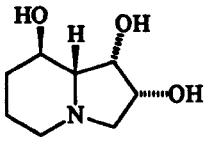

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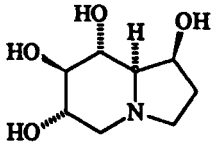

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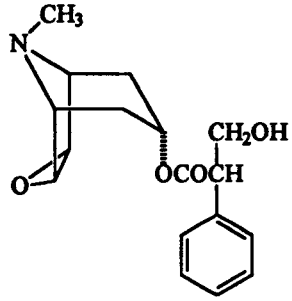
Until recently, five structural classes were encompassed by these properties: pyrrolidines, e.g. 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) (1); piperidines, e.g. deoxynojirimycin (2); pyrrolizidines, e.g. australine (3) and alexine (4); and indolizidines, e.g. swainsonine (5) and castanospermine (6) [3-8]. An entirely new class has now be added to this group with the discovery of the calystegines[1], bicyclic alkaloids which possess a *nor*tropane structure bearing hydroxyl groups varying in position and stereochemistry [9,10]. Alkaloids of the tropane class are a well-established group with valuable pharmacological properties which have been frequently and comprehensively reviewed [11,12]. The most familiar representative is scopolamine (7) which is widely used as a mydriatic in ophthamology, to suppress motion sickness, as a sedative and preanesthetic, and to prevent muscle spasm. Scopolamine and related alkaloids occur in the plant family Solanaceae and bear a methyl substituent on the nitrogen atom. In contrast, *nor*tropane alkaloids (i.e. those lacking the *N*-methyl group) have rarely been isolated although they occasionally occur as minor constituents in plants containing the familiar tropane alkaloids.



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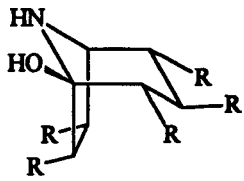

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This Chapter is designed to review the chemistry and biological activity of the calystegines, which at the present time are rarely found to occur in association with the correspondingly substituted tropane alkaloids. However, new sources of calystegines are rapidly being discovered and the occurrence of additional polyhydroxy tropane alkaloids cannot be excluded.

---

[1]These compounds were originally named as "calystegins" but the term "calystegines" has subsequently been adopted by certain research groups. In order to conform with the more usual convention for naming of alkaloids and to emphasize their relationship to other polyhydroxy alkaloids, the latter term will be used throughout this Chapter.

2. ISOLATION AND STRUCTURAL ELUCIDATION.

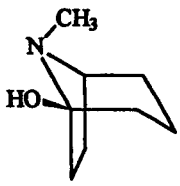
In 1988, Tepfer *et al.* [9] reported the discovery of a group of novel compounds in root exudates of the bindweed or morning glory, *Calystegia sepium* (Convolvulaceae). Their presence was also detected in another bindweed, *Convolvulus arvensis*, as well as in *Atropa belladona* (Solanaceae) but not in 102 other species from twenty-four plant families that were examined. These compounds, named calystegins (in the context of this review, calystegines), were proposed as nutritional mediators for rhizosphere bacteria, establishing and maintaining specific plant-bacterium relationships. Experiments showed that the calystegines provided selective nutrition to *Rhizobium meliloti* 41, which was the only bacterium of forty-two tested that could catabolize and utilize them as a sole source of carbon and nitrogen (Section 7.2).

The structures of these calystegines were subsequently established as a series of closely related polyhydroxy-*nor*tropanes of the general structure (**8**, R= -H or -OH) [10], classified into two groups, calystegines A and B, on the basis of their relative mobility on paper electrophoresis. Sufficient quantities for structural elucidation were obtained by genetic transformation with *Agrobacterium rhizogenes* of *C. sepium* roots, which were then grown *in vitro* in fermenter cultures to produce kilogram quantities of transformed roots. The calystegine A group was separated by HPLC into four components (A_1, A_2, A_3 and A_4), of which the structure of the major compound, calystegine A_3, was established by a combination of high-resolution mass spectrometry and ^1H and ^{13}C NMR spectroscopy [10]. The calystegine B group was separated into two constituents, B_1 and B_2, and the structures of both determined in a similar manner.

The calystegines isolated from *C. sepium* possess three structural features in common: a *nor*tropane ring system; two or three hydroxyl group substituents on either the five- or six-membered rings; and a novel aminoketal functionality, which generates a tertiary hydroxyl group at the bicyclic ring bridgehead. The latter had been discovered previously in physoperuvine (**9**), isolated from *Physalis peruviana* (Solanaceae), but this compound differs from the calystegines in bearing an *N*-methyl group and therefore is classified as a tropane rather than a *nor*tropane alkaloid. The aminoketal grouping in physoperuvine was recognized as existing either as the bridged bicyclic tropane ring system (**9**), or as the 4-aminocycloheptanone (**10**), in tautomeric equilibrium [13,14]. Evidence from CD measurements suggested that the bicyclic form predominated by ca. 45:1, and an X-ray crystal structure of the hydrochloride salt established that this also had the tropane structure [14]. Recent syntheses of physoperuvine (**9**) and *nor*physoperuvine (**11**) have enabled ^{13}C NMR spectroscopic studies to be performed, with particular reference to the bridgehead quaternary carbon, which showed that at low temperatures (223°K) the bicyclic:monocyclic ratio for (**9**) and for (**11**) is 98:2 and ca. 100:0 respectively [15]. At ambient temperatures however, the signals in both the ^1H and ^{13}C spectra were too broad and complex to be analysable, while at higher temperatures they were indicative of the presence of some of the monocyclic tautomer. The ease with which such tautomerism can occur must be taken into consideration when dealing with the structures and

reactivity of the calystegines in solution since the major tautomeric form may be dependant upon the pH and the number, regiochemistry and stereochemistry of substituent hydroxyl groups.

An additional tropane alkaloid having the aminoketal moiety, 1-hydroxytropacocaine (**12**), has recently been isolated from varieties of *Erythroxylum novogranatense* (Erythroxylaceae) but the NMR evidence indicates that this alkaloid exists entirely in the bicyclic form [16]. Another alkaloid with certain similarities to the calystegines is Bao Gong Teng A (**13**), a monoacetylated dihydroxy *nor*tropane isolated from the Chinese herb *Erycibe obtusifolia*, which has been shown to be effective for the treatment of glaucoma [17]. However this compound lacks the bridgehead hydroxyl group and therefore cannot tautomerize to an aminocycloheptanone. The structure and absolute configuration have been confirmed by synthesis [18,19].



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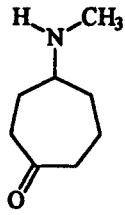

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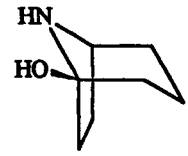

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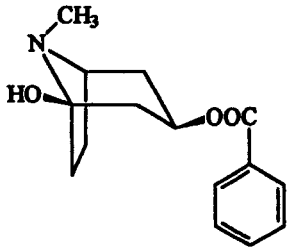

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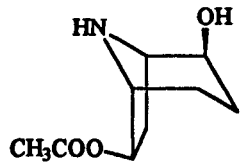

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2.1 Isolation of Calystegines

Calystegines, in common with other classes of polyhydroxy alkaloids, are highly water-soluble and cannot be isolated by conventional alkaloid separation techniques which employ acid-base partioning methods with non-hydroxylic organic solvents [20]. Ion-exchange chromatography is therefore extensively employed for separation of the alkaloid fraction from neutral and acidic compounds present in the extract [21,22].

Calystegines were originally obtained by Tepfer *et al.* [9] from transformed root cultures of *Calystegia sepium* grown in a nutrient medium, into which they are exuded. The alkaloids are also present in the roots themselves, from which they can be extracted with water. The aqueous solution was fractionated on Dowex 50W-X8 (H^+ form) to give a mixture of the calystegines and amino acids, from which neutral sugars had been removed. The alkaloids were enriched by treatment with a culture of *Agrobacterium tumefaciens* B6 806 which catabolized the amino acids present, removing most of the compounds showing ninhydrin-positive spots on high-voltage paper electrophoresis. Elution from a GF 05 gel filtration column at pH 7 with the volatile buffer trimethylammonium carbonate separated the calystegines into two major fractions, A and B. Each of these fractions was purified to homogeneity by HPLC on a Zorbax NH_2 column eluted with acetonitrile-water (4:1), the calystegine A and B fractions being separated into four (A_1, A_2, A_3 and A_4) and two (B_1 and B_2) components, respectively.

Using similar techniques, calystegines A_3 and B_2 were subsequently isolated by Nash *et al.* [23] from certain species of the plant family Solanaceae, including several varieties of potatoes, and from moths and butterflies which feed on *Datura* and *Solanum*. A 75% ethanol extract of either plant material or insects was subjected to ion-exchange chromatography on Amberlite CG-50 (H^+ form). Elution with 0.1M acetic acid permitted the alkaloids to be separated, with calystegine B_2 preceding calystegine A_3.

Recent investigations by Asano and coworkers have resulted in the isolation and characterization of calystegines from the plant families Moraceae and Solanaceae, including additional members of the A and B classes, a pentahydroxy alkaloid (calystegine C_1), and a calystegine with a bridgehead amino group (calystegine N_1) [24-27]. Purification was achieved by extensive ion-exchange chromatography of the hot water extract of the plant material under examination which was treated with an equivalent volume of methanol to precipitate the less water-soluble components. The filtered solution was then applied to an Amberlite IR-120B (H^+ form) column and the total alkaloid fraction eluted with 0.5N ammonium hydroxide. Subsequent chromatography on Amberlite CG-50 (NH_4^+ form) eluted with water, followed by 0.5N ammonium hydroxide, gave a series of four fractions each of which was further fractionated. Typically, each fraction was chromatographed on columns of Dowex 1-X2 (OH^- form) with water, Dowex 50W-X8 (pyridinium form) eluted with a pH 6 pyridinium acetate buffer, and CM Sephadex C-25 (NH_4^+ form) eluted with 0.01N ammonium hydroxide. Sephadex LH-20 in the reverse-phase mode was also employed, the alkaloids being eluted

with butanol-acetic acid-water-methanol (10:10:90:15).

By the use of these techniques as many as eighteen alkaloids have been isolated from a single plant, including polyhydroxy-pyrrolidines and -piperidines and their glycoside derivatives, and the calystegines [24,25]. It should be noted that the initial Amberlite CG-50 separation yields the more highly hydroxylated compounds, especially the glycosides, in the first fraction whereas the calystegines occur primarily in the middle two fractions. The final fraction generally contains the simple monocyclic alkaloids such as deoxynojirimycin (2).

Another technique which has been applied is the use of multilayer coil countercurrent chromatography (MLCCC) for separation of the partially purified extract obtained from ion-exchange chromatography into calystegine A and B fractions [28]. A column (120 m X 1.66 mm i.d.) was pre-equilibrated with a stationary phase of 0.1M ammonia in water, saturated with *n*-butanol and a mobile phase of *n*-butanol, saturated with 0.1M ammonia in water. The sample was applied and the system operated at 800 rpm and a flow rate of 1 mL/minute for 24 hours and fractions collected and monitored by TLC. The calystegine A fraction eluted in the butanol phase after about 20 hours (ca. 1200 mL) while the calystegines B were retained in the aqueous stationary phase. Rechromatography in the same system permitted calystegine A_3 to be isolated, but the technique is too time-consuming for general application.

In situations when analysis is required to establish the presence and identity of calystegines in a plant extract it is not necessary to utilize a complex series of chromatographic separations. The plant material can be extracted with methanol or methanol-water and passage over a Dowex 50W-X8 column with dilute ammonium hydroxide as eluant provides substantial purification, yielding the alkaloid fraction, including other polyhydroxy alkaloids, contaminated only by basic amino acids. The extract can thus be rapidly prepared for analysis [29,30].

2.2 Structural Elucidation of Calystegines

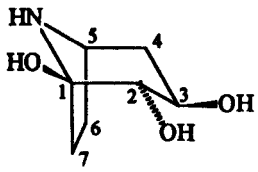
All of the calystegines isolated to date have been obtained as amorphous white solids and melting points have therefore not been reported. Structures have been determined by spectroscopic methods, in particular ^1H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry. Additional confirmatory evidence has been obtained from analysis of the alkaloids as trimethylsilyl ether derivatives by gas chromatography - mass spectrometry (Section 4.4). In certain cases the structures and absolute configurations have been confirmed by synthesis (Section 6).

2.2.1 Calystegine A_3 (14)

The molecular formula of calystegine A_3 (1α,2β,3α-trihydroxy*nor*tropane) was first established as $C_7H_{13}NO_3$, 159.0900, by high resolution EI mass spectrometry. Ammonia ion CIMS

gave a value of *m/z* 160 $[M+H]^+$, with a major fragment ion at *m/z* 142, corresponding to loss of H_2O [10,31]. Subsequent FAB-MS experiments confirmed these values and the specific rotation was recorded as $[\alpha]_D$ -17.3° (*c* 0.47, H_2O) [27]. The absence of evidence for double bonds in the molecule required a bicyclic structure for the alkaloid, which could best be accommodated by either a pyrrolizidine or *nor*tropane ring system.

^{13}C NMR in D_2O solution established the presence of a single quaternary carbon at δ93.6 ppm, three methine carbons (δ54.3, 72.8 and 82.5 ppm), and three methylene carbon atoms (δ29.4, 31.8 and 42.6 ppm) (10,27,31). The relatively low-field shifts of the quaternary and two of the methine carbons were indicative of the presence of oxygen substituents on these atoms and the *nor*tropane system was thus established, since a pyrrolizidine ring would require four methylene carbon resonances. ^1H NMR confirmed this structure, the six methylene protons occurring as a complex pattern ca. δ1.45-2.12 ppm, and a methine proton (δ3.46, H(5)) showing coupling to one proton each on two different methylene groups. One of the remaining methine protons (δ3.40, H(2)) showed long-range coupling (J=1.5Hz) to a proton of a methylene group and was also coupled to the other methine proton at δ 3.70 (H(3)). The value of the coupling (J=8.4Hz) was indicative of a *trans*-diaxial relationship, so that the substituent hydroxyl groups have to be *trans*-diequatorial in disposition.



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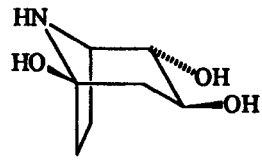

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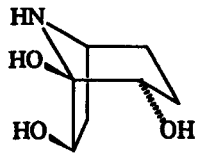

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2.2.2 Calystegine A_5 (15)

The MS of calystegine A_5 (1α,3α,4β-trihydroxy*nor*tropane), $[\alpha]_D$ +68.7° (*c* 0.60, H_2O) established that this alkaloid is isomeric with calystegine A_3 [26]. The chemical shifts in the ^{13}C NMR spectrum were quite similar to those in the spectrum of the latter, suggesting that the alkaloid was a stereoisomer or that the two secondary hydroxyl groups occupied different positions on the six-membered ring of the *nor*tropane system. However, the ^1H NMR showed significant differences, in particular the coupling of the bridgehead proton at C(5) to a downfield methine proton at δ3.47 ppm (H(4)) and to one proton of a single methylene group, which thus established the presence of a hydroxyl group at C(4). The proton at this position was coupled to another methine proton at δ3.72 ppm (H(3)), the magnitude of the coupling (J=8.8Hz), indicating a *trans*-diaxial arrangement so that the substituent hydroxyl groups at positions 3 and 4 must also be *trans* and diequatorial to each other.

2.2.3 Calystegine A_6 (16)

The FAB-MS of calystegine A_6 (1α,2β,7α-trihydroxy*nor*tropane), $[\alpha]_D$ -27.6° (*c* 0.37, H_2O), showed that this alkaloid was isomeric with the previously identified members of the calystegine A series [27]. Although the quaternary carbon showed a similar chemical shift in all three isomers, significant upfield shifts for one of the methylene carbons and one of the hydroxyl-substituted methines indicated that the two secondary hydroxyl groups do not occupy adjacent positions.

Analysis of the ^1H NMR spectrum, in concert with decoupling and ^1H-^{13}C COSY experiments, located the two secondary hydroxyl groups on C(2) and C(7). The coupling constants for H(2) (J=11.4 and 6.2Hz) established an axial orientation for this proton and a significant nOe between H(3ax) and H(7) indicated an *endo* orientation for the latter. A unique feature of calystegine A_6 relative to all other calystegines having a hydroxylated five-membered ring is the location of this substituent adjacent to the bridgehead hydroxyl group rather than at C(6). This was confirmed by the complex multiplet for H(5) at δ3.45, rather than the simpler pattern to be expected if this proton were adjacent to a methylene and a methine group.

2.2.4 Calystegine B_1 (17)

The presence of an additional oxygen atom in calystegine B_1 (1α,2β,3α,6α-tetrahydroxy*nor*tropane) relative to the trihydroxylated calystegine A series was established by the high resolution MS molecular formula, $C_7H_{13}NO_4$, 175.0848, and confirmed by ammonia CI and FAB-MS [10,26,31]. The alkaloid is levorotatory with $[\alpha]_D$ -13.0° (*c* 0.64, H_2O).

^{13}C NMR confirmed the presence of the bridgehead hydroxyl group through the typical quaternary carbon at δ93.8 ppm, and an extra secondary hydroxyl group by the presence of four

methine carbons (δ62.9, 72.7, 76.0 and 81.3 ppm), but only two methylene carbon atoms (δ38.9 and 43.6 ppm). The large disparity in chemical shifts between the latter suggested that they were located in different rings and in particular that the one most downfield must be between two hydroxyl groups. This conclusion can only be accomodated by a hydroxyl substituent at C(6), with the methylene group at C(7). In the ^{1}H NMR spectrum the signal assigned to the bridgehead proton H(5) showed no coupling to the H(6) proton, indicating a dihedral angle approaching 90° which requires an *endo* configuration for the latter. Decoupling experiments established the presence of a methylene group at C(4) and the remaining two hydroxyl groups are therefore located at C(2) and C(3), with chemical shifts similar to those observed for calystegine A_3. Coupling constants for the H(2) and H(3) protons, virtually identical to those of the latter alkaloid, indicate that the vicinal hydroxy groups must have the same *trans*-diequatorial configuration.

The structure of a 3-*O*-β-D-glucopyranoside of calystegine B_1 was determined by the use of 2D NMR spectroscopy [32]. The linkage site was established by a correlation peak in the HMBC spectrum between the anomeric proton and the aglycone C(3) carbon. A downfield shift for the C(3) resonance and upfield shifts for the C(2) and C(4) resonances in the ^{13}C NMR spectrum, relative to the aglycone, provided additional confirmation for the structure.

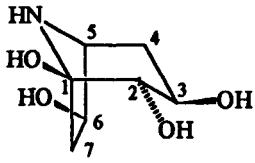
2.2.5 Calystegine B_2 (18)

High resolution MS determinations established calystegine B_2 (1α,2β,3α,4β-tetrahydroxy*nor*tropane), $[\alpha]_D$ +27.2° (*c* 0.50, H_2O), as an isomer of calystegine B_1 [10,31]. The ammonia CIMS gave a peak at *m/z* 158 corresponding to loss of water from the parent ion. Treatment of the alkaloid with acetic anhydride in pyridine gave an acetyl derivative, $[\alpha]_D$ -18.8° (*c* 0.53, $CHCl_3$), with a molecular ion at *m/z* 343 in the EIMS. The ^{1}H NMR spectrum of the latter showed four acetyl signals at δ2.03, 2.08, 2.09, and 2.24 ppm, revealing the presence of one -*N*Ac and three -*O*Ac groups, while a broad singlet at δ6.30 which disappeared on exchange with D_2O indicated that the tertiary hydroxyl group remained unacetylated [24].

The ^{13}C NMR of the parent compound exhibited two methylene resonances with much closer chemical shifts than those in calystegine B_1, suggesting that both groups are located in the five-membered ring moiety. This was confirmed by ^{1}H-^{1}H decoupling experiments on the H(5) proton which showed couplings of 1.5 and 7.0 Hz respectively to H(6*endo*) and H(6*exo*) [27]. Further analysis of the spectrum indicated equivalent, large (8.4 Hz) coupling constants for the H(2), H(3) and H(4) protons thus establishing an axial configuration for each. The chair conformation of the six-membered ring, with the secondary hydroxyl substituents in *trans*-equatorial relationships was verified by the nOe between H(2) and H(4), while H(3) showed similar interactions with H(6*endo*) and H(7*endo*).

It should be noted that this alkaloid was originally named nortropanoline by Asano and coworkers but its structural identity to the previously discovered calystegine B_2 [10] was

subsequently recognized and the similarity in specific rotations suggested that both alkaloids were of the same absolute configuration [24].



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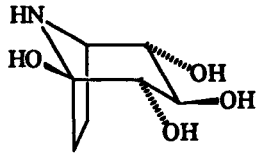

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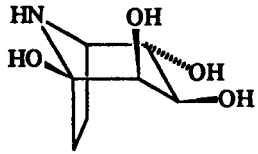

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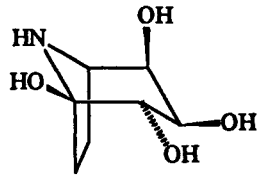

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2.2.6 Calystegine B_3 (19)

The FAB-MS of calystegine B_3 (1α,2α,3α,4β-tetrahydroxy*nor*tropane), $[\alpha]_D$ +82.8° (*c* 0.50, H_2O), showed that this alkaloid was a third member of the calystegine B group [26]. The resonances in its ^{13}C NMR spectra were much closer in chemical shift values to those for calystegine B_2 than for calystegine B_1 suggesting that the alkaloid was an epimer of the former. This supposition was confirmed by complete analysis of the ^1H NMR spectrum which showed a coupling of 3.6Hz between H(2) and H(3), so that the hydroxyl substituent on C(2) must have an axial orientation, whereas the C(3) and C(4) substituents retain the equatorial configuration.

2.2.7 Calystegine B_4 (20)

The fourth member of the tetrahydroxylated group, calystegine B_4 (1α,2β,3α,4α-tetrahydroxy*nor*tropane), $[\alpha]_D$ -63.0° (*c* 0.65, H_2O), had a consistent high-resolution FAB-MS value of 176.0923 $[M+H]^+$ ($C_7H_{14}NO_4$ requires 176.0923). The similarity in ^{13}C NMR resonances to those of calystegines B_2 and B_3, rather than calystegine B_1, supported a 1,2,3,4- pattern of hydroxyl

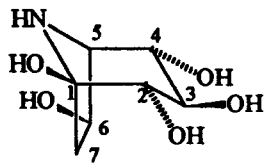
substituents.

The ^{1}H NMR spectrum in D_2O provided little useful information due to overlapping signals for H(2) and H(3). When the spectrum was determined in pyridine-d_5/D_2O (4:1) these signals, and all others, were sufficiently well resolved for the coupling constants to be determined. A large value of 8.8Hz for the coupling between H(2) and H(3) established that these protons were *trans*-diaxial, and H(4) was therefore equatorial. The epimeric nature of the 4-hydroxyl substituent in calystegine B_4, relative to calystegine B_2, was thus confirmed [33]. The addition of pyridine-d_5 to the NMR solution may be a useful technique for other calystegines with poorly resolved proton signals in D_2O.

2.2.8 Calystegine C_1 (21)

The molecular weight of calystegine C_1 (1α,2β,3α,4β,6α-pentahydroxy*nor*tropane), $[\alpha]_D$ +23.1° (*c* 0.80, H_2O), was determined to be 16 amu greater than that of the calystegine B series by FAB-MS. ^{13}C NMR showed the presence of only a single methylene group which was located at the 7-position, with the additional hydroxyl group at the C(6), by two-dimensional COSY experiments [25].

In the ^{1}H NMR spectrum, decoupling experiments showed the *J* values for H(2), H(3), and H(4) to be large and equivalent (8.8Hz), as in calystegine B_2. The substituent hydroxyl groups at these positions must therefore all be *trans* and equatorial. The orientation of the hydroxyl group at C(6) was determined to be α through observation of an nOe between H(3ax) and H(6*endo*). Similar effects were observed between H(3ax) and H(7*endo*), and between the H(2ax) and H(4ax) protons [26].



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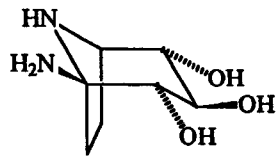

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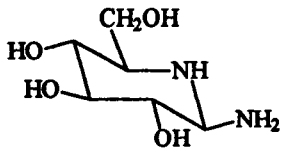
2.2.9 Calystegine N_1 (22)

Calystegine N_1 (1α-amino-2β,3α,4β-trihydroxy*nor*tropane), $[\alpha]_D$ +59.4° (*c* 0.18, H_2O), was assigned to an entirely new group of calystegines (the N series) because the FAB-MS gave an odd-

numbered $[M+H]^+$ ion of m/z 175, indicative of replacement of a hydroxyl group by an amino group relative to the calystegine B series [27]. Acetylation of the compound with acetic anhydride in pyridine gave a derivative shown to be an *N,O*-pentaacetate by ^1H and ^{13}C NMR, and FAB-MS.

The additional amino group was located on C-1 in the parent alkaloid by the chemical shift of the sole quaternary carbon at δ78.3 in the ^{13}C NMR, in contrast to all other calystegines in which the hydroxyl-substituted quaternary carbon resonance occurs at an essentially invariant value of δ93-94 ppm. The complete connectivity of the remaining carbon and hydrogen atoms was determined by a combination of homonuclear decoupling experiments and two-dimensional HMBC and HMQC. In general the chemical shifts in the ^1H NMR, with the exception of H(2) and H(*7exo*) which showed upfield shifts, were very similar to those in calystegine B_2. As in the latter, the coupling constants for H(2), H(3), and H(4) were large (8.5Hz), establishing the same relative stereochemistry for the hydroxyl groups at these positions.

It is noteworthy that calystegine N_1 undergoes approximately 40% conversion into calystegine B_2 on storage for six months at 4°C. This suggests that *nor*tropane alkaloids with an amino substituent at the bridgehead position may be quite labile at ambient temperatures, or may undergo facile conversion to the hydroxy derivatives during isolation. Conversely, calystegine N_1 may be an artifact formed from calystegine B_2 during ion-exchange chromatographic purification using ammonium hydroxide as an eluent. Supporting evidence for this alternative may reside in the quantitative formation of 1-β-amino-1-deoxynojirimycin (**23**) upon dissolving the bisulfite adduct (**24**) of nojirimycin in ammonia-saturated methanol at room temperature [34].



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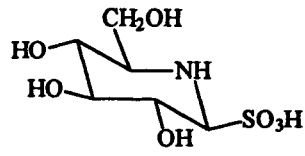

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The NMR data accumulated for the calystegines identified to date is quite comprehensive and should enable novel structures in the class to be elucidated fairly easily by comparison with established structures. The ^1H and ^{13}C NMR chemical shifts and coupling constants determined for the above nine alkaloids are tabulated in Table 1 and Table 2, respectively.

Table 1. [1]H NMR Assignments[†] for Calystegines A_3 (14), A_5 (15), A_6 (16), B_1 (17), B_2 (18), B_3 (19), B_4 (20), C_1 (21), and N_1 (22).

	Calystegine				
	A_3 (14)	A_5 (15)	A_6 (16)	B_1 (17)	B_2 (18)
Proton Number			δ, ppm (J, Hz)		
2ax	3.40 (1.5,8.4)	1.68 (11.0,12.3)	3.70 (6.2,11.4)	3.34 (1.8,8.4)	3.42 (1.8,8.4)
2eq	-	2.26 (6.4,12.3)	-	-	-
3ax	3.70 (6.6,8.4,11.0)	3.72	1.28	3.45 (6.6,8.4,11.0)	3.35 (8.4,8.4)
3eq	-	-	1.97	-	-
4ax	1.45-1.57	3.47 (4.0,8.8)	1.56	1.47 (4.0,11.0,13.2)	3.58 (1.5,4.0,8.4)
4eq	1.99 (2.6,6.6,13.2)	-	1.50	2.03 (2.6,6.6,13.2)	-
5eq	3.46 (2.6,3.6,6.6)	3.34 (4.0,7.0)	3.45	3.26	3.32 (1.5,4.0,7.0)
6*endo*	2.01-2.12	1.76-1.96	2.12 (7.7,14.3)	4.11 (2.6,7.3)	1.76
6*exo*	1.45-1.57	1.76-1.96	1.87 (1.5,3.3,7.7,14.3)	-	1.95
7*endo*	1.45-1.57	1.76-1.96	4.08 (3.3,7.7)	2.52 (7.3,14.7)	2.00
7*exo*	2.01-2.12	1.76-1.96	-	1.40 (1.8,2.6,14.7)	1.54

[†]Data from Asano *et al.* [26,27], spectra obtained in D_2O at 400 MHz using sodium 3-(trimethylsilyl)propionate as an internal standard; c.f. [10, 24, 25, 31].

Table 1. (Continued)

Proton Number	Calystegine B_3 (19)	B_4 (20)[‡]	C_1 (21)	N_1 (22)
	δ, ppm (J, Hz)			
2ax	-	4.32 (1.7,8.8)	3.35 (1.8,8.8)	3.23 (1.5,8.5)
2eq	3.83 (3.6)	-	-	-
3ax	3.61 (3.6,9.5)	4.04 (4.4,8.8)	3.15 (8.8,8.8)	3.28 (8.5,8.5)
3eq	-	-	-	-
4ax	3.65 (3.6,9.5)	-	3.54 (4.7,8.8)	3.52 (4.0,8.5)
4eq	-	4.02 (2.9,4.4)	-	-
5eq	3.28 (3.6,6.6)	3.71 (2.9,7.8)	3.20 (1.5,4.7)	3.26 (4.0,7.0)
6endo	1.74	1.35 (5.1,9.9,13.2)	4.29 (1.5,2.9,7.4)	1.73
6exo	1.92	2.24 (4.4,7.8,9.9,13.2)	-	1.85
7endo	1.76-1.81	2.43 (4.4,9.9,13.2)	2.55 (7.4,14.3)	1.91
7exo	1.76-1.81	1.98 (1.7,5.1,13.2)	1.47 (1.8,2.9,14.3)	1.34

[‡]Data from Asano *et al.* [33], spectrum obtained in pyridine-d_5/D_2O (4:1) at 400 MHz using tetramethylsilane an internal standard.

Table 2. ^{13}C NMR Assignments† for Calystegines A_3 (14), A_5 (15), A_6 (16), B_1 (17), B_2 (18), B_3 (19), B_4 (20), C_1 (21), and N_1 (22).

	Calystegine								
	A_3 (14)	A_5 (15)	A_6 (16)	B_1 (17)	B_2 (18)	B_3 (19)	B_4 (20)	C_1 (21)	N_1 (22)
Carbon Number	δ (ppm)								
1	93.6	94.1	93.1	93.8	93.2	93.0	92.5	93.6	78.3
2	82.5	47.9	74.5	81.3	80.4	77.2	79.6	79.3	80.7
3	72.8	71.7	28.6	72.7	77.7	73.0	73.8	77.7	77.7
4	42.6	78.2	32.5	38.9	77.6	75.2	74.8	75.4	77.5
5	54.3	59.1	53.7	62.9	58.6	58.3	59.1	67.4	59.5
6	29.4	24.8	40.6	76.0	24.5	23.0	25.1	71.7	25.8
7	31.8	36.6	70.2	43.6	31.5	34.1	29.8	43.6	32.9

†Data from Asano *et al.* [26,27,33], spectra obtained in D_2O at 100 MHz using sodium 3-(trimethylsilyl)propionate as an internal standard; c.f. [10, 24, 25, 31].

The absolute configuration of the calystegines has not been determined *a priori* although the class as a whole would appear to be susceptible to configurational analysis by the exciton chirality method, which has been applied to a number of polyhydroxylated natural products including methyl α-D-glucopyranoside [35] and the piperidine alkaloid, deoxymannojirimycin [36]. The absolute configuration of calystegine B_2 has been established by stereospecific synthesis of both (+)- and (-)-enantiomers, with specific rotations of +17.1° and -17.5°, respectively [37]. Although a higher value of +27.2° has been reported for naturally occurring calystegine B_2 [26], its identity with the dextrorotatory synthetic compound is established by the fact that both are catabolized by *Rhizobium meliloti* and are potent inhibitors of α-galactosidase and β-glucosidase, whereas synthetic (-)-calystegine B_2 exhibits none of these properties [38]. Calystegine B_2 is thus defined as 1*R*,2*S*,3*R*,4*S*,5*R*-1,2,3,4-tetrahydroxy*nor*tropane (**18**).

3. OCCURRENCE

3.1 Occurrence in Convolvulaceae

Calystegines were first isolated from the bindweed or morning glory, *Calystegia sepium*, from which they derive their name, in the course of a search for compounds produced by root structures which could possibly act as nutritional mediators of specific plant-bacterium relationships [9]. Of a total of 105 plant species from twenty-four plant families examined, compounds having such potential were detected in two members of the Convolvulaceae, *C. sepium* and *Convolvulus arvensis*, and a Solanaceous plant (*Atropa belladonna* - see Section 3.3). The calystegines were subsequently extracted from root cultures of *C. sepium* transformed by *Agrobacterium rhizogenes*, grown *in vitro*. Three alkaloids were isolated and characterized from this extract: calystegines A_3 (**14**), B_1 (**17**), and B_2 (**18**). The latter comprised 40% and 60% respectively of the B fraction, but calystegine A_3 accounted for only 60% of the A fraction and three additional uncharacterized alkaloids were observed in this group by HPLC. Two new calystegines, A_5 and A_6, have since been isolated from other plant families by Asano and coworkers [26,27] but whether or not either of these correspond to the unidentified calystegines in *C. sepium* remains in question. Analysis of *Convolvulus arvensis* growing as an adventitious weed in California has shown calystegine B_2 to be the major constituent of the crude alkaloid fraction, which comprises 1.93% of the dry weight of the roots [39]. Calystegines A_3, B_1 and B_4 are also present, together with some unidentified isomers of the B group.

The only other genus of plants in the Convolvulaceae which has been examined for the presence of calystegines is *Ipomoea*. Seeds of Weir Vine (*Ipomoea* sp. Q6 [aff. *calobra*]) growing in a very limited area of Queensland, Australia were analyzed by GC-MS and shown to contain calystegine B_2 (**18**) and possibly also calystegine C_1 (**21**), co-occurring with the polyhydroxyindolizidine alkaloid swainsonine (**5**). A related species, *I. polpha*, from the Northern

Territories, contained the same complex of alkaloids [29]. Recent experiments have also identified calystegines and swainsonine in the leaves of *I. carnea* from Mozambique [40]. In contrast, calystegines were not detected in roots, leaves or seeds of *I. tricolor* and only trace amounts in seeds of *I. alba* [40], so that elaboration and accumulation of these alkaloids cannot be regarded as a general feature of the genus.

3.2 Occurrence in Moraceae

The polyhydroxy piperidine alkaloid deoxynojirimycin (**2**) has long been known to be a constituent of the leaves and roots of certain *Morus* (mulberry) species [41]. A recent very thorough examination of *M. bombycis* and *M. alba* has resulted in the discovery of many additional polyhydroxy-pyrrolidine and -piperidine alkaloids, including glycoside derivatives, and in addition certain calystegines. The only polyhydroxy*nor*tropane isolated from *M. bombycis* was calystegine B_2 (**18**) [24] but *M. alba* was found to contain both calystegines B_2 and C_1 (**21**) [25]. No genera in the Moraceae other than *Morus* have so far been reported to contain calystegines, nor have tropane alkaloids been isolated from this plant family, so their occurrence therein is unique at the present time.

3.3 Occurrence in Solanaceae

The greatest number of plant species in which the calystegines have been detected belong to the family Solanaceae. This may partly be a reflection of the importance which this family holds as a source of foodstuffs and medicinals to human society, so that once the alkaloids were discovered in one member of the family there was an incentive to search for them in other genera.

In the course of their initial screening, Tepfer *et al.* [9] detected calystegines in *Atropa belladonna* but although the paper electrophoretic properties of the extract appeared identical with that of *Calystegia sepium*, the identity of the specific alkaloids present was not determined. Recent studies by Dräger and coworkers have identified calystegines A_3 (**14**), B_1 (**17**) and B_2 (**18**) in this plant, and the same alkaloids were also shown to occur in *Mandragora officinarum* (mandrake), *Scopolia carniolica*, and *Hyoscyamus niger* [30]. All of these species, together with *A. belladonna*, belong to the Atropoideae, a subfamily of the Solanaceae well known for the occurrence of tropane ester alkaloids. Low amounts of calystegines were detected in *Datura stramonium* (Jimson-weed) but none in *Nicotiana* species. The only glycoside isolated so far is the 3-*O*-β-D-glucoside of calystegine B_1, from *Nicandra physalodes* [32], but additional derivatives of this type will undoubtedly be discovered.

Relatively high levels of calystegine B_2 and somewhat lower levels of A_3 were found in the sprouts of green potato tubers (*Solanum tuberosum*, cultivar not identified) [30]. The same two calystegines had earlier been identified in the skins of the potato cultivar 'Estima', with an A_3:B_2 ratio of approximately 1:2, whereas calystegine B_2 alone was found in leaves of the cultivars 'King

Edward', 'Dunluce', 'Desiree', and 'Cara' and calystegines A_3, B_1 and B_2 were found in *S. melongena* (eggplant) fruits [23]. In the same study the poisonous plant *S. dulcamara* (bittersweet) contained predominantly calystegine B_2 in the leaves, and the alkaloid was also detected in leaf fragments from herbarium samples of *S. dimidiatum* and *S. kwebense*. A GC-MS analysis of fruits of *S. dimidiatum* (potatoweed) collected from Texas showed that the alkaloid was also present in this part of the plant [42].

The extract of the whole plant of *Hyoscyamus niger* has now been shown by Asano *et al.* to contain calystegines, namely A_3 (14), A_5 (15), A_6 (16), B_1 (17), B_2 (18), B_3 (19) and N_1 (22); only calystegine C_1 (21) was not found [27]. The same research group has isolated calystegines A_3, A_5, B_1, B_2 and B_3 from *Physalis alkekengi* var. *francheti* (Chinese lantern plant). In addition, the roots of *Scopolia* species which are used as a crude drug, have been shown to contain calystegines A_3, A_5, B_1, B_2, B_3 and C_1 together with the novel tetrahydroxy alkaloid, calystegine B_4 (20) [33].

The detection of calystegines in so many genera of the Solanaceae suggests that additional investigation of this plant family could prove fruitful, if only to attempt to define the subfamilies and tribes in which these alkaloids occur. However, caution should be exercised in adopting this approach due to the fact that calystegines also occur in the taxonomically disparate families Convolvulaceae and Moraceae.

4. DETECTION AND ANALYSIS

4.1 Paper Electrophoresis

Prior to the structural identification of the calystegines as *nor*tropane alkaloids, plant extracts were screened by the use of high-voltage paper electrophoresis with silver staining for the presence of compounds which could be capable of serving as selective nutritional sources in the rhizosphere [9]. The samples were spotted on Whatman 3MM paper and subjected to 3 kV for 15 minutes in a pH 1.9 buffer comprised of formic acid/acetic acid/water (30:60:910). The dried electrophoretogram was visualized by dipping it in a solution of silver nitrate in aqueous acetone, followed by sodium hydroxide in aqueous ethanol, and finally in photographic paper fixer. This technique was originally devised for detection of sugars on paper chromatograms [43] but fortunately it is equally capable of reacting with the calystegines, which can be regarded as structurally related to aminosugars. While reasonably sensitive, resolution of the individual calystegines cannot be achieved, and the calystegine A and B groups are only just visible as separate spots at low concentrations. At higher concentrations the two groups merge into an elongated spot.

4.2 Thin-Layer Chromatography

Calystegines are not particularly well-suited for analysis by thin-layer chromatography due to their high polarity and strong hydrophilicity. Asano's group has employed high-performance silica gel plates developed with propanol/acetic acid/water (4:1:1) but all of the calystegines have similar, and some identical, R_f values in this solvent system [24-27]. Observed values range from 0.41 to 0.51, rendering discrimination between certain alkaloids difficult, if not impossible. In this solvent system there appears to be no correlation between R_f and degree of hydroxylation.

Dräger has explored the potential of silica gel, polyamide and kieselguhr as stationary phases, developed with various solvent systems, for analysis of calystegines [28]. The less polar substrates gave excessive tailing or diffuse spots but silica gel was suitable when aqueous ammonia was included in the developing solvent. The optimum resolution was obtained with a mobile phase consisting of methanol/0.6M ammonium hydroxide/chloroform (6:2:1) in which calystegine A_3 had an R_f of 0.45 and calystegines B_1 and B_2 had a value of 0.61. However, the latter alkaloids remained unresolved under any of the conditions employed.

As with other polyhydroxy alkaloids [20-22], the calystegines fail to respond to alkaloid detection sprays such as Dragendorff's reagent or iodoplatinate, except at high concentrations. A pink color is generated with ninhydrin but the detection limit is ca. 20μg and the strong purple color produced by any amino acids present may obscure the response. The silver nitrate method used for paper electrophoresis enables the alkaloids to be visualized at a level of 0.5μg but suffers from the disadvantage that a two-step treatment is necessary [28]. Chlorine/*o*-tolidine reagent, a two-part spray consisting of dilute sodium hypochlorite solution, followed by a mixture of *o*-tolidine in dilute acetic acid and aqueous potassium iodide, has been routinely used by Asano for screening extracts and purified calystegines [24-27]. Blue-black spots are generated with primary or secondary amines but the detection limit of this reagent has not been reported.

4.3 High Performance Liquid Chromatography

HPLC, using a reversed phase system consisting of a Zorbax NH_2 column eluted with acetonitrile/water (4:1), was employed for the preparative separation of the calystegines [10]. The lack of a chromophore renders the use of UV or fluorescence detection impossible without pre- or post-derivatization of the sample and the relatively low sensitivity of refractive index (RI) detection severely limits this technique for routine analysis. However, the rapid evolution of LC-MS methods may lead to their application in the future.

4.4 Gas Chromatography - Mass Spectrometry

The most effective analytical technique for the calystegines employed to date has been gas

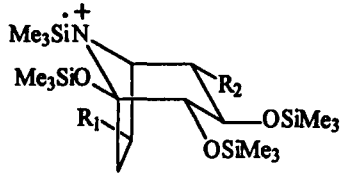
chromatography in concert with mass spectrometric (MS) detection. The latter offers a significant advantage over all other methods in that valuable structural information can be acquired for each peak in the sample. For quantitative analysis the higher sensitivity obtainable with a flame ionization detector (FID) or phosphorus-nitrogen detector (PND) is advantageous.

The high polarity of calystegines requires that a volatile derivative be prepared, for which purpose trimethylsilylation has been used exclusively. A wide choice of derivatization reagents is available and Molyneux *et al.* [39] reported the formation of per-trimethylsilylated derivatives in which even the tertiary hydroxyl and the secondary amino groups were derivatized on treatment with *N*-methyl-*N*-(trimethylsilyl)-fluoracetamide (MSTFA). Gas chromatographic separation was performed on a 60 m X 0.32 mm i.d. SE-30 fused silica column, with calystegines A_3, B_1 and B_2 eluting at 15.55, 16.51 and 18.11 minutes. The mass spectral fragmentation pattern of the per-TMS derivatives was analogous to that observed for tropane alkaloids which bear a methyl substituent on the nitrogen atom [44] and proceeds through a tricyclic ion (25) to ultimately give the dihydropyrrolinium ion (26) base peak, as illustrated in Figure 1. In addition to these fragments the MS also shows an ion corresponding to loss of a $-CH_3$ group but the molecular ion is observed [39].

In subsequent experiments using the same reagent the per-TMS derivative was not formed, even under forcing conditions, the MS indicating that the -NH group remained underivatized [29]. The reason for this discrepancy is unclear but may be due to trace contaminants in the sample. The GC retention times for these derivatives of calystegines A_3, B_1, B_2, and C_1 on the same SE-30 column were somewhat shorter than for the corresponding per-TMS derivatives, with values of 13.45, 15.23, 16.28 and 17.46 minutes, respectively, and the fragmentation patterns were quite different [29, 40]. In particular, the base peak was usually quite weak and sometimes not even observed, while peaks which are typical of TMS ether derivatives, corresponding to loss of $-CH_3$ (-15 amu) and TMS-OH (-90 amu), were readily apparent. A strong base peak with *m/z* 217 was observed for calystegines B_2 and C_1. This fragment, which commonly occurs in TMS derivatives of sugars, inositols or piperidine alkaloids such as deoxynojirimycin (2), is due to the ion TMSO-CH=CH-CH=$^+$OTMS (27) and thus is characteristic of calystegines having three adjacent secondary hydroxyl groups. The other major fragment arising from the calystegine trimethylsilyl ethers appears to be a 2-substituted pyrrolinium ion (28) resulting from direct cleavage [28] of the six-membered ring, as outlined in Figure 2, instead of a dihydropyrrolinium ion (26) formed from the tricyclic ion generated by the pertrimethylsilyl derivatives (Figure 1).

The same trimethylsilyl ether derivatives are produced on treatment of the alkaloids with a mixture of hexamethyldisilazane and trichlorosilane (10:1) [23,28], but the use of MSTFA may be advantageous in that all byproducts of the reaction are volatile. Gas chromatography on a 30 m X 0.25 mm i.d. DB5 column with simultaneous detection by flame-ionization (FID) and phosphorus-nitrogen (PND) gave retention times of 12.01, 15.32 and 17.22 minutes for calystegines A_3, B_1 and B_2 [28]. The detection limits were ca. 10 pmol with PND, while FID was approximately six times less sensitive. A quantitative GC analysis method has been developed using either octadecane or deoxynojirimycin (2) as an internal standard. The latter has certain advantages in that it is detectable by the more sensitive PND whereas the hydrocarbon is not. The technique has been applied to the

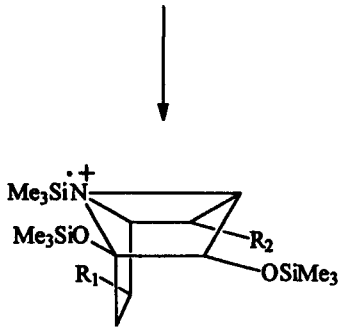
determination of individual alkaloids in various plant parts, with quantification in the range of 5-400µg/g of tissue [30].



Calystegine A3 (14), $R_1 = R_2 = H$; *m/z* 447

Calystegine B_1 (17), $R_1 = OSiMe_3$, $R_2 = H$; *m/z* 535

Calystegine B_2 (18), $R_1 = H$, $R_2 = OSiMe_3$; *m/z* 535



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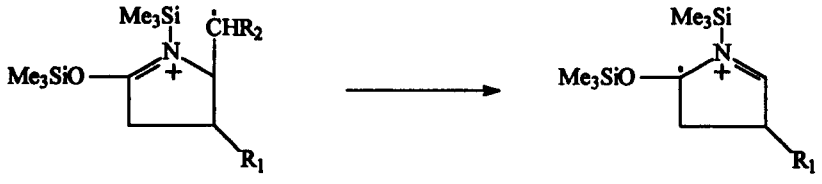

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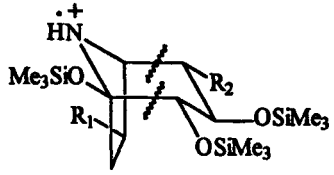
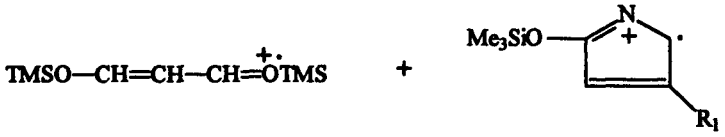
Figure 1: Mass spectrometric fragmentation of per-TMS derivatives of calystegines A_3 (14), B_1 (17) and B_2 (18).



Calystegine A_3 (14), $R_1 = R_2 = H$; *m/z* 375

Calystegine B_1 (17), $R_1 = OSiMe_3$, $R_2 = H$; *m/z* 463

Calystegine B_2 (18), $R_1 = H$, $R_2 = OSiMe_3$; *m/z* 463



27 **28**

Figure 2: Mass spectrometric fragmentation of *O*-TMS derivatives of calystegines A_3 (14), B_1 (17) and B_2 (18).

5. BIOSYNTHESIS

The biosynthesis of the calystegines has not been elucidated but a reasonable working hypothesis would involve modification of the tropane alkaloid biosynthetic pathway which commences with tropinone (29), derived from L-ornithine and L-arginine. Dräger [28] has proposed that pseudotropine (30), formed from tropinone through reduction by tropinone reductase II, could be the intermediate for generation of all of the calystegines (Figure 3), since a common feature is the

presence of an α-hydroxyl group at the 3-position. Only one of the alkaloids discovered to date is an exception to this rule, namely calystegine A_6 (16), which lacks an hydroxyl group at the 3-position. Reduction of tropinone by tropinone reductase I would give tropine (31), an established precursor of typical tropane ester alkaloids such as hyoscyamine and scopolamine which have a β-substituent at the 3-position.

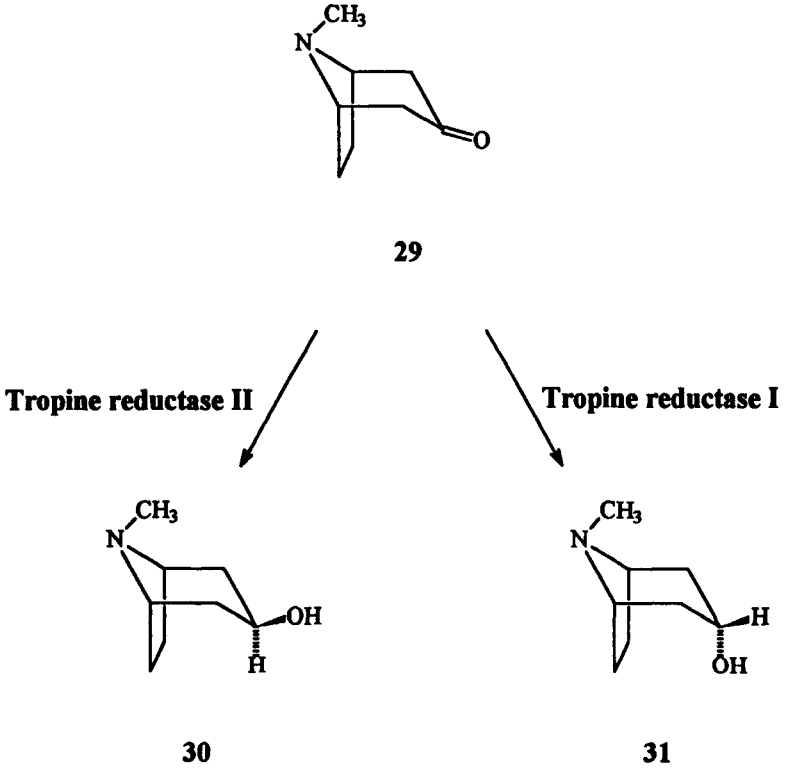
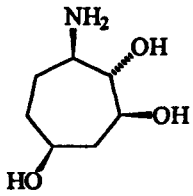


Figure 3: Hypothetical biosynthetic route to the calystegines from tropinone (29) *via* reduction to pseudotropine (30) by tropinone reductase II. Reduction by tropinone reductase I yields tropine (31), the precursor of tropane ester alkaloids.

A number of difficulties exist with this postulate. Stermitz' group has shown that fresh aerial parts of field bindweed (*Convolvulus arvensis*) contain pseudotropine as the major alkaloid, together with trace amounts of tropine and tropinone, as well as the pyrrolidine alkaloids hygrine and

cuscohygrine [45]. However, no calystegines were detected in this plant sample, despite GC-MS analysis. In contrast to this, tropine reductases I and II have been identified in *Atropa belladonna*, *Datura stramonium*, and *Hyoscamus niger* [46-48], all of which have now been shown to produce both typical tropane alkaloids and the calystegines [28]. A more profound problem is the lack of *N*-methylation in most of the calystegines and the high degree of hydroxylation. In the tropane alkaloid group *N*-methylation occurs at a very early stage of the biosynthetic pathway and appears to be a prerequisite for subsequent transformations. Since *N*-demethylation is an unlikely biosynthetic transformation, it may be that calystegines are derived either by a divergent pathway which has escaped *N*-methylation or else from an entirely different precursor. Consideration of these factors suggests that a more probable precursor might be pipecolic acid, as was found to be the case for swainsonine (**5**) [49].

The 1β-amino-2α,3β,5β-trihydroxycycloheptane (**32**), recently isolated from *Physalis alkekengi* var. *francheti* [50], may be a biosynthetic precursor to the calystegines which occur in this plant. Alternatively, the compound could result from enzymic reduction of the 5-aminocycloheptanone tautomer (**33**) of calystegine A_3 (**15**) and thus be a termination point of the biosynthetic process, providing little useful information as to the origin of the alkaloids.



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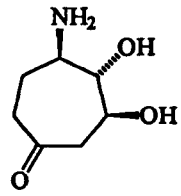

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One of the advantages of the ability to isolate calystegines from transformed root cultures is that this should enable experiments to be conducted much more easily than in whole plants and the biosynthetic pathway may therefore be elucidated in the near future.

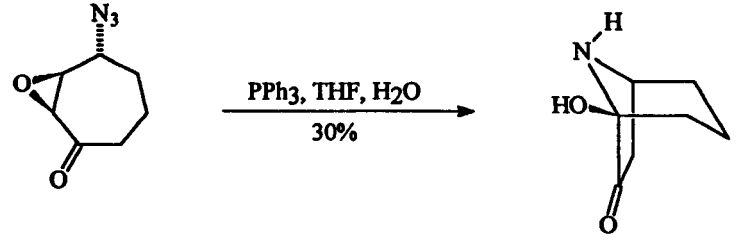
6. SYNTHESIS

Several enantiospecific and racemic syntheses of calystegines have been reported, as well as those of some non-natural derivatives. All of these have proceeded via the formation of an

appropriately substituted 5-aminocycloheptanone intermediate (e.g **34**) which is tautomerized in the later stages of the synthesis into the bicyclic aminoketal structure, e.g. calystegine B_2 (**18**). Since the novelty of specific approaches resides in the methods used to generate the appropriate aminocycloheptanone, it is not within the purview of this chapter to discuss in detail the individual synthetic routes and only the broad outlines will be presented.

34 **18**

The earliest synthetic report of a calystegine-type ring system by Lallemand *et al.* [51] described the synthesis, by reduction of the key intermediate 2,3-epoxy-4-azidocycloheptanone (**35**), of racemic 1-hydroxy-7-keto*nor*tropane (**36**), which existed entirely in the bicyclic ring form, possibly stabilized by hydrogen-bonding between the adjacent hydroxyl and keto groups. No attempt was made to reduce the keto functionality to form a dihydroxycalystegine.

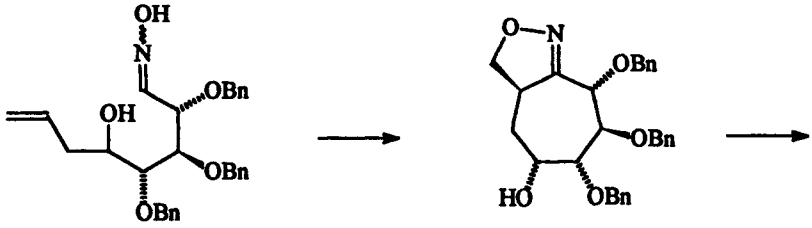


35 **36**

The same group subsequently synthesized racemic calystegine A_3 (**14**) and three stereoisomers, as well as the *N*-methylated 1-hydroxytropane, physoperuvine (**9**), by use of the same general approach [52]. The *trans*-compound with diequatorial hydroxyl groups at the 2- and 3-positions was shown to be identical with calystegine A_3 by ^1H and ^{13}C NMR, so that the other *trans*-isomer had to have the same groups diaxial. The *cis*-isomer in which the C(2) and C(3) hydroxyl

groups were axial and equatorial, respectively, was distinguished by coupling constants for H(3) of 3.5 and 14.5 Hz; by default the remaining isomer had the opposite configuration for these two substituents. Whereas the former was shown to exist entirely in the bicyclic form the latter showed equilibrium between the mono- and bi-cyclic structures with slow incorporation of deuterium at the 2- and 7-positions when treated with D_2O. An analogous synthetic sequence generated 1,3-dihydroxy*nor*tropane isomers with the 3-hydroxyl group in the axial and equatorial configurations. Unfortunately, the glycosidase inhibitory properties were not reported for any of these isomers, other than calystegine A_3, so that structure-activity relationships for the series are not available.

A derivative of enantiomeric calystegine B_2, bearing a hydroxymethyl group at the 7-position (40), has been synthesized stereoselectively through a protected polyhydroxy aminocycloheptanone (38) and the corresponding azide (39) obtained by intramolecular cycloaddition of an olefinic oxime (37) derived from D-glucose [53].



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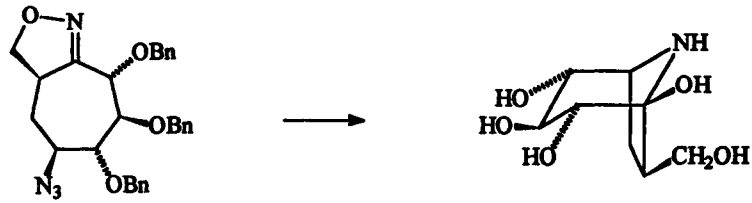
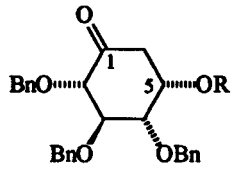


39 40

This reaction sequence was subsequently extended, through oxidation of the hydroxymethyl group and deformylation, to the synthesis of both enantiomers of calystegine B_2 (18), which had specific

rotations of +12° and -14°, respectively [54], while the value measured for natural calystegine B_2 was +2.9° [31]; a value of +27.2° has subsequently been recorded [26]. Although the precise identity of the natural compound could therefore not be established by comparison of the specific rotations, the synthetic (+)-enantiomer showed glycosidase inhibitory properties comparable with those of the natural product while the (-)-enantiomer was a non-inhibitor [38].

The same pair of enantiomers was synthesized through ring enlargement of the protected, pseudosymmetric polyhydroxycyclohexanone (41), derived from D-glucose, by introduction of the nitrogen atom at the 1- or 5-position to yield the corresponding azides (42 or 43) [55]. Reduction of each azide and deprotection yielded the desired aminocycloheptanones. The NMR spectrum of these products showed only broad signals, consistent with an equilibrium between the monomer, oligomers, and the aminoketal form [37]. Workup of the reaction under strongly basic conditions (pH 11) however gave either (+)- or (-)-calystegine B_2 exclusively, indicating that protonation of the amine group favors formation of the monocyclic form. The (+)- and (-)-enantiomers of calystegine B_2 had specific rotations of +17.1° and -17.5°, respectively, but only (+)-calystegine B_2 was catabolized by *Rhizobium meliloti* [38], confirming the identity of the natural compound with the latter.



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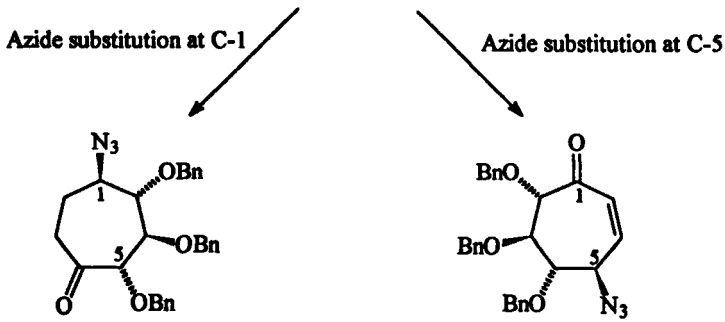
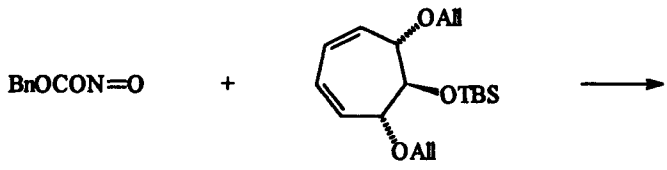


42 43

Lallemand's group has also investigated an alternative route to the crucial aminocycloheptanone intermediate (46) via a Diels-Alder addition between an acylnitroso derivative (44) and a protected polyhydroxy-cycloheptadiene (45) [56]. Due to an inability to remove the allyl groups chosen for protection of the hydroxyl functionality, this approach has yielded a propyl ether derivative of calystegine B_2; other protecting groups which can be removed with more facility should render the parent alkaloid accessible.



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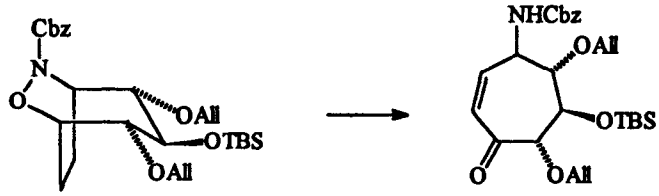

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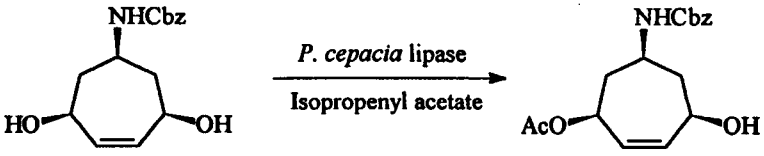
A recent synthesis of both (+)- and (-)-enantiomers of calystegine A_3 has been achieved, commencing from the 6-azido derivative of *meso*-2-cycloheptene-1,4-diol [57]. The amine derived from this compound was protected as the benzyl carbamate (47) and subjected to asymmetrization using *Pseudomonas cepacia* lipase to give the mono-acetate (48). The product of this reaction was elaborated in a series of steps to give the calystegine A_3 enantiomers via the usual polyhydroxy aminocycloheptanone. When treated at pH 11 with sodium hydroxide in D_2O the hydrochloride salts gave ^1H and ^{13}C NMR spectra identical with those reported for the natural product, but the free base could not be isolated from this solution without decomposition.



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The specific rotations for the synthetic calystegine A_3 enantiomer hydrochlorides were +12.4° and -12.2°, respectively. Since the rotation of the hydrochloride salt of calystegine A_3 has not been reported the synthesis does not unequivocally define the absolute configuration of the natural product, but the assumption was made that the alkaloid has the same absolute configuration as calystegine B_2, i.e. 1*R*,2*S*,3*R*,5*R* as shown in structure (14).

7. BIOLOGICAL ACTIVITY

7.1 Glycosidase Inhibition

The structural similarities of the calystegines to known polyhydroxy alkaloid glycosidase inhibitors suggested that they might also have similar properties. For example, comparison of the stereochemical structures of castanospermine (6) and calystegine B_2 (18) showed a remarkable similarity in the disposition in space of the nitrogen atom and the four hydroxyl groups (Figure 4).

Castanospermine, (6) **Calystegine B_2, (18)**

Figure 4: Stereochemical comparison of castanospermine (6) and calystegine B_2 (18) indicating similarity in spatial disposition of nitrogen atom and hydroxyl groups.

Initial assays, using the alkaloid extract from the roots of *Convolvulus arvensis*, purified by ion-exchange chromatography, showed significant activity towards β-glucosidase and α-galactosidase, but no inhibition of amyloglucosidase, β-galactosidase, α- or β-mannosidase, α-fucosidase and β-xylosidase. Further experiments with pure alkaloids isolated from *Calystegia sepium*, confirmed these results, calystegine A_3 being a moderately good inhibitor of β-glucosidase ($K_i = 4.3 \times 10^{-5}$ M) but only a weak inhibitor of α-galactosidase ($K_i = 1.9 \times 10^{-4}$ M). A mixture of calystegines B_1 (27%) and B_2 (73%), however, was a potent inhibitor of the same enzymes with K_i values of 3×10^{-6} and 7×10^{-6}, respectively [39], values comparable to those measured for australine (3), swainsonine (5), and castanospermine (6) towards other glycosidases [1,5].

These initial results were subsequently confirmed with pure calystegines B_1 and B_2 [23,24], and extended to other members of the series as they were discovered [25-27]. The IC_{50} and K_i values have been summarized by Asano *et al.* [26,27]. Comparison of the extent of inhibitory activity with variation in structure or stereochemistry allows certain broad conclusions to be drawn, as follows:

- An increase in degree of hydroxylation of the *nor*tropane ring generally results in enhanced potency of inhibition. Thus, members of the calystegine A group are either non-inhibitory or show only low activity against the enzymes tested. Calystegines B_1 and B_2 are active in the μM range with similar activities towards β-glucosidase, but the B_1 isomer is an order of magnitude better as an inhibitor of β-galactosidase. In contrast, calystegine B_2 is a potent inhibitor of α-galactosidase, whereas B_1 shows no activity towards this enzyme. Calystegine C_1 shows activity towards β-glucosidase and β-galactosidase at 10^{-7} and 10^{-6}M, respectively, but has only a weak effect on α-galactosidase. An exception to the trend for higher activity with increased hydroxylation is calystegine B_3 which is even less effective as an inhibitor than calystegine A_3 [26]. While the tendency holds for the common glycosidases, it fails for porcine kidney trehalase, which is strongly inhibited by calystegines A_3, B_2 and B_4, but only weakly by C_1 [33].

- An equatorial hydroxyl substituent at the 2-position appears to be required for optimal activity. Absence of this group, as in calystegine A_5, results in loss of all inhibitory function relative to A_3, whereas epimerization to the axial configuration, as in calystegine B_3, markedly decreases or eliminates activity relative to B_1 or B_2 [26].

- Replacement of the bridgehead hydroxyl group at the 1-position by an amino group, as in calystegine N_1, reduces the inhibitory activity by one or two orders of magnitude in comparison to calystegine B_2 which otherwise has the same substitution pattern and stereochemistry. Moreover the inhibition produced by the latter is of the competitive type, whereas the N analog is a noncompetitive inhibitor [27].

Asano *et al.* have rationalized certain of these observations for β-glucosidase inhibition in terms of the generally accepted model for glycosidase activity, which invokes the presence of two carboxylic acid groups at the active site of the enzyme, one responsible for generation, and the other for stabilization, of the glycosyl cation intermediate [26]. They speculate that for calystegines B_1 and C_1, the *exo* hydroxyl group at the 6-position is protonated by the acidic group responsible for catalytic activity within the active site, in an analogous manner to the inhibitor conduritol B epoxide [58]. In contrast, calystegine B_2, which shows a similar level of inhibitory activity towards β-glucosidase is supposed to be bound to the glucosyl cation binding site through the hydroxyl group at the 4-position. The essential requirement of equatorial hydroxyl groups at the 2- and 3-positions is in accord with earlier studies of interaction of other inhibitors with β-glucosidase [59]. Thus the interaction of inhibitory calystegines with glycosidases can be envisioned as binding, to the sites determining specificity and to the catalytic center, through specific hydroxyl groups and through the imino group.

The mechanism of galactosidase inhibitory activity is less apparent. Calystegines B_1, B_2 and C_1 are potent inhibitors of either α- or β-galactosidase, yet calystegine B_3, with a much closer configurational similarity to D-galactose than any of the former, has no inhibitory activity against these enzymes. A similar observation has been made in the castanospermine series in which 6-*epi*castanospermine, while having a structural similarity to mannose, does not inhibit either α- or β-mannosidase but rather the same enzyme as castanospermine, namely α-glucosidase [60]. Obviously, a much larger set of natural or synthetic isomers and epimers is needed before a complete understanding of structure-activity relationships can be applied to prediction of inhibitory activity. Some progress in this direction has been made through a comparison of glycosidase inhibition by synthetic analogs and derivatives of (+)-calystegine B_2. The non-natural (-)-enantiomer showed no glycosidase inhibitory properties, whereas *N*-methylation of natural B_2 suppressed inhibition of β-glucosidase while activity towards α-galactosidase was retained [38].

7.2 Rhizobial Interactions

Calystegines were first isolated as a consequence of a search for plant root constituents which might be biosynthesized and exuded as signals to influence the ecology of the rhizosphere [9]. A particularly well investigated example of such a relationship is the *Rhizobium*-legume symbiosis which results in the initiation and growth of root nodules proficient at fixing nitrogen [61,62]. A number of flavonoids produced by legumes have now been shown to be capable of inducing rhizobial nodulation genes [63].

The precise role of the calystegines has not so far been elucidated but their occurrence in a limited number of plants from several different plant families indicates that they are not involved in a family-specific role such as nodulation. Instead, they might be suspected of providing an exclusive carbon and nitrogen source to soil bacteria that carry genes for the catabolism of calystegines. If so,

the rhizospheres of plants that produce the alkaloids should contain calystegine-catabolizing (Cac^+) bacteria. Recent experiments have shown that out of fifty-one rhizosphere isolates from *C. sepium* and *C. arvensis*, 22% were Cac^+. In contrast no Cac^+ bacteria were found among forty-two isolates from the dicot *Alliaria petiolata* and a monocot, a domesticated lawn grass, two species that do not produce calystegines. Furthermore, the catabolism is uniquely associated with the natural (+)-calystegine B_2 enantiomer which is degraded by the Cac^+ bacterium *Rhizobium meliloti* 41, while the synthetic (-)-enantiomer is not [38]. These results provide a preliminary insight into the obviously complex role played by calystegines in rhizobial ecology which will only be elucidated by further research.

7.3 Plant Interactions

The ability of calystegines to inhibit enzymes involved in glycoprotein processing suggested that these alkaloids might have potential allelopathic effects upon plants which do not biosynthesize the alkaloids. A precedent exists in the ability of the glucosidase inhibitor castanospermine to significantly suppress root length elongation of seedlings of the dicotyledons lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*), and to a lesser extent the monocotyledons red millet (*Panicum miliaceum*) and barnyard grass (*Echinochloa crusgalli*) [64]. The natural (+)-calystegine B_2 enantiomer has been shown to inhibit germination of alfalfa seeds by 50% at a 5mM concentration and to significantly suppress elongation of transformed root cultures of the same species. The synthetic (-)-enantiomer, however, had no effect on seed germination and although exhibiting a modest inhibitory effect on root elongation after 19 hours, ultimately had a slight stimulatory effect after 43 hours of growth [38]. No effects on other plant species have so far been reported and the relationship of these findings to allelopathic properties of calystegine-producing plants under natural conditions remains to be demonstrated.

7.4 Mammalian Toxicity

7.4.1 Livestock Poisonings

A particularly interesting aspect of the biological activity of calystegines is their potential toxicity towards animals or humans which may ingest them. As with other glycosidase inhibitors, phenotypes of specific lysosomal storage diseases may be produced, each depending upon the identity of the glycosidase which is inhibited [65]. In the case of swainsonine (5) from *Astragalus*, *Oxytropis* or *Swainsona* species, the signs of poisoning are analogous to those of genetic mannosidosis, a rare disease in humans but a not uncommon occurrence in Angus cattle [66]. Australine (3) and castanospermine (6), which occur in seeds of the Moreton Bay chestnut (*Castanospermum australe*), are both inhibitors of α-glucosidase and signs of poisoning of livestock and humans are similar to

those observed for Pompé's disease, arising from a genetic deficiency of the same enzyme [42,67]. It would not be unreasonable therefore to predict that the calystegines, having the ability to inhibit β-glucosidase and α-galactosidase, would produce syndromes that mimic the genetic absence or diminution of such activities, namely Gaucher's and Fabry's diseases, respectively [42,65].

7.4.1.1 Poisonings by Convolvulaceae

The bindweeds, *Calystegia sepium* and *Convolvulus arvensis*, in which calystegines were first identified [9,39] have on occasion been reported to be poisonous to livestock, but the toxic principle has never been identified. A recent investigation by Stermitz and coworkers of the cause of weight loss, abdominal pain and intestinal fibrosis of horses grazing pastures in northern Colorado heavily infested with field bindweed (*C. arvensis*) failed to show any calystegines present in the above-ground parts of the plant. However, the tropane alkaloids tropine, pseudotropine, and tropinone and the pyrrolidine alkaloids hygrine and cuscohygrine were identified [45]. The evidence thus suggests that the symptoms observed on consumption of this plant are not due to calystegines.

Certain species belonging to another genus of the Convolvulaceae, *Ipomoea*, have been associated with poisoning episodes. In parts of Queensland, Australia a poorly defined species growing in a very limited area, *Ipomoea* sp. Q6 [aff. *calobra*], known as Weir vine, has been reported to produce nervous derangement in sheep, cattle and horses that consume the plants. Controlled feeding experiments with sheep reproduced the clinical signs reported from field cases and were consistent with a lysosmal storage disease, including muscle-twitching, trembling, and "star-gazing" [68]. Histological examination of brain tissue revealed cytoplasmic vacuolation of neurons similar to lesions seen in locoweed and *Swainsona* poisoning [66]. Analysis of Weir vine seeds by GC-MS established the presence of calystegine B_2 (**18**), a second B isomer which is not B_1, and C_1 (**21**), and the indolizidine alkaloid, swainsonine (**5**) [29]. Since Weir vine has sometimes been regarded as a subspecies of *I. polpha*, which grows in other parts of Queensland and the Northern Territory, seeds of the latter were also analyzed and found to contain the same alkaloids although in different proportions [29]. While some of the symptoms of toxicity are very consistent with induced mannosidosis caused by ingestion of swainsonine, those related to epileptiform seizures are not, and may therefore be caused by the glycosidase inhibitory properties of the calystegines. Unfortunately the presence of swainsonine in these plant species confounds the resolution of the problem and a clear-cut association of toxicity with the presence of calystegines is not possible at this time.

The toxicity of *Ipomoea* species to livestock is not confined to the Australian continent. Poisoning of goats by *I. carnea*, an introduced species used as a windbreak around houses and kraals, has recently been reported in Mozambique [69]. Analysis of leaf material from this plant also showed the presence of calystegine B_2 (**18**), calystegine C_1 (**21**), and swainsonine (**5**) at similar levels to those found in the Australian samples [40] and a histopathological examination of brain tissue from affected animals was consistent with a lysosomal storage disease [69].

7.4.1.2 Poisonings by Solanaceae

An entirely different toxic situation known as "Crazy Cow Syndrome" is produced in cattle upon consumption of potato-weed (*Solanum dimidiatum*), a plant endemic to parts of Texas. Poisoned animals exhibit neurological signs characteristic of a cerebellar malfunction, in particular incoordination, staggering gait and epileptiform seizures induced by various stimuli. Histopathological lesions include cellular vacuolation and degeneration of Purkinje cells [70]. All of this evidence is suggestive of an induced lysosomal storage disease, the seizures being characteristic of Gaucher's disease, and the Purkinje cell insult analogous to Fabry's disease, in humans. The involvement of calystegines through their known occurrence in the Solanaceae and ability to inhibit β-glucosidase and α-galactosidase is therefore possible. The presence of calystegines in fragments of leaf from herbarium specimens of *S. dimidiatum* has subsequently been established by GC-MS [23], and extraction of leaves and fruits of fresh plant material collected in Texas gave an alkaloid fraction which was shown to contain calystegine B_2 and related alkaloids when analyzed by the same technique [42]. These results provide circumstantial evidence for the implication of the polyhydroxy *nor*tropane alkaloids in the syndrome.

Similar poisonings have been reported to arise from related plant species, *S. kwebense*, in South Africa, where the problem is known as "Maldronksiekte" (Crazy-drunk Disease), and *S. fastigiatum* and *S. bonariensis* in Brazil and Uruguay [71,72]. Although the presence of calystegines in *S. kwebense* herbarium samples was established by GC-MS analysis [23], none could be detected in air-dried, freshly collected samples of this species, nor in *S. fastigiatum* [40]. This discrepancy is not likely to be due to lability of calystegines, since the herbarium samples were many years old, but the recent studies of Dräger *et al.* show that for other members of the Solanaceae the levels are much higher in the first aerial parts to appear in the spring, or in developing leaves and leaf buds [30]. Possibly therefore plant samples collected at other growth stages may not contain sufficient alkaloid to be detectable, a hypothesis in accord with the often erratic nature of poisoning episodes.

Indirect evidence for the potential toxicity of calystegines is their detection in Lepidoptera which feed upon Solanaceae [23]. Specimens of death's-head hawk-moth (*Acherontia atropus*), feeding naturally on *Datura*, were raised in captivity and their caterpillars fed upon *Solanum tuberosum*. Both pupae and adults were shown by GC-MS to contain glycosidase-inhibiting polyhydroxy*nor*tropane alkaloids but not steroidal glycoalkaloids, which also occur in potatoes. Similar analysis of dried specimens of the butterfly, *Mechanitis polymnia*, which feeds on *Solanum* species during the larval stage, also identified sequestered calystegines. It seems probable that these alkaloids confer a protective function upon the insects during vulnerable stages of development, possibly by rendering them indigestible to predators.

7.4.2 Human Poisonings

The occurrence of calystegines in the leaves, skins and sprouts of potatoes (*Solanum tuberosum*), in fruits of the egg-plant (*S. melongena*) and in roots of sweet potato (*Ipomoea batatas*) [23,30,73], raises concerns regarding the safety of these vegetables in the human diet. Even with due consideration given to the Axiom of Paracelsus: *Sola dosis facit venenum* (Only the dose makes the poison), situations can be visualized in which ingestion of excessive levels of calystegines could have adverse effects. Thus, individuals with a genetic predisposition to low levels of β-glucosidase or α-galactosidase might suffer adverse effects from inhibition of these residual enzymes. The measurements of Nash *et al.* showed calystegine levels of 0.01% of the fresh weight of skins of the potato cultivar 'Estima', while the concentration of the rest of the tuber was one-tenth of this amount [23]. Recent analyses have shown levels up to 1mg/g fresh weight in potato skins [73] and although these are not normally eaten to a great extent, they may be consumed quite avidly by some individuals. In comparison, it has been estimated that swainsonine levels in excess of 0.001% of the dry weight are sufficient to produce intoxication in livestock eating the plants containing it [42]. The K_i value for swainsonine inhibiting α-mannosidase is ca. 70nM [2], whereas the values for calystegines B_1, B_2, and C_1 acting upon β-glucosidase are 430, 550, and 290 nM respectively [26]. Preliminary studies [74] have shown that calystegines A_3, B_2 and C_1 are potent inhibitors of human liver β-glucosidase and also good inhibitors of β-xylosidase, while calystegine B_2 alone is a good inhibitor of α-galactosidase. Based upon extrapolations from the swainsonine situation one could reasonably anticipate that these alkaloids, ingested at the levels detected in potato skins, could certainly cause gastrointestinal problems if not more serious effects. They are also not destroyed by cooking and have been shown to be present in processed potato products [73].

Interestingly, a different class of alkaloids known to occur in potatoes, the steroidal glycoalkaloids, have long been regarded as responsible for infrequent deaths and episodes of severe illness requiring hospitalization in groups of individuals eating potatoes, especially when these are baked in their skins. Documentation of illness in ca. 2000 people with thirty resulting deaths has been provided [75]. A consistent pattern in such episodes is nausea and vomiting, diarrhoea, and severe abdominal pain, with occasional neurological disturbances. However, a recent review of the situation has stated that "the current toxicological status of the glycoalkaloids present in an important dietary item is surprisingly poorly defined" [76], and no evidence has ever been presented that accounts for such glycoalkaloid toxicity. The potato glycoalkaloids α-chaconine and α-solanine are definitely teratogenic in laboratory animals [77] but they are not inhibitors of any of the common glycosidases [40]. In contrast, calystegines, as potent inhibitors of glycosidases essential for proper digestive function, are associated with biochemical mechanisms capable of causing digestive problems. The measurement of glycoalkaloid content in commercial potato varieties and in new cultivars is now a routine procedure. It would appear prudent to require a similar screening procedure for the calystegines since the analytical methodology already available is capable of fulfilling such a task. The

demonstrated presence of calystegines (and swainsonine) in several wild *Ipomoea* species and in the edible sweet potato (*I. batatas*) suggests that varieties and new cultivars of the latter should also be routinely analyzed for such alkaloids. The ultimate proof of involvement of calystegines in poisoning episodes, whether of livestock or humans, will depend upon confirmation by animal feeding experiments with the pure alkaloids.

CONCLUSIONS

In contrast to other classes of glycosidase inhibitors which by now have been extensively investigated, an understanding of the chemistry and biological activity of the calystegine group of alkaloids is only partially developed. The structural intricacies of the *nor*tropane ring system permit a considerable variation in the regio- and stereo-specificity of substituent groups and additional calystegines will undoubtedly be discovered. The occurrence of the known members of the group in three different plant families is suggestive of a wide taxonomic distribution and investigation of other genera in the Convolvulaceae, Moraceae and Solanaceae, as well as other plant families, should prove rewarding. In addition the biosynthesis of this class of compounds remains to be established.

The role of calystegines as nutritional mediators for rhizosphere bacteria may be only a partial explanation of their function in plants and other possibilities for their presence need to be investigated. Almost certainly the alkaloids will be found to be responsible for additional cases of livestock poisonings and their potential for toxicity to humans, particularly where they occur in food plants, mandates thorough investigation. Finally, the glycosidase inhibitors swainsonine and castanospermine have shown considerable potential as therapeutic agents in the treatment of cancer and viral diseases. The possibilities for the existence of equivalent properties among the calystegines should be vigorously explored.

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Chapter Five

Polyhydroxylated Alkaloids That Inhibit Glycosidases

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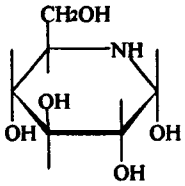
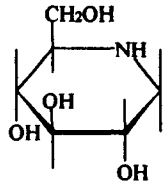
1.1. Introduction

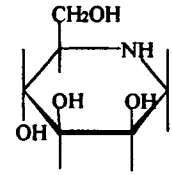
A remarkable range of monocyclic and bicyclic polyhydroxylated alkaloids that are specific and potent glycosidase inhibitors have been isolated from plants, microbial filtrates, and insects, though the latter have probably sequestered them from their food-plants. They include polyhydroxylated derivatives of pyrrolidine, piperidine, pyrrolizidine, octahydroindolizine (indolizidine) and *nor*tropane ring systems. An increasing number are being found which also occur as glycosides. These alkaloids are very widespread but long remained undetected because they do not react with reagents commonly used for alkaloid detection such as Dragendorff's and iodoplatinate reagents and do not readily dissolve in solvents such as chloroform. The first natural glycosidase-inhibiting alkaloid to be detected was the piperidine alkaloid nojirimycin (1), which was isolated from a *Streptomyces* filtrate in 1966 [1] but most have been discovered since 1983, during which time they have aroused increasing interest as potential anti-viral, anti-cancer and anti-diabetic agents, and as agrochemicals. Due to the potential applications several hundred related alkaloids have been synthesised but only a small proportion are available in the public domain. There are now a vast number of publications on synthesis and this is beyond the scope of this review but will be considered in brief in Section 1.7.



1
Nojirimycin
5-Amino-5-deoxy-D-glucopyranose



2
1-Deoxynojirimycin (DNJ)
1,5-Dideoxy-1,5-imino-D-glucitol



3
1-Deoxymannojirimycin (DMJ)
1,5-Dideoxy-1,5-imino-D-mannitol

These alkaloids can be considered as analogues of monosaccharides in which the ring oxygen has been replaced by nitrogen. They inhibit glycosidases by mimicking the pyranosyl and furanosyl moiety of the natural substrates, e.g., nojirimycin corresponds to glucose in the pyranose configuration and is an inhibitor of α- and β-glucosidases from various sources. Systematically these alkaloids have been described in the literature as derivatives of the parent heterocyclic compounds or sugars. 1-Deoxynojirimycin (2), prepared by removal of the anomeric hydroxyl of nojirimycin in 1968 [2] but later isolated from microbial and plant sources, is more stable than nojirimycin and has become a model glycosidase-inhibiting alkaloid, giving

rise to a trivial nomenclature for 1-deoxy analogues of other alkaloids of this type. Thus 1-deoxynojirimycin (DNJ) is 2*S*-hydroxymethyl-3*R*,4*R*,5*S*-trihydroxypiperidine or 1,5-dideoxy-1,5-imino-D-glucitol, and the 1-deoxy piperidine analogue of mannose has been given the trivial name of 1-deoxymannojirimycin (DMJ) (3). The nomenclature used most commonly for particular compounds and common abbreviations will be used here.

A distinction will be made between the glycosidase-inhibiting alkaloids and the amino sugars, amino acids and other miscellaneous compounds which also inhibit glycosidases. These will be considered briefly in Section 1.9.

1.2. Distribution

Table 1.1. shows the micro-organisms, plants and insects reported to contain polyhydroxylated alkaloids which have a structural resemblance to carbohydrates. There is no doubt that many more alkaloids of this type will be found from diverse sources, including plants in many taxa not previously considered to be alkaloid-producers. Some of the alkaloids such as 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) (9) are being reported from many disparate species of plants and more recently from micro-organisms, which would indicate that this is clearly a common metabolite. Others, such as the octahydroindolizine alkaloid castanospermine (28), have only been found in two closely related legume genera and the polyhydroxylated pyrrolizidine alkaloids also appear to be restricted in distribution. Although such patterns are appearing it is probably too early to discuss the significance of the distribution of most of these alkaloids until wider screens have been conducted.

Table 1.1. Naturally-Occurring Alkaloid Analogues of Carbohydrates.

Alkaloid	**Source and Reference**
Pyrrolines	
Nectrisine (FR-900483) (4)	*Nectria lucida* F-4490 (ATCC 20722) (Ascomycetes) [3]
Pyrrolidines	
2*R*-Hydroxymethyl-3*S*-hydroxypyrrolidine (CYB-3) (5)	*Castanospermum australe* (Leguminosae) seeds/leaves [4]
N-(Hydroxyethyl)-2-(hydroxymethyl)-3-hydroxypyrrolidine (6)	*Castanospermum australe* (Leguminosae) seeds [5]

Table 1.1. Continued.

Alkaloid	Source and Reference
1,4-Dideoxy-1,4-imino-D-arabinitol (D-AB1) (7)	*Angylocalyx* spp. (Leguminosae) seeds/leaves/bark [6] *Arachniodes standishii* (Polypodiaceae) leaves [7] *Eugenia* spp. (Myrtaceae) leaves/bark [8] *Hyacinthoides non-scripta* (Hyacinthaceae) bulb/leaves [8] *Morus bombycis* (Moraceae) leaves [9]
1,4-Dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol	*Morus alba* (Moraceae) roots [10] *Morus bombycis* (Moraceae) leaves [9]
1,4-Dideoxy-1,4-imino-D-ribitol (8)	*Morus alba* (Moraceae) roots [10]
2*R*,5*R*-Dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) (9)	*Derris elliptica* (Leguminosae) leaves [11] *Lonchocarpus* spp (Leguminosae) seeds/leaves [12] *Aglaonema* spp (Araceae) leaves [13] *Nephthytis poissoni* (Araceae) fruit/leaves [8,13] *Hyacinthoides non-scripta* (Hyacinthaceae) bulb/leaves [8] *Campanula rotundifolia* (Campanulaceae) leaves [8] *Omphalea diandra* (Euphorbiaceae) leaves [14] *Endospermum* sp. (Euphorbiaceae) leaves [15] *Urania fulgens* (Lepidoptera) body [14] *Streptomyces* sp. KSC-5791 [16]
Gualamycin (10)	*Streptomyces* sp. NK11687 [17]
Piperidines	
Nojirimycin (11)	*Streptomyces roseochromogenes* R-468 [1,2,18] *Streptomyces lavandulae* SF-425 [1,2,18] *Streptomyces nojiriencis* SF-426 [1,2,18]
Nojirimycin B (12) (Mannojirimycin)	*Streptomyces lavandulae* SF-425[19]
Galactostatin (13)	*Streptomyces lydicus* PA-5726 [20]
1-Deoxynojirimycin (DNJ) (14)	*Streptomyces lavandulae* subsp. *trehalostaticus* no. 2882 [21] *Bacillus amyloliquefaciens, Bacillus polymyxa, Bacillus subtilis* [22] *Morus* sp. (Moraceae) roots [10,23] *Morus bombycis* (Moraceae) leaves [9]

Table 1.1. Continued.

Alkaloid	Source and Reference
N-Methyl-1-deoxynojirimycin	*Morus alba* (Moraceae) roots [10]
1-Deoxynojirimycin-2-*O*-, 3-*O*-, 4-*O*-* α-D-glucopyranosides and 2-*O*-, 6-*O*-α-D-galactopyranosides and 2-*O*, 3-*O*-, 4-*O*-, 6-*O*-β-D-glucopyranosides	*Morus alba* (Moraceae) roots [10] **Streptomyces lavandulae* GC-148 [24]
1-Deoxymannojirimycin (DMJ) (15)	*Lonchocarpus sericeus* (Leguminosae) seeds/leaves [25] *Angylocalyx* spp. (Leguminosae) seeds/leaves/bark [8] *Omphalea diandra* (Euphorbiaceae) leaves [26] *Streptomyces lavandulae* GC-148 [27]
α-Homonojirimycin (16)	*Omphalea diandra* (Euphorbiaceae) leaves [14] *Nephthytis poissoni* (Araceae) leaves [8] *Urania fulgens* (Lepidoptera) body [14]
α-Homonojirimycin-7-*O*-β-D-glucopyranoside (MDL 25,637)	*Omphalea diandra* (Euphorbiaceae) leaves (tentative) [14] *Nephthytis poissoni* (Araceae) leaves [8] *Lobelia sessilifolia* (Campanulaceae) leaves [8]
Fagomine (17)	*Fagopyrum esculentum* (Fagaceae) seeds [28] *Xanthocercis zambesiaca* (Leguminosae) seeds [29] *Morus bombycis* (Moraceae) leaves [9] *Morus alba* (Moraceae) roots [10]
3-*Epi*fagomine (18)	*Morus alba* (Moraceae) roots [10]
Fagomine-4-*O*-β-D-glucopyranoside	*Xanthocercis zambesiaca* (Leguminosae) seeds [29]
Pyrrolizidines	
Alexine (19)	*Alexa* spp. (Leguminosae) seeds/leaves [30]
3,7a-Di*epi*alexine (20)	*Castanospermum australe* (Leguminosae) seeds/leaves [31]
7a-*Epi*alexine (Australine) (21)	*Castanospermum australe* (Leguminosae) seeds/leaves [32]
1,7a-Di*epi*alexine (22)	*Alexa* spp./*C. australe* (Leguminosae) seeds/leaves [33]

Table 1.1. Continued.

Alkaloid	Source and Reference
7,7a-Di*epi*alexine (23)	*Alexa* spp./*C. australe* (Leguminosae) seeds/leaves [33]
7a-*Epi*alexaflorine (24)	*Alexa grandiflora* (Leguminosae) leaves [34]
Casuarine (25)	*Casuarina equisetifolia* (Casuarinaceae) bark [35] *Eugenia jambolana* (Myrtaceae) leaves [8]
Casuarine-6-*O*-α-D-glucopyranoside	*Casuarina equisetifolia* (Casuarinaceae) bark [35] *Eugenia jambolana* (Myrtaceae) leaves [8]
Octahydroindolizines	
Swainsonine (26)	*Swainsona canescens* (Leguminosae) leaves [36] *Astragalus* spp. (Leguminosae) leaves/stems [37] *Oxytropis* spp. (Leguminosae) leaves/stems [37] *Ipomoea* sp. aff. *calobra* (Convolvulaceae) seeds [38] *Rhizoctonia leguminicola* (Basidiomycetes) [39] *Metarhizium anisopliae* (Deuteromycetes) [40]
Swainsonine *N*-oxide	*Astragalus lentiginosus* (Leguminosae) [37]
Lentiginosine (27)	*Astragalus lengtiginosus* (Leguminosae) leaves [41]
Castanospermine (28)	*Castanospermum australe* (Leguminosae) seeds/leaves/bark [42] *Alexa* spp. (Leguminosae) seeds/leaves/bark [43]
6-*Epi*castanospermine (29)	*Castanospermum australe* (Leguminosae) seeds/leaves/bark [44,45]
6,7-Di*epi*castanospermine (30)	*Castanospermum australe* (Leguminosae) seeds [5]
7-Deoxy-6-*epi*castanospermine (31)	*Castanospermum australe* (Leguminosae) seeds [46]
Tropanes	
Calystegine A_3 (32)/ Calystegine B_2 (33)	*Calystegia sepium* (Convolvulaceae) leaves/roots [47,48] *Calystegia japonica* (Convolvulaceae) roots [8] *Convolvulus arvensis* (Convolvulaceae) leaves/roots [47,49] *Ipomoea* sp. aff. *calobra* (Convolvulaceae) seeds [38] *Atropa belladonna* (Solanaceae) leaves/roots [47,48]

Table 1.1. Continued.

Alkaloid	Source and Reference
Calystegine A_3 (32)/ Calystegine B_2 (33) (continued)	*Solanum spp.* (Solanaceae) tubers/leaves [50] *Datura wrightii* (Solanaceae) leaves [50] *Physalis alkekengi* var. *francheti* (Solanaceae) roots [51] *Scopolia carniolica* (Solanaceae) leaves/roots [52] *Mandragora officinarum* (Solanaceae) leaves/roots/fruits [52] *Hyoscyamus niger* (Solanaceae) leaves/roots [52]
Calystegine A_5 (34)	*Physalis alkekengi* var. *francheti* (Solanaceae) [51]
Calystegine B_1 (35)	*Calystegia sepium* (Convolvulaceae) leaves/roots [48] *Convolvulus arvensis* (Convolvulaceae) leaves/roots [47,49] *Ipomoea batatus* (Convolvulaceae) leaves/roots [8] *Physalis alkekengi* (Solanaceae) roots [51] *Scopolia carniolica* (Solanaceae) leaves/roots [52] *Mandragora officinarum* (Solanaceae) leaves/roots/fruits [52] *Hyoscyamus niger* (Solanaceae) leaves/roots [52]
Calystegine B_3 (36)	*Physalis alkekengi* (Solanaceae) roots [51]
Calystegine C_1 (37)	*Morus alba* (Moraceae) roots [10] *Ipomoea batatus* (Convolvulaceae) roots [8]
Calystegine B_1-3-*O*-β-D-glucopyranoside	*Nicandra physalodes* (Solanaceae) [8]
Miscellaneous	
Nagstatin (38)	*Streptomyces amakusaensis* MG846-fF3 [53]
Trehazolin (39)	*Micromonospora* strain SANK 62390 (Actinomycetes) [54]
Kifunensine (40)	*Kitasatosporia kifunense* 9482 (Actinomycetes) [55]

While there may be phylogenetic reasons for particular distributions of the polyhydroxylated alkaloids in plants and micro-organisms caution should be exercised in using the presence of these compounds as taxonomic markers in plants. One reason is that these alkaloids can be released into the soil by producer plants and micro-organisms from whence some such as DMDP and castanospermine can be readily taken up and accumulated in plant

tissues of completely unrelated neighbouring species (see Section 1.4.). It may also be the case that micro-organisms (*Rhizobium*, other rhizosphere organisms, or endophytes) closely associated with specific plants may also produce polyhydroxylated alkaloids which could then be taken up by the plant. However, in the case of castanospermine and the alexines (australines) in *Castanospermum* and *Alexa* there is a definitive taxonomic relationship which was highlighted by the presence of these compounds [43].

1.3. Glycosidase Inhibition

Most of the polyhydroxylated alkaloids listed in Section 1.2. which have been studied in detail have been shown to inhibit glycosidases in a reversible and competitive manner [56]. Some can be extremely potent and specific inhibitors of glycosidases, e.g., swainsonine which has a K_i for human lysosomal α-D-mannosidase of $7x10^{-8}$M [57]. The inhibition of certain enzymes can be predicted from the number, position and configuration of the hydroxyl groups but there can be marked differences in the inhibition of isozymes of a glycosidase in different species [58,59] and even within the same cell. For example Golgi α-mannosidase I is preferentially inhibited by polyhydroxylated alkaloids in the pyranose form, e.g., 1-deoxymannojirimycin, whereas Golgi α-mannosidase II is more susceptible to analogues in the furanose form, e.g., swainsonine [56]. The inhibition is often pH-dependent and it has been suggested that the alkaloids inhibit by the formation of an ion pair between the protonated inhibitor and an anionic group, probably a carboxylate anion, in the active site [60].

There is now a vast amount of literature on inhibition of glycosidases by particular polyhydroxylated alkaloids and related compounds (both natural and synthetic) and an overview of this area is given in some reviews [56, 60, 61, 62]. A large amount of research has been conducted on the inhibition of *exo*-glycosidases involved in glycoprotein formation and catalysis which has been shown to be important in both the toxicity and anti-viral and anti-cancer activities of these compounds. While not all natural polyhydroxylated alkaloids have been tested on a wide range of glycosidases, Table 1.2. lists some of the glycosidases which the compounds are reported to inhibit. It should be emphasised, however, that the variations in isozymes make it difficult to state, for example, that a potent inhibitor of many α-D-glucosidases will inhibit all very strongly.

Table 1.2. Inhibition of Glycosidases by Natural Polyhydroxylated Alkaloids.

Alkaloid	Glycosidases Inhibited
Pyrrolines	
Nectrisine (FR-900483)	α-glucosidase, α-mannosidase [3,63]
Pyrrolidines	
2*R*-Hydroxymethyl-3*S*-hydroxypyrrolidine (CYB-3)	weak inhibitor of α-glucosidase [64]
1,4-Dideoxy-1,4-imino-D-arabinitol (D-AB1)	potent inhibitor of α-glucosidase [64,65] and α-D-arabinosidase [66]
1,4-Dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol	weak inhibitor of α- and β-glucosidases [10]
1,4-Dideoxy-1,4-imino-D-ribitol	weak inhibitor of α-glucosidase [62]
2*R*,5*R*-Dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP)	invertase [12] and α- and β-glucosidase [58,59], trehalase [16], human β-mannosidase [56]
Piperidines	
Nojirimycin	α- and β-glucosidases [67]
Nojirimycin B	α-mannosidases [19]
Galactostatin	β-galactosidases [20]
1-Deoxynojirimycin (DNJ)	trehalase, α- and β-glucosidases, invertase, exo-β-1,3-glucanase [21]
N-Methyl-1-deoxynojirimycin	more potent inhibitor of α-glucosidase than DNJ [68]
1-Deoxynojirimycin-4-*O*-α-D-glucopyranoside	β-amylase [69]
1-Deoxynojirimycin-2-*O*-, 3-*O*-, 4-*O*-α-D-glucopyranosides and 2-*O*-, 6-*O*-α-D-galactopyranosides and 2-*O*-, 3-*O*-, 4-*O*-, 6-*O*-β-D-glucopyranosides	varying levels of inhibition of α-glucosidase [10]
1-Deoxymannojirimycin (DMJ)	α-mannosidases, and α-fucosidase [18], Golgi α-mannosidase [70]
α-Homonojirimycin	α-glucosidases [58,59,64]
α-Homonojirimycin-7-*O*-β-D-glucopyranoside	processing α-glucosidase II [71]
Fagomine	weak inhibitor of α-glucosidases [64], moderate inhibitor of β-galactosidase [61]

Table 1.2. Continued.

Alkaloid	Glycosidases Inhibited
3-*Epi*fagomine	β-galactosidase [61]
Fagomine-4-*O*-β-D-glucopyranoside	weak inhibitor of β-galactosidase [64]
Pyrrolizidines	
Alexine	trehalase and amyloglucosidase [33]
3,7a-Di*epi*alexine	amyloglucosidase [33]
7a-*Epi*alexine (Australine)	amyloglucosidase [32]
1,7a-Di*epi*alexine	α-glucosidase and amyloglucosidase [33]
7,7a-Di*epi*alexine	α-glucosidase and amyloglucosidase [33]
7a-*Epi*alexaflorine	weak inhibitor of amyloglucosidase [34]
Casuarine	α-glucosidases, trehalase, amyloglucosidase [72]
Octahydroindolizines	
Swainsonine	lysosomal and other α-mannosidases [73,74]
Lentiginosine	amyloglucosidase [41]
Castanospermine	α- and β-glucosidases [58,59,75]
6-*Epi*castanospermine	amyloglucosidase [44], neutral α-mannosidase [76]
6,7-Di*epi*castanospermine	amyloglucosidase and fungal β-glucosidase [5]
7-Deoxy-6-*epi*castanospermine	amyloglucosidase and yeast α-glucosidase [46]
Tropanes	
Calystegine A_3	β-glucosidase and trehalase [49,51]
Calystegine A_5	not inhibitory [51]
Calystegine B_1	β-glucosidase and β-galactosidase [51]
Calystegine B_2	β-glucosidase, α-galactosidase and trehalase [51]
Calystegine B_3	weak inhibitor of β-glucosidase and α-galactosidase [51]
Calystegine C_1	β-glucosidase and β-galactosidase [10,51]
Miscellaneous	
Nagstatin	β-*N*-acetyl-glucosaminidase [53]
Trehazolin	trehalase [54]
Kifunensine	Golgi α-mannosidase I [55]

In addition to the inhibition of enzymes, it has also been reported that activation of some enzymes can occur in the presence of polyhydroxylated alkaloids, for example, in addition to

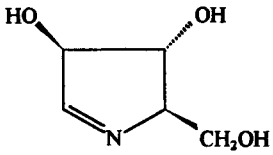
inhibiting α- and β-glucosidases, glycoprotein processing α-glucosidase I and lysosomal β-mannosidase, DMDP, a β-D-fructofuranose analogue, activates β-galactosidase (220%), α-L-arabinosidase (60%) and β-D-xylosidase (70%) from preparations of human fibroblasts and liver [77].

1.4. Ecological Significance and Toxicity

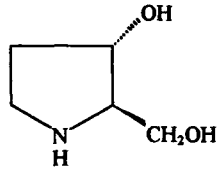
As might be expected for a group of compounds which affect very basic metabolic processes, the polyhydroxylated alkaloids have been reported to have a wide range of effects on other organisms and some cause serious livestock poisonings. In view of their inhibition of glycosidases it may be assumed that, at the least, they would be effective as anti-nutritional compounds in plants and insects, which would deter potential predators who might not only be unable to obtain full nutritional value from eating food containing them, but may also suffer digestive discomfort from inhibition of digestive enzymes. Making oneself indigestible is a good defence strategy and it is becoming increasingly clear that the production of these often highly active glycosidase inhibitors is widespread in plants and micro-organisms.

1.4.1. Effects on Bacteria and Fungi

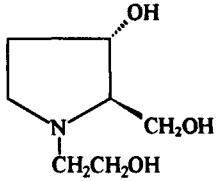
The production of glycosidase-inhibiting alkaloids by micro-organisms could well have the role of inhibiting the growth of competing micro-organisms. This is supported by the early work on nojirimycin [18], in which it was the anti-microbial activity against a drug-resistant strain of *Shigella flexneri* 2a, and also *Sarcina lutea* and *Xanthomonas oryzae* which led to its isolation. It has been shown that due to this activity nojirimycin is effective in preventing the rice plant disease caused by *X. oryzae* [18]. However, nectrisine (4) showed no anti-microbial activity to bacteria and fungi [3] and castanospermine, 2-hydroxymethyl-3-hydroxypyrrolidine (5), 1,4-dideoxy-1,4-imino-D-arabinitol (7), DMDP and 1-deoxynojirimycin did not inhibit the growth of either *Enterobacter aerogenes* or a range of fungi [78]. Castanospermine and DMDP did slow the vegetative growth of *Pythium ultimum* and *Botrytis cinerea* [78]. It has been suggested [47] that calystegines, which are often accumulated in the roots of the Convolvulaceae and Solanaceae, act as an exclusive carbon and nitrogen source for micro-organisms in the rhizosphere possessing a plasmid which encodes for their catabolism. In a screen of forty-two rhizosphere bacteria only *Rhizobium meliloti* 41, which contained the plasmid, was able to utilise them.



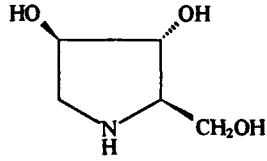
4
3,4-Dihydroxy-5-hydroxymethyl-1-pyrroline
(Nectrisine)
(FR-900483)



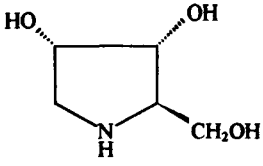
5
2-Hydroxymethyl-3-hydroxypyrrolidine
(CYB3)



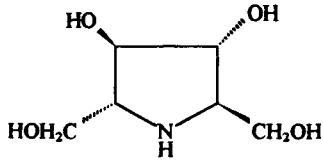
6
N-Hydroxyethyl-2-hydroxymethyl-3-hydroxypyrrolidine



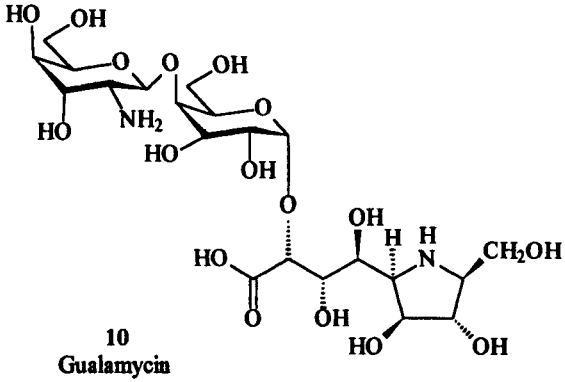
7
1,4-Dideoxy-1,4-imino-D-arabinitol
(D-AB1)



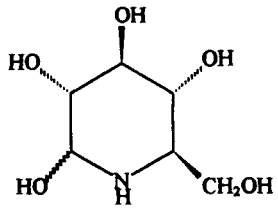
8
1,4-Dideoxy-1,4-imino-D-ribitol



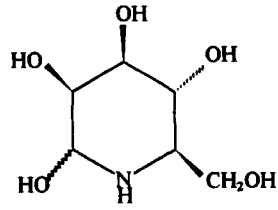
9
2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine
(DMDP)



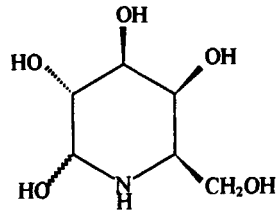
10
Gualamycin



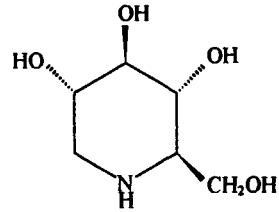
11
Nojirimycin



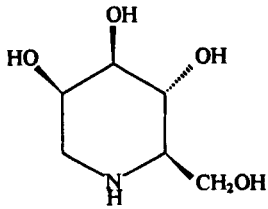
12
Nojirimycin B



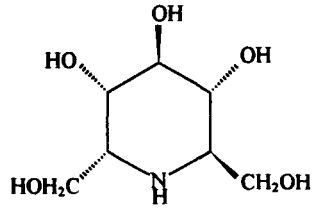
13
Galactostatin



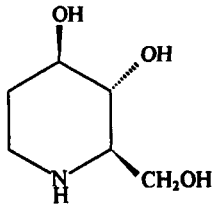
14
1-Deoxynojirimycin (DNJ)



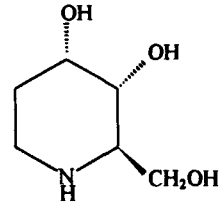
15
1-Deoxymannojirimycin (DMJ)



16
α-Homonojirimycin (HNJ)



17
Fagomine



18
3-*Epi*fagomine

1.4.2. Effects on Plant Growth

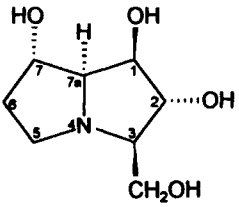
Nojirimycin [79] and DMDP [80] are inhibitory to auxin-stimulated cell extension of *Avena* and *Triticum* coleoptiles and *Pisum sativum* stem segments. Rosenfield and Matile [81] suggested that auxin-stimulated modification of cell wall polysaccharides by *exo*-β-glucanases was inhibited. Nojiirimycin has also been shown to inhibit the growth of *Pyrus communis* pollen tubes. Castanospermine, 2-hydroxymethyl-3-hydroxypyrrolidine, 1,4-dideoxy-1,4-imino-D-arabinitol, and 1-deoxynojirimycin inhibit the growth of seedlings of *Lactuca sativa* and *Allium porrum* [78]. Castanospermine was the strongest inhibitor followed by 1,4-dideoxy-1,4-imino-D-arabinitol. Castanospermine is absorbed by roots of *Triticum aestivum* and *L. sativa*. In the latter plant it reduced growth and was lethal at a concentration of 4×10^{-5}M. but in *T. aestivum* the plant survived this concentration; leaf and root length decreased while the number of leaves increased [78]. Stevens and Molyneux [82] have also shown castanospermine but not swainsonine is phytotoxic to *Medicago sativa*, *Panicum miliaceum* and *Echinochloa crusgalli*. Phloem mobility has been demonstrated for swainsonine [83] and DMDP [84]. The presence of castanospermine in bark and roots of *Castanospermum* and *Alexa*, several polyhydroxylated alkaloids in *Morus* root bark [10] and DMDP in *Lonchocarpus* root bark, raises the possibility that these compounds may affect the growth of other plants as the result of leaching from bark and leaf litter.

1.4.3. Effects on Nematodes

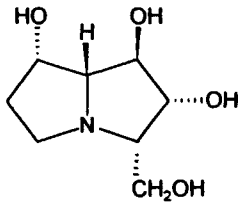
Castanospermine has a marked effect on population growth of the fungal feeding nematode *Aphelenchus avenae* but it is not toxic on immersion of nematodes in a 0.1% (w/v) solution [78]. DMDP has been shown to be active against several plant parasitic nematode species (including *Globodera pallida* and *Meloidogyne* spp) *in vitro* and *in vivo*. It was effective when applied as a soil drench or seed dressing [84]. Applied as a soil drench at 30μg ml^{-1} it inhibited virus acquisition and transmission and root galling by *Xiphinema diversicaudatum*. The mode of action of castanospermine and DMDP is not known, but they lack the rapid *in vitro* toxic effect characteristic of synthetic nematocides.

1.4.4. Effects on Insects

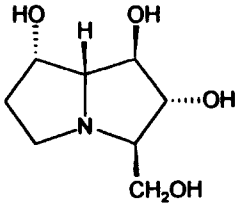
Castanospermine [83] and DMDP [84] have both been shown to have feeding deterrent activity against aphids and castanospermine reduced the survival rate. However, appreciable levels of swainsonine were detected in the honeydew from a pea aphid (*Acyrthosiphon pisum*) colony opportunistically feeding on *Astragalus lentiginosus* and it was not a deterrent to this species [83].



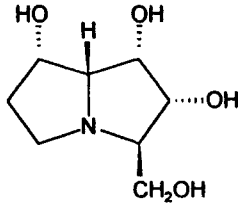
19
Alexine



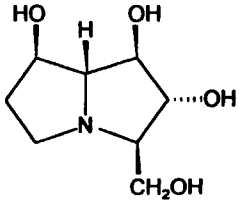
20
3,7a-Di*epi*alexine



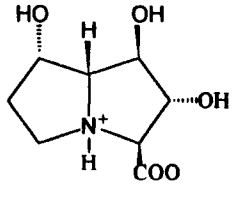
21
7a-*Epi*alexine
(Australine)



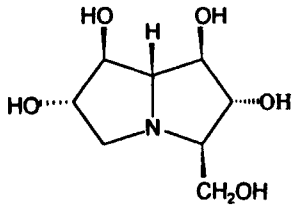
22
1,7a-Di*epi*alexine



23
7,7a-Di*epi*alexine



24
7a-*Epi*alexaflorine



25
Casuarine

DMDP inhibited feeding of nymphs of *Schistocerca gregaria* and *Locusta migratoria* at concentrations below 0.001% w/w but did not appear to be toxic to them when force-fed [85].

Antifeedancy studies using *Spodoptera littoralis* have shown that swainsonine, castanospermine, DMDP, and 1,4-dideoxy-1,4-imino-D-arabinitol are antifeedant, but that 1-deoxynojirimycin, 1-deoxymannojirimycin, fagomine (17), fagomine-4-*O*-β-D-glucoside and 2-hydroxymethyl-3-hydroxypyrrolidine were not antifeedants [86]. In contrast to *S. littoralis*, *S. exempta* is more sensitive to many antifeedant compounds and this species was deterred from feeding by 1-deoxynojirimycin and 1-deoxymannojirimycin. After exposure to the fructofuranose analogue DMDP, the ability of a glucose sensitive lateral styloconic sensillum of *S. littoralis* to respond to glucose was observed to be blocked for several hours. Preliminary studies indicated that castanospermine interacted with the glucose receptor in a similar way. The blocking of responses of sensillae by plant alkaloids may serve to render insects unable to detect phagostimulatory sugars.

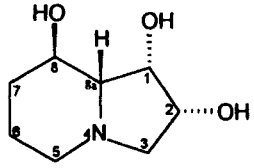
In view of the effects of DMDP on insects, interestingly the gut α-glucosidase activity of the bruchid beetle *Ctenocolum tuberculatum*, which has a host range limited to DMDP-containing genera of the legume subtribe Lonchocarpinae, is 100 times less sensitive to DMDP than the corresponding activity in the gut of larvae of another bruchid beetle, *Callosobruchus maculatus*, which does not feed on DMDP-containing legumes [78]. In the same study castanospermine was shown to be strongly inhibitory to the development of *C. maculatus* and *Tribolium confusum* when incorporated into the diet. As the brightly-coloured day-flying moth *Urania fulgens* feeds on *Omphalea diandra* which contains DMDP, α-homonojirimycin (16) and 1-deoxymannojirimycin, and accumulates the two former compounds in its body [14], it must presumably have some means of dealing with the glycosidase inhibition of these compounds. Similarly, the Death's head hawk-moth (*Acherontia atropus*) can store calystegines from its food plants in the Solanacae [50] but in contrast the Convolvulus hawk-moth (*Agrius convolvuli*) excretes high concentrations of the calystegines it consumes as a larva and they are not found in the adults [8]. The Lepidoptera which store glycosidase inhibitors may use them as defences against predators such as birds, but perhaps the alkaloids may also serve as defences against diseases (Section 1.5).

Gualamycin (10) has been shown to have acaricidal activity [17].

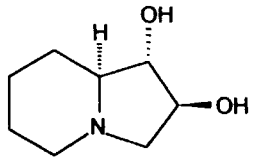
1.4.5. Toxicity to Mammals

The toxicity to livestock of the legumes *Swainsona canescens* and *Castanospermum australe* in Australia led to the isolations of the toxic principles swainsonine [36] and castanospermine [42]. Cattle eating *Swainsona* species developed a syndrome called "pea struck" which is due to decreased α-mannosidase activity resulting in accumulation of mannose-rich oligosaccharides in lysosomes causing neuronal vacuolation, axonal dystrophy, loss of cellular function and death [87]. The disorder "locoism" in the western United States was also found to be due to swainsonine in locoweeds (*Astragalus* and *Oxytropis* species). Clinically,

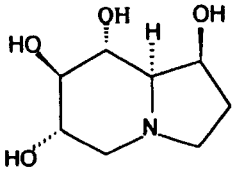
swainsonine poisoning is characterised, amongst other things, by depression, tremors, nervousness, emaciation and gastrointestinal malfunction [88]. Poisoning of animals in China by *Oxytropis ochrocephala* and *O. kansuensis* has also been shown to be due to the presence of swainsonine at concentrations of 0.012% and 0.021% (dry weight), respectively [89]. Such potent glycosidase inhibitors do not need to be present at high concentrations for toxic effects. In the case of swainsonine the low concentration ingested is made more effective by its ability to permeate the plasma membrane freely, but once inside lysosomes it becomes protonated due to the low pH and becomes concentrated [90]. A threshold of toxicity is difficult to establish but a conservative estimate of the concentration in the diet which should be of concern could be as low as 0.001% [91].



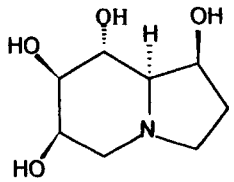
26
Swainsonine



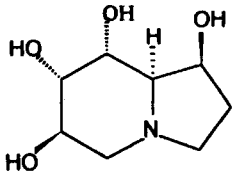
27
Lentiginosine



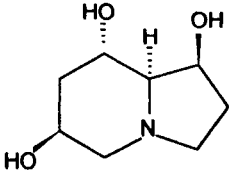
28
Castanospermine



29
6-*Epi*castanospermine



30
6,7-Di*epi*castanospermine



31
7-Deoxy-6-*epi*castanospermine

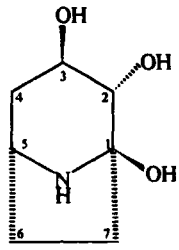
Castanospermum australe contains the potent α- and β-glucosidase inhibitor castanospermine and this has been shown to induce a deficiency of lysosomal β-glucosidase [92]. Feeding experiments with rodents resulted in glycogen accumulation [93] and in rats it

causes degenerative vacuolation of hepatocytes and skeletal myocytes [88]. This resembles the genetic disorder glycogenosis type II (Pompe's disease), in the same way that swainsonine intoxication resembles the genetic deficiency of lysosomal α-D-mannosidase activity (mannosidosis). Pigs, cattle and horses are reported to be poisoned by *C. australe* with the chief symptom being gastroenteritis [94]. Several cases of human poisoning have occurred [95].

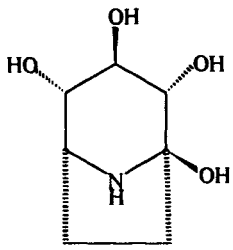
As knowledge on the distribution and activity of polyhydroxylated alkaloids grows it is likely that more cases of livestock problems may be recognised as being due to these compounds. For example, in the U.K. livestock have been reported to suffer abdominal pain, dysentery, lethargy and dullness after grazing *Hyacinthoides non-scripta* (Liliaceae) but the toxic principles remained unknown [96]. We have now found that the plant contains high concentrations of DMDP and related alkaloids which may be the cause [8].

Recently, swainsonine has also been detected in seeds of the Australian plant *Ipomoea sp* (aff. *calobra*) (Weir Vine) (Convolvulaceae) [38]. This plant is reported to produce a neurological disorder in livestock with clinical signs similar to those caused by other swainsonine-containing plants. This species also contains the *nor*tropane calystegine B_2 (**33**) which inhibits β-glucosidase and α-galactosidase and so there may be a combination of toxic effects. The calystegines are common in the Solanaceae and there are several *Solanum* species which produce cases of poisoning very similar to locoism [91]. For example, *S. dimidiatum* causes "Crazy Cow Syndrome" in Texas and *S. kwebense* which causes "Maldronksiekte" in South Africa [97], both characterised by neurological signs of cerebellar disorder, including staggering, incoordination, with severe cellular vacuolation and degeneration of Purkinje cells. Both plants contain calystegine B_2 and other calystegines [50]. It seems likely that these syndromes are lysosomal storage disorders caused by glycosidase inhibition produced by the calystegines. The presence of calystegines in human foods such as potatoes, aubergines and sweet potatoes [50] poses the question of what effect do these compounds have on humans?

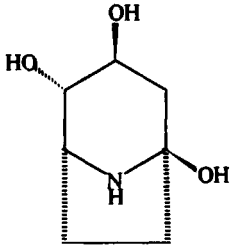
Poisoning by polyhydroxylated alkaloids generally takes several weeks of ingestion before it becomes apparent. Although lysosomal function will return to normal after the intoxication ends as the alkaloids are excreted and many of the lesions will disappear, some long term effects may result in reduced animal performance [98]. The selective advantage to the plant of containing glycosidase-inhibiting alkaloids such as swainsonine and castanospermine is not entirely clear because of the relatively long time period required for poisoning to become apparent and also affected animals appear to actively seek out the plants containing them [37,94].



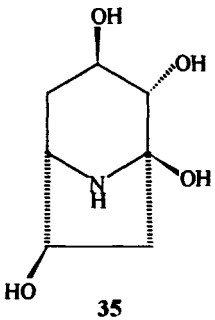
32
Calystegine A_3



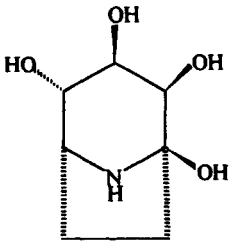
33
Calystegine B_2



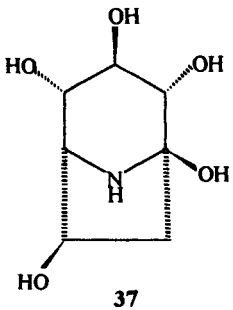
34
Calystegine A_5



35
Calystegine B_1



36
Calystegine B_3



37
Calystegine C_1

1.5. Potential Therapeutic Activity

The potential pharmaceutical benefits of polyhydroxylated alkaloids as anti-viral and anti-cancer agents appear to largely result from their ability to alter glycoprotein structure by

interfering with processing of the oligosaccharide moiety. These effects are also responsible for the neurological damage caused by swainsonine and castanospermine but it has been found that the doses required for beneficial effects may be below those causing damage, or that allowing a period of lysosomal clearance between doses may limit toxicity. Symptoms of gastroenteritis caused by inhibition of gut glycosidases can be limited by chemically modifying the natural products to increase the rate of uptake.

Castanospermine has been shown to have *in vitro* antiviral activity against human immunodeficiency virus (HIV) [99] and human cytomegalovirus (CMV) [100], which is an opportunistic pathogen in AIDS. Alterations in viral coat glycoproteins in the presence of castanospermine are associated with a loss of infectivity. 6-*O*-acyl derivatives of castanospermine are more potent inhibitors of HIV growth than the natural product with the 6-*O*-butyryl-derivative (MDL 28,574) currently in clinical trials for AIDS. The lipophilic nature of the acyl derivative improves uptake by cells but the compound appears to be intracellularly converted to castanospermine [101].

1-Deoxynojirimycin also has been shown to have anti-HIV activity and the pharmacokinetic properties of 1-deoxynojirimycin have been improved by producing *N*-substituted derivatives such as *N*-butyl-deoxynojirimycin (SC-48334) which has enhanced anti-HIV activity [102].

Nectrisine also has anti-viral activity and inhibits the retrovirus Friend leukaemia virus *in vivo* in mice and can potentiate the activity of AZT (zidovudine) [103]. Synergistic activity has also been shown when castanospermine and AZT are used in combination therapy *in vitro* [104]. *In vivo* combination studies with *N*-butyl-deoxynojirimycin and AZT in human patients indicated anti-viral activity but caused diarrhoea, abdominal pain and weight loss [105]. Chemical modification of *N*-butyl-deoxynojirimycin to make it better tolerated was suggested.

Castanospermine, swainsonine and nectrisine have been shown to have anti-cancer activity. Castanospermine inhibits experimental metastasis in mice [106]. Nectrisine (FR-900483) has been reported to induce Ia antigen and restore the immune response of immunosuppressed mice and these effects were assumed to be due to α-mannosidase inhibition [107].

Most of the anti-cancer work using these alkaloids has centred around swainsonine which has been shown to enhance the activities of the mouse immune system *in vitro* [40] and *in vivo* [108]. The antimetastatic effect of swainsonine has been shown to be largely due to augmentation of natural antitumour defences such as killer T-cells. Swainsonine has a slow effect after administration, with a peak of activity after 2 to 3 days, the effect being retained 5-7 days after treatment stopped. Swainsonine also increases susceptibility of cancer cells to natural killer cells and lymphokine activated killer cell cytotoxicity [109]. Direct anti-tumour activity is also suggested as transformed human tumour cells revert to normal when treated with swainsonine [110]. A phase I study of swainsonine in patients with advanced malignancies

showed that minimal toxicity was caused when administered intravenously to cancer patients at dosages that inhibit both Golgi α-mannosidase II and lysosomal α-mannosidases [111]. In a preliminary pharmacokinetic evaluation in mice it appeared that central nervous system toxicity may not be a problem at swainsonine levels preventing metastasis [112].

Mannostatin A, a pentasubstituted amino-cyclopentane produced by *Streptoverticillium verticillus* var *quintum* ME3-AG3 [113], is also a specific inhibitor of α-D-mannosidase and has shown anti-metastatic activity [114].

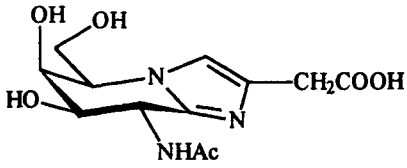
Research has continued to consider alkaloid glycosidase inhibitors such as 1-deoxynojirimycin as potential anti-diabetic agents. *N*-Hydroxyethyl-deoxynojirimycin (miglitol, BAY m 1099) has improved retention in the small intestine which increases its potential *in vivo* antidiabetic activity, not by hypoglycaemic activity like insulin or sulfonylureas but by reducing postprandial insulin secretion, lessening diabetic glucosuria and reducing carbohydrate-driven synthesis of low density lipoproteins [115]. Fagomine and 2-*O*-α-D-galactopyranosyl-deoxynojirimycin also have potent anti-hyperglycaemic effects in streptozocin-diabetic mice [116].

Castanospermine also has been shown to have anti-inflammatory activity, modifies the expression of adhesion molecules and prolongs allograft survival in a dose dependent manner [117].

1.6. Isolation

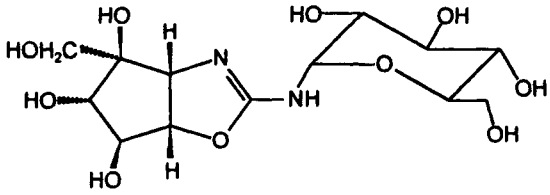
Ishida and co-workers in 1967 [18] noted that it was not possible to extract nojirimycin from the culture filtrate of *Streptomyces* using organic solvents. They found that the antibiotic was adsorbed from the filtrate by strong cation exchange resins such Dowex 50 and IR-120 and purification could be achieved by chromatography on these resins. This method has remained the most widely used. Molyneux has reviewed techniques used for the isolation, characterisation and analysis of polyhydroxylated alkaloids [118].

Eighteen polyhydroxylated alkaloids (aglycones and glycosides) have been isolated from hot water extracts of *Morus alba* roots (equivalent volume of MeOH added after cooling) by the use of Celite, Amberlite IR-120 (H^+ form), Amberlite CG-50 (NH_4^+ form) and Dowex 1 anion exchange resin (OH^- form) for the aglycones, and Dowex 50W (pyridine form), Dowex 1 (OH^- form) and CM Sephadex C-25 (NH_4^+ form) for the glycosides [10]. The alkaloids were monitored by HPTLC Silica Gel-60F254 (Merck) using the solvent system 4:1:1 (PrOH-AcOH-H_2O), with detection by spraying with chlorine-*o*-tolidine reagent. Preparative centrifugal tlc has also been used to isolate alkaloids from *Castanospermum australe* and purity checked by tlc on silica gel plates developed with $CHCl_3$-MeOH-NH_4OH-H_2O (70:26:2:2) and alkaloids detected with Ac_2O followed by Ehrlich's reagent [32].

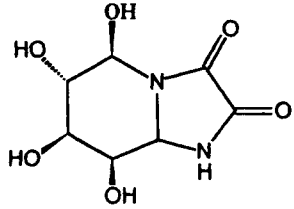


38
Nagstatin

Purification of the particularly potent glycosidase inhibitors can be monitored by enzyme assays as used initially for swainsonine isolation from *Swainsona canescens* [36], and this has also been used for microbial compounds such as trehazolin [54] although ion exchange is an important component of these purifications. A combination of screening for trehalase inhibition, preparative HPLC and ion exchange chromatography was used recently for the isolation of DMDP from *Streptomyces* [16]. Ion exchange was also used for the isolation of nagstatin with assays for β-*N*-acetylglucosaminidase inhibition [38]. The screening of micro-organisms for immunoactive compounds by their competitive action against inhibition of mouse spleen cells by immunosuppressive serum led to the isolation of swainsonine from *Metarhizium anisopliae* [40]. This purification involved a combination of column chromatographic separations on activated charcoal, CM-Sephadex and silica gel and fractions were monitored using the above assay



39
Trehazolin



40
Kifunensine

High pressure liquid chromatography with electrochemical detection has been used for castanospermine, 1-deoxynojirimycin, 1-deoxymannojirimycin and swainsonine using a cation exchange column. With this technique it was possible to detect the compounds when added to urine and plant extracts [119].

Gas chromatography of trimethylsilyl-ether derivatives of the alkaloids [120] achieves good separations of all the natural alkaloids, including epimers, by using capillary columns such as BPX5 (SGE), but packed OV-1 columns can also achieve good resolution. A great advantage of GC analysis is that it is highly sensitive and when coupled to a mass spectrometer gives useful structural information [91].

1.7. Synthesis

Interest in glycosidase inhibition has led to many efficient chemical syntheses of the polyhydroxylated alkaloids and their derivatives [121,122]. For example, castanospermine has been synthesised [123], along with a series of epimers and deoxy derivatives [124] and acyl derivatives such as 6-*O*-butyrylcastanospermine [125]. Burgess and Henderson [126] have reviewed syntheses of castanospermine and analogues, and Furneaux *et al.* have reported the synthesis of australine and analogues from castanospermine [127]. *N*-Butyl-deoxynojirimycin has been synthesised and shows better anti-HIV activity than the natural product [102]. Duclos *et al.* have synthesised a hydroxymethyl analogue of the tropane alkaloid calystegine B_2 [128].

Chemical syntheses have allowed investigations of structure-activity relationships, e.g., for mannosidase inhibitors [129,130]. Some potent new inhibitors have been produced such as 1,4-dideoxy-1,4-imino-D-mannitol (DIM) (**41**), an azafuranose analogue of mannose [131] which is a good mannosidase inhibitor but unlike 1-deoxymannojirimycin it does not inhibit Golgi α-mannosidase I. 1-Deoxymannojirimycin is a more potent inhibitor of α-L-fucosidase than of α-D-mannosidase [132]. 1-Deoxyfuconojirimycin (1,5-dideoxy-1,5-imino-L-fucitol) (DFJ) (**42**) is a synthetic potent and specific inhibitor of human liver α-L-fucosidase.

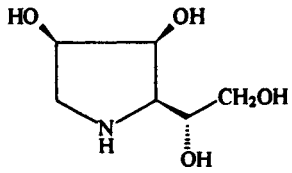
The α-glucosidase inhibitory 7-O-β-D-glucopyranosyl derivative of α-homonojirimycin was designed to be a transition state analogue of sucrose [133] before it and its aglycone were discovered as natural products. The synthesis of more disaccharide analogues containing 1-deoxymannojirimycin as endomannosidase inhibitors for chemotherapeutic applications has aroused interest as this group of inhibitors may be more effective at preventing formation of fully mature *N*-linked oligosaccharides [134,135]. The reason for the interest in endoglycosidases is that they are not inhibited by inhibitors of exoglycosidases such as swainsonine, castanospermine, 1-deoxymannojirimycin and 1-deoxynojirimycin. Cells grown in the presence of exoglycosidase inhibitors can form fully matured glycans by using endo-α-mannosidases which bypass the early trimming reactions of exoglycosidases.

Recently *N*-containing analogues of rhamnose have been proposed for treating diseases such as leprosy and tuberculosis caused by mycobacteria which contain rhamnose in the cell wall since rhamnose has no role in mammalian metabolism. Design and synthesis of specific inhibitors of rhamnosidase have been reported [136,137].

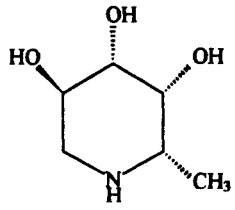
Because elevated levels of β-*N*-acetylglucosaminidases occur in serum of animals with malignant disease and in malignant cells, there is an interest in producing inhibitors of this enzyme as anti-cancer agents [138]. Polyhydroxylated alkaloids have been used as models to produce inhibitors for this purpose. Specific and potent inhibitors of β-*N*-acetylglucosaminidases such as 6-acetamido-6-deoxy-castanospermine [139] and the piperidine 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol have been synthesised [140].

Clearly glycosides of the polyhydroxylated alkaloids occur widely in nature and it has been possible to produce α- and β-glucosides of 1-deoxynojirimycin by the use of native and immobilised glucosidases from rice and *Rhodotorula lactosa* [141]. Transglucosylation between 1-deoxynojirimycin and soluble starch has also been possible by using bacterial saccharifying amylase. The α-1,4-glucosyl-deoxynojirimycin produced was shown to be a potent inhibitor of β-amylase [69].

Most probably, many of the synthesised inhibitors will be found to be produced by micro-organisms or plants in the future.



41
1,4-Dideoxy-1,4-imino-D-mannitol
(DIM)



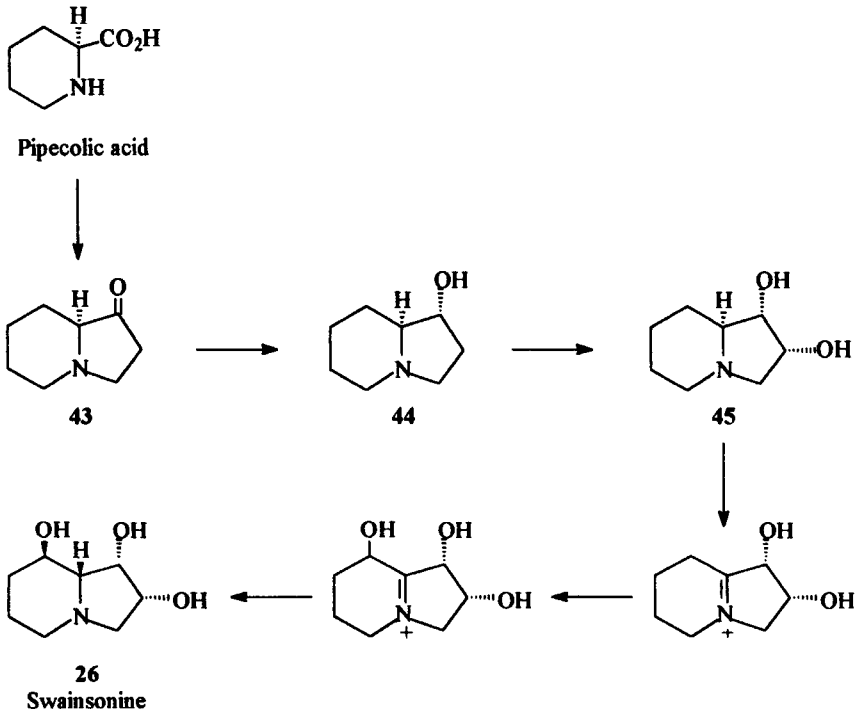
42
Deoxyfuconojirimycin
(DFJ)

1.8. Biosynthesis

Biosynthetic studies have been largely restricted to microbial products. An early study of nojirimycin indicated that it is derived from glucose with head C(1) and tail C(6) inversion, and suggested that an amino group is introduced via 5-ketoglucose [2]. Recently, in *Bacillus subtilis* var *niger* (ATCC 9372), nojirimycin B (12) and nojirimycin have been suggested as intermediates in the biosynthesis of 1-deoxynojirimycin [142]. 1-Deoxymannojirimycin was also derived from nojirimycin B in *Bacillus subrutilis* (ATCC 27467). *Agrobacterium* sp strain 19-1 has been shown to be able to epimerise 1-deoxynojirimycin to 1-deoxymannojirimycin [143].

The biosynthesis of swainsonine was studied in the fungus *Rhizoctonia leguminicola*; The piperidine ring was shown to be derived from lysine via pipecolic acid. A noteworthy

feature of the pathway (Scheme 1.1) is that the carboxyl carbon atom of pipecolate is retained in the alkaloid, whereas the normal course of events in the biosynthesis of alkaloids involves loss of the carboxyl group. Two additional carbons are contributed from acetate to the side-chain which cyclises to oxo-octahydroindolizine (**43**) which gives rise by reduction to (1,8a-*trans*)-1-hydroxyoctahydroindolizine (**44**) and is then hydroxylated at C(2) to give (1,8a-*trans*-1,2-*cis*)-1,2-dihydroxyoctahydroindolizine (**45**) before being converted to swainsonine [144]. Swainsonine biosynthesis in *Astragalus oxyphysus* occurs by a very similar pathway [145].

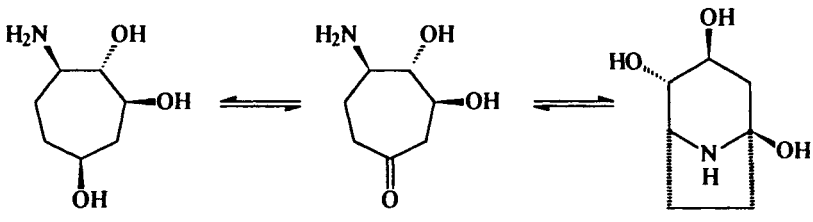


Scheme 1.1

Molyneux *et al.* [5] hypothesize that the *N*-(hydroxyethyl)-2-(hydroxymethyl)-3-hydroxypyrrolidine (**6**) isolated from *Castanospermum australe* might be a precursor of the octahydroindolizines (castanospermines) in that plant by insertion of an additional carbon atom between the 2-(hydroxymethyl) group and the terminal carbon atom of the *N*-(hydroxyethyl) group and of the pyrrolizidines (australines and alexines) by insertion of the extra carbon

between the 2-(hydroxmethyl) group and the methylene group adjacent to the nitrogen atom of the *N*-(hydroxyethyl) group.

The bridgehead hydroxyl of the calystegines is unusual in tropane and *nor*tropane alkaloids and the presence of calystegine C_1 (37) in *Morus alba* [10], a genus not noted for tropane alkaloids, may indicate that at least in this plant there is a different pathway to this *nor*tropane alkaloid. Recently *Physalis alkekengi* var *franchetii*, which accumulates several calystegines in its roots, has been found to contain 1β-amino-2α,3β,5β-trihydroxycycloheptane (46) and it is suggested that it may be a precursor of calystegine A_5 (Scheme 1.2) [146].



46 34

Calystegine A_5

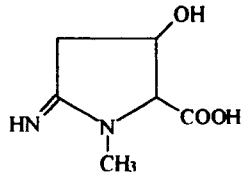
Scheme 1.2

1.9. Other *N*-containing and Miscellaneous Glycosidase Inhibitors

The polyhydroxylated alkaloids are not the only nitrogen-containing compounds which can inhibit glycosidases and in particular some amino acids and amino sugars also act as analogues of carbohydrates. A few of these analogues will be mentioned here as similar compounds may be encountered in screening material for glycosidase-inhibiting alkaloids.

2*S*-Carboxy-3*R*,4*R*,5*S*-trihydroxy-piperidine from *Baphia racemosa* (Leguminosae) seeds is an inhibitor of human β-D-glucuronidase and α-L-iduronidase [147]. The all *trans*-dihydroxyproline, (2*S*,3*R*,4*R*)-2-carboxy-3,4-dihydroxypyrrolidine, from *Amanita virosa* is structurally analogous at C(2), C(3) and C(4) to D-glucuronic acid and it is also a moderate inhibitor of human β-D-glucuronidase [148].

Siastatin B, 2*R*-acetamido-3*R*,4*S*-dihydroxypiperidine-5*S*-carboxylic acid, produced by *Streptomyces verticillus* var. *quintum* MB695-A4, is an inhibitor of neuraminidase [149]. Pyrostatin A (47) and B are specific inhibitors of β-*N*-acetylglucosaminidase isolated from filtrates of *Streptomyces sp.* SA-3501 [150].



47
Pyrostatin A

Acarbose (Glucobay) and homologs isolated from *Actinoplanes* strains are inhibitors of α-glucosidases which have been investigated as potential therapeutic agents for treating diabetes [115]. Acarbose is a pseudo-tetrasaccharide comprising the amino-sugar acarviosine and one maltose unit. Validoxylamine A and glucosides (*Streptomyces hygroscopicus* subsp. *limoneus*) are part of the validamycin complex. They inhibit trehalase and also have insecticidal activity [151]. Valiolamine and 5-*epi*valiolamine are also produced by *Streptomyces hygroscopicus* subsp. *limoneus* under aerobic conditions. These compounds, but especially the former, inhibit α-glucosidases [152]. Salbostatin is a trehalase inhibitor produced by *Streptomyces albus* (ATCC 21838). It is a pseudodisaccharide consisting of valienamine linked to 2-amino-1,5-anhydro-2-deoxyglucitol [153]. Pyridindolol, a β-galactosidase inhibitor has been isolated from the Actinomycetes [154]. There are also other classes of microbial products which inhibit glycosidases and these include carbohydrates, polypeptides and glycopeptides [155].

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