# ALKALOIDS OF *THALICTRUM XXXIV.*[1] THREE NEW ALKALOIDS, THALMIRABINE, THALISTINE, AND *O*-METHYLTHALIBRINE, AND OTHERS FROM ROOTS OF *THALICTRUM MINUS* RACE B

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ABSTRACT.—Three new antimicrobial bisbenzylisoquinoline alkaloids, thalmirabine (**3**), thalistine (**11**), and *O*-methylthalibrine (**18**) were isolated from the roots of *T. minus* L. Race B and characterized by spectral and chemical methods. Additional alkaloids, not previously reported from this source, are *N*-methylcorydaldine (**1**), thalrugosine (**2**), thalphenine (**22**), columbamine (**23**), thalifendine (**24**) and jatrorrhizine (**25**).

To date, sixteen alkaloids have been reported from the roots of *Thalictrum minus* L. race B (Ranunculaceae) (1, 2, 3), some of which possess hypotensive and antimicrobial properties. In this paper we present the isolation and identification from this source of nine additional alkaloids, of which three are new natural products. Five were obtained from the ether-soluble tertiary nonphenolic fraction, the so-called Fraction F of an earlier publication (2); while four were quaternary alkaloids derived from Fraction E. The compounds are presented in the order of elution from the first chromatographic column.

*N*-Methylcorydaldine (**1**) was isolated from the mother-liquor residue of adiantifoline (1) in a very minute amount and was characterized by comparison of physical properties with those of a known sample. As a natural product, it was first obtained from *T. fendleri* (4). Thalrugosine (**2**) another known alkaloid, was likewise identified by direct comparison with an authentic sample (5).

Thalmirabine (**3**), an optically active amorphous solid, $[\alpha]_D+116°$, a new alkaloid, exhibited in the mass spectrum a molecular ion peak at *m/e* 668 (37%), which from the value and intensity suggested a bisbenzylisoquinoline with two diphenyl ether bridges and a probable molecular formula of $C_{39}H_{44}N_2O_8$. The nmr spectrum exhibited peaks for two *N*-methyls, five *O*-methyls, eight aromatic protons, one of which was at an upfield position ($\delta$ 6.00) characteristic of H–8 protons, and one phenolic hydroxyl, which was lost in the presence of $D_2O$. The lost group was supported by the ir peak at 3530 $cm^{-1}$ and the preparation of *O*-methylthalmirabine (**4**) by methylation with diazomethane. Since neither thalmirabine (**3**) nor its methyl ether **4** appeared to be known compounds, a total structure proof was undertaken. Reductive cleavage of *O*-ethylthalmirabine (**5**) with sodium/ammonia gave (*S*)-(+)-*O*-methylarmepavine (**6**) as the sole nonphenolic base, and two phenolic bases. One, (*S*)-5-hydroxy-2-methyl-4',6,7-trimethoxybenzyltetrahydroisoquinoline (**7**), was characterized from spectral data and by conversion to the ethyl ether **8** which was identical with one of the degradation products of *O*-ethylthalidezine (6). The other, (*S*)-6,7-dimethoxy-5-ethoxy-4'-hydroxy-2-methylbenzyltetrahydroisoquinoline (**9**), was identified by direct comparison with one of the degradation products common to *O*-ethylthaligosine and

[1]For paper XXXIII see W.-N. Wu, J. L. Beal and R. W. Doskotch, *J. Nat. Prod.*, **43** (1980).
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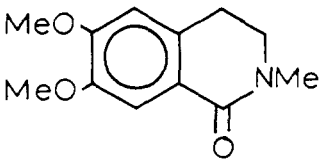
*O*-ethylthalirugine (7). Methylation of product **9** formed the methyl ether **8**, thereby confirming the original assignment.

The $Na/NH_3$ degradation products require that the diphenyl ether group joining the head to head subunits of thalmirabine (**3**) must be from the C–5 position of one to the C–8 of the other, and that the phenolic hydroxy be located at the C–5 position of the tetraoxygenated isoquinoline. Exact location of the other

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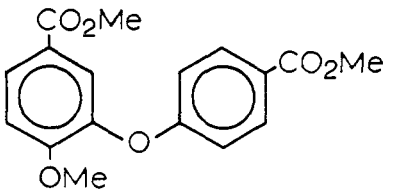


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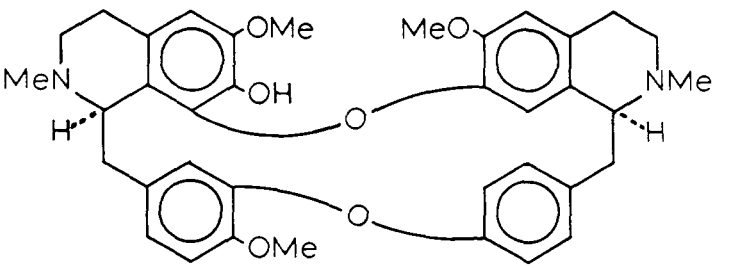


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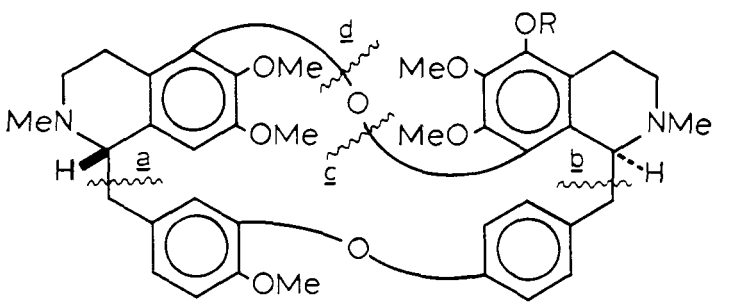
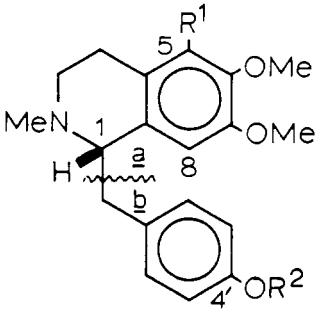


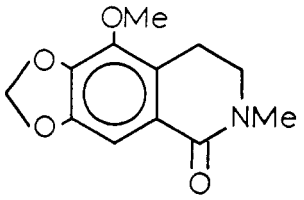

3; R = H

4; R = Me

5; R = Et

diphenyl ether bridge was not possible for one unit, but from biogenetic consideration, the most favored location would be *ortho* to the methoxy. Experimental proof that this is the case was made available by isolation of 2-methoxy-4',5-dicarboxydiphenyl ether (characterized as the dimethyl ester **10**), from the potassium permanganate oxidation of thalmirabine (**3**). Since the cd spectra of the degradation products supported *S*-configuration for both asymmetric centers (8), the unique structure for thalmirabine is as drawn in **3**. Thalmirabine is the third member of a type of bisbenzylisoquinoline, designated by the numerical system of Shamma and Moniot (9) as 5*,6,7,11†,12-5,6,7,8*12†, previously represented by thalfine and thalfinine (10), and also isolated from *T. minus* race B (2).

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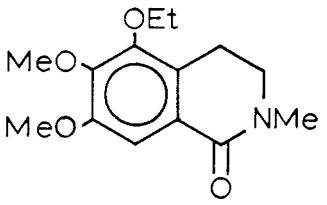
6; $R^1$ = H, $R^2$ = Me

7; $R^1$ = OH, $R^2$ = Me

8; $R^1$ = OEt, $R^2$ = Me

9; $R^1$ = OEt, $R^2$ = H

19; $R^1$ = $R^2$ = H

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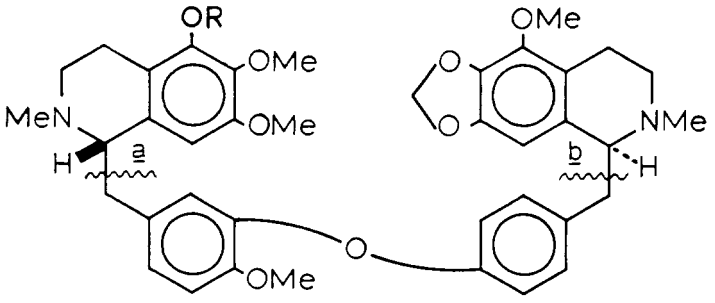
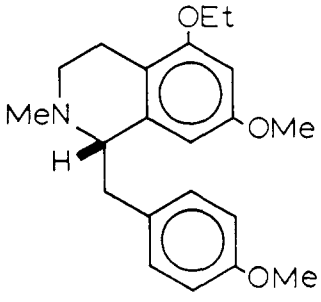



11; R = H

12; R = Me

13; R = Et

Thalfinine differs from thalmirabine in possessing 5'-methoxy-6',7'-methylenedioxy substituents in place of the 5'-hydroxy-6', 7'-dimethoxy. The experimental proof for the configuration at C–1' of thalfinine had not been presented, although *S*-stereochemistry appears the most probable (2). The cd curves of thalmirabine (**3**) and *O*-methylthalmirabine show a closer relationship to thalfinine than to epithalfinine (2), although there are some differences, and an unequivocal answer to settle the question of configuration at the second center is not possible from this analysis.

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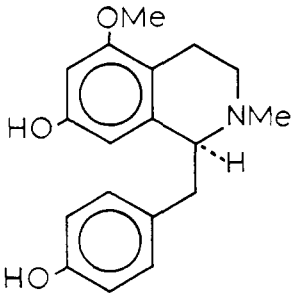


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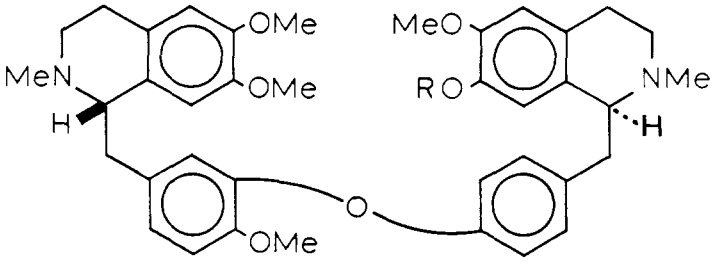
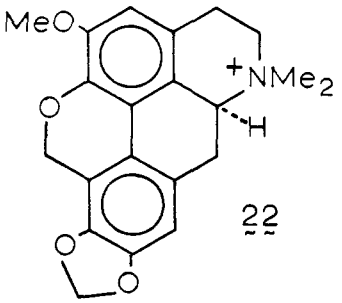



18; R = Me

20; R = H

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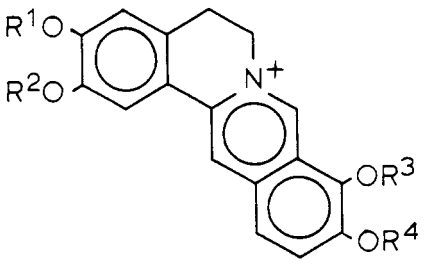
Thalistine (**11**), the second new alkaloid is also amorphous, optically active, and a bisbenzylisoquinoline, but unlike thalmirabine (**3**) contains only one diphenyl ether; the mass spectrum showed a weak (5%) molecular ion at *m/e* 668 corresponding to formula $C_{39}H_{44}N_2O_8$. The nmr spectrum indicated the presence of two *N*-methyls, four *O*-methyls, one methylenedioxy, and nine aromatic protons, two of which are at the high field position ($\delta$ 5.76) expected for H-8 protons of *N*-methylbenzyltetrahydroisoquinolines and their derivatives. In addition, there was one $D_2O$ exchangeable proton for a phenolic group, that was also suggested by the ir peak at 3520 $cm^{-1}$, and confirmed by the preparation of the methyl ether **12** with diazomethane. Analysis of the mass spectral peaks of thalistine (**11**) supported a structure in which one of the isoquinoline units contained two methoxys and a phenolic hydroxyl [fragment **11***a*, *m/e* 222 (100%)], while the other isoquinoline possessed the methylenedioxy group and one methoxy [fragment **11***b*, *m/e* 220 (91%)]; the fourth methoxy would be with the diphenyl ether component. Thalistine ethyl ether (**13**) no longer contained in the mass spectrum the peak at *m/e* 222 but, instead, a new peak at *m/e* 250 (96%) for fragment **13***a* was present; the *m/e* 220 (100%) remained intact.




**21**; $R^1 + R^2 = CH_2$, $R^3 = R^4 = Me$

**23**; $R^1 = R^3 = R^4 = Me$, $R^2 = H$

**24**; $R^1 + R^2 = CH_2$, $R^3 = Me$, $R^4 = H$

**25**; $R^1 = H$, $R^2 = R^3 = R^4 = Me$

Two degradations were performed on *O*-ethylthalistine (**13**). First, $Na/NH_3$ cleavage produced (*S*)-4',7-dimethoxy-5-ethoxy-2-methylbenzyltetrahydroisoquinoline (**14**) as the nonphenolic product, identified by direct comparison with one of the degradation products of *O*-ethylthalidezine (11), and (*S*)-4',7-dihydroxy-5-methoxy-2-methylbenzyltetrahydroisoquinoline (**15**) as the phenolic product, also identified by comparison with a known sample (11, 12). Those products confirmed the *S*-configuration for both centers, as was suggested by the cd spectrum of thalistine (**11**), and placed the methoxy of the diphenyl ether on the same benzylisoquinoline unit bearing the phenolic group. Although it is well-documented (2, 12) that trioxygenated tetrahydroisoquinolines do not survive intact the $Na/NH_3$ reaction, the products that result do allow for formulation of the correct structure of thalistine, except for the location of the diphenyl ether. The second degradation reaction, $KMnO_4$ oxidation, settled the remaining uncertainty and also provided the isoquinolines with the substituents unaltered. Two iso-

quinolones, 5-methoxy-2-methyl-6,7-methylenedioxy-1-oxo-1,2,3,4-tetrahydroisoquinoline (**16**) and 6,7-dimethoxy-5-ethoxy-2-methyl-1-oxo-1,2,3,4-tetrahydroisoquinoline (**17**) were obtained, which firmly established the nature of the corresponding units in thalistine (**11**). The other product, 2-methoxy-4',5-dicarboxydiphenyl ether, characterized as the dimethyl ester **10**, completed the study on thalistine (**11**).

Thalistine (**11**) is related to the thalistyline group of alkaloids (bisbenzylisoquinoline type with one diphenyl ether formed from two 4',5,6,7-tetraoxygenated benzyltetrahydroisoquinoline units) and brings the total to five (2, 11, 12). Thalirabine, the only other thalistyline-type alkaloid from *T. minus* race B, is related to thalistine as the monoquaternary *N*-methyl derivative with the synonymous name *N*-methylthalistine (2).

The third new alkaloid, *O*-methylthalibrine (**18**), an amorphous optically active bisbenzylisoquinoline alkaloid with one diphenyl ether (mass spectral features), has formula $C_{39}H_{46}N_2O_6$ and exhibits peaks in the nmr spectrum for two *N*-methyls, five *O*-methyls, and eleven aromatic protons, two of which are at upfield positions ($\delta$ 6.10 and 6.16) characteristic of H–8 protons. Since no phenolic characteristics were observed, the alkaloid was directly cleaved by $Na/NH_3$, forming two major products *S*-(+)-*O*-methylarmepavine (**6**) and *S*-(+)-armepavine (**19**). These and the $KMnO_4$ oxidation products of 6,7-dimethoxy-2-methyl-1-oxo-1,2,3,4-tetrahydroisoquinoline (**1**) and 2-methoxy-4',5-dicarboxydiphenyl ether (identified as the ester **10**) led to unambiguous structure **18** for the compound. The literature records this substance as a methylation product of thalibrine (**20**) (13), and direct comparison of the synthesized material with the natural product confirmed the structural assignment.

The quaternary alkaloid fraction yielded four protoberberine alkaloids common to *Thalictrum*; berberine (**21**), [already reported (3) ] columbamine (**23**), thalifendine (**24**) and jatrorrhizine (**25**), as well as the aporphine, thalphenine (**22**).

The three new alkaloids were evaluated in the antimicrobial screen routinely employed in our laboratory (14) and gave the following results. The concentrations are the minimal inhibitory values: thalmirabine (**3**) active against *Mycobacterium smegmatis* (100 $\mu$g/ml) thalistine (**11**) active against *M. smegmatis* (100 $\mu$g/ml) and *Staphyloccocus aureus* (1000 $\mu$g/ml), and *O*-methylthalibrine (**18**) active against *M. smegmatis* (100 $\mu$g/ml) and *Candida albicans* (500 $\mu$g/ml).

## EXPERIMENTAL[4]

EXTRACTION AND INITIAL FRACTIONATION.—The fractions utilized in this paper originate from the separation technique reported earlier (2) and from fractions of a subsequent publication (1).

*N*-METHYLCORYDALDINE (**1**).—The adiantifoline mother-liquor residue (1.2 g) (1) from the ether-soluble tertiary nonphenolic bases (Fraction F) of column fractions no. 30–33 was rechromatographed on silica gel (50 g) with chloroform (100 ml), 0.5% (300 ml), 1% (500 ml) and 2% methanol in chloroform (500 ml) as eluents. A 3 mg residue was eluted with 0.5% methanol in chloroform which formed tiny colorless needles (1.5 mg) of *N*-methylcorydaldine (**1**) from methanol, mp 122–3° [lit. (15) mp 125–6°] and showed physical properties (mp, mmp, tlc, uv, ir, and nmr) identical with a known sample (16).

The 1% and 2% methanol in chloroform eluates gave 570 mg of adiantifoline.

THALRUGOSINE (**2**).—The column fraction no. 46 (1.25 g) of the ether-soluble tertiary nonphenolic bases was rechromatographed on 60 g of neutral alumina with benzene-chloroform (1:1, 500 ml) and chloroform (500 ml) as eluents. The first solvent and early fractions of the second solvent gave 85 mg of a crystalline residue that was recrystallized from ether to give thalrugosine (**2**), mp 211–3° [lit. (5) mp 212–4°]. Identification was by direct comparison of physical properties (tlc, uv, ir, nmr, cd and mmp) with authentic thalrugosine (**2**).

[4]For details of reagents, instruments and conditions used, see paper XXXII, W.-N. Wu, J. L. Beal and R. W. Doskotch, *J. Nat. Prod.*, **43**, 270 (1980).

THALMIRABINE (**3**).—The column fraction no. 46 that gave thalrugosine (**2**) on rechromatography yielded from the late chloroform effluent 208 mg of an amorphous but homogeneous solid, named thalmirabine (**3**): $R_f$ 0.59 on tlc with silica gel G and benzene-acetone-ammonium hydroxide solution (20:20:0.5); $[\alpha]^{20}D$ +116° (*c* 0.2, MeOH); cd (C 3.0 x $10^{-3}$M, MeOH) $[\theta]_{300}$ 0, $[\theta]_{285}$ −11,000, $[\theta]_{269}$ 0, $[\theta]_{230}$ +65,100; uv λ max 314 nm (shld, log ε 3.34) and 280 (3.95); ir ν max 3530 $cm^{-1}$ (OH); nmr (60 MHz, $CDCl_3$) δ 2.36, 2.60 (2s, 2 NMe), 3.38, 3.42, 3.72, 3.80 and 3.86 (5s, 5 OMe), 5.20 (brs, OH, lost in $D_2O$), 6.00 (s, H-8), 6.4–7.3 (m, 7 ArH); and ms *m/e* 668 (37%, $M^+$, $C_{39}H_{44}N_2O_8$), 442 (4, $C_{24}H_{30}N_2O_6$, *a* + *b* cleavage), 222 (56, $C_{12}H_{16}NO_3$, cleavage at *b* + *c* + H), 221 (100, double ion of 442 and/or $C_{12}H_{15}NO_3$, cleavage at *b* + *c*) and 206 (18, $C_{12}H_{16}NO_2$, cleavage at *a* + *d* + H).

Anal. Calcd for $C_{39}H_{44}N_2O_8 \cdot 1.5\ H_2O$: C, 67.32; H, 6.81.
Found: C, 67.29; H, 6.49.

Thalmirabine (**3**) gave a positive test with phosphomolybdic acid for a phenol and a negative Gibbs' Test for a *para* unsubstituted phenol.

*O*-METHYLTHALMIRABINE (**4**).—Thalmirabine (**3**, 10 mg) was dissolved in 2.5 ml methanol and mixed with ethereal diazomethane prepared from 0.3 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide and 4 ml of 0.1N KOH in methanol. After 6 days at ambient temperature, the reaction residue was chromatographed on 1 g of silica gel with chloroform (50 ml), 1% and 2% methanol in chloroform (100 ml each). The 1% methanol in chloroform effluent afforded 7.5 mg of *O*-methylthalmirabine (**4**): $R_f$ 0.7 on tlc under the same conditions as thalmirabine; cd (C 4.9 x $10^{-3}$M, MeOH) $[\theta]_{305}$ 0, $[\theta]_{285}$ −6,650, $[\theta]_{263}$ 0, $[\theta]_{230}$ +43,000; and nmr (90 MHz, $CDCl_3$) δ 2.37, 2.64 (2s, 2 NMe), 3.38, 3.42, 3.74, 3.83 (double intensity), 3.88 (5s, 6 OMe), 5.99 (s, H-8), 6.4–7.5 (m, 7 ArH);

O-ETHYLTHALMIRABINE (**5**).—Thalmirabine (120 mg) in 5 ml of MeOH was mixed with ethereal diazoethane prepared from 2 g of *N*-ethyl-N'-nitro-N-nitrosoguanidine and 4 ml of 50% aq. KOH. After 1 wk at ambient temperature, the residue after evaporation of solvent was chromatographed on 6 g of silica gel with chloroform (50 ml), 1% and 2% methanol in chloroform (100 ml each) as eluents. The 1% methanol in chloroform effluent gave 75 mg of *O*-ethylthalmirabine (**5**) as an amorphous solid: $R_f$ 0.78 on tlc under the same conditions as thalmirabine; nmr (60 MHz, $CDCl_3$) δ 1.40 (t, *J* 7, $OCH_2CH_3$), 2.38, 2.63 (2s, 2 NMe), 3.40, 3.42, 3.79, 3.83, 3.88 (5s, 5 OMe), 4.04 (q, *J* 7, $OCH_2CH_3$), 6.03 (s, H-8), 6.4–7.4 (m, 7 ArH); and ms *m/e* 696 (70, $M^+$, $C_{41}H_{48}N_2O_8$), 470 (23, $C_{26}H_{34}N_2O_6$, cleavage at *a* + *b*), 250 (65, $C_{14}H_{20}NO_3$, cleavage at *b* + *c* + H), 235 (100, double ion of 470) and 205 (60, cleavage at *a* + *d*).

SODIUM/AMMONIA CLEAVAGE OF O-ETHYLTHALMIRABINE (**5**).—To 15 ml of liq. $NH_3$ containing 150 mg Na maintained below −50° was added dropwise during 0.5 hr, 5 ml of tetrahydrofuran solution containing 52 mg of *O*-ethylthalmirabine (**5**). Reaction was allowed to proceed for 2.5 hr. After the $NH_3$ evaporated at ambient temp., the unreacted Na was decomposed with excess methanol. The mixture was concentrated to ~2 ml, taken up in 125 ml of ether and extracted with 1N NaOH to separate the products into phenolic and nonphenolic fractions.

The ether layer was washed ($H_2O$), dried ($Na_2SO_4$) and evaporated to give 8 mg of a colorless oil that was chromatographed on 1 g of neutral alumina with benzene (25 ml), benzene-chloroform (1:1, 50 ml) and chloroform (50 ml) as eluents. The benzene-chloroform (1:1) effluent afforded 5 mg of a colorless oil, that was identified as (*S*)-(+)-*O*-methylarmepavine (**6**) by comparison of physical data (tlc, uv, ir, nmr and cd) with those of an authentic sample.

The alkaline solution containing the phenolic products was treated with excess $NH_4Cl$ to pH 9 and extracted with 250 ml of ether. The washed ($H_2O$), dried ($Na_2SO_4$) and evaporated ether extract left 18 mg of a pale yellow residue that showed two spots on tlc, $R_f$ 0.68 and 0.42 under the same conditions as for thalmirabine. The residue was chromatographed on 1.5 g of silica gel with chloroform (50 ml), 1%, 2% and 3% methanol in chloroform as eluents. The 1% methanol in chloroform effluent contained 9 mg of a colorless solid, identified as (*S*)-5-hydroxy-2-methyl-4',6,7-trimethoxybenzyltetrahydroisoquinoline (**7**): mp 85–6°; $R_f$ 0.68 on tlc; cd (C 6.1 x $10^{-3}$M, MeOH) $[\theta]_{285}$ 0, $[\theta]_{272}$ +2,900, $[\theta]_{250}$ 0, $[\theta]_{230}$ +70,000; uv λ max 283 (log ε 3.85); ir ($CHCl_3$) ν max 3520 $cm^{-1}$ (OH); nmr (60 MHz, $CDCl_3$) δ 2.52 (s, NMe), 3.57, 3.78, 3.86 (3s, 3 OMe), 5.70 (s, H-8), 6.80 and 7.02 (AA'BB' q, *J* 9, 4 ArH); and ms *m/e* 343 (0.2%, $M^+$, $C_{20}H_{25}NO_4$), 222 (100, *a*) and 121 (7, *b*). *O*-Ethylation of product **7** with diazoethane gave the ethyl ether **8** identical (tlc, uv, ir, nmr and cd) with one of the nonphenolic products formed from *O*-ethylthalidezine (**6**), and prepared by $Na/NH_3$ cleavage for a direct comparison.

The 2% methanol in chloroform effluent yielded 6.5 mg of a colorless crystal identified as 6,7-dimethoxy-5-ethoxy-4'-hydroxy-2-methylbenzyltetrahydroisoquinoline (**9**): mp 113–4°; $[\alpha]^{20}D$ +103° (c 0.308, MeOH); cd (C 8.6 x $10^{-3}$M, MeOH) $[\theta]_{300}$ 0, $[\theta]_{284}$ +7,000, $[\theta]_{262}$ (shld) +1,300, $[\theta]_{250}$ (min) +600, $[\theta]_{230}$ +56,000; uv λ max 282 nm (log ε 3.54); ir ($CHCl_3$) ν max 3595 $cm^{-1}$ (OH); nmr (60 MHz, $CDCl_3$) δ 1.36 (t, *J* 7, $OCH_2CH_3$), 2.53 (s, NMe), 3.57, 3.83 (2s, 2 OMe), 4.07 (q, *J* 7, $OCH_2CH_3$), 5.91 (s, H-8), 5.9 (brs, OH, lost in $D_2O$), 6.66 and 6.91 (AA'BB' q, $J_{AB}$ 8.5, 4 ArH); and ms (ci, i-butane) *m/e* 358 (10%, $MH^+$, $C_{21}H_{25}NO_4$), 250 (100, *a*) and 107 (2, *b*). This product was identical with one of the $Na/NH_2$ cleavage products of *O*-ethylthaligosine and *O*-ethylthalirugine (**7**). *O*-Methylation of base **9** with diazomethane yielded 5-ethoxy-2-methyl-4',6,7-trimethoxybenzyltetrahydroisoquinoline (**8**).

$KMnO_4$ OXIDATION OF THALMIRABINE (**3**).—A 85 mg sample of thalmirabine (**3**) dissolved in 20 ml of acetone was treated portionwise with 200 mg of $KMnO_4$ during 1 hr while being stirred. After an additional 3 hr of stirring, the excess reagent was decomposed with methanol, and the $MnO_2$ precipitate was removed by filtration. The filtrate was concentrated to 5 ml, 10 ml of $H_2O$ was added and then acidified with 1N HCl. After exhaustive extraction with $CHCl_3$ (250 ml), and removal of solvent, the 53 mg residue was treated overnight with ethereal diazomethane prepared from 1 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide and 5 ml of 0.1N KOH in methanol. The reaction residue after evaporation of solvent was purified on 5 g of neutral alumina with benzene as eluent. The white crystalline product, mp 78–9°, was identical (tlc, mmp, ir and nmr) with a known sample of the dimethyl ester (**10**) of 2-methoxy-4',5-dicarboxydiphenyl ether.

THALISTINE (**11**).—The residue (2 g) from column fraction no. 47 of the ether-soluble nonphenolic tertiary alkaloid partition fraction was rechromatographed on silica gel (60 g) with chloroform (100 ml), 1% (200 ml), 2% (200 ml), 3% (600 ml) and 4% methanol in chloroform (500 ml) as eluents. The 3% and 4% methanol in chloroform eluate gave 631 mg of a pale yellow residue, which was further separated on a 30 g neutral alumina column with benzene (100 ml), benzene-chloroform (1:1, 300 ml) and chloroform (300 ml) as eluents. The chloroform effluent left a 570 mg amorphous base named thalistine (**11**): $R_f$ 0.6 on tlc with silica gel G and benzene-acetone-ammonium hydroxide solution (5:5:0.2); $[\alpha]^{20}D$ +104° (*c* 0.35, MeOH); cd $[\theta]_{290}$ −1,530, $[\theta]_{226}$ +64,000; uv λ max 278 (log ε 3.90); ir ($CHCl_3$) ν max 3520 $cm^{-1}$ (OH), nmr (60 MHz, $CDCl_3$) δ 2.47, 2.50 (2s, 2 NMe), 3.60, 3.63 (2s, 2 OMe), 3.78 (s, 2 OMe), 5.76 (s, H-8 and H-8'), 5.88 (s, $OCH_2O$), 6.4–7.2 (m, 7 ArH), 5.8 (brs, OH, lost with $D_2O$); and ms *m/e* 668 (5%, $M^+$, $C_{39}H_{44}N_2O_8$), 667 (1), 236 (15), 222 (100, *a*), 221 (82), 220 (91, *b*), 205 (33), 204 (31), 192 (50) and 176 (10). Positive tests were obtained with phosphomolybdic acid and Gibbs' reagents for a phenolic unit with an unsubstituted *para* position.

The dimethiodide salt was prepared from 5 mg of thalistine, 1 ml of acetone and 0.5 ml of methyl idodide. After reacting 2 hrs, the precipitate was crystallized from methanol to give 2 mg of rosettes, mp 205–7° (dec), $[\alpha]^{23}D$ +105° (*c* 0.01, MeOH). The product was identical (mp, mmp, specific rotation and ir) with thalirabine methodiiodide (2).

*O*-METHYLTHALISTINE (*N*-DESMETHYLTHALISTYLINE) (**12**).—A 60 mg sample of thalistine (**11**) in 5 ml of methanol was treated for 3 days with ethereal diazomethane prepared from 1 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide and 5 ml of 0.1N KOH in methanol. The residue after removal of solvent was chromatographed on 3.5 g of neutral alumina with benzene (50 ml) and the following mixtures of benzene-chloroform (2:1, 50 ml), (1:1, 100 ml), (1:2, 100 ml) and $CHCl_3$ (50 ml) as eluents. The benzene-chloroform (1:1) effluent produced 25 mg of *O*-methylthalistine (**12**), $R_f$ 0.73 on tlc with the system used for thalistine. The compound was identical (tlc, uv, ir, nmr, cd and specific rotation) with *N*-desmethylthalistyline, an alkaloid isolated from *T. longistylum* DC (12).

*O*-ETHYLTHALISTINE (**13**).—Thalistine (262 mg) in 10 ml of methanol was treated for 3 days with ethereal diazoethane generated from 2 g of *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine and 10 ml of 50% aq. KOH. The reaction residue after removal of solvent was chromatographed on 15 g of neutral alumina (activity 1) with 100 ml each of benzene, benzene-chloroform (3:1), (1:1) and chloroform as eluents. The last two solvents gave effluents from which was obtained 130 mg of the *O*-ethyl derivative **13**: $R_f$ 0.73 on tlc with silica gel G and benzene-acetone-ammonium hydroxide solution (10:10:0.3); nmr (60 MHz, $CDCl_3$) δ 1.33 (t, *J* 7, $OCH_2CH_3$), 2.44, 2.48 (2s, 2 NMe), 3.60, 3.62, 3.77, 3.79 (4s, 4 OMe), 4.01 (q, *J* 7, $OCH_2CH_3$), 5.73, 5.94 (2s, H-8 and H-8'), 5.88 (s, $OCH_2O$) and 6.6–7.1 (m, 7 ArH); and ms *m/e* 696 (1%, $M^+$, $C_{41}H_{48}N_2O_8$), 695 (0.5, M–H), 667 (1), 250 (96, *a*), 221 (32), 220 (100, *b*), 205 (16), 204 (11), 192 (46), and 176 (10).

SODIUM/AMMONIA CLEAVAGE OF O-ETHYLTHALISTINE (**13**).—The reductive cleavage of *O*-ethylthalistine (120 mg) was conducted as described for *O*-ethylthalmirabine (**5**) using 20 ml $NH_3$ containing Na and 10 ml of tetrahydrofuran. The nonphenolic bases (47 mg) showed two spots on tlc, $R_f$ 0.90 (major) and 0.85 (minor) with silica gel G and benzene-acetone-ammonium hydroxide solution (10:10:0.3). These nonphenolic bases were chromatographed on silica gel (5 g) with benzene (50 ml), benzene-chloroform (1:1, 75 ml) and chloroform (50 ml) as eluents. The major product (11 mg), as an oil, was identified as 4',7-dimethoxy-5-ethoxy-2-methylbenzyltetrahydroisoquinoline (**14**) by direct comparison (tlc, uv, ir, nmr and cd) with one of the degradation products from *O*-ethylthalidezine (11).

The phenolic base fraction contained one major component $R_f$ 0.53 on tlc with the same system used for the nonphenolic fraction. Chromatography on silica gel (4 g) with 50 ml each of chloroform, 1%, 2% and 3% methanol in chloroform as eluents, gave from the 3% methanol in chloroform eluate after crystallization from methanol, 12 mg of 4',7-dihydroxy-5-methoxy-2-methylbenzyltetrahydroisoquinoline (**15**), mp 222° [lit. (12) mp 221–2°]. Identification was made by direct comparison of physical data (tlc, mp, mmp, uv, ir, nmr and cd) with those of a known sample (11, 12).

$KMnO_4$ OXIDATION OF O-ETHYLTHALISTINE (**13**).—A 200 mg sample of *O*-ethylthalistine (**13**) dissolved in 25 ml of acetone was stirred at ambient temperature and treated with 400 mg of $KMnO_4$ over 1 hr, at which time the solution showed a purple color. Stirring was continued an additional 3 hrs, and then excess methanol was added to decompose the unused reagent. The $MnO_2$ precipitate was removed by filtration and the filtrate concentrated to 10 ml and mixed with 10 ml of $H_2O$. After being acidified with 1N HCl, the mixture was extracted with chloroform (250 ml) and the chloroform residue (185 mg) was chromatographed on 5 g of silica gel with benzene (50 ml), benzene-chloroform (3:1, 50 ml), chloroform (50 ml) and 5% methanol in chloroform (100 ml) as eluents. The early benzene-chloroform (3:1) effluent contained 16 mg of 5-methoxy-2-methyl-6,7-methylenedioxy-1-oxo-1,2,3,4-tetrahydroisoquinoline (**16**) as colorless needles from methanol, mp 140° [lit. (12) mp 136–137°]. Identification was by direct comparison (mp, mmp, tlc, uv, ir and nmr) with a known sample previously obtained from thalistyline (12).

The late benzene-chloroform (3:1) effluent produced 12 mg of a colorless oil which was identified as 6,7-dimethoxy-5-ethoxy-2-methyl-1-oxo-1,2,3,4-tetrahydroisoquinoline (**17**) by direct comparison (tlc, uv, ir and nmr) with an authentic sample prepared from *O*,*O*-diethylthalirugine (7).

The 5% methanol in chloroform and methanol effluent contained 20 mg of a solid that showed identical tlc behavior as 4',5-dicarboxy-2-methoxydiphenyl ether and on methylation with diazomethane gave 12 mg of crystalline dimethyl ester **10** from methanol, mp 77–8°, after purification on neutral alumina (2 g) with benzene as eluent. Identity was by direct comparison (mp, mmp, tlc, ir and nmr) with a known sample.

O-METHYLTHALIBRINE (**18**).—The residue (2.3 g) from column fractions no. 48–52 of the ether-soluble tertiary nonphenolic alkaloids was dissolved in a small amount of chloroform and added to a neutral alumina column (120 g), then eluted with benzene (200 ml), mixtures of benzene-chloroform (4:1, 250 ml), (1:1, 500 ml), (1:2, 500 ml) and chloroform (400 ml). The benzene-chloroform effluent left 450 mg of an amorphous base, named *O*-methylthalibrine (**18**): $R_f$ 0.69 on tlc with silica gel G and benzene-acetone-ammonium hydroxide solution (20:20:0.7); $[\alpha]^{20}D$ +109° (*c* 0.22, MeOH) [lit. (13) $[\alpha]D$ +82° (*c* 0.36, $CHCl_3$)]; cd (C 3.4 x $10^{-3}$M, MeOH) $[\theta]_{300}$ 0, $[\theta]_{287}$ +15,500, $[\theta]_{270}$ 0, $[\theta]_{250}$ 0, $[\theta]_{228}$ +75,500; uv λ max 285 nm (shld, log ε 4.01), 280 (4.02); nmr (60 MHz, $CDCl_3$) δ 2.49 and 2.53 (2s, 2 NMe), 3.60, 3.63, 3.78, 3.80 and 3.83 (5s, 5 OMe), 6.10 and 6.16 (2s, H-8 and H-8'), 6.53 and 6.56 (2s, H-5, H-5'), and 6.6–7.2 (m, 7 ArH); and ms *m/e* 638 (0.03%, $M^+$, $C_{39}H_{46}N_2O_6$), 206 (100, *a*), 191 (9, *a*-Me) and 190 (9).

SODIUM/AMMONIA CLEAVAGE OF *O*-METHYLTHALIBRINE (**18**).—The reaction was performed as described for *O*-ethylthalmirabine (**5**). A 100 mg sample of *O*-methylthalibrine (**18**) in 5 ml of tetrahydrofuran was added over 0.5 hr to 20 ml liq. $NH_3$ containing enough Na to persist at least 0.5 hr. After reacting for an additional 2 hr, the reaction mixture was separated into the phenolic and nonphenolic bases. The nonphenolic fraction (45 mg) containing one spot on tlc, $R_f$ 0.67 on tlc with silica gel and benzene-acetone-ammonium hydroxide solution (20:20:0.5), was purified on 5 g of neutral alumina with benzene (50 ml), benzene-chloroform (1:1, 100 ml) and chloroform (50 ml) as eluents. The second solvent eluted 35 mg of a colorless oil, which was identified as *S*-(+)-*O*-methylarmepavine (**6**) on the basis of physical properties identical (tlc, uv, ir, nmr and cd) with those of an authentic sample.

The phenolic fraction (40 mg) was chromatographed on 2 g of silica gel with chloroform (25 ml) 1%, 2% and 3% methanol in chloroform (50 ml each) as eluents. The 3% methanol in chloroform effluent gave 20 mg of a second phenolic base, $R_f$ 0.45 on tlc with the same system as for *O*-methylarmepavine, and with physical properties (tlc, uv, ir, nmr, ms and cd) identical with *S*-(+)-armepavine (19).

$KMnO_4$ OXIDATION OF O-METHYLTHALIBRINE (**18**).—A 200 mg sample of *O*-methylthalibrine in 20 ml of acetone was treated with 400 mg of $KMnO_4$. After being stirred 5 hrs the reaction was worked up as described for the oxidation of thalmirabine (**3**) to yield 25 mg of isoquinoline **1**, and 42 mg of 2-methoxy-4',5-dicarboxydiphenyl ether as the dimethyl ester **10**. Both products were identified by direct comparison of physical properties (mp, tlc, ir, and nmr) to those of known samples.

METHYLATION OF THALIBRINE (**20**).—A 350 mg sample of thalibrine (**20**) in 3 ml of methanol was added to an ethereal diazomethane generated from 0.5 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide and 2.5 ml of 0.1N KOH in methanol. After at 3 days at ambient temperature, the residue after evaporation of solvent was chromatographed on 2 g of neutral alumina with benzene, benzene-chloroform (1:1) and chloroform (50 ml each) as eluents. The second solvent eluted 18 mg of the amorphous methyl ether **18**, which showed identical tlc mobility, specific rotation, uv, ir, nmr and cd spectra as the *O*-methylthalibrine isolated from the plant.

CHROMATOGRAPHY OF THE QUATERNARY ALKALOID FRACTION.—The quaternary alkaloid reineckates (23 g) was dissolved in 2 liters of 50% aq. acetone, and mixed with 150 g of Amberlite™ IRA 400 (Cl form) ion exchange resin for 3 days. Collection of the resin by filtration and evaporation of the filtrate left 12 g of the quaternary alkaloid chlorides. Chromatog-

raphy on 0.5 kg of neutral alumina (activity 1) with chloroform (0.5 liter), the following mixtures of methanol in chloroform 2.5% (2 liters), 5 (2), 10 (2), 20 (2), 40 (3), 50 (2), and methanol (2 liters) as eluents. Effluent fractions of 20 ml were evaporated; residue weights were determined and analyzed by tlc.

BERBERINE (**21**) AND THALPHENINE (**22**).—The yellow crystalline residue from column fractions no. 60–195 gave 415 mg of crystalline berberine (**22**) chloride, which was identified from physical properties. Fractions no. 196–230 (175 mg) also gave crystalline berberine (**21**) chloride. The mother liquor residue (130 mg) was chromatographed on 13 g of neutral alumina with chloroform (50 ml), 1% (125 ml), 2% (100 ml) and 4% (100 ml) methanol in chloroform as eluent. The early 2% methanol in chloroform effluent gave 20 mg of berberine, and the late fractions plus the 4% MeOH in $CHCl_3$ eluate left a residue, which crystallized from methanol-acetone to give thalphenine (**22**) chloride: mp 186–8° [lit. (17) mp 185–186°]; $[\alpha]^{21}D+71°$ (*c* 0.28, MeOH) [lit. (17) $[\alpha]D+69$ (*c* 1.3, EtOH)], and other physical properties (tlc, mmp, uv, ir, nmr and cd) identical with those of an authentic sample.

COLUMBAMINE (**23**).—The residue (945 mg) of column fractions no. 231–262 was rechromatographed on 30 g of silica gel with chloroform (50 ml), 2.5% (200 ml), 5% (500 ml), 10% (750 ml) and 15% (250 ml) methanol in chloroform as eluent. The 2.5, 5, and 10% methanol in chloroform gave more berberine (316 mg), and the later 10% methanol in chloroform afforded a residue that was dissolved in 1 ml of methanol and with KI gave a yellow precipitate that recrystallized from methanol to give micro-needles of columbamine (**23**) iodide, mp 206–8° and other physical data (mmp, tlc, uv and ir) identical with an authentic sample.

THALIFENDINE (**24**).—The residue (953 mg) of column fractions no. 263–363 was rechromatographed on silica gel (30 g) and chloroform (50 ml), 2.5% (200 ml), 5% (500 ml) 10% (750 ml) and 20% methanol in chloroform as eluent. The later 10% methanol in chloroform effluent afforded 17 mg of a yellow-orange crystalline residue that was converted to the iodide salt and crystallized from methanol to give thalifendine (**24**) iodide, mp 210° (dec) identified by comparison (mmp, tlc, uv, ir and nmr) with an authentic sample.

JATRORRHIZINE (**25**).—The residue (1.3 g) of column fractions no. 363–532 was rechromatographed on 40 g of silica gel with the following mixtures of methanol in chloroform, 2.5% (250 ml), 5 (750), 10 (500), 15 (750), 20 (500) and 30 (500). The residue (156 mg) of the 10% and 20% effluent was converted to the iodide salt, crystallized from methanol to give yellow-orange needles of jatrorrhizine (**25**) iodide, mp 207–9° (dec), identified by direct comparison (mmp, tlc, uv and ir) with a known sample.

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