ALKALOIDS OF *THALICTRUM*. XXXIII.[1] ISOLATION AND CHARACTERIZATION OF ALKALOIDS FROM THE ROOT OF *THALICTRUM ALPINUM*

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ABSTRACT.—The roots of *T. alpinum* L. yielded twelve tertiary alkaloids of which two are new natural products, thalpindione (**1**), the second reported dioxobisbenzyltetrahydroisoquinoline alkaloid, and *N*-desmethylthalrugosidine (**5**); the others were thalidasine (**3**), thalrugosidine (**4**), thalicarpine (**9**), thalrugosaminine (**10**), neothalibrine (**11**), *O*-methylisoboldine (**12**), isoboldine (**13**), *N*-methyl-6,7-dimethoxyisoquinolone (**14**), oxyberberine (**15**), and noroxyhydrastinine (**16**). Thalpindione was *O*-methylated to thalrugosinone (**2**), a recently reported new alkaloid, and *N*-desmethylthalrugosidine (**5**) was *N*-methylated to thalrugosidine (**4**). Location of the unmethylated nitrogen in **5** was established by Na/NH_3 cleavage of the *O*-ethyl derivative **6** to fragments **7** and **8**. From the quaternary alkaloid fraction six alkaloids were obtained, palmatine (**17**), berberine (**18**), columbamine (**19**), jatrorrhizine (**20**), thalifendine (**21**), and magnoflorine.

In the continuing study of the alkaloids from *Thalictrum*, we have examined *T. alpinum* L. (Ranunculaceae), a species that is indigenous to Europe, Asia, and northern North America. It is a relatively small plant, (about 15 cm average height), which grows in humid places on rich soils at higher elevations or in colder climates. Our plants were grown from seed in the Medicinal Plant Garden. In order to obtain enough material for study, seven years of growth were required.

The alkaloids were extracted from the dried and powdered roots by percolation with ethanol and then separated from the nonalkaloid material by a solvent partition sequence that utilizes the basic nature of the desired materials. In this manner three tertiary alkaloid fractions were obtained, the ether-soluble nonphenolic, the ether-soluble phenolic, and the chloroform-soluble alkaloids. The quaternary alkaloids were isolated by precipitation as the reineckate salts and then regenerated as the chlorides for chromatography. The major fractions were resolved separately by adsorption chromatography.

The ether-soluble nonphenolic alkaloids were chromatographed on silica gel and effluent fractions rechromatographed to yield pure constituents. The alkaloids obtained are given in the order of elution. Thalpindione (**1**), an amorphous base, was assigned the formula $C_{37}H_{36}N_2O_9$ by high resolution mass spectrometry. The molecular size suggested it belonged to the bisbenzylisoquinolines, and the high intensity of the molecular ion pointed to the possibility of two diphenyl ether linkages. The nmr spectrum supported the presence of one *N*-methyl, four *O*-methyls and one hydroxyl. The latter was confirmed by the 3530 cm^{-1} peak in the ir spectrum which also exhibited carbonyl absorption at 1663 cm^{-1}. The presence of two carbonyls would complete the nine oxygens required by the formula and would make thalpindione (**1**), the second example of a dioxobisbenzyltetrahydroisoquinoline alkaloid; the first is thalrugosinone (**2**), recently reported from *T. rugosum* (1). Methylation of thalpindione (**1**) with diazomethane gave thalrugosinone (**2**), proving that the starting material was monophenolic and that its complete structure, as argued for thalrugosinone (**2**) (1) except for the location

[1]For paper XXXII see J. Wu, J. L. Beal, W.-N. Wu and R. W. Doskotch, *J. Nat. Prod.*, **43**, 270 (1980).

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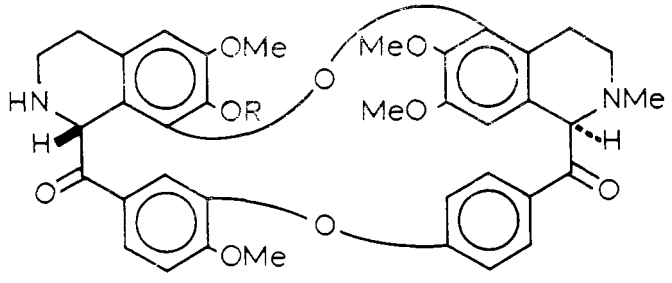
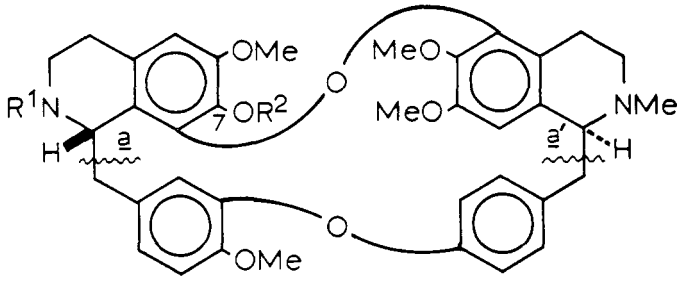
of the phenolic group, can be given as in **1**. The cd spectrum is little changed in going from thalpindione (**1**) to thalrugosinone (**2**) and is very similar to that of thalidasine (**3**), (2, 3) and thalrugosidine (**4**) (4), thus underscoring a similar conformation for these alkaloids. Consequently, the chemical shifts of *N*-methyls and *O*-methyls in the nmr spectra would have relatively fixed positions and could be used for location of substituents. For example, the methoxy at C-7 of thalidasine (**3**) is found at δ 3.27, since in thalrugosidine (**4**) this peak is missing while the other peaks remain invariant. Similarly, the peak at δ 3.33 of thalrugosinone



1; R = H

2; R = Me

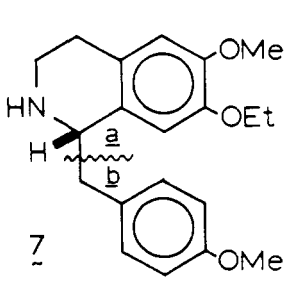


3; $R^1 = R^2$ = Me

4; R^1 = Me, R^2 = H

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

6; R^1 = H, R^2 = Et



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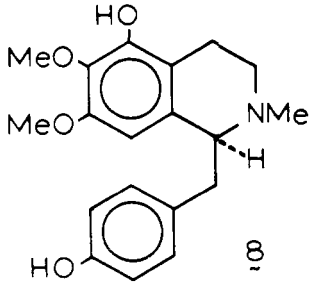

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(**2**) is absent from the spectrum of thalpindione (**1**) while the other positions are little changed. In fact, the peak locations for the substituents common to the four alkaloids deviate at most 0.05 ppm and are located within ±0.02 ppm on the average. From this evidence, the phenolic group of thalpindione (**1**) was placed at C–7.

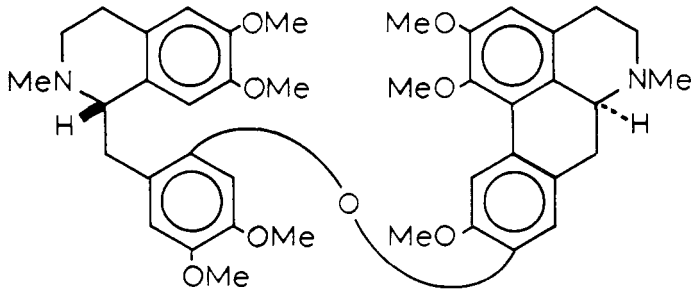
The second and third alkaloids obtained from the ether-soluble nonphenolic fraction were thalidasine (**3**) (2, 3) and thalrugosidine (**4**) (4), well-characterized known compounds, with the latter being only the second reported from nature. The fourth was a new alkaloid, named *N*-desmethylthalrugosidine (**5**), as will be seen, from its relationship to the parent compound. The structure was established from spectral data and chemical degradation. The mass spectrum with an intense molecular ion peak at *m/e* 624 (85%) corresponded to a bisbenzylisoquinoline with two diphenyl ethers and the formula $C_{37}H_{40}N_2O_7$. The nmr spectrum identified one *N*-methyl, four *O*-methyls, and nine aromatic protons, while the ir spectrum showed hydroxyl absorption at 3535 cm^{-1}. The cd curve was similar to that of thalidasine (**3**). *N*-methylation of *N*-desmethylthalrugosidine with formaldehyde and sodium borohydride produced thalrugosidine (**4**), thereby establishing the structure including stereochemistry for this alkaloid, except for the position of the *N*-methyl group. This uncertainty was resolved by sodium-ammonia cleavage of *O*-ethyl-*N*-desmethylthalrugosidine (**6**) to the fragments **7** and **8**, representing the "left-" and "right-side" portions. Compound **8**, (*S*)-4',5-dihydroxy-6,7-dimethoxybenzyltetrahydroisoquinoline, was found to be identical with the corresponding fragment produced in a similar way from thalidasine (**3**). Clearly, the methylated nitrogen must be associated with the "right-side" portion. Confirmation was obtained from compound **7**, (*S*)-4',6-dimethoxy-7-ethoxybenzyltetrahydroisoquinoline, which is a new substance with the structure assigned from spectral properties and knowledge of the starting material **6**. The fact that product **7** exhibited in the nmr spectrum the methyl triplet and the methylene quartet of an ethyl group and no *N*-methyl singlet required that the unmethylated nitrogen of *N*-desmethylthalrugosidine (**5**) be present on the same benzylisoquinoline unit as the phenolic hydroxyl.

This result allowed identification of the individual *N*-methyls for thalidasine (**3**) and related substances. Those with chemical shifts in the nmr spectrum near δ 2.2 are associated with the "left-side" (as drawn in **3**), while the other appears near δ 2.6. Confirmation for placement of the *N*-methyl group of thalrugosinone (**2**) and thalpindione (**1**) to the "right-side" is now available; the alkaloids exhibit peaks at δ 2.64 and 2.63, respectively. It is interesting to note that *N*-desmethylthalrugosidine (**5**) on oxidation of the α-benzylic methylenes to ketones would give thalpindione (**1**). This may be the biogenetic relationship between them.

Three additional compounds were isolated from the ether-soluble nonphenolic tertiary alkaloid fraction, namely, thalicarpine (**9**) (5, 6), thalrugosaminine (**10**) (7), and neothalibrine (**11**) (1), which were identified by direct comparison of physical properties with those of authentic samples. Of the seven alkaloids from the so-called ether-soluble nonphenolic fraction, four contained phenolic groups. It is clear that the partition system is not dividing the alkaloids cleanly into discrete groups; but it is providing, however, less complex mixtures that are more easily separated by chromatography. The ether-soluble phenolic alkaloid fraction yielded four alkaloids, (*S*)-*O*-methylisoboldine (also known as thaliporphine or thalicmidine) (**12**) (8, 9), (*S*)-isoboldine (**13**) (10), thalrugosidine (**4**)

and neothalibrine (**11**). The last two were isolated from the nonphenolic fraction as well. All were identified by comparison of physical properties with those of known samples.

The chloroform-soluble tertiary alkaloids after chromatographic separations yielded in minute quantities three known compounds, *N*-methyl-6,7-dimethoxy-



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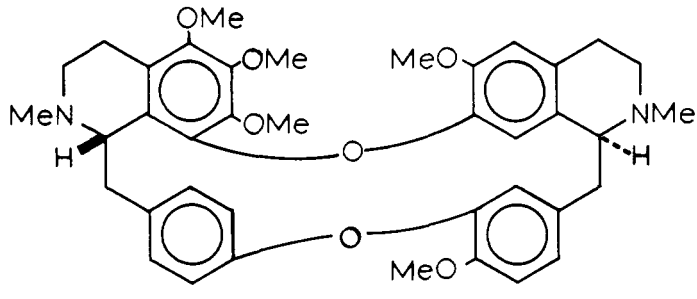

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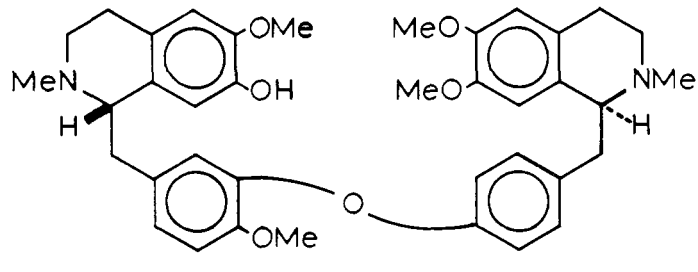

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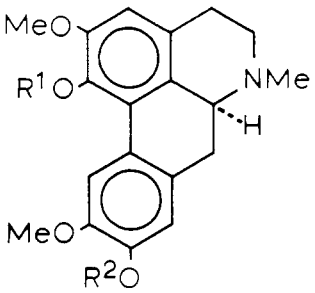
isoquinolone (**14**) (11), oxyberberine (**15**) and noroxyhydrastinine (**16**). Recently, an efficient synthesis of compound **14** was reported (12).

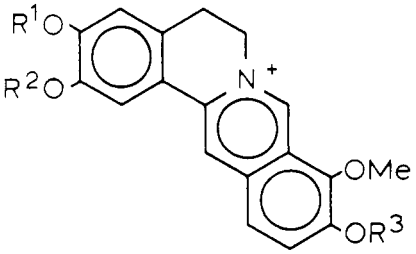
The quaternary alkaloid fraction, as the chlorides, after chromatography and preparation of crystalline iodides led to the identification of six alkaloids rather common to *Thalictrum*,—the five protoberberines, palmatine (**17**), berberine (**18**),

columbamine (**19**), jatrorrhizine (**20**) and thalifendine (**21**), and the ubiquitous aporphine, magnoflorine. All were identified by direct comparison with authentic samples. Information on these compounds can be found in a review on *Thalictrum* (13).



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

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



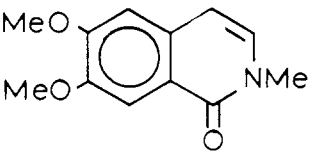
17; $R^1 = R^2 = R^3 = Me$

18; $R^1 + R^2 = CH_2, R^3 = Me$

19; $R^1 = R^3 = Me, R^2 = H$

20; $R^1 = H, R^2 = R^3 = Me$

21; $R^1 + R^2 = CH_2, R^3 = H$



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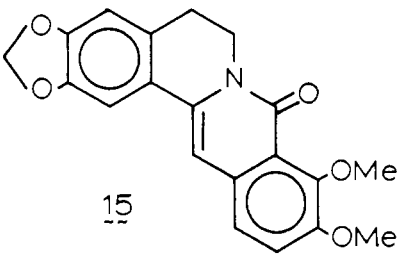

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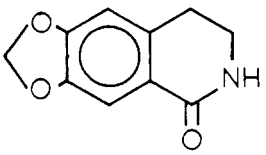

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EXPERIMENTAL[3]

PLANT MATERIAL.—The roots for this research were harvested in August, 1976, from seven-year-old plants grown in The Ohio State University College of Pharmacy Medicinal Plant

[3]Melting points are uncorrected. Nmr spectra were determined in stated solvents with tetramethylsilane as internal standard on Varian A-60A or Bruker HX-90E instruments, the latter equipped for Fourier transform analysis. Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. Ir spectra were taken in KBr windows on a Beckman IR 4230. Uv spectra were taken in methanol on a Cary 15 instrument. Mass spectra were obtained on an AEI MS-9 or DuPont 21-491 instruments by direct inlet probe at 70eV. Optical rotations were measured on a Perkin-Elmer 241 photoelectric polarimeter and cd spectra in methanol on a Durrum-Jasco ORD/UV-5 spectropolarimeter with Sproul Scientific SS-20 modification. Tlc was performed on silica gel HF and column chromatography on silica gel PF 254 of E. Merck, Darmstadt, Germany.

Garden. They were grown from seed obtained from the Department of Botany, University of Turku, Turku, Finland. Voucher specimens are on file in the herbarium of the Division of Pharmacognosy and Natural Products Chemistry of The Ohio State University College of Pharmacy.

EXTRACTION AND INITIAL ISOLATION PROCEDURE.—Powdered roots (1.8 kg) were extracted by percolation with ethanol at room temperature. Evaporation of the solvent at reduced pressure and 40° left a residue of 230 g, which was suspended in 6 liters of 2% aq. citric acid and extracted with 6 liters of chloroform. The aqueous acid solution was basified to pH 9–10 with conc. NH_4OH and extracted successively with ether and chloroform (15 liters each) to remove the tertiary alkaloids. The ether phase was extracted with 6 liters of 5% aq NaOH to remove the phenolic alkaloids. The aqueous alkaline solution was treated with excess solid NH_4Cl to produce a cloudy suspension and extracted with ether (16 liters). The ether extract was washed with water, dried (Na_2SO_4) and evaported to dryness to give 3.94 g of the ether-soluble tertiary phenolic fraction as a yellow-brown residue.

The ether extract, from which the phenolic alkaloids were removed, was washed with water, dried (Na_2SO_4) and evaporated to dryness, left 6.26 g of the ether-soluble nonphenolic alkaloid fraction as a pale yellow residue. The chloroform extract was washed with water, dried (Na_2SO_4) and evaporated to dryness to give 2.36 g of the chloroform-soluble tertiary alkaloids.

The aqueous solution, after removal of the tertiary alkaloids, was acidified with citric acid to pH 4–5, and treated with a saturated aqueous solution of ammonium reineckate (2%) to excess. The precipitate of alkaloid reineckates (27 g) was collected by filtration, and mixed with 2 liters of acetone-water (1:1). The insoluble material was removed by filtration; the filtrate was stirred with 190 g of anion exchange resin Amberlite™ IRA 400 (Cl^- form) for 3 days and then filtered. The filtrate was evaporated to dryness and extracted with methanol; evaporation of the methanol solution left 14 g of quaternary alkaloid chlorides.

CHROMATOGRAPHY OF THE ETHER-SOLUBLE NONPHENOLIC ALKALOIDS.—The ether-soluble nonphenolic alkaloid fraction (6.2 g) was chromatographed on 310 g of silica gel with the following eluting solvents: $CHCl_3$ (2 liters), mixtures of methanol in chloroform as 1% (3 liters), 2 (3), 5 (5), 7.5 (2), 10 (2), 20 (2), 30 (1), 50 (1), MeOH (1 liter) and 5% HCl in MeOH (1 liter). Effluent fractions of 100 ml were evaporated; residue weights were determined and examined by tlc. The compounds isolated are reported below.

THALPINDIONE (**1**).—The residue (55 mg) from column fractions no. 41–48 of the ether-soluble nonphenolic alkaloids was rechromatographed on 3 g of neutral alumina with benzene (50 ml), benzene-chloroform (1:1, 75 ml) and $CHCl_3$ (50 ml) as eluents. The benzene-chloroform (1:1) effluent gave 25 mg of thalpindione (**1**) of a pale yellow amorphous, but homogeneous, base which would not crystallize: R_f 0.8 on tlc with silica gel G and benzene-acetone-ammonium hydroxide solution (20:20:0.5); $[\alpha]^{21}D$ −41.5° (*c* 0.29, MeOH); cd (C 4.48 x 10^{-3}M, MeOH) $[\theta]_{505}$ 0, $[\theta]_{285}$ −22,900, $[\theta]_{270}$ 0, $[\theta]_{265}$ +2.230, $[\theta]_{258}$ (min) +560, $[\theta]_{240}$ +78,000, $[\theta]_{227}$ 0, and $[\theta]_{215}$ (end) −80,000; uv λ max 283 nm (shld, log ε 3.77) and 275 (3.78) with no change in 0.01N NaOH or HCl; ir ($CHCl_3$) ν max 3530 (OH) and 1663 cm^{-1} (C=O); nmr (90 MHz, $CDCl_3$) 2.63 (s, NMe), 3.47, 3.80, 3.89 and 3.90 (4s, 4 OMe), 4.4–4.7 (m, 2 methine) 6.1–7.7 (m, 9 ArH), and 5.2 (br, OH, lost in D_2O); and ms *m/e* 652.2436 (26%, M^+, $C_{37}H_{36}N_2O_9$ requires 652.2421).

O-METHYLATION OF THALPINDIONE (**1**) TO THALRUGOSINONE (**2**).—A 4 mg sample of thalpindione (**1**) in 1.5 ml of methanol was mixed with ethereal diazomethane prepared from 0.1 g of *N*-methyl-*N*-nitroso-p-toluenesulfonamide and 2 ml 0.1N KOH in methanol. After reacting 1 wk at ambient temperature, the solvent was evaporated at reduced pressure and the residue chromatographed on 1 g of silica gel with $CHCl_3$ (20 ml), 1% and 2% methanol in chloroform (50 ml each) as eluents. The 1% methanol in chloroform effluent gave 2.2 mg of a pale yellow amorphous base, R_f 0.82 on tlc with silica gel G and benzene-acetone (1:1) and spectral properties (ir, uv, nmr and cd) identical with thalrugosinone (**2**) a recently reported alkaloid from *T. rugosum* (1).

THALIDASINE (**3**).—The residue (590 mg) from column fractions no. 49–52 of the ether-soluble nonphenolic tertiary alkaloids was rechromatographed on 30 g of neutral alumina with benzene (200 ml), benzene-chloroform (2:1, 250 ml) and (1:2, 400 ml) as eluents. The last two solvents eluted 485 mg of a homogeneous base that gave identical specific rotation, tlc mobility, and uv, ir, nmr and cd spectra as authentic thalidasine (**3**) (2, 3).

THALRUGOSIDINE (**4**).—*A*. Both column fractions no. 58–64 (858 mg) and 65–69 (1.5 g) of the ether-soluble nonphenolic tertiary alkaloid fraction afforded thalrugosidine (**4**); the first by crystallization from methanol gave 500 mg, mp 175–6° [lit. (4) 172–4°]. The second fraction was chromatographed on 75 g of neutral alumina with 200 ml of benzene, benzene-chloroform (1:1), $CHCl_3$ and 1% methanol in chloroform as eluents. The benzene-chloroform (1:1) effluent gave a residue (850 g) that yielded 520 mg of crystalline thalrugosidine (**4**) mp 174–6°, from methanol. Identity was established by comparison of physical properties (mp, mmp, tlc, ir, uv, nmr and cd) with those of an authentic sample.

B. The residue (79 mg) of column fractions no. 284–292 of the ether-soluble phenolic tertiary alkaloids was rechromatographed on neutral alumina (4 g) with benzene (50 ml), benzene-chloroform (4:1, 50 ml), (3:1, 75 ml), (1:1, 75 ml), (3:1, 50 ml) and $CHCl_3$ (25 ml) as eluents. The benzene-chloroform (4:1) effluent gave 10 mg of a crystalline residue which was recrystallized from methanol, mp 175–7° and was identified as thalrugosidine (**4**) by direct comparison with an authentic sample.

N-DESMETHYLTHALRUGOSIDINE (**5**).—The residue (311 mg) from column fractions no. 70–76 was chromatographed on 16 g of neutral alumina (activity 1) with benzene (100 ml), benzene-chloroform (1:1, 200 ml) and chloroform (250 ml) as eluents. The benzene-chloroform (1:1) effluent gave 35 mg of a pale yellow powder that formed colorless needles from methanol: mp 205–6°, R_f 0.67 on tlc with silica gel G and benzene-acetone-ammonium hydroxide (20:20:0.3); $[\alpha]^{21}D$ −57° (*c* 0.23, MeOH); cd (C 3.6 x 10^{-3}M, MeOH) $[\theta]_{300}$ 0, $[\theta]_{285}$ −24,000, $[\theta]_{272}$ 0, $[\theta]_{270}$ +6,400, $[\theta]_{258}$ (min) +3,600, $[\theta]_{242}$ +67,000, $[\theta]_{232}$ 0 and $[\theta]_{225}$ −21,000; uv λ max 283 (log ε 3.91) and 278 (3.90) with no shift in 0.01N NaOH or HCl; ir ($CHCl_3$) ν max 3535 cm^{-1} (OH); nmr (60 MHz, $CDCl_3$) δ 2.62 (s, NMe), 3.52, 3.77, 3.88 and 3.92 (4s, 4 OMe), and 6.2–7.7 (m, 9 ArH); and ms *m/e* 624 (85%, M^-, $C_{37}H_{40}N_2O_7$ requires 624), 623 (17, M-1), 398 (33, $C_{22}H_{23}N_2O_5$, cleavage at *a* and *a'*), 397 (100, 398–H), 383 (6, 398–Me), 199 (55, double ion of 398), 222 (4, $C_{12}H_{16}NO_3$), 206 (8, $C_{12}H_{16}NO_2$) and 178 (4, $C_{10}H_{12}NO_2$). The alkaloid gave a positive test for a phenolic group with phosphomolybdic acid.

N-METHYLATION OF *N*-DESMETHYLTHALRUGOSIDINE (**5**) TO THALRUGOSIDINE (**4**).—A 25 mg sample of *N*-desmethylthalrugosidine (**5**) was added to 2 ml of a formaldehyde solution (1 ml of 37% formaldehyde and 10 ml methanol) and stirred 1 hr at ambient temperature. Then 80 mg of $NaBH_4$ was added gradually and stirred 1 hr; the 3 ml of acetone was added and stirred 1 hr. The residue obtained on evaporation of the reaction mixture was dissolved in 2 ml of 5% aq. NaOH and excess NH_4Cl was added followed by extraction with ether (125 ml). The ether extract was washed with water, dried (Na_2SO_4) and evaporated to dryness. The 27 mg white residue was chromatographed on 2 g of neutral alumina with benzene (50 ml), benzene-chloroform (1:1, 50 ml) and chloroform (25 ml) as eluents. The second solvent gave 23 mg of a product that crystallized from methanol, mp 173–5°. The compound was identified as thalrugosidine (**4**) on the basis that the physical properties (tlc, mp, uv, ir, nmr, specific rotation and cd) were identical with an authentic sample. A mixture mp was undepressed.

O-ETHYL-*N*-DESMETHYLTHALRUGOSIDINE (**6**).—*N*-desmethylthalrugosidine (30 mg) in 2 ml of methanol was treated with ethereal diazoethane, prepared from 0.5 g of *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine and 2 ml of 50% aq. KOH, for 4 days at ambient temperature. Evaporation of the solvent left a residue (31 mg) that was chromatographed on 3 g of neutral alumina with benzene (25 ml), benzene-chloroform (3:1) and (1:1) each 50 ml and chloroform (25 ml) as eluents. The benzene-chloroform (3:1) effluent gave 21 mg of compound **6** as an amorphous, but homogeneous base: R_f 0.94 on tlc with silica gel G and benzene-acetone-ammonium hydroxide solution (10:10:0.3); ir ($CHCl_3$) no OH peaks; nmr (90 MHz, $CDCl_3$) δ 0.73 (t, *J* 7, OCH_2CH_3), 2.61 (s, NMe), 3.54, 3.74, 3.86 and 3.91 (4s, 4 OMe); and ms *m/e* 652 (40%, M^+, $C_{39}H_{44}N_2O_7$ requires 652), 637 (7, M–Me), 621 (8, M–OMe), 426 (27, $C_{22}H_{29}N_2O_5$, cleavage at *a* and *a'*), 425 (100, 426–H), 411 (7, 426–Me), 222 (8), 213 (63, double ion of 426), 206 (10), 204 (17), 198 (10), 192 (7), 190 (17), and 188 (3).

SODIUM/AMMONIA CLEAVAGE OF *O*-ETHYL-*N*-DESMETHYLTHALRUGOSIDINE (**6**).—A 15 mg sample of compound **6** was dissolved in 3 ml of tetrahydrofuran and cleaved with Na in liq. ammonia (10 ml) in the usual manner (4). After reaction for 3 hrs and evaporation of ammonia, the residue was treated with excess methanol, concentrated to 3 ml and mixed with 100 ml Et_2O. The ether extract was extracted successively with 100 ml of 5% aq. NaOH and water, then dried (Na_2SO_4) and evaporated to dryness to give 4.5 mg of the nonphenolic material, R_f 0.75 on tlc with silica gel G and benzene-acetone-ammonium hydroxide solution (10:10:0.3). Chromatography on neutral alumina (1 g) with benzene, benzene-chloroform (1:1) and chloroform (20 ml each) as eluents, gave from the second effluent, 2.5 mg of an amorphous solid characterized as (*S*)-6,4'-dimethoxy-7-ethoxy-1-benzyl-1,2,3,4-tetrahydroisoquinoline (**7**): cd (C 3.06 x 10^{-3}M, MeOH) $[\theta]_{295}$+6,540 and $[\theta]_{256}$+16,400; nmr (90 MHz, $CDCl_3$) δ 1.43 (t, *J* 7, OCH_2CH_3), 4.03 (q, *J* 7, OCH_2CH_3), 2.6–3.3 (m, methylene protons), 3.80 and 3.84 (2s, 2 OMe), 6.58 and 6.65 (2s, H–5 and H–8), and AA'BB' 'quartet' 6.86, 7.15 (2H each, J_{AB} 9); and ms *m/e* (chemical ionization, i-butane) 328 (100%, MH^+, $C_{20}H_{25}NO_3$ requires 327), 326 (25, M–H), 223 (29), 206 (69, *a*), 192 (10), 177 (31), 153 (13) and 121 (8, *b*).

The NaOH solution was treated with NH_4Cl to produce a cloudy suspension and then extracted with 125 ml ether. When ether was washed with water, dried (Na_2SO_4) and evaporated to dryness, 4 mg of a pale yellow residue remained as the phenolic products (major spot R_f 0.5 with the same system as used for nonphenolic material). Chromatography on silica gel (1 g) with chloroform (25 ml) and 4% methanol in chloroform (50 ml) as eluents gave from the last solvent 2 mg of phenolic product **8** [(*S*)-4'-5-dihydroxy-2-methyl-6,7-dimethoxy-1-benzyl-1,2,3,4-tetrahydroisoquinoline or (*S*)-5-hydroxyarmepavine] identical (tlc, uv, ir, nmr and cd) with the cleavage product obtained from *O*-ethylthalrugosidine (**4**).

THALICARPINE (**9**).—The column separation that gave *N*-desmethylthalrugosidine (**5**) yielded from the chloroform eluates a pale yellow solid (17 mg), which crystallized from methanol to give thalicarpine **9**: mp 122–4°C [lit. (5) mp 129–30° from Et_2O]; $[\alpha]^{20}D+130°$ (*c* 0.28, MeOH) [lit. (6) $[\alpha]D+131°$ (MeOH)]. The product was identical (tlc, uv, ir, nmr and cd) with authentic thalicarpine.

THALRUGOSAMININE (**10**).—The residue (185 mg) from column fractions no. 80–96 of the ether-soluble nonphenolic tertiary alkaloids was rechromatographed on 10 g of neutral alumina (activity 1) with benzene (25 ml), benzene-chloroform (1:1, 100 ml) and chlorform (100 ml) as eluents. The benzene-chloroform (1:1) and early $CHCl_3$ effluents gave 71 mg of a homogeneous amorphous base, that was identified as thalrugosaminine (**10**) by direct comparison (tlc, uv, ir, nmr, specific rotation and cd) with an authentic sample (**7**).

NEOTHALIBRINE (**11**).—*A*. The residue (75 mg) from column fractions no. 116–120 of the ether-soluble nonphenolic tertiary alkaloids was rechromatographed on 3.8 g of neutral alumina (activity 1) with benzene-chloroform (1:1, 50 ml), chloroform (100 ml) and 1% methanol in chloroform (75 ml) as eluents. The sample was placed on the column as a small volume chloroform solution. The 1% methanol in chloroform effluent gave 30 mg of a white amorphous base, R_f 0.2 on tlc with silica gel G and benzene-acetone-ammonium hydroxide (4:4:0.1), that was identified as neothalibrine (**11**) by comparison of physical properties (tlc, ir, uv, nmr and cd) with those of an authentic sample (1).

B. The residue (165 mg) from column fractions no. 480–499 of the ether-soluble phenolic tertiary alkaloids was rechromatographed on 8 g of neutral alumina with chloroform, 1% methanol in chloroform and 2% methanol in chloroform (100 ml each) as eluents. The first two effluents contained 109 mg of an amorphous base that had physical properties identical to neothalibrine (**11**).

CHROMATOGRAPHY OF THE ETHER-SOLUBLE PHENOLIC TERTIARY ALKALOIDS.—The phenolic alkaloid fraction (3.9 g) was chromatographed on 200 g of silica gel with the following eluents: chloroform (1 liter) and mixtures of methanol in chloroform, 2% (3 liters), 4 (2), 6 (1), 10 (1), 20 (1), 30 (1) and 50 (1), then methanol (1 liter) and 5% HCl in methanol (1 liter). Effluent fractions of 15 ml were collected and residue weights were determined and analyzed by tlc. Fractions were pooled according to composition and further separated, as given below, except for thalrugosidine (**4**) and neothalibrine (**11**), which are described earlier as subsections for these alkaloids isolated from the nonphenolic fraction.

(*S*-)*O*-METHYLISOBOLDINE (THALIPORPHINE, THALICMIDINE) (**12**).—The residue (1.0 g) from column fractions no. 121–143 of the phenolic alkaloids, after attempts at rechromatography on alumina and silica gel failed, was separated on preparative tlc (0.5 mm silica gel HF) with benzene-acetone-ammonium hydroxide solution (10:10:0.4). The first product (32 mg), R_f 0.55 with the same solvent system but composition (10:10:0.3), crystallized from methanol to give *O*-methylisoboldine (**12**), mp 184–5° [lit. (8) mp 172°]; $[\alpha]^{21}D+57°$ (*c* 0.16, MeOH) [lit. (9) $[\alpha]^{17}D+44°$ (EtOH)]; cd (C 4.6 x 10^{-3}M, MeOH) $[\theta]_{330}$ 0, $[\theta]_{306}-36{,}600$, $[\theta]_{276}-25{,}800$, $[\theta]_{256}$ 0, $[\theta]_{235}+153{,}000$; uv λ max 305 nm (log ϵ 4.20), 280 (4.18) and 270 (4.08, shld); ir ($CHCl_3$) ν max 3520 cm^{-1} (phenolic OH) and positive phosphomolybdic acid test; and nmr (60 MHz, $CDCl_3$) δ 2.55 (s, NMe), 3.88 (s, OMe), 3.91 (s, 2 OMe), 6.53 (s, H-3), 6.77 (s, H-8) and 8.03 (s, H-11).

(*S*)-ISOBOLDINE (**13**).—The residue (120 mg) from column fractions no. 255–283 of the phenolic alkaloids was rechromatographed on 6 g of neutral alumina with benzene (50 ml), benzene-chloroform (1:1, 200 ml) and chloroform (125 ml) as eluents. The benzene-chloroform effluent gave 25 mg of a residue that formed from chloroform 6 mg of crystalline isoboldine (**13**); mp 122–3°C [lit. (10) mp 122–3°C]; R_f 0.37 on tlc with silica gel G and benzene-acetone-ammonium hydroxide solution (10:10:0.3); $[\alpha]^{22}D+67°$ (*c* 0.15, MeOH); cd (C 4.6 x 10^{-3}M, MeOH) $[\theta]_{330}$ 0, $[\theta]_{306}-21{,}800$, $[\theta]_{290}$ (min)$-11{,}000$, $[\theta]_{275}-17{,}400$, $[\theta]_{255}$ 0, $[\theta]_{240}+71{,}900$; uv λ max 313 nm (shld, log ϵ 4.19), 306 (4.21), 281 (4.18) and 271 (shld, 4.08), with shift to 320 (4.35) in 0.01N NaOH; ir ($CHCl_3$) ν max 3520 cm^{-1} (phenolic OH) and positive phosphomolybdic acid test; and nmr δ 2.54 (s, NMe), 3.93 (s, 2 OMe), 6.10 (brs OH, lost with D_2O), 6.54, 6.82 (2s, H-3 and H-8) and 8.02 (s, H-11).

CHROMATOGRAPHY OF THE CHLOROFORM-SOLUBLE TERTIARY ALKALOIDS.—The alkaloid fraction (2.36 g), dissolved in a minimum quantity of chloroform was placed on a column of neutral alumina (120 g, activity 1) and eluted with benzene-chloroform (1:1, 0.5 liter), chloroform (0.5) and the following mixtures of methanol in chloroform 1% (0.75 liter), 2 (0.75), 4 (1.0) and 5 (1.0). Effluent fractions of 20 ml were collected and evaporated to dryness; residue weights were determined and analyzed by tlc. The following alkaloids were isolated.

N-METHYL-6,7-DIMETHOXYISOQUINOLONE OR 1,2-DIHYDRO-6,7-DIMETHOXY-2-METHYL-1-OXOISOQUINOLINE (6,7-DIMETHOXY-2-METHYLISOCARBOSTYRIL) (**14**).—The residue (257 mg) from fractions no. 42–68 of the chloroform-soluble tertiary alkaloids was rechromatographed on 13 g of silica gel with chloroform (250 ml), 1% (300 ml) and 2% methanol in chloroform (250 ml) as eluents.

The early 1% methanol in chloroform effluent gave 15 mg of a pale yellow residue that crystallized from methanol-chloroform (1:1) as colorless needles of isoquinolone **14**: mp 112–3°C [lit. (12) mp 109–110°C]; R_f 0.53 on tlc with silica gel and benzene-acetone (1:1); uv λ max 335 nm (log ε 3.53), 321 (3.64), 310 (shld, 3.59), 293 (3.87), 281 (3.85) and 270 (3.84) with no shift in acid or base, ir ($CHCl_3$) ν max 1658 cm^{-1} (C=O); nmr (90 MHz, $CDCl_3$) δ 3.55 (s, NMe), 3.93, 3.96 (2s, 2 OMe), 6.37, 6.97 (ABq, *J* 7.5, H-3 and H-4), 6.83 (s, H-5) and 7.80 (s, H-8); and ms *m/e* 219 (100%, M^+, $C_{12}H_{13}NO_3$), 204 (26, M–Me) and 176 (14, M–Me–CO). The physical properties were compared with those of a known sample and found to be identical.

OXYBERBERINE (**15**).—The mother-liquor residue from crystallization of isoquinolone **14** was separated on preparative tlc on silica gel (0.25 mm) with benzene-chloroform to give 1.5 mg of oxyberberine (**15**), mp 199–201° from methanol. Identification was by direct comparison (tlc, mp, ir and nmr) with an authentic sample (1).

NOROXYHYDRASTININE (**16**).—The later 1% methanol in chloroform effluent from the rechromatography of fractions no. 42–68 gave a residue that crystallized from methanol to give 0.8 mg of noroxyhydrastinine (**16**), R_f 0.4 on tlc with silica gel and benzene-acetone (1:1). Identification was by direct comparison (tlc, ir and nmr) with a known sample (1).

CHROMATOGRAPHY OF QUATERNARY ALKALOIDS.—The crude quaternary alkaloid chlorides (14 g) were chromatographed on 400 g of silica gel with the following eluting solvents: chloroform (1 liter) and mixtures of methanol in chloroform, 5% (3 liter), 7.5 (1), 10 (3), 20 (2), 40 (2) and methanol (2 liters). Effluent fractions of 100 ml were collected; residue weights were determined and examined by tlc. Pooled fractions were handled as described below to afford pure compounds.

PALMATINE (**17**) and BERBERINE (**18**).—The yellow residue (110 mg) of column fractions no. 21–22 was rechromatographed on neutral alumina (5 g) with chloroform (50 ml), and mixtures of methanol in chloroform, 1% (100 ml), 2 (50) and 4 (50) as eluents. The early 1% methanol in chloroform effluent gave 10 mg of a yellow crystalline residue that formed an iodide salt, mp 140–2° (dec) that was identified as palmatine (**17**) iodide by comparison (tlc, uv, ir and nmr) with an authentic sample.

The later 1% methanol in chloroform as well as the 2% and 4% effluents afforded 65 mg of a yellow crystalline residue that gave a crystalline iodide salt, mp 265–7° (dec), which was identified as berberine (**18**) iodide by comparison (mp, uv and ir) with an authentic sample. A major amount of berberine was obtained from column fractions 23–41 (1.2 g).

COLUMBAMINE (**19**), JATRORRHIZINE (**20**) AND THALIFENDINE (**21**).—The residue (401 mg) of column fractions no. 42–47 was rechromatographed on 16 g of silica gel with chloroform (50 ml), 2.5% (200 ml), 5 (600) and 10 (500) of methanol in chloroform as eluents. The 2.5% and early 5% methanol in chloroform effluents gave berberine (**18**) chloride, while the later 5% methanol in chloroform afforded 69 mg of a yellow crystalline product and converted to the crystalline iodide that was identified as columbamine (**19**) iodide by comparison (tlc, mp, uv and ir) with an authentic sample.

The later 5% methanol in chloroform effluent yielded 15 mg of a residue that with KI in methanol crystallized as yellow needles, mp 208–10° (dec). The compound was identified as jatrorrhizine (**20**) iodide by comparison (mp, mmp, tlc, ir, uv and nmr) with an authentic sample.

The 10% methanol in chloroform effluent residue (50 mg) produced an iodide (30 mg) that was identified as thalifendine (**21**) iodide by comparison (mp, mmp, tlc, ir and nmr) with an authentic sample.

MAGNOFLORINE.—The residue (1 g) from column fractions no. 57–71 was rechromatographed on neutral alumina (50 g) with chloroform (50 ml), 2.5% (200 ml), 5 (900), 10 (200) and 20 (300) of methanol in chloroform as eluents. The 10% and 20% methanol in chloroform effluents contained the magnoflorine residue that formed a crystalline iodide with KI from methanol, mp 250–2°C (dec). Identification was made by comparison (mp, mmp, tlc, ir and nmr) with an authentic sample.

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