THE ALKALOIDS OF *THALICTRUM FOLIOLOSUM*[1]

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ABSTRACT.—N,O,O-Trimethylsparsiflorine (**1**), a new aporphine alkaloid; known tertiary alkaloids thalicarpine (**3**), thalidasine (**4**), thalrugosidine (**5**) and reticuline (**6**); and the quaternary alkaloids magnoflorine, berberine and palmatine were isolated from the rhizome of *Thalictrum foliolosum* DC.

Thalictrum foliolosum DC. (Ranunculaceae), a tall perennial herb, is one of the seven species of genus *Thalictrum* found in India (1). Extracts of the roots of the plant have been used by the natives in many ailments (1,2). The quaternary alkaloids, magnoflorine (3,4), jatrorrhizine (5), berberine (5), and palmatine (5) were isolated earlier from the plant. *Thalictrum* species have been known to produce a variety of alkaloids of chemical and biological interest (6–11). The alcoholic extract of the leaves, stems, and roots of *T. foliolosum* exhibited spasmolytic activity in a broad biological screen (12). This activity was concentrated, in the follow up studies, in the alkaloidal fraction of the extract. This prompted us to reinvestigate the alkaloidal constituents of *T. foliolosum*. The investigation resulted in the isolation of a new aporphine alkaloid, N,O,O-trimethylsparsiflorine (**1**), two known bisbenzylisoquinoline alkaloids, thalidasine (**4**) and thalrugosidine (**5**), one 1-benzylisoquinoline aporphine alkaloid, thalicarpine (**3**), one 1-benzyltetrahydroisoquinoline alkaloid, reticuline (**6**), two protoberberine alkaloids, berberine and palmatine and a quaternary aporphine alkaloid, magnoflorine. The tertiary alkaloids (**3**), (**4**), (**5**) and (**6**) were isolated for the first time from this species, whereas the quaternary alkaloids had been previously isolated from the rhizome of the plant.

N,O,O-Trimethylsparsiflorine (**1**) was assigned the molecular formula $C_{20}H_{23}O_3N$ on the basis of high resolution mass spectrometry. The ir spectrum of the base (ν max. 1600, 1560, 1490, 1360 and 1210 cm^{-1})in conjunction with its uv spectrum (λ max. 216, 274 and 298 nm, no change in alkali) suggested the presence of an aporphine system in the molecule.

The nmr spectrum of the base integrated for 23 protons. A signal at δ 2.30 (s, 3H) was assigned to one N-methyl group. The signals for three aromatic methoxyl groups were at δ 3.49 (s, 3H), 3.64 (s, 3H) and 4.00 (s, 3H), respectively. In the aromatic region there were four protons. A singlet at δ 6.32 was assigned to C–3 proton. A double doublet for an *ortho* and *meta* coupled proton centered at δ 6.54 (dd, $J_1=8.0$ and $J_2=2.0$ Hz) was due to the proton at C–9. A doublet for one proton at δ 6.86 (d, $J=8.0$ Hz) was assigned to an *ortho* coupled proton at C–8. The characteristic low field signal for the C–11 proton appeared as a doublet at δ 7.74 (d, $J=2.0$ Hz). In the mass spectrum of the base, the molecular ion peak appeared at *m/e* 325 (M^+). Other significant peaks in the mass spectrum were at *m/e* 324 (M^+–1), 310 (M^+–15), 294 (M^+–31) and 282 (M^+–43). The mass fragmentation pattern of the base was that of aporphine alkaloid type (13).

The base remained unchanged on treatment with Ac_2O/pyridine or diazomethane. When refluxed with CH_3I, it formed a methiodide. The structure (**1**) for the base emerged from the data given above. The physical and chemical constants of the base methiodide were very close to that of N,O,O-trimethylsparsiflorine methiodide (14). Direct comparison (mp, mmp, ir, uv and nmr) of the base methiodide with an authentic sample of N,O,O-trimethylsparsiflorine methiodide prepared from sparsiflorine (**2**), an aporphine alkaloid isolated from *Croton sparsiflorus* morong (15), established the identity.

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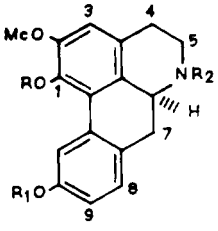
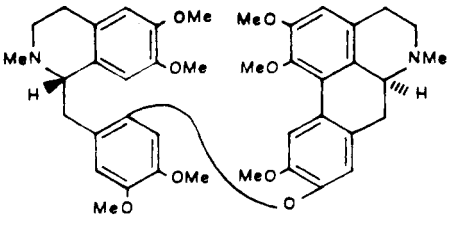
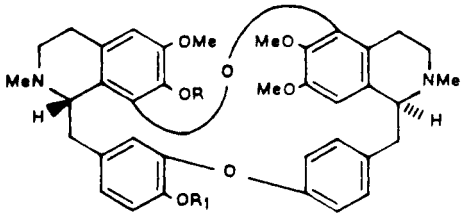
The second, third, and fourth alkaloids obtained from the chloroform soluble alkaloid fraction were thalicarpine (**3**), thalidasine (**4**) and thalrugosidine (**5**). Thalicarpine was characterized by spectroscopic data and direct comparison (mp, mmp, co-tlc, nmr, ms, ir and uv) of the base with an authentic sample of thalicarpine. This alkaloid was first isolated from extracts of *T. dasycarpum* (16) in 1963. Since then, it has been isolated from many other *Thalictrum* species (17–20). Thalicarpine produced transient hypotensive effects in cats (16) and exhibited significant inhibitory activity against Walker carcinosarcoma 256 in rats (18).



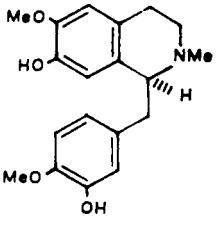
1. R = R_1 = R_2 = Me
2. R = R_1 = R_2 = H



(3)



4. R = R_1 = Me
5. R = H; R_1 = Me



(6)

The nmr spectrum showed the presence of two N-methyl groups both in thalrugosidine (**5**) and thalidasine (**4**). There were five methoxy groups in thalidasine (**4**) and four methoxy groups in thalrugosidine (**5**). Methylation of thalrugosidine with ethereal diazomethane gave thalidasine (**4**), thus revealing that thalrugosidine was a monophenolic base. The base peak at *m/e* 206 in the mass spectrum of thalrugosidine suggested that the phenolic group was in the isoquinoline moiety rather than in the benzylic portion. The physical constants and spectroscopic data of the phenolic base were almost identical with reported data for thalrugosidine (**5**) (21) and that for the nonphenolic base with thalidasine (**4**). Thalrugosidine was first isolated from extracts of *T. rugosum* in 1972 (21). It has recently been isolated from *T. alpinum* as well (22). This alkaloid has been reported to exhibit antimicrobial activity (21). Thalidasine (**4**) was first isolated from extracts of *T. dasycarpum* in 1967 (6). Since then, it was also been isolated from many other *Thalictrum* species (23–26). This alkaloid showed significant inhibitory activity against Walker intramuscular carcinosarcoma 256 in rats at 200 mg/kg (6). It also exhibited hypotensive and antimicrobial activities.

Reticuline (**6**), palmatine, berberine and magnoflorine were isolated from more polar fractions and were identified by direct comparison (mp, mmp, ir and uv) with authentic samples. 1-Benzyltetrahydroisoquinoline base, reticuline, is the key biological precursor of a large number of alkaloids (27). The quaternary aporphine alkaloid, magnoflorine, showed marked hypotensive activity (28). Thalidasine (**4**), thalicarpine (**3**), thalrugosidine (**5**) and N,O,O-trimethylsparsi-

florine (1) were tested for antibacterial and antifugal activities against *Streptococcus faecalis, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aurens, Candida albicans, Cryptococcus neoformans, Sporotrichum schenckii, Trichophyton mentagrophytes* and *Aspergillus fumigatus* and were all found to be inactive (29).

EXPERIMENTAL[2]

PLANT MATERIAL.—The plant material used in this study was collected from Hazaribagh, Bihar, in February, 1979 and was identified by Dr. K. K. Singh of Central Drug Research Institute, Lucknow. A herbarium specimen is on deposit in the herbarium of the botany section of the institute.

EXTRACTION.—Air dried powdered plant material (2.5 kg) was exhaustively extracted with EtOH (5x31) at room temperature. The solvent from the percolate was removed under reduced pressure below 40° to give a viscous mass which was extracted with 5% hydrochloric acid. The aqueous acidic solution was defatted with petroleum ether (5x200 ml) and then basified with aqueous Na_2CO_3 to pH 9. The liberated bases were extracted with $CHCl_3$ (6x150 ml), washed with water and dried and the solvent removed to give the alkaloidal mixture A (4 g). The remaining aqueous alkaline solution was extracted with chloroform-methanol (80:20) to give the polar alkaloidal mixture B (8 g).

ISOLATION OF BASES.—The chloroform soluble alkaloid mixture A (4 g) was chromatographed on neutral alumina (200 g). The column was successively eluted with benzene, benzene-chloroform (1:1), chloroform and chloroform-methanol with an increasing proportion of methanol. Each fraction of 100 ml was collected, and the elution was followed by tlc. A total of 100 fractions were collected.

N,O,O-TRIMETHYLSPARSIFLORINE (1).—Fractions 10 to 25 (elution with benzene-chloroform, 3:2) gave a crude base which was subjected to preparative tlc (plate: silica gel-G; *solvent*: chloroform-methanol, 96:4). The major band on the plate was cut and extracted with chloroform-methanol (4:1). Removal of the solvent from the extract furnished the nonphenolic base (1) (120 mg), mp 124–26°; λ max (MeOH) 216 (log ε 4.56), 27% (4.01) and 298 (4.13) nm, no change in NaOH; ν max. 2900, 2800, 1600, 1560, 1490, 1440, 1420, 1360, 1320, 1310, 1315 and 1210 cm^{-1}; nmr (90 Hz, $CDCl_3$): δ 2.30 (s, NMe), 3.49 (s, OMe), 3.64 (s, OMe), 4.00 (s, OMe), 6.32 (s, C_5-H), 6.54 (dd, $J_1=8$ and $J_2=2$ Hz, C_9-H), 6.86 (d, $J=8$ Hz, C_8-H) and 7.74 (d, $J=2$ Hz, C_{11}-H); ms: *m/e* 325 (M^+), 324 (M^+–1), 310 (M^+–15), 294 (M^+–31) and 283 (M^+–43) (Found: C, 86.45; H, 7.22; N, 4.28%; 325.2342. $C_{20}H_{23}NO_3$ requires C, 86.15; H, 7.07, N, 4.30%. $C_{20}H_{23}NO_3$ requires 325.2366).

A mixture of base (40 mg), methanol (3 ml), and methyl iodide (0.5 ml) was refluxed for 1 h. When the solvent from the resulting mixture was removed and the product was crystallized from methanol, base methiodide (24 mg) mp 210–12° (decomp.) was obtained.

N,O,O-TRIMETHYLSPARSIFLORINE METHIODIDE.—A mixture of sparsiflorine (2) (15) (100 mg), methanol (3 ml), chloroform (1 ml) and MeI (1 ml) was refluxed for 1 h. When resulting mixture was worked up, N,O,O-trimethylsparsiflorine methiodide (14) (78 mg) mp 211–213° (decomp.) was obtained.

The base methiodide was found identical (mp, mmp, ir and uv) to N,O,O-trimethylsparsiflorine methiodide.

THALICARPINE (3).—Fraction 26 to 40 (elution with benzene-chloroform, 1:1) gave a crude base which was subjected to preparative tlc (plate: silica gel-G; *solvent*: chloroform-methanol, 94:6). The major band, when cut and extracted with chloroform-methanol (80:20), gave the nonphenolic base (3) (80 mg), mp 158–60°; $[\alpha]_D$+130° (c, 1.00 in MeOH). The base was found identical (mp, mmp, co-tlc, uv, ir, nmr and ms) to an authentic sample of thalicarpine (3) (16) mp 160–61°; $[\alpha]^{25}_D$+133° (c, 0.83 in MeOH).

THALIDASINE (4) AND THALRUGOSIDINE (5).—The fractions 41 to 70 (elution with chloroform-methanol, 95:5) were mixed, and the mixture of the bases was subjected to preparative tlc (plate: silica gel-G; *solvent*: chloroform-methanol, 9:1). The two major bands so resolved were cut and extracted with chloroform-methanol (4:1). Extraction of the less polar band furnished the nonphenolic base (4) (127 mg), mp 143–44°; $[\alpha]^{25}_D$−68° (c, 1.2 in $CHCl_3$); λ max.

[2]Melting points were taken in a sulfuric acid bath and are uncorrected. The uv spectra in MeOH were obtained on a Perkin-Elmer model 202 recording spectrophotometer and the ir spectra were determined on a Perkin-Elmer model 337 or 577 grating recording spectrophotometer in KBr pellets. The optical rotations were measured on a JASCO DIP 180 polarimeter. The nmr spectra were recorded in deuterated chloroform, unless otherwise stated, on a Varian A-60D and R-32 spectrometer with tetramethylsilane as internal standard and chemical shifts recorded in δ (ppm) units. The mass spectra were taken with JMS D-300 mass spectrometer fitted with a direct inlet system. Silica gel (60–120 mesh) (HDH) and neutral alumina (Sarabhai M) were used for column chromatography and silica gel GF-254 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 below 40°.

273 and 281 nm; nmr (90 Hz, $CDCl_3$) δ 2.27 (s, NMe), 2.68 (s, NMe), 3.31 (s, OMe), 3.48 (s, OMe), 3.82 (s, OMe), 3.96 (s, OMe) and 3.98 (s, OMe). Thalidasine (**4**) (16) had mp 144–45°; $[\alpha]^{25}D-70°$ (c, 1.0 in $CHCl_3$).

The more polar band, on extraction and subsequent removal of the solvent, furnished the phenolic base (**5**) (137 mg), mp 170–71°; $[\alpha]^{30}D-180°$ (c, 0.88 in MeOH). Thalrugosidine (**5**) (21) had mp 172–70°; $[\alpha]^{25}D-185°$ (MeOH).

O-Methylthalrugosidine (**4**).—Thalrugosidine (**5**) (80 mg) in methanol (1 ml) was treated with an excess of ethereal CH_2N_2. The resulting mixture, when worked up after 2 days, gave O-methylthalrugosidine (**4**) (45 mg) mp 143–44°; $[\alpha]^{27}D-68°$ (c, 0.72 in MeOH).

O-Methylthalrugosidine was found identical (mp, mmp, co-tlc, nmr, uv, ir and mass) to thalidasine (**4**) (10).

Reticuline (**6**).—The fractions 71 to 85 (elution with chloroform-methanol, 9:1) were mixed and the solvent removed. The residue so obtained was chromatographed on a column of silica gel. Elution with chloroform-methanol (9:1) gave the phenolic base (**6**) as amorphous powder, $[\alpha]^{28}D+128°$ (c, 2.0 in MeOH) base hydrochloride $[\alpha]^{28}D+72°$ (c, 0.5 H_2O) identical (co-tlc, nmr, ms, uv and ir) to reticuline (**6**) (12), $[\alpha]^{25}D+132°$ (MeOH). Base hydrochloride had $[\alpha]^{25}D+73°$ in H_2O.

The fractions 86 to 100 (elution with chloroform-methanol, 3:1) were mixed and the solvent removed. The residue, when mixed with the polar alkaloidal mixture (mixture B) and subjected to preparative tlc (plate: silica gel-G; solvent: chloroform-methanol, 85:15), gave magnoflorine, berberine and palmatine, chlorides identified by direct comparison (mp, mmp, co-tlc, ir, uv and nmr) with authentic samples.

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