solution with chloroform and ether. Spaeth and Becke (8) also have reported such a behavior for the peyote phenolic alkaloids. According to them, these compounds were at least in part extracted with ether from an aqueous potassium hydroxide solution even though they used concentrated solution (50%) of aqueous potassium hydroxide.

In conclusion, it may be indicated that although the β-phenylethylamine quaternary alkaloids, candicine and coryneine (≡ 3-hydroxycandicine), have been isolated from some cacti (1,20), *Lophophora williamsii* appears to be the first cactus plant from which simple quaternary tetrahydroisoquinolines have been isolated and identified. Occurrence of such compounds in other cacti could be expected, and as in peyote, these compounds may be present at least as minor basic constituents.

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Keyphrases

Peyote alkaloids
Alkaloids, quaternary–*Lophophora williamsii*
Anhalotine, lophotine, and peyotine–isolation
IR spectrophotometry–structure
UV spectrophotometry–structure
NMR spectrometry

Thalictrum Alkaloids V

Isolation

By MAURICE SHAMMA and BERNARD S. DUDOCK

The experimental procedure which led to the isolation of the new alkaloids thalifendlerine (I), thalifendine (II), thalidastine (III), thaliporphine (IV), preocoteine (V), and thalidezine (VI), from *Thalictrum fendleri* is described in detail. Known alkaloids also found in *T. fendleri* are berberine (VII), jatrorrhizine (VIII), glaucine (IX), magnoflorine (X), ocoteine (XI), hernandezine (XII), and thalicarpine (XIII). A completely new dimeric aporphinebenzylisoquinoline alkaloid of only partially elucidated structure is thaldimerine (XIV). A novel procedure for the separation of tertiary alkaloids consists of partition chromatography of the mixture of hydrochloride salts using a cellulose column in the system methyl ethyl ketone–water.

THE STRUCTURAL ELUCIDATION of a variety of new isoquinoline-type alkaloids from *Thalictrum fendleri* has previously been reported. The alkaloids involved were thalifendlerine (I), thalifendine (II), thalidastine (III), thaliporphine (IV), preocoteine (V), and thalidezine (VI) (1–4). In addition, the presence of the following known alkaloids in *T. fendleri* was indicated: berberine (VII), jatrorrhizine (VIII), glaucine (IX), magnoflorine (X), ocoteine (XI), hernandezine (XII) (3), and thalicarpine (XIII). The purpose of the present paper is to describe the isolation procedure employed to obtain these 13 compounds. Additionally, the isolation of the completely new alkaloid thaldimerine (XIV) will also be discussed, although lack of sufficient material has so far precluded the complete structural elucidation.

Coarsely ground *T. fendleri* was extracted with methanol and the solvent was evaporated. The oily residue obtained was taken up in dilute acetic acid and filtered. The filtrate was extracted with chloroform and ether to remove acidic and neutral components. The aqueous acidic layer was then made just basic with concentrated ammonium

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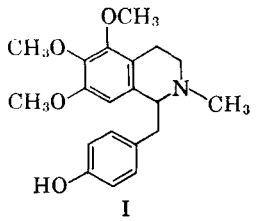
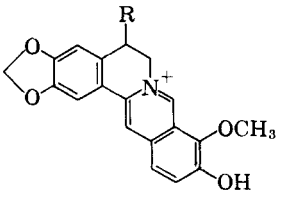
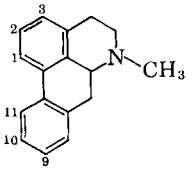
The authors thank Professor Jack L. Beal for alkaloidal samples. The assistance of Dr. Richard M. Sheeley and Mr. Richard Moon in collecting the plant material is gratefully acknowledged.

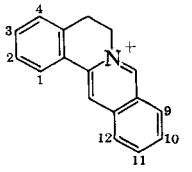


I

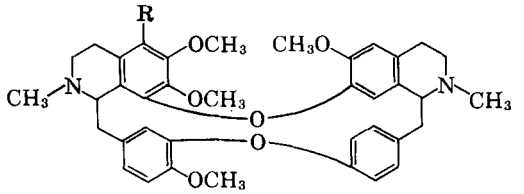


II, R = H
III, R = OH

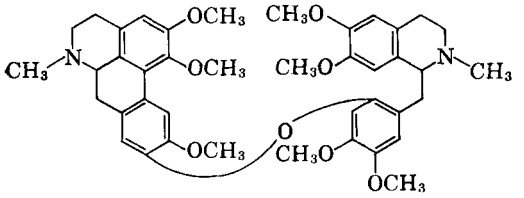




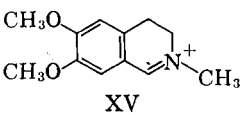
IV, 1-Hydroxy-2,9,10-trimethoxy
V, 1-Hydroxy-2,3,9,10-tetramethoxy
VII, 2,3-Methylenedioxy-9,10-dimethoxy
IX, 1,2,9,10-Tetramethoxy
VIII, 2,9,10-Trimethoxy-3-hydroxy
X, 1,11-Dihydroxy-2,10-dimethoxy-*N*-methyl salt
XI, 1,2-Methylenedioxy-3,9,10-trimethoxy



VI, R = OH
XII, R = OCH_3



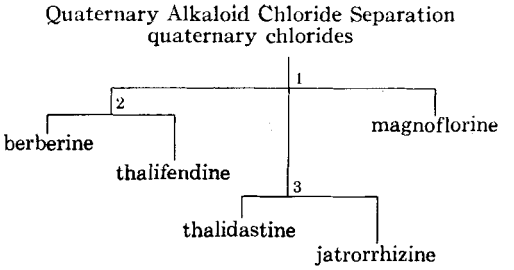
XIII



XV

hydroxide, and reextracted with chloroform and ether to remove the tertiary alkaloids. The aqueous solution was acidified and the quaternary alkaloids were precipitated as their reineckate salts, which were then converted to the alkaloid chloride salts by ion exchange.

Quaternary Alkaloid Chloride Separation



1, partition chromatography
2, alumina chromatography
3, preparative TLC

Scheme I

Preliminary attempts to fractionate the quaternary alkaloid chloride mixture, employing fractional crystallization and adsorption chromatography on columns of alumina, silica gel, and magnesium silicate,[1] gave relatively poor separations. Partition chromatography, previously shown to be successful in fractionating highly complex quaternary alkaloid mixtures (5), was therefore employed. Methyl ethyl ketone saturated with water was chosen as the partition system for the large-scale column chromatography on cellulose. This solvent combination gave a good separation, and had the advantage of being fast running, reasonably stable, and odorless, and low boiling so that it could easily be removed without alkaloid decomposition.

The partition column used cellulose powder as the inert support. The cellulose was first saturated with the stationary phase (water saturated with methyl ethyl ketone) then "dry-packed" (6) into the column and finally equilibrated with the mobile phase. The quaternary chloride mixture, dissolved in the stationary phase bound to the cellulose powder, was then added to the top of the equilibrated column and the separation was begun by allowing the mobile phase to slowly flow down the column. Efforts were made to control the temperature, and the packing and drip-rate fluctuations. The complete procedure is described in detail under *Experimental.*

The quaternary alkaloid mixture was thus separated into distinct fractions as summarized in Scheme I. Fractions were combined on the basis of their paper and thin-layer chromatographic pattern, and usually further purified by adsorption chromatography on alumina or preparative thin-layer chromatography (TLC).

Due to the success of partition chromatography in fractionating the quaternary alkaloids, the same procedure was employed to separate the

[1] Florisil. Floridin Co., Pittsburgh, Pa.

TABLE I—FRACTIONATION OF TERTIARY ALKALOIDS BY PARTITION CHROMATOGRAPHY[a]

Fractions	Flasks[b]	Alkaloids isolated
1	1–6	No well-defined alkaloids isolated
2	7–15	Thalifendlerine (I), 3 Gm.[c]
3	16–20	Thalifendlerine (I), 200 mg. Ocoteine (XI), 200 mg. Glaucine (IX), 5 mg. Preocoteine (V), 60 mg.
4	21–28	Thaliporphine (IV), 70 mg.
5	29–35	Thaliporphine (IV) 30 mg. and other minor alkaloids
6	36–55	Not investigated to date[d]
7	56–61	
8	62–89	
9	90–105	
10	106–115	
11	Methanol washing	Hernandezine (XII), 50 mg. Thalicarpine (XIII), 20 mg. Thaldimerine (XIV), 20 mg Thalidezine (VI), 300 mg.

[a] The column was 180 cm. in height, 10 cm. wide, and contained 1,500 Gm. of cellulose powder. [b] Each flask contained about 100 ml. of solution. [c] The weight refers to the alkaloid free base obtained from 15 Kg. of dried plant. [d] TLC revealed the presence of hernandezine and thalicarpine.

tertiary bases. The total tertiary alkaloid mixture, which was only half as prevalent as the quaternary alkaloid fraction, was converted to hydrochloride salts and fractionated on large cellulose columns with methyl ethyl ketone saturated with water, in the same manner as previously described. To our knowledge, this is the first time such an isolation procedure has been employed for the fractionation of tertiary alkaloids. Upon completion of the partition chromatography, the column was washed thoroughly with methanol to insure complete removal of the alkaloids. Similar fractions were combined on the basis of their TLC patterns. This procedure gave 11 fractions which are indicated in Table I.

The general procedure for separating the components of each fraction was to convert the tertiary alkaloid salts back to the free bases, and then to employ adsorption chromatography on neutral alumina with chloroform, containing increasing amounts of methanol, as solvent. The fractions from this chromatographic separation were further purified by preparative TLC.

It was repeatedly found in the course of the present investigation that the true complexity of the crude alkaloid mixtures could not be determined prior to the large-scale partition chromatography on cellulose. It is therefore felt that the use of large partition columns as the first step in alkaloid fractionation is decidedly advantageous, and facilitates the isolation of minor alkaloids.

The new tertiary alkaloid thaldimerine (XIV) which has not previously been described was obtained as a transparent light brown oil which was homogeneous by TLC. The UV spectrum with $\lambda_{max.}^{EtOH}$ 303 and 283 mμ showed a marked bathochromic shift upon the addition of base, so that the alkaloid must be phenolic. The NMR spectrum of a deuteriochloroform solution exhibited two *N*-methyl groups superimposed at 2.48δ (six protons), and six *O*-methyls at 3.57 (three protons), 3.79 (nine protons), and 3.92δ (six protons), underscoring the fact that the alkaloid must be dimeric. The mass spectrum with a molecular ion at *m/e* 699 pointed to the formula $C_{40}H_{46}O_9N_2$, indicating the alkaloid to be an aporphine-benzylisoquinoline dimer. The base peak at *m/e* 206 represents the moiety XV. Other intense peaks were situated at *m/e* 491, 411, 397, 340, 222, and 192. It is quite tempting to present a complete structural expression for thaldimerine on the basis of these data. The authors shall refrain, however, from doing so, pending the isolation of additional thaldimerine for further study.

EXPERIMENTAL

All melting points were taken on a block manufactured by the Nalge Co. and are uncorrected. Infrared spectra were taken on a Beckman IR-5 spectrophotometer, and the ultraviolet spectra were measured in distilled 95% EtOH on a Cary model 14 spectrophotometer. The nuclear magnetic resonance spectra were taken on a Varian Associates A-60 instrument unless indicated otherwise, in deuteriochloroform or trifluoroacetic acid with tetramethylsilane as internal standard.

Mass spectra were obtained with a Nuclide 12-90-G1.1 mass spectrometer using the following procedure. The sample was placed into the heated inlet and the temperature was gradually raised until a sufficient ion current was obtained. The probe was then removed, thoroughly cleaned, and a fresh alkaloid sample added. It was then placed into the heated inlet at the previously determined temperature and the spectrum was taken immediately after a sufficient ion current was reached. In no case was a spectrum used after the sample had been in the heated inlet for more than 2 min. because of rapid alkaloid decomposition at the extreme temperatures employed.

Thin-layer chromatography was performed on a purified silica gel.[2] Preparative TLC was run on plates 8 × 8 in., (20 × 20 cm.) coated with a 1-mm.-thick adsorbent layer. Paper chromatography was performed by the descending procedure on Whatman No. 1 paper. Cellulose powder refers to Whatman ashless grade.[3] The ion-exchange resin[4] was chromatographic grade, 100–200 mesh, chloride form. Methyl ethyl ketone (MEK) was purchased from Eastman Organic Chemicals and purified by refluxing in Zn dust for 8 hr., followed by distillation from Zn dust. Ammonium reineckate was purchased from Matheson, Coleman and Bell and dissolved in water only prior to immediate use.

The following procedures were strictly adhered to:

[2] Adsorbosil I. Applied Science Laboratories, State College, Pa.
[3] Chromedia CF 11.
[4] Amberlite CG 400. Matheson, Coleman and Bell.

(*a*) solvent evaporations were performed below 60°, either at reduced pressure or under a nitrogen stream, (*b*) samples were stored under nitrogen in the dark, (*c*) compounds were removed from the preparative TLC adsorbent as soon as the solvent had evaporated, (*d*) quaternary alkaloid reineckate salts were converted to their chloride form within 4 hr. of their preparation, (*e*) after use, all chromatographic columns were washed with methanol to insure complete alkaloid removal.

Plant Extraction and Preliminary Separation—The *T. fendleri* samples used in this research were harvested during three consecutive summers in the area surrounding Tabiona, Utah. They were air dried and shipped to State College, Pa. The plant material (a total of 40 Kg. was used for this investigation) was ground to a fine powder and extracted in a Soxhlet extractor with MeOH, until the MeOH was clear and gave a negative Mayer's test (7) (usually 3–5 days). The extracts, upon evaporation of the MeOH, gave a thick, viscous residue which was dissolved as much as possible in dilute (3–5%) HOAc and filtered with the aid of Filtercel. Approximately 1 L. of acid solution was used to dissolve the extract from each 1 Kg. of dried plant. The solution was then extracted with an equal volume of $CHCl_3$ to remove nonalkaloidal components. The aqueous layer was basified (pH 8) with concentrated NH_4OH, and reextracted with an equal amount of $CHCl_3$, followed by an equal amount of Et_2O. The $CHCl_3$ and Et_2O layers were combined and the solvent was removed to give a black oil containing the total tertiary alkaloid mixture. It was found preferable not to keep alkaloids in $CHCl_3$ solution due to slow decomposition.

The aqueous layer was now freed of organic solvent at reduced pressure, and acidified (pH 2) with concentrated HCl. Freshly prepared, warm (50–60°), aqueous ammonium reineckate solution was added until the alkaloids were completely precipitated. After cooling to 0°, the precipitate was removed by suction filtration. It was immediately dissolved in acetone giving a deep red solution. This solution was diluted with an equal volume of MeOH and the alkaloids were converted to the chloride form by ion exchange as described below. Slight differences in relative alkaloid concentrations were noted in plant batches of different years.

Ion-Exchange Conversion of Alkaloid Reineckates to Chlorides—The yellow ion-exchange resin was completely converted to the chloride form by soaking in 1 *N* aqueous HCl for 10 min. and drying by suction filtration. It was then washed once in distilled water and redried. The resin was now washed three times with acetone–MeOH (1:1) and finally suspended in acetone–MeOH (1:1) for 30 min., with occasional stirring. This mixture was stirred to form a slurry which was poured slowly into a chromatographic column containing a glass wool plug. After the resin had settled (30 min.) the stopcock was opened, and the resin was washed repeatedly with acetone–MeOH (1:1) until the eluate was clean and gave no residue upon evaporation of 25 ml. of eluant. The deep red alkaloid reineckate solution in acetone–MeOH (1:1) was now placed on the column. A successful conversion was indicated by a yellow effluent of alkaloidal chlorides if concentrations are low, or more generally by a deep red band at the top of the column. The completeness of the conversion could also be checked by the disappearance of the 310 mμ reineckate anion peak (8) in the effluent. The longer alkaloid reineckate solutions were permitted to stand, the poorer was the subsequent conversion to the chloride form.

Chromatography—*Adsorption Chromatography*—All adsorption chromatography columns were prepared as a slurry. Alumina, which was found to be superior to other adsorbents, was Baker neutral alumina, 100–200 mesh, slurry pH 6–8. It was stored tightly sealed and was not activated before use. Silica gel and magnesium silicate were found to give poorer results due to the difficulty of desorbing some of the alkaloids from their surface.

Partition Chromatography—The large partition columns of cellulose, which were the major means of fractionation employed in this study, were carefully prepared and equilibrated prior to use. They were packed dry using a procedure modified from Kupchan (6) as follows.

Dry cellulose powder (500 Gm.) was placed in a 5-L. round-bottom flask equipped with a tight-fitting stopper covered with aluminum foil. Water saturated with MEK (250 ml.) (the lower phase of the MEK–H_2O system, *i.e.*, the stationary phase) was added, and the mixture was shaken vigorously to break up all lumps. The cellulose powder, appearing dry at this stage, had now been impregnated with the stationary phase. Approximately 10 Gm. of this cellulose powder was placed into the chromatography column and tamped down lightly. This procedure was repeated usually 20–30 times, until the proper size column was built. The cellulose powder was now topped with 3 or 4 filter paper disks cut to fit. Care was taken so that at no stage was the cellulose powder exposed to the air for more than a few minutes because the stationary phase impregnated on it could easily evaporate.

After the column had been built, a large amount of mobile phase, MEK–H_2O (MEK–H_2O = methyl ethyl ketone saturated with water), was added and allowed to run through. This washing and equilibration was continued for at least 8–48 hr., whereby the pressure ridges became less pronounced. A successfully equilibrated column gives a clear eluate, a cloudy eluate indicating incomplete equilibration. The equilibration of the column can be destroyed by even moderate temperature fluctuations, in which case it has to be reequilibrated prior to use.

The placing of the sample on the column was usually the most critical step in the chromatographic separation. Since alkaloid salts are generally only slightly soluble in the mobile phase (MEK–H_2O), the following procedure was used to place them on the column.

The alkaloid chlorides (5 Gm.) were dissolved in 10 ml. of stationary phase (water saturated with methyl ethyl ketone) and the solution was added to 20 Gm. of dry cellulose powder and shaken vigorously to break up all lumps. This cellulose powder was then carefully placed on the top of the previously prepared and equilibrated column, and tamped down lightly. This was topped with 3 or 4 filter paper disks, and the chromatography was started by the addition of the mobile phase.

In general, 1 Gm. of crude alkaloid chloride per 100 Gm. of dry cellulose powder was used to insure an optimum separation. The large cellulose columns employed contained 1,500 Gm. of cellulose and

TABLE II—INITIAL QUATERNARY ALKALOID SEPARATION

Fraction	Alkaloids Isolated[a]
I	Berberine chloride (VII), 1 Gm.
	Thalifendine chloride (II), 200 mg.
II	Jatrorrhizine chloride (VIII), 150 mg.
	Thalidastine chloride (III), 700 mg.
III	Magnoflorine chloride (X) 10 Gm.[b]

[a] The weight refers to the amount of alkaloid, in the chloride form, that was obtained from 15 Kg. of dried plant. [b] This weight is approximate since only a small fraction of the crude magnoflorine chloride was purified.

TABLE III—ALUMINA COLUMN CHROMATOGRAPHY OF FRACTION I (TABLE II)

Solvent	Alkaloids Present
Acetone–methanol (9:1)	Berberine
Acetone–methanol (1:1)	Uncharacterized alkaloid
Methanol	Thalifendine

were 180 cm. in height and 10 cm. wide, and a maximum of 50 Gm. of alkaloid chloride was added at one time. No column was used twice to insure against cross-contamination of alkaloid fractions.

Occasionally another procedure of sample application was employed for the separation of protoberberine alkaloids. In this case the column was prepared and equilibrated as previously described. Solid crude quaternary chlorides were now added to a 250-ml. conical flask containing 100 ml. of the mobile phase (MEK–H_2O). The flask was stoppered tightly and shaken vigorously. After 2 hr. the mobile phase was decanted off and 100 ml. of fresh mobile phase was added and the procedure was repeated until the desired amount of quaternary chloride was dissolved. This mobile phase saturated with alkaloids, sometimes amounting to 2 L. in volume, was now added to the column and the separation was begun. Despite the large volume used in the sample application, an excellent separation of protoberberine alkaloids could be achieved. This procedure was not tried with other complex quaternary mixtures.

Fractionation of Quaternary Alkaloids—The quaternary alkaloid chloride mixture was first separated on a large partition column in MEK–H_2O as previously described. The eluate was separated into three fractions, the first two of which were bright yellow and were shown by TLC in MeOH to contain three alkaloids each. The final fraction was colorless, exhibited a brilliant blue-white fluorescence under UV light, and consisted of primarily one component. Table II summarizes the alkaloids isolated from these three fractions.

Fraction I (see Table II) upon evaporation of the MEK–H_2O gave a yellow oil showing a number of spots on thin-layer chromatography. A neutral alumina column was prepared as a slurry in the solvent acetone–methanol (9:1). The yellow oil was dissolved in acetone–methanol (1:2), carefully placed on the alumina column, and elution was begun with acetone–methanol (9:1), which brought down the first yellow band. Elution was continued with acetone–methanol (1:1), which brought down another yellow band, and was completed with pure methanol, which brought down the final, yellow-orange band. The results of this alumina column are summarized in Table III.

Berberine (VII)—Upon evaporation of the acetone–methanol (9:1), the residue crystallized readily from methanol and was shown to be identical to authentic berberine chloride in terms of melting point; found 205°, reported 205° (9), mixed m.p. 205°, superimposable IR spectra (KBr pellet and Nujol mull), NMR spectra in trifluoroacetic acid and UV spectra. TLC and paper chromatography confirmed the identity of the isolated berberine chloride with authentic material.

Thalifendine (II)—Upon elution of the alumina column containing fraction I with methanol, an orange and very diffuse band emerged. Since this band had no sharply defined boundaries, a large volume of methanol was used to insure complete elution. After evaporation of the solvent, an orange solid remained. This solid showed only one spot on TLC and was converted to a yellow solid by addition of dilute hydrochloric acid. The yellow solid crystallized readily from methanol, and was recrystallized to give fine yellow needles sintering above 230°. The structural elucidation of thalifendine has been reported (1).

Thalidastine (III)—The second fraction obtained from the large cellulose column, fraction II (see Table II), gave a yellow viscous oil upon evaporation of the solvent. This oil gave three spots on TLC, all of which appeared to be protoberberine-like from their yellow-orange color. The oil was separated by preparative TLC in methanol into two easily distinguishable bands as follows: top band, R_f 0.4–0.5 thalidastine; bottom band R_f 0.0–0.1 jatrorrhizine and uncharacterized yellow alkaloid.

Both bands were eluted from the TLC plate with methanol which also dissolved some of the adsorbent. The alkaloids were freed of this impurity by alumina column chromatography with absolute ethanol. On evaporation of the ethanol, thalidastine appeared as an orange oil and jatrorrhizine and the uncharacterized yellow alkaloid as a yellow-orange oil.

The orange oil from the top band mentioned above was dissolved in methanol, aqueous HCl was added until the solution turned yellow, and upon evaporation of the solvent yellow crystalline thalidastine chloride was obtained. The structural study of this alkaloid has been described (2).

Jatrorrhizine (VIII)—Fraction II (see Table II), in addition to containing the previously mentioned thalidastine, also contained two other protoberberine alkaloids, the known alkaloid jatrorrhizine and a yellow uncharacterized salt. These two alkaloids were easily separated from thalidastine by preparative TLC in methanol, but it was found very difficult to separate them from each other. After repeated failures it was decided to employ a magnesium silicate column from which jatrorrhizine could be eluted, but not the uncharacterized salt. Using this column with acetone–methanol (1:1) as solvent, pure jatrorrhizine (one spot on TLC) was obtained as a red-brown oil. This oil was dissolved in methanol, hydrochloric acid was added until the color turned yellow, and upon evaporation jatrorrhizine chloride crystallized as fine needles.

The isolated material was shown to be identical with authentic jatrorrhizine chloride by comparison

of NMR spectra in trifluoroacetic acid and UV spectra, both of which were superimposable. Additionally TLC and paper chromatography confirmed the identity of the two samples. The isolated material was reduced in a microhydrogenator with Adams catalyst in methanol for 24 hr. A white crystalline reduction product was obtained in quantitative yield. This was shown to be identical to authentic tetrahydrojatrorrhizine by melting point comparison; found 210°, reported 216° (10) mixed m.p. 210°, practically superimposable IR ($CHCl_3$) and mass spectra, and TLC R_f values in several solvent systems.

Magnoflorine (X)—Fraction III (see Table II) was the last and largest fraction eluted from the large cellulose column. Upon evaporation of the solvent a thick brown oil was obtained. This oil gave one spot on TLC and paper chromatography, with very few minor spots also present. A small amount of this oil was rechromatographed on a long thin cellulose column with MEK–H_2O as solvent. Part of the eluate from this column crystallized upon evaporation of solvent.

The isolated material and magnoflorine chloride were shown to be identical through IR (Nujol mull) and UV spectral comparisons. Additional TLC and paper chromatography confirmed the spectral results. Magnoflorine is the most prevalent alkaloid in *T. fendleri*.

Preliminary Separation of the Crude Tertiary Alkaloids—The solid crude tertiary alkaloids (50 Gm.) were dissolved in 50 ml. of 2 *N* methanolic HCl, the pH being kept highly acidic by dropwise addition of concentrated HCl when needed. The MeOH and excess acid were removed very thoroughly under a nitrogen steam and *in vacuo*. The tertiary hydrochloride salts were then subjected to partition chromatography (1,500 Gm. cellulose powder) in the same manner as described above for the quaternary chloride salts. Upon elution from the column and combination of like fractions the solvent was removed and the tertiary hydrochloride salts were converted back to their free bases. This was done by dissolving the salts in 10–20 ml. of water followed by dropwise addition of concentrated NH_4OH until the solution was just basic and then repeated extraction with $CHCl_3$. The $CHCl_3$ layer was removed, dried with Na_2SO_4, filtered, and evaporated to dryness. All adsorption chromatography and structural elucidation was done on the tertiary free bases. The large partition separation of the tertiary hydrochloride mixture is summarized in Table I.

Thalifendlerine (I)—Fraction 2 (see Table I) gave a light brown oil upon evaporation of the solvent. This oil, showing one spot on TLC, crystallized upon the addition of methanol. After several recrystallizations from the same solvent and then from acetonitrile, large white rods were obtained. The chemistry of this interesting benzylisoquinoline alkaloid has been described (1).

Separation of Tertiary Alkaloid Fractions 3 and 4—Fraction 3 (see Table I) gave a brown oil upon evaporation of the solvent. TLC showed it to be a highly complex mixture composed of at least 10 alkaloids, although some were present to only a minor extent. TLC also indicated that many components of this fraction were present in the succeeding fraction, designated fraction 4. Fraction 4 was further purified in the same manner as will be described for fraction 3, and will therefore not be discussed in detail.

TABLE IV—SEPARATION OF FRACTION 3

Flasks	Solvents	Alkaloids
1–9	$CHCl_3$	Ocoteine (XI), glaucine (IX)
10–20	$CHCl_3$	None
21–27	1% MeOH	None
28–35	1% MeOH	Preocoteine (V)
36–39	3% MeOH	Preocoteine (V), thaliporphine (IV)
40–44	3% MeOH	Thaliporphine (IV), thalifendlerine (I)
44–53	5% MeOH	Thalifendlerine (I), plus other alkaloids
54–64	10% MeOH	Several alkaloids present

The brown oily fraction 3 (1.2 Gm.) was dissolved in chloroform, filtered, and chromatographed on a neutral alumina column (90 Gm.) prepared as a slurry in chloroform. After the addition of 400 ml. of chloroform, methanol was added in increasing increments (gradient elution chromatography) until a 10% methanol solution in chloroform was reached. The results of this adsorption chromatography are given in Table IV.

Flasks 1–27 contained 25 ml. each and 28–64 ml. each of solvent.

Flasks 1–9 (see Table IV) were combined and evaporated to dryness, giving a light yellow oil which failed to crystallize. TLC showed this oil to be composed of at least three components which were easily separated by preparative TLC in acetone. The top band was not characterized, the middle band was identified as ocoteine (XI), and the lower band corresponded to glaucine (VII).

Ocoteine (XI)—The middle band from the preparative TLC, gave a UV spectrum with $\lambda_{max.}^{EtOH}$ 314, 302, 283, and 232 mμ (log ε 4.1, 4.2, 4.2, and 4.4). The NMR spectrum showed one *N*-methyl at 2.54 δ, three *O*-methyl groups at 4.01 (three protons) and 3.92 δ (six protons), one methylenedioxy as two doublets at 6.05 and 5.92 δ (J = 1.5 c.p.s.), and two aromatic protons at 6.78 and 7.62 δ. The UV and NMR spectra were practically superimposable with those of an authentic sample of known ocoteine. TLC confirmed the identity of the two samples. In addition, the mass spectrum of the isolated alkaloid showed a molecular ion (M^+) at m/e 369 ($C_{21}H_{23}NO_5$) and other large peaks at 354 (M-15), 338 (M-31), and 326 (M-43), all characteristic of aporphine alkaloids, and in agreement with the reported structural assignment (11).

Glaucine (IX)—The lower band from the preparative TLC gave a colorless oil, λ_{max}^{EtOH} 300, 280, and 218 mμ, showing no shift on addition of base. This UV spectrum indicated the isolated alkaloid to be a 1, 2, 9,10- substituted, nonphenolic aporphine, and strongly suggested the alkaloid was glaucine (12). This assumption was strengthened by the mass spectrum, which showed a large molecular ion (M^+) at m/e 355 ($C_{21}H_{25}NO_4$), and the other expected peaks at 340 (M-15), 324 (M-31), and 312 (M-43) (11). The isolated alkaloid was then shown to be the known base glaucine by means of a direct comparison with an authentic sample in terms of UV and mass spectral comparisons and TLC R_f values. The structural assignment was performed on less than 5 mg. of isolated material.

Preocoteine (V)—Flasks 28–35 (Table IV) were combined and upon evaporation of the solvent gave a light green oil. This oil was shown by TLC to contain some nonalkaloidal material, and this impurity was separated by preparative TLC in methanol–acetone (5:3). The alkaloid moved to the middle of the plate and the impurity was located at the solvent front. Upon removal from the plate with chloroform–methanol (9:1), preocoteine exhibited $\lambda_{max.}^{EtOH}$ 314, 304, 279, and 222 mμ, with a marked bathochromic shift upon addition of base. The NMR spectrum in deuteriochloroform at 100 Mc. showed the presence of one *N*-methyl at 2.52 δ, four *O*-methyl groups at 3.92 δ (six protons), and 3.88 δ (six protons), and two aromatic protons, one downfield at 7.99 δ characteristic of a C-11 proton, and the other at 6.74 δ (12). The absence of a relatively upfield methoxyl indicates the phenolic function is at C-1 (12). The UV spectrum of preocoteine strongly resembles that of ocoteine (XI). The structural elucidation of preocoteine has been recorded (4).

Thaliporphine (IV)—Flasks 36–39 (see Table IV) were combined and upon solvent evaporation gave an oil showing two spots on TLC. These two components were easily separated by preparative TLC in chloroform–methanol (19:2). The top band proved to be preocoteine (V). The lower band was eluted from the purified silica gel with chloroform–methanol (9:1), and crystallized with difficulty from methanol. The chemistry of this new aporphine alkaloid, designated thaliporphine, has been discussed (4).

Flasks 40–44 (see Table IV) were combined and the solvent removed. TLC showed it to contain thaliporphine (IV) and thalifendlerine (I). These two alkaloids were separated by preparative TLC in methanol–chloroform (2:19), the top band being thaliporphine, and the bottom band thalifendlerine.

Flasks 44–53 and 54–64 (see Table IV) after combination and solvent evaporation were shown to be complex mixtures of at least eight components each by TLC in methanol–chloroform (1:1). Preparative TLC in this system allowed the isolation of some thalifendlerine from flasks 44–53. The other components of both fractions were present in too small amounts to be isolated.

Fractions 5 and 6 were chromatographed on a column of neutral alumina, using chloroform with increasing amounts of methanol as the eluent. Additional quantities of thaliporphine were isolated from this procedure together with other minor alkaloids which were not characterized.

Hernandezine (XII) and Thalicarpine (XIII)—The methanol washing from the large cellulose column (see Table I) proved to be rich in alkaloids. After solvent evaporation the oily residue was dissolved in chloroform and chromatographed on a neutral alumina column. The solvent polarity was gradually increased by the addition of methanol, until pure methanol was used. Fractions were combined on the basis of their TLC patterns in methanol–acetone (5:3). This chromatographic procedure gave two distinct fractions: an early fraction eluted with pure chloroform, and a late fraction eluted with chloroform–methanol. The results of this chromatographic procedure are summarized in Table V.

Table V—Separation of Methanol Washing

Early fraction (eluted with chloroform)	Hernandezine (VIII) Thalicarpine (XIII)
Late fraction (eluted with chloroform containing methanol)	Thaldimerine (XIV) Thalidezine (II)

The early fraction showed three spots on TLC either in methanol–acetone (5:3) or in methanol–chloroform (1:1). Preparative TLC in these solvents was used to separate these three components. The top band was identified as the known alkaloid hernandezine (VIII), the middle band was shown, by comparative TLC to be thalicarpine (XIII), and the bottom band was present in too small amounts to be investigated.

Hernandezine, m.p. 158° (MeOH), was obtained upon washing the purified silica gel with methanol–chloroform (1:9). A direct comparison of the isolated material with an authentic sample showed the two materials to be identical in terms of IR (chloroform), UV, and mass spectral comparisons, and TLC R_f values. A structural reevaluation of hernandezine has been reported (3).

Thalicarpine (XIII) obtained by washing the purified silica gel with methanol–chloroform (1:9) was identified by spectral comparison with an authentic sample, and comparative TLC.

Thaldimerine (XIV)—The late fraction (see Table V) showed two spots on TLC in methanol–acetone (5:3). After many trials it was decided that methanol–ether (7:3) was a suitable solvent combination for preparative TLC. A complete separation of the two alkaloids was achieved only after repeated use of preparative TLC. The upper band proved to be a new alkaloid, designated thalidezine (VI). The lower band, which was oily and turned brown on exposure to iodine vapor, was also a new alkaloid and was named thaldimerine (XIV).

Thalidezine (VI)—The new bisbenzylisoquinoline alkaloid thalidezine (Table V) was obtained in crystalline form by repeated preparative TLC separations from the crude thaldimerine fraction above. Recrystallization from acetone gave 300 mg. of white needles, m.p. 158–159°. The structural elucidation of thalidezine has been reported (4).

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Keyphrases

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Alkaloid isolation–*Thalictrum fendleri*
Chromatography, partition and adsorption–separation
TLC and paper chromatography–separation
NMR spectrometry
IR spectrophotometry–structure
UV spectrophotometry–structure

Inhibitors of Monoamine Oxidase

Influence of Methyl Substitution on the Inhibitory Activity of β-Carbolines

By BENG T. HO, WILLIAM M. MCISAAC, KENNETH E. WALKER, and VICENTE ESTEVEZ

A number of tetrahydro and aromatic β-carbolines, mostly with a methyl substituent at various positions, were synthesized and their *in vitro* inhibitory activities on monoamine oxidase evaluated. Substitution of a methyl group at the N-9 nitrogen of tetrahydro-β-carboline gave a potent competitive inhibitor of the enzyme. Methyl groups at C-1 of both tetrahydro and aromatic β-carbolines generally reduced the potency, whereas introduction of a methyl group at the N-2 nitrogen of tetrahydro-β-carboline gave a compound of equal activity.

FOR MANY YEARS, since the discovery of iproniazid, inhibitors of the enzyme monoamine oxidase have been used as chemotherapeutic agents for the treatment of depression. However, the many potent antidepressants of the hydrazine class are hepatotoxic, and for this reason have fallen into disuse. Attempts, therefore, have been made to search for other antidepressants with more rapid onset and lower toxicity.

A number of β-carbolines have been found to be potent inhibitors of monoamine oxidase (1–3), yet little is known about the way these compounds bind to the enzyme. In this phase of these studies, a number of tetrahydro and aromatic β-carbolines, mostly with methyl substituents at various positions, were synthesized and their *in vitro* inhibitory activities evaluated. Information on the mode of binding of these compounds to the enzyme will be useful in the design and synthesis of more potent inhibitors which may become useful in the treatment of depression.

DISCUSSION

A fivefold decrease in inhibitory activity was observed when a methyl group was placed on C-1 of the tetrahydro-β-carbolines I and IV (Table I) and the aromatic β-carboline (XX)(Table II) to give II, V, and XXI, respectively. Ethyl substitution on C-1 of I and XX resulted in an even greater decrease in activity; III was seven times less active as an inhibitor than I, and XIII was 16 times less than XX. This loss of inhibitory activity could be attributed to steric hindrance by the bulk of the alkyl group. When a larger group such as COOH was substituted on C-1 of tetrahydro-β-carbolines, an even greater loss of activity occurred; the activity of XV was about 18 times less than I and four times less than II. However, the possibility of a repulsion between COO^- of XV and an anion site on the enzyme could not be entirely ruled out. Likewise, a methyl substituent on C-3 caused a threefold decrease in activity when I was compared with XVII. A COOH group on C-3 gave an inactive compound (XVIII).

Introduction of a methoxy group to C-6 of the ring decreased the inhibitory activity; a fourfold loss in activity was observed in both cases when I was converted into IV, or II into V. An eightfold decrease in activity was observed when 1,9-dimethyl-β-carboline (XII) was compared to its 6-methoxy derivative (XIII). If the indole nucleus binds as an electron donor to an electron-poor locus of the enzyme in a charge-transfer complex (4), the electron-donating methoxy group should make IV and V bind better. Thus, the decreased activity of 6-methoxytetrahydro-β-carbolines can be attributed either to the bulk of the methoxy group or to its polar nature, which is unfavorable for the binding. Studies to obtain greater binding of the indole nucleus by other substituents are in progress.

Neither methyl nor propyl substitution on N-2 of II affected the inhibitory activity; VI and VII were equally as active as II, indicating that a long alkyl

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