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ENZYMIC CONTROL OF STEREOCHEMISTRY AMONG THE THALICTRUM BISBENZYLISOQUINOLINE ALKALOIDS

Hélène Guinaudeau,[1] Alan J. Freyer and Maurice Shamma,
Department of Chemistry, The Pennsylvania State University,
University Park, Pennsylvania 16802, U.S.A.

and Kemal Hüsnü Can Başer,
Faculty of Pharmacy, Anadolu University,
Eskişehir, Turkey

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Abstract: The new bisbenzylisoquinolines (+)-thaligrisine (1) and (+)-thaliphylline (6) have been isolated from Thalictrum minus var. microphyllum. Four rules are described which correlate the structures of Thalictrum bisbenzylisoquinolines with their stereochemistry at C-1 and C-1'. As a result, (+)-thalrugosamine is shown to be identical with (+)-homoaromoline (14). (+)-Thalisamine and (+)-N'-norhernandezine are also identical and are represented by expression 18. The sole exception to the rules is (-)-isothalidezine (15).

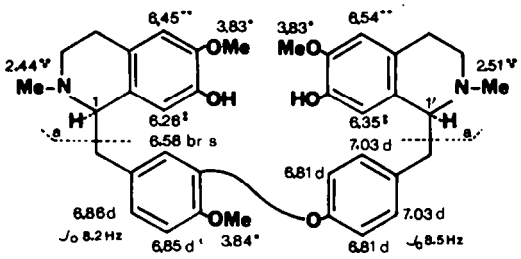
The bisbenzylisoquinolines are the largest subgroup among the isoquinoline alkaloids, numbering over 260 members.[2] As implied by their name, bisbenzylisoquinolines are formed from *in vivo* condensation of two tetrahydrobenzylisoquinolines through phenolic oxidative coupling. Initial bonding may occur either in a tail to tail or head to tail mode. In some cases, this is followed by a second oxidative coupling, or even a third, so that several bisbenzylisoquinolines are known which incorporate not just one, but two or three linkages connecting the two tetrahydrobenzylisoquinoline moieties. What is more, the stereochemistry at the two asymmetric centers, C-1 and C-1', appears not to have any specific preference. One is thus inclined, *prima facie*, to believe that dimers of the 1R,1'R, 1S,1'S, 1S,1'R and 1R,1'S configurations are equally possible.

We would now like to attempt to demonstrate, at least in part, that the realm of bisbenzylisoquinolines is not one of disorder, but is a rational discipline which obeys firm and well-defined biogenetic rules. Consequently, one may be able not only to classify the bisbenzylisoquinolines in a systematic manner, but also to verify some of the past structural assignments.

We propose to limit ourselves, for the time being, mainly to the bisbenzylisoquinolines of the genus Thalictrum (Ranunculaceae). We have done so because Thalictrum species are extremely rich in the varieties of isoquinoline alkaloids they can provide.[3] Additionally, more than 22 Thalictrum species have been investigated, leading to an impressive array of 58 different bisbenzylisoquinoline alkaloids.

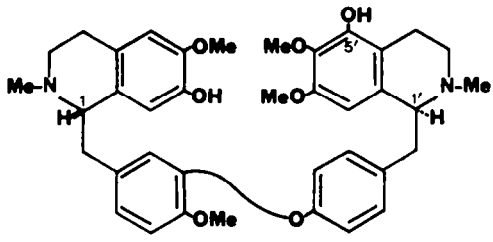
Our study started with the small plant Thalictrum minus var. microphyllum which grows in the vicinity of Eskişehir, in western Anatolia, and which had previously yielded the unusual aporphine-benzylisoquinolines (+)-istanbulamine[4] and (+)-uskudaramine.[5,6] In the course of the work on the isolation of these alkaloids, it was observed that the plant also produced several bisbenzylisoquinolines. It was decided to go through the somewhat elaborate procedure of separation and purification of these bisbenzylisoquinolines in order to complete the systematic study of the alkaloids present, with the hope of gaining some understanding of the prevailing biogenetic trends.

Nine bisbenzylisoquinolines were thus obtained, two of which are new. The first hitherto unreported alkaloid is (+)-thaligrisine (1), $C_{37}H_{42}N_2O_6$, $[\alpha]_D^{28}$ +57°(c 0.13, MeOH), whose more sig-



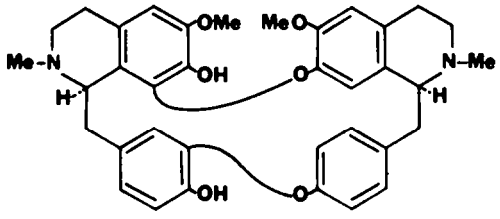
(+)-Thaligrisine (1)

1R-1'S

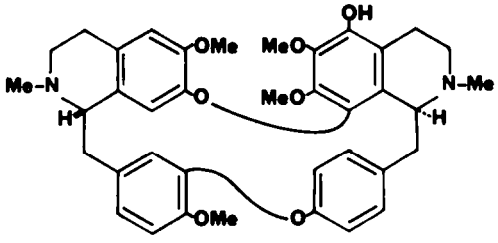


(+)-Thalirugine (2)

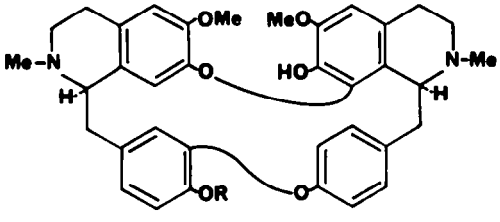
1S-1'S



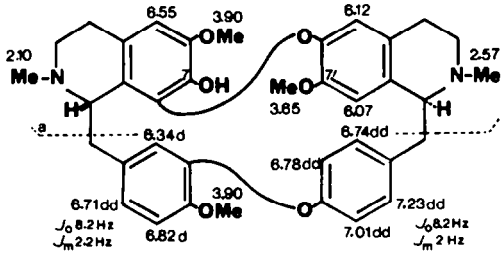
(+)-Obamegine (3)



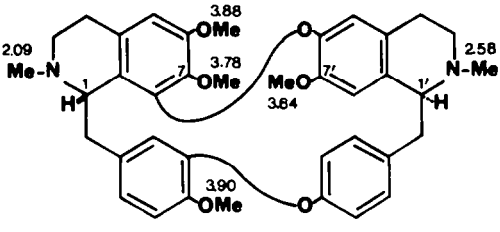
(+)-Thaligosine (5)



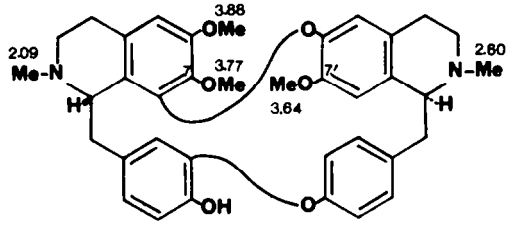
(+)-Aromoline (4)



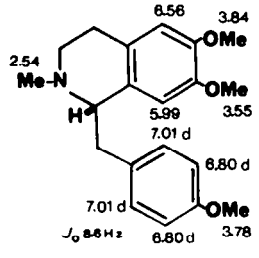
(+)-Thaliphylline (6)



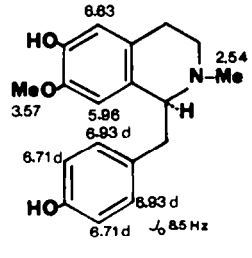
(+)-O-Methylthalicberine (7)



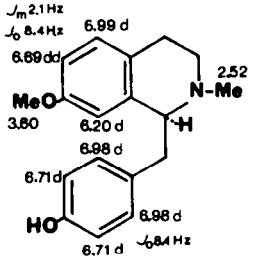
(+)-Thalicberine (8)



(+)-O-Methylarmepavine (9)



(+)-N-Methylisococlaurine (10)



(11)

nificant ^{1}H NMR chemical shifts have been summarized around expression 1. The mass spectrum shows a small $(M - 1)^+$ peak m/z 609, while the base peak m/z 192 is due to ion a (see expression 1). (+)-Thaligrisine is, in fact, the enantiomer of (-)-grisabine which had previously been obtained from Abuta grisebachii (Menispermaceae),[7] and the two alkaloids show essentially the same ^{1}H NMR and mass spectral characteristics.

The structural elucidation of the second new alkaloid, (+)-thaliphylline (6), $C_{37}H_{40}N_2O_6$, $[\alpha]_D^{25}$ +198°(c 0.12, MeOH), posed a somewhat greater challenge. The ^{1}H NMR spectrum showed two N-methyl and three methoxyl singlets, and the salient chemical shifts have been outlined around expression 6. The alkaloid is monophenolic, and upon diazomethane O-methylation yielded a compound which we had reason to believe was (+)-O-methylthalicberine (7).[8] This same compound 7 was also found as an alkaloid in the plant. The phenolic function of thaliphylline (6) had to be located in the upper part of the dimer, given that the mass spectrum shows a strong molecular ion m/z 608, and a base peak m/z 381 due to the upper portion a of the molecule as represented in expression 6.

Since we did not possess a sample of authentic O-methylthalicberine (7)[8] on hand for comparison, it was decided to run a sodium in liquid ammonia cleavage on our compound 7 in order to confirm its identity and absolute configuration. By extension, we would also be ascertaining some of the structural features and stereochemistry of thaliphylline (6). Eight milligrams of our O-methylthalicberine (7) were subjected to an improved sodium in liquid ammonia reduction procedure, the details of which are given in the Experimental. Two major as well as one minor products were identified from this reaction. The two major compounds were (+)-O-methylarmepavine (9) and (+)-N-methylisococlaurine (10) obtained in 2 mg and 2.2 mg amounts, respectively. The minor product (1 mg) was the tetrahydrobenzylisoquinoline (+)-11. Characteristic ^{1}H NMR chemical shifts have been cited around expressions 9-11. The cleavage results were as expected, and indicated to us that we were indeed dealing with (+)-O-methylthalicberine (7) with the stereochemistry at C-1 and C-1' as indicated.

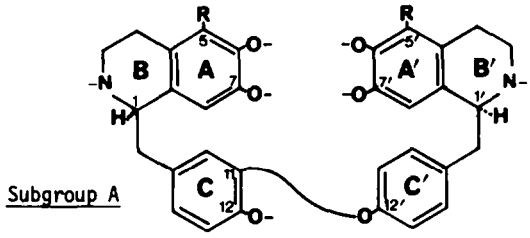
Another accompanying alkaloid is the known (+)-thalicberine (8)[8,9] whose ^{1}H NMR spectrum is represented around expression 8. Comparison of the spectra of (+)-thaliphylline (6), (+)-O-methylthalicberine (7) and (+)-thalicberine (8) showed that the δ 3.77-3.78 singlet absorption associated with a methoxyl group at C-7 in that series is missing in the spectrum of thaliphylline (6). The phenolic function of this alkaloid must, therefore, reside at that site. Such an assignment for 6 would also be in agreement with the mass spectral data for that alkaloid, which requires the phenolic group to be in the upper half of the molecule.

The known dimers found in our plant proved to be (+)-thalirugine (2), (+)-obamegine (3), (+)-aromoline (4) and its O-methyl derivative homoaromoline, and (+)-thaligosine (5), in addition to the aforementioned (+)-O-methylthalicberine (7) and (+)-thalicberine (8).[2]

The general pathway for the biogenesis of the bisbenzylisoquinolines of T. minus var. macrophyllum now became apparent. Disregarding the presence of methoxyl substituents as against hydroxyls, it is possible to state that through phenolic oxidative coupling thaligrisine (1) may act as a precursor to obamegine (3) on the one hand, and aromoline (4) and homoaromoline on the other. Furthermore, thalirugine (2) may be the precursor of thaligosine (5). Phenolic analogs of thalirugine (2), but lacking the C-5' hydroxyl, are the precursors for thaliphylline (6), O-methylthalicberine (7) and thalicberine (8).[10]

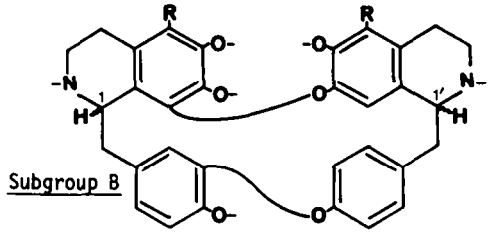
At this stage, it was decided to consider all of the bisbenzylisoquinolines obtained from the genus Thalictrum, to determine what general overview could be derived. To do this, the Thalictrum bisbenzylisoquinolines were first uniformly drawn with their two lower aromatic rings in the fashion indicated in our drawings, i.e. with the phenolic or methoxyl group residing in ring C at C-12 in the lower left hand side of the molecules, and the termini of the diaryl ether linkage located at C-11 and C-12'. An effort was then made to draw conclusions from well over 150 papers written on the subject of Thalictrum dimeric alkaloids.[2,3]

Table I: Bisbenzylisoquinoline Alkaloidal Subgroups
(R substituents may be H, OH, or OMe)



1R,1'S: (+)-Thaligrisine

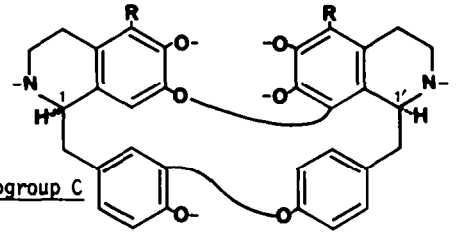
1S,1'S: (+)-O-Methylthalibrine, (+)-thalibrine, (+)-northalibrine, (+)-neothalibrine, (+)-thalirugine, (+)-thaliruginine, (+)-thaliracebine, (+)-thalistyline, (+)-thalirugidine, (+)-methostyline, (+)-thalirabine, (+)-thalistine



1R,1'S: (+)-Isotetrandrine, (+)-berbamine, (+)-thalrugosine, (+)-obamegine.

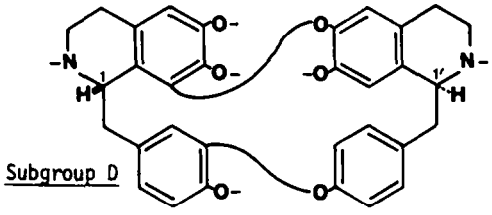
1S,1'S: (+)-Hernandezine, (+)-hernandezine N-oxide, (+)-N'-norhernandezine, (+)-thalidezine, (+)-N-desmethylthalidezine, (+)-N'-northalibrunine, (+)-thalibrunine

1S,1'R: (-)-Isothalidezine

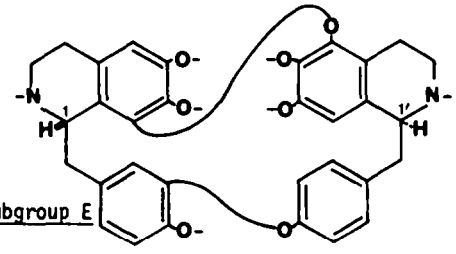


1R,1'S: (+)-Obaberine, (+)-oxyacanthine, (+)-homoaromoline, (+)-aromoline

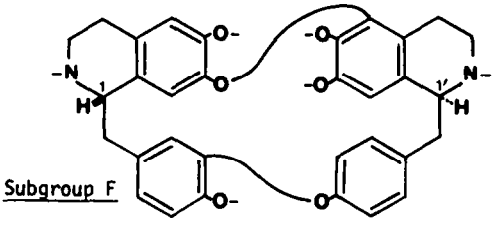
1S,1'S: (-)-Thalrugosaminine, (-)-thaligosine, (-)-thalisopidine



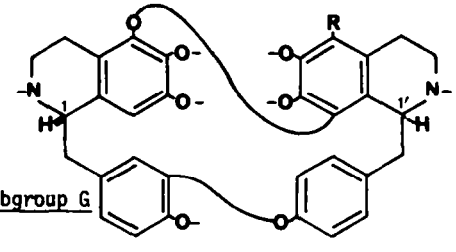
1S,1'S: (+)-O-Methylthalicberine, (+)-thalicberine, (+)-thaliphylline



1S,1'S: (-)-Desmethylthalidasine, (-)-thalidasine, (-)-thalrugosinone, (-)-thalfoetidine, (-)-thaligosidine, (-)-thalrugosidine, (-)-N-desmethylthalrugosidine, (-)-thalpindione



1S,1'S: (-)-Thalmine, (-)-thalictine, (-)-thalabadensine.



1S,1'S: (+)-Thalfinine, (+)-thalmirabine

The following four rules were found to govern the formation of bisbenzylisoquinolines in Thalictrum species:

(1) The dimers may belong to any of seven different structural subgroups represented by general formulas A to G (Table I). (For convenience, the alkaloids belonging to each of these subgroups have been further separated in Table I on the basis of their stereochemistry.)
(2) When a benzylisoquinoline moiety is oxygenated at C-5 or C-5', it has the S configuration.
(3) The right hand benzylisoquinoline moiety of a dimer incorporates the S configuration at C-1'.
(4) The left hand benzylisoquinoline moiety has the S configuration at C-1, except in subgroups A, B and C where it may be R.

A corollary of rules 3 and 4 above is that, on the one hand, bisbenzylisoquinolines whose occurrence is restricted to the genus Thalictrum (Ranunculaceae), or to its chemically and botanically close relative the genus Hernandia (Hernandiaceae), possess the 1S,1'S configuration. On the other hand, Thalictrum alkaloids with the 1R,1'S configuration are also encountered in other botanical families and include such familiar compounds as isotetrandrine, berbamine, obamegine, obaberine, oxyacanthine, aromoline and homoaromoline.

Furthermore, when an imine function is present, it is found to lie on the right hand side of the dimer, while the left hand benzylisoquinoline moiety has the S configuration.[11] Alkaloids with imine functions are: Subgroup B: (+)-thalsimine, (+)-thalsimidine, (+)-thalibrunimine, (-)-dihydrothalictrinine,[12] (-)-thalictrinine, (-)-oxothalibrunimine and (-)-O-methylthalibrunimine; Subgroup D: (+)-thalmethine and (+)-O-methylthalmethine; and Subgroup G: (+)-thalphine.[2]

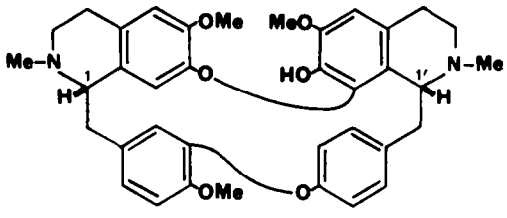
Once the Thalictrum alkaloids had been classified in this systematic fashion, it became of interest to consider which alkaloids reported in the literature seemed to deviate from the rules, or at least needed further clarification. Three such examples were found:

(a) (+)-Thalrugosamine: This dimer, $C_{37}H_{40}N_2O_6$, was obtained from T. rugosum, and was assigned structure 12 with the 1S,1'R configuration - a conclusion which conflicts with rule 3 above.[13] A reevaluation of the structural work revealed that the alkaloid had been duly O-methylated and the resulting (+)-O-methylthalrugosamine found to be identical with the known (+)-O-methyloxyacanthine. The assignment was then further refined with a sodium in liquid ammonia cleavage of (+)-O-ethylthalrugosamine, followed by characterization of the reduction fragments by comparison with those obtained from similar reduction of (+)-O-methyloxyacanthine.[13]

Confusion in the structural assignment arose simply because (+)-oxyacanthine was inadvertently considered to be represented by expression 13, when in reality it is the enantiomer of 13.[2] We can now state that (+)-thalrugosamine is represented by expression 14, in which the C-1 center is R, and C-1' is S. In other words, (+)-thalrugosamine (14) does not break the aforementioned rules. Furthermore, since it possesses the 1R,1'S configuration, it should also be of common occurrence, and its distribution should not be limited to the Thalictrums. Indeed, (+)-thalrugosamine (14) corresponds to the known dimer (+)-homoaromoline which has been isolated from several botanical families.[2]

(b) (-)-Isothalidezine (15): The characterization of (-)-isothalidezine, $C_{38}H_{42}N_2O_7$, found in T. podocarpum, can easily stand as the epitome of what a rational and systematic study of a bisbenzylisoquinoline should be.[14] The structural assignment as well as the stereochemical conclusions are irrefutable. The alkaloid was O-ethylated, and then subjected to sodium in liquid ammonia reduction. Additionally, it was cleaved with potassium permanganate in acetone, and the main oxidation fragment was characterized through synthesis. Finally, CD spectra were recorded at each critical stage.[14]

(-)-Isothalidezine (15) has the 1S,1'R configuration. It is, therefore, the sole authenticated exception to the rules enunciated above, whereas the other 57 Thalictrum dimers were found to comply with these rules. The reason as to why isothalidezine does not follow the biogenetic



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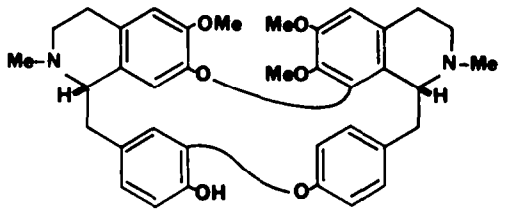

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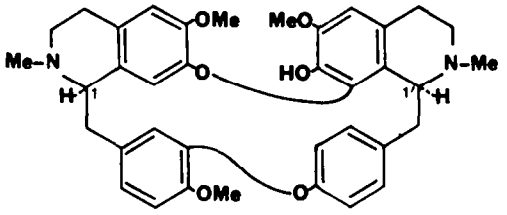
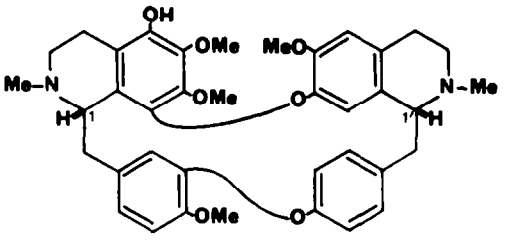
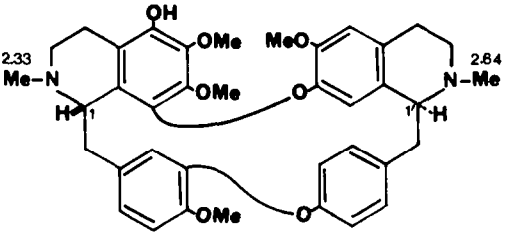


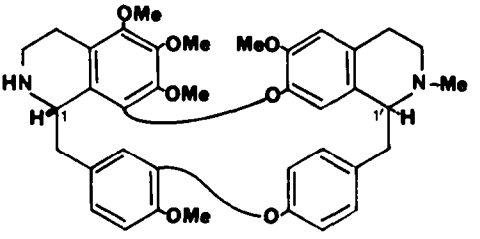
(+)-Thalrugosamine (14)
= (+)-Homoaromoline



(-)-Isothalidezine (15)



(+)- Thalidezine (16)



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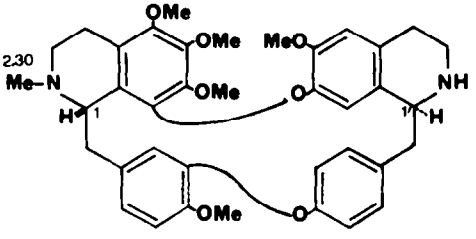
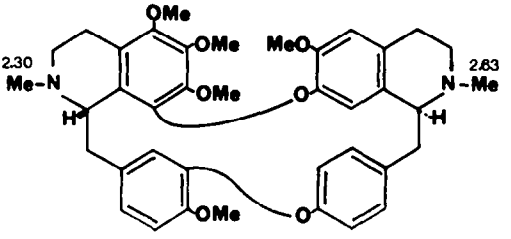
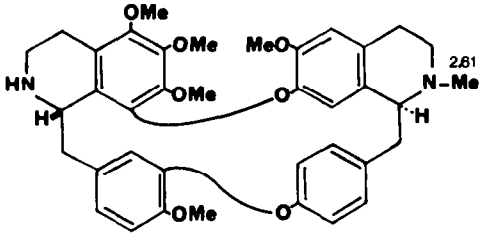


(+)-N'- Norhernandezine (18)



(+)-Hernandezine (19)



(+)-N-Desmethylthalidezine (20)

trend can only be adumbrated. A possible clue is that it is accompanied in T. podocarpum by its diastereomer (+)-thalidezine (16). From seven kilograms of the powdered roots, as much as 1.79 g of thalidezine (16) were obtained, while only 92 mg of isothalidezine (15) could be found. It is possible, therefore, that isothalidezine (15) is not an "original" alkaloid, but is derived biogenetically from thalidezine (16) by oxidation at C-1' to form an iminium cation, followed by enzymic reduction of the iminium bond from the beta side of the dimer. In any case, the biogenesis of (-)-isothalidezine (15) warrants further investigation, preferably using labeled precursors.

(c) (+)-Thalisamine: This alkaloid, $C_{38}H_{42}N_2O_7$, was originally isolated from Thalictrum simplex and was studied in the 1960's.[15] With the use of the low resolution NMR spectroscopy available at that time, it was assigned structure 17.[2] In 1980, the alkaloid (+)-N'-norhernandezine, also analyzing for $C_{38}H_{42}N_2O_7$, was reported present in T. rochebrunianum, and was conclusively shown to possess structure 18.[16] Both structures incorporate the 1S,1'S configuration, and are in accord with rules 3 and 4. Since they are close structural analogs of the well known (+)-hernandezine (19), they should both be dextrorotatory. At this juncture, however, we are left with the realization that the two compounds possess nearly identical spectra. In particular, the ^1H NMR N-methyl singlet absorption appears at δ 2.31 for thalisamine[15] and at δ 2.30 for (+)-N'-norhernandezine.[16] In (+)-hernandezine (19), however, which possesses two N-methyl groups, the N-methyl singlets are far apart, falling at δ 2.30 and 2.63.[2] Furthermore, in the related (+)-N-desmethylthalidezine (20) of clearly established structure, the N-methyl singlet is at δ 2.61.[14] In other words, in the hernandezine-thalidezine series, the left hand N-methyl group appears between δ 2.30 and 2.33, while the right hand N-methyl usually falls between δ 2.61 and 2.64. We conclude that thalisamine and N'-norhernandezine are identical alkaloids, and are represented by structure 18.

As new Thalictrum bisbenzylisoquinolines are isolated, it will be interesting to observe just to what extent the above rules will apply.

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Experimental

Isolation: Four kg of dried roots and rhizomes was extracted with cold ethanol. The chloroform soluble alkaloidal fraction was chromatographed on Merck Silica Gel H for TLC, elution being with $CHCl_3$-MeOH-NH_4OH (90:10:0.2). Further purification was by TLC on Merck Silica Gel F-254 using the system CH_3CN-C_6H_6-EtOAc-MeOH-NH_4OH (40:30:20:5:5). NMR spectra were recorded at 360 MHz in deuteriochloroform solution.

(+)-Thaligrisine (1), 17 mg, λ max MeOH 226 sh, 284 nm (log ϵ 4.48, 3.98); MS m/z 609 $(M-1)^+$ (0.2), 608 (0.6), 418 (0.1), 381 (0.4), 364 (0.1), 206 (1), 192 (100), 175 (2.3); CD MeOH $\Delta\varepsilon$ (nm) 0 (300), +2.5 sh (254), +15 (244), +2(228), positive tail at 215 nm; $[\alpha]_D^{23}$ +57° (0.13, MeOH).

(+)-Thaliphylline (6), 20 mg, λ max MeOH 211, 279, 290 sh nm (log ϵ 4.71, 3.89, 3.75); MS m/z 608 $(M)^+$ (38), 607 (24), 593 (4.5), 577 (1.7), 381 (100), 367 (16), 204 (2.6), 192 (21), 191 (89), 190 (22), 176 (33), 174 (48); CD MeOH $\Delta\varepsilon$ (nm) 0 (300), +21 (286), 0 (270), -4.2 (250), 0 (246), +61 (214); $[\alpha]_D^{23}$ +198° (0.12, MeOH).

Known alkaloids isolated and characterized spectrally or by comparison with known samples are: (+)-thalirugine (2), 2.5 mg; (+)-obamegine (3), 27 mg; (+)-aromoline (4), 21 mg; (+)-thaligosine (5), 8 mg; (+)-O-methylthalicberine (7), 2 mg; (+)-thalicberine (8), 12 mg; (+)-homoaromoline (14), 3 mg.

O-Methylation of Thaliphylline (6): Compound 6 (11 mg) was dissolved in 2 mL MeOH. To the soln. was added ethereal diazomethane (2 mL). After 24 h near 0° C, the soln. was evaporated under a hood, and the residue purified by TLC. The product was (+)-O-methylthalicberine (7).

Sodium in Liquid Ammonia Cleavage of O-Methylthaliphylline(7): To fresh, doubly distilled, dry liquid ammonia (10 mL), was added a soln. of 8 mg of O-methylthaliphylline. The soln. was maintained near -78° C, under a dry nitrogen atmosphere. A minimum amount of sodium (≈ 15 mg) was added with stirring so as to produce a stable blue color which lasted for about a half hour. The mixture was allowed to warm up to room temperature. Excess methanol was added to destroy any residual sodium. Following removal of the solvent, the residue was treated with water, and acidified with dil. hydrochloric acid. The mixture was then basified with ammonium hydroxide, and extracted with chloroform. The residue after evaporation of the chloroform was subjected to TLC. In this fashion, tetrahydrobenzylisoquinolines (+)-9,[8] (+)-10,[8] and 11 were obtained.

Tetrahydrobenzylisoquinoline 11: Obtained as above, MS m/z 283 $(M)^+$ for $C_{18}H_{21}NO_2$ (0.1), 282 (0.3), 222 (1), 206 (1.7), 192 (0.2), 177 (18), 178 (100). Polarimetric measurements indicated this compound to be dextrorotatory, but an exact determination of its specific rotation was not possible due to paucity of material.

References and Footnotes

1. Permanent address: Faculté de Médecine et de Pharmacie, Université de Limoges, 87032 Limoges Cedex, France; and ERA 317, UER de Chimie Thérapeutique, Centre d'Etudes Pharmaceutiques, 99290 Chatenay-Malabry, France.
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