THE ALKALOIDS OF *THALICTRUM FOLIOLOSUM*

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ABSTRACT.—A defatted ethanolic extract of the roots of *Thalictrum foliolosum* DC. (Ranunculaceae) afforded the alkaloids thalrugosidine (**1**), thalrugosaminine (**2**), thalisopine (thaligosine) (**3**), thalirugidine (**4**), oxyberberine (berlambine) (**5**), and noroxyhydrastinine (**6**) after the usual systematic partitioning and chromatographic procedures.

Thalictrum foliolosum DC. (Ranunculaceae) is a tall perennial rigid herb indigenous to the temperate Himalayas (5000–8000 ft) and the Khasia hills (4000–6000 ft) of India. Extracts of the roots have been used by the natives as a tonic, febrifuge, a diuretic, a cathartic, and a collyrium for the improvement of eyesight as well as in the treatment of flatulence, jaundice, and visceral obstructions (1, 2).

Although the literature is abundant with numerous chemical and phytochemical studies of various *Thalictrum* species (3–5), the only references to previous work on *T. foliolosum* reported the isolation of the quaternary protoberberine alkaloids berberine, jatrorrhizine and palmatine (6) and the quaternary aporphine alkaloid magnoflorine (7, 8) from extracts of the rhizomes. The genus *Thalictrum* has been a particularly rich source of benzylisoquinoline-derived alkaloids with over 100 different alkaloids having been isolated and characterized by the end of the last decade (9). The multiplicity of types of benzylisoquinoline-derived alkaloids isolated from *Thalictrum* species include monobenzylisoquinolines (4), isoquinolones (4), protoberberines (4), aporphines (4, 10), dehydroaporphines (10), oxoaporphines (4, 10), pavines (4), isopavines (4), protopines (4), phenanthrenes (4, 10), bisbenzylisoquinolines (3, 5), aporphine-benzylisoquinoline-dimers (4, 11), dehydroaporphine-benzylisoquinoline dimers (4, 11), proaporphine-benzylisoquinoline dimers (11), and aporphine-pavine dimers (11). A number of these bases have been found to exhibit hypotensive activity in normotensive animals and antimicrobial activity against selected mycobacteria and fungi (12–14).

It was decided to undertake a phytochemical investigation of the alkaloids of *T. foliolosum* because of the paucity of reports in the literature concerning this species and to seek a source of compounds with potential biological activity.

This paper is to report the isolation and identification of thalrugosidine (**1**), thalrugosaminine (**2**), thalisopine (thaligosine) (**3**), thalirugidine (**4**), oxyberberine (berlambine) (**5**), and noroxyhydrastinine (**6**) from extracts of the roots of *T. foliolosum*. The dried, powdered roots of *T. foliolosum* were defatted with petroleum ether and extracted by percolation with ethanol. The ethanol extract was evaporated to a thick syrup, treated with aqueous citric acid, and filtered. The filtrate was partitioned with chloroform (Fraction A), alkalinized with ammonium hydroxide and repeatedly extracted with ether (Fraction B). The combined ether

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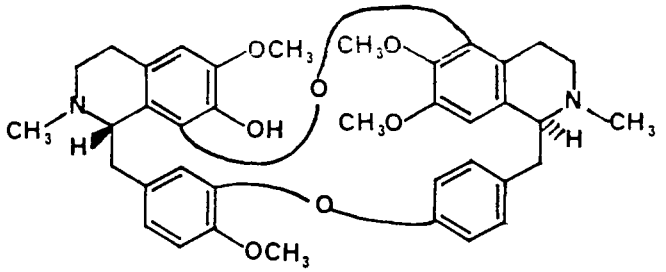
extracts (Fraction B) were chromatographed over neutral alumina in chloroform to afford the bisbenzylisoquinoline alkaloid thalrugosidine (**1**). This alkaloid was first isolated from extracts of *T. rugosum* in 1972 (12) and, to our knowledge, this constitutes only the second reported isolation of this base from nature.[2] Thalrugo-



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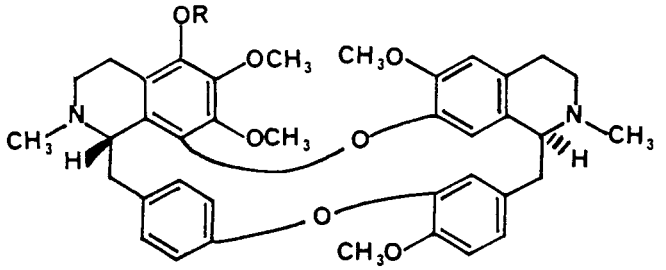
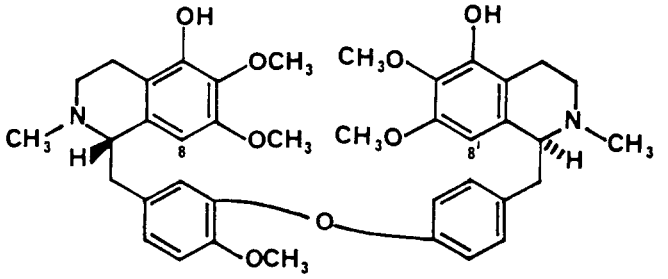


2 R = CH_3
3 R = H



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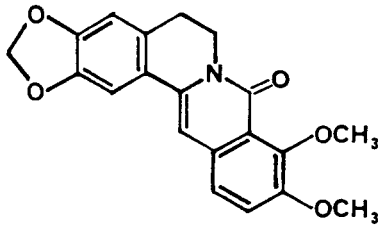

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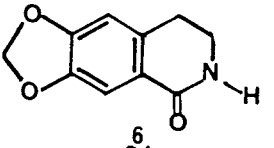

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[2]Note added in press—Thalrugosidine has now been isolated from *Thalictrum alpinum* as well (W.-N Wu, J. L. Beal and R. W. Doskotch, *J. Nat. Prod.*, **43**, 372 (1980).

sidine exhibited weak antimicrobial activity over a narrow spectrum and was not considered a serious candidate for possible clinical use (12).

Continued elution with chloroform afforded the bisbenzylisoquinoline alkaloid thalrugosaminine (**2**) whose presence was first suggested by thin-layer chromatographic detection in extracts of *T. isopyroides* (15), where the base was tenatively named O-methylthalisopine. However, thalrugosaminine was not isolated until 1976 from extracts of *T. rugosum* (16) and shortly thereafter from extracts of *T. revolutum* (14) and *T. minus* race B (17). Thalrugosaminine exhibited both hypotensive activity in rabbits (14) and antimicrobial activity against the acid-fast rod *Mycobacterium smegmatis* (14, 16). The occurrence of thalrugosaminine appears to be restricted to the genus *Thalictrum* to this date.

Elution of the column with 1% methanol in chloroform yielded the bisbenzylisoquinoline alkaloid thalisopine (**3**), which was first isolated from extracts of *T. isopyroides* in 1968 (15). Thalisopine was recently isolated from extracts of *T. rugosum* where it was mistakenly characterized as a new alkaloid called thaligosine (9). Thalisopine has recently been reported to exert significant cardiac antiarrhythmic effects in various animals (18). To our knowledge, this is only the third species of *Thalictrum* known to contain thalisopine, which has not been found outside of the genus to date. Elution of the column with 2% methanol in chloroform next afforded thalirugidine (**4**) which was also only recently isolated from extracts of *T. rugosum* (9). To our knowledge, the isolation of this bisbenzylisoquinoline alkaloid from *T. foliolosum* represents only the second reported occurrence in nature.

Finally, chromatography of Fraction A over neutral alumina and elution with benzene-chloroform (1:1) mixture afforded oxyberberine (berlambine) (**5**). This protoberberine alkaloid has been previously isolated from extracts of *T. minus* race B (19), *T. lucidum* (13), and *T. podocarpum* (20), as well as species of the genera *Berberis* (Fam. Berberidaceae) and *Mahonia* (Fam. Menispermaceae) (23). Later fractions of benzene-chloroform (1:1) eluate gave noroxyhydrastinine (**6**). This isoquinolone alkaloid was first isolated from extracts of *T. minus* var. *adiantifolium* in 1969 along with the closely related structural analog thalifoline (21). Isoquinolone alkaloids have been reported in other genera (22) and their occurrence in plants containing isoquinoline derived alkaloids is not unusual.

In summary, the isolation of thalrugosidine (**1**), thalrugosaminine (**2**), thalisopine (**3**), thalirugidine (**4**), oxyberberine (**5**), and noroxyhydrastinine (**6**) from *T. foliolosum* constitutes the first report of any nonquaternary alkaloid from this species and further supports the overall chemotaxonomic character hitherto established for the genus *Thalictrum*.

EXPERIMENTAL[3]

[3]Melting points were taken on a Thomas-Hoover apparatus or a Fisher-Johns apparatus and are uncorrected. The uv spectra were obtained on a Perkin-Elmer model 202 recording spectrophotometer and the ir spectra were determined on a Perkin-Elmer model 257 recording spectrophotometer in KBr pellets or $CHCl_3$ solutions. The nmr spectra were recorded in deuterated chloroform on a Hitachi Perkin-Elmer model R-24 high resolution spectrometer with tetramethylsilane as internal standard and chemical shifts recorded in δ(ppm) units. The mass spectra were taken with a LKB-9000 mass spectrometer. The optical rotations were measured on a Perkin-Elmer model 241 polarimeter. Cd curves were taken in methanol solution on a Jasco ORD-UV-5 instrument. Silica Gel (60–120 mesh) (BDH) and neutral alumina (Sarabhai M) were used for column chromatography and Silica Gel G (Centron Res. Lab or Fisher Scientific) was used for thin-layer chromatography. The solvent system benzene-acetone-ammonium hydroxide (8:10:0.1) was used unless otherwise noted. Anhydrous sodium sulfate was routinely used for drying organic solvents and all solvents were evaporated under reduced pressure at 40°.

PLANT MATERIAL.—The plant material used in this study was collected in the Western Himalayas (30.5° lat. and 78° long.) in June, 1978 and identified by Mr. O. P. Misra of Central National Herbarium at Shibpur, West Bengal. A herbarium specimen is on deposit in the Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University.

EXTRACTION AND FRACTIONATION.—Powdered dried roots (4 kg) of *Thalictrum foliolosum* DC. were defatted with petroleum ether (10 liters), dried and extracted by percolation with ethanol (95%) (2 x 12 liters). The extract residue (100 g) was stirred with aqueous citric acid (7%) (1 liter), filtered and the filtrate extracted with chloroform (1 liter) in a liquid-liquid extractor to afford a dark residue (Fraction A) (1.5 g). The citric acid solution was subsequently basified with ammonium hydroxide to pH 9 and extracted with ether (3 x 1 liter) to afford a brown residue (Fraction B) (3 g).

CHROMATOGRAPHY OF FRACTION B.—Fraction B (3 g) was dissolved in chloroform, adsorbed on alumina (30 g) and the adsorbed material was placed on a column of alumina (80 g) in benzene. The column was first eluted with benzene (0.4 liter), then with chloroform (1 liter) and finally with varying proportions of methanol in chloroform. Fifty ml fractions were collected.

ISOLATION OF THALRUGOSIDINE (**1**).—Elution of the column with chloroform (Frs. 9–11) afforded a white residue, which upon treatment with ethanol gave thalrugosidine (**1**) (190 mg) as white needles, mp 176–178; R_f 0.68; $[\alpha]^{26}D$ −79° (c 0.24, MeOH); uv, λ max (MeOH) 275 nm (sh) (log ε 3.85) and 283 (3.87); cd, $[\theta]_{285}$ −29,400, $[\theta]_{266}$ +6150, $[\theta]_{242}$ +73,300 and $[\theta]_{226}$ −45,400; ir, ν max (KBr) 3430 cm^{-1}, 1500 and 1120; nmr, δ2.21 and 2.61 (2s, $2NCH_3$), 3.50, 3.76, 3.86 and 3.88 (4s, $4OCH_3$), 6.33–7.73 (m, 9ArH); ms, *m/e* 638 (M^+, 64%, $C_{38}H_{42}N_2O_7$), 623 (11),608(6), 417(2), 416(2), 412(15), 411(48), and 206(100). This alkaloid was identical to an authentic sample of thalrugosidine (**1**) by direct comparison (uv, ir, nmr, ms, sp. rotn, cd, mp, mmp).

ISOLATION OF THALRUGOSAMININE (**2**).—Continued elution of the column with chloroform (Frs. 17–27) yielded a white amorphous residue, which upon treatment with chloroform-hexane gave thalrugosaminine (**2**) (350 mg) (amorphous), mp 92–97°; R_f 0.10; $[\alpha]^{26}D$ −103° (c 0.60, MeOH); uv λ max (MeOH) 222 nm (log ε 4.79) and 284(3.96); cd, $[\theta]_{288}$ +3100, $[\theta]_{272}$ −10,900, $[\theta]_{240}$ −57,400 and $[\theta]_{225}$ +79,900; ir ν max (KBr) 1503 cm^{-1}; nmr, δ2.51 and 2.56 (2s, $2NCH_3$), 3.10, 3.42, 3.80, 3.85, and 3.96 (5s, $5OCH_3$), 6.40–7.38 (m, 9ArH); ms, *m/e* 652 (M^+, 41%, $C_{39}H_{44}N_2O_7$) 637(10), 545(6), 461(2), 426(10), 425(35), 411(23), 213(100), 175(4), and 174(15). The identity was confirmed by direct comparison (uv, ir, nmr, ms, sp. rotn, cd, mp) with an authentic reference sample of thalrugosaminine (**2**).

ISOLATION OF THALISOPINE (**3**).—Elution of the column with 1% methanol in chloroform (0.5 liter) afforded a white residue (Frs. 30–32) which upon treatment with ether gave thalisopine (**3**) (300 mg) as white microneedles mp 140–145°; R_f 0.08; $[\alpha]^{27}D$ −96° (c, 0.28, MeOH); uv, λ max (MeOH) 235 nm (log ε 4.22) and 285 (3.76); ir, ν max (KBr) 3420 cm^{-1} and 1507; nmr, δ2.53 and 2.58 (2s, $2NCH_3$), 3.13, 3.44, 3.83 and 3.99 (4s, $4OCH_3$), 6.47–7.35 (m, 9ArH); ms, *m/e* 638 (M^+, 68%, $C_{38}H_{42}N_2O_7$), 623(6), 531(5), 447(1), 412(23), 411(77), 206(100), 175(13), and 174(37). A direct comparison (uv, nmr, ms, sp. rotn, mp) with an authentic reference sample served to confirm the identity of the alkaloid as thalisopine (thaligosine) (**3**).

ISOLATION OF THALIRUGIDINE (**4**).—Elution of the column with 2% methanol in chloroform (0.5 liter) yielded a white amorphous residue from Frs. 40–42 which was rechromatographed over a short bed of Silica Gel (5 g) and eluted first with chloroform and then with increasing percentage of methanol in chloroform. Elution with 10% methanol in chloroform afforded thalirugidine (**4**) (80 mg) as an amorphous base; R_f 0.14; $[\alpha]^{24}D$ +114° (c, 0.07, MeOH); uv, λ max (MeOH) 222 nm (log ε 4.58) and 282(4.05); ir, ν max ($CHCl_3$) 3520 cm^{-1}, 3020, 1505, 1205, and 1170; nmr, δ2.48 and 2.51 (2s, $2NCH_3$), 3.57, 3.59, 3.82 (3s, $3OCH_3$) and 3.78 (s, $2OCH_3$), 5.73 and 5.78 (2s, H-8 and H-8'), 6.70–7.30 (m, 7ArH); ms, *m/e* 670 (M^+, 1%, $C_{39}H_{46}N_2O_8$), 222(100), 221(7), 220(10), 207(8), 206(13), 192(19) and 178(4). The identity was confirmed by direct comparison (uv, ir, nmr, ms, sp. rotn.) with an authentic reference sample of thalirugidine (**4**).

CHROMATOGRAPHY OF FRACTION A.—Fraction A (1.5 g) was dissolved in benzene and chromatographed over a dry packed column of alumina (40 g) and eluted first with benzene (0.5 liter) and then with chloroform-benzene (1:1) mixture (1.5 liters). Fractions of 25 ml were collected.

ISOLATION OF OXYBERBERINE (BERLAMBINE) (**5**).—Elution of the column with benzene-chloroform (1:1) afforded a yellow residue from Frs. 24–40 which upon treatment with methanol yielded oxyberberine (berlambine) (**5**) as yellow needles (100 mg), mp 197–198°; R_f 0.62; uv, λ max (MeOH) 225 nm (log ε 4.69), 344(4.45), 370(sh) (4.26) and 389(sh) (4.09); ir, ν max ($CHCl_3$) 3000 cm^{-1}, 1650, 1603, 1485, and 1280; nmr, δ2.87 and 4.31 (2t, 4H, CH_2CH_2) (J=6 Hz), 3.96 and 4.02 (2s, $2OCH_3$), 6.01 (s, CH_2O_2), 6.72 and 7.28 (2s, 4ArH), and 7.20(s, ArH); ms, *m/e* 351 (M^+, 100%, $C_{20}H_{17}NO_5$), 336(89), 322(35) and 308(31). The identity was established by direct comparison (uv, ir, nmr, ms, mp) with an authentic reference sample of oxyberberine (**5**).

ISOLATION OF NOROXYHYDRASTININE (**6**).—Continued elution of the column with benzene-chloroform (1:1) mixture yielded a pale yellow residue from Frs. 56–68 which upon treatment with methanol gave noroxyhydrastinine (**6**) (30 mg) as white prisms, mp 183–185°; R_f 0.40; uv, λ max (MeOH) 224 nm (log ε 4.48) 263(3.88) and 306(3.99); ir, ν max (KBr) 3190 cm^{-1}, 3050 and 1660; nmr, δ2.85 and 3.52 (2t, 4H, CH_2CH_2) (J=7 Hz), 5.92 (s, CH_2O_2), 6.60 and 7.47 (2s, 2ArH); ms, *m/e* 191 (M^+, 88%, $C_{10}H_9NO_3$), 162(80), 134(100), 104(20), and 76(43). The identity was established by direct comparison (uv, ir, nmr, ms, mp, mmp) with an authentic reference sample (21).

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