CONSTITUENTS OF *PACHYGONE OVATA* AND PHARMACOLOGICAL ACTION OF ITS MAJOR LEAF ALKALOID

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ABSTRACT.—*Pachygone ovata* (Menispermaceae) grows in the sandy sea shores of southern India, and its fruits are used as fish poison. Systematic fractionation of ethanolic extracts of the leaves, stems, and roots of the plant afforded five alkaloids: *N*-methylcrotsparine (**1**), reticuline (**2**), liriodenine (**5**), trilobine (**6**), and coclaurine (**7**). In addition there was isolated a new amine oxide, reticuline *N*-oxide (**3**), and a cyclitol, characterized as (+)-quercitol (**4**). The pharmacological actions of reticuline were investigated. It showed central stimulant, hyperthermic, and spinal convulsant actions, the profile closely resembling that reported with thebaine.

Pachygone, a member of the family Menispermaceae, is a small genus of scandent shrubs which are distributed mainly in the Indo-Malaysian region. *Pachygone ovata* is the only species available in India, and it grows in the sandy seashores of the Coromandel coast. The dried fruits of this plant are used by local people for destroying vermin and stupefying fish (1, 2). No phytochemical work on any of the *Pachygone* species has yet been reported. The chemical constituents isolated from different parts of the plant and identified are *N*-methylcrotsparine (**1**), reticuline (**2**), reticuline *N*-oxide (**3**), (+)-quercitol (**4**), liriodenine (**5**), trilobine (**6**) and coclaurine (**7**). Of these compounds, reticuline *N*-oxide is a new amine oxide which has not been reported earlier either as a natural product or as a synthetic compound.

The dried and powdered leaves of *P. ovata* were extracted with 95% ethanol by percolation. The ethanol extract was concentrated under reduced pressure, and the syrupy concentrate was treated with 5% aqueous citric acid, and the acid solution was filtered from non-basic materials. The filtrate was washed with chloroform, alkalinized with ammonium hydroxide and extracted, first with benzene and then with chloroform. The benzene extract was concentrated and chromatographed over a bed of alumina. Elution of the column with benzene yielded an alkaloid which was characterized as *N*-methylcrotsparine (**1**) from detailed spectral analysis of the alkaloid and its dideuterio derivative (**1a**), by its conversion to the known aporphine alkaloid nuciferine (**8**) and, finally, by its direct comparison (mp, co-tlc) with an authentic sample. *N*-Methylcortsparine-d_2 (**1a**), obtained by treatment of alkaloid **1** with alkaline D_2O, showed in its pmr spectrum the presence of the lone aromatic proton of the molecule [which permitted differentiation of the alkaloid from homolinearisine (**1b**) (3)] but the absence of olefinic protons at $\alpha\alpha'$-positions of the cyclohexadienone system. The reaction is considered to be characteristic of a 4,4-disubstituted cyclohexadienone system and presumably involves addition of D_2O to, and elimination of HOD from, the molecule. This is the second report of the isolation of *N*-methylcrotsparine and the first from a

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Menispermaceous plant. The alkaloid was earlier isolated from *Croton sparsiflorus* (4), a member of the family Euphorbiaceae.

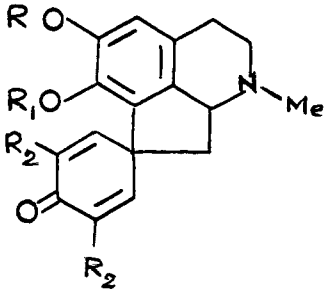
The chloroform extract of tertiary alkaloids of *P. ovata* leaves was concentrated and chromatographed over silica gel. Elution of the column with benzene-chloroform (1:1) furnished an oil which formed a cyrstalline perchlorate, mp 202°. The alkaloid was characterized as reticuline (**2**) by spectral (uv, ir, pmr) analysis. This was confirmed by methylation with diazomethane to (+)-laudanosine (**2a**) and also by direct comparison with an authentic sample of reticuline perchlorate (5). This appears to be the first report of the occurrence of reticuline in a plant of the family Menispermaceae.

The water-soluble alkaloid reticuline *N*-oxide (**3**) was isolated through Mayer's complex and, on treatment with anion exchange resin (IRA 400, Cl^-), gave an amorphous hydrochloride. The amorphous free base, $C_{19}H_{23}NO_5$, generated from its salt, showed physical and spectral properties comparable to reticuline and, on reduction with sulfurous acid (6), yielded an oily base which gave a crystalline perchlorate identical with reticuline perchlorate (mp, mmp, ir). Oxidation of reticuline (**2**) with *m*-chloroperbenzoic acid (7) furnished an amorphous base identical with reticuline-*N*-oxide (ir, co-tlc). There is no previous report of the occurrence of this compound in nature.

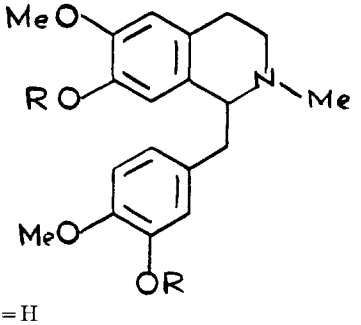
The ethanolic extract of the stems of this plant furnished on concentration a crystalline solid which was identified as (+)-quercitol (**4**) from a comparison of physical and spectral properties of the compound and its benzoyl derivative with those of (+)-quercitol and its pentabenzoate (**4a**) (8). The alkaloid fraction, isolated from this extract following the usual procedure, was chromatographed over alumina, and petroleum ether-ethyl acetate (1:1) eluate afforded a yellow crystalline alkaloid which was identified as liriodenine (**5**) by direct comparison (uv, ir, pmr, ms, mp, mmp, co-tlc) with an authentic sample (9).

The total alkaloidal fraction of the ethanolic extract of the roots was fractionated into phenolic and non-phenolic bases by the usual procedure. The non-phenolic fraction on chromatography over a bed of alumina and elution with a petroleum ether-benzene (3:1) mixture yielded a crystalline alkaloid which was characterized as trilobine (**6**) from spectral (uv, pmr, ms) analysis and also by direct comparison of N-methylderivative with isotrilobine (**6α**). The phenolic fraction on chromatography over silica gel and elution with chloroform-methanol (9:1) afforded co-claurine (**7**) identical by direct comparison (mp, mmp, co-tlc) with an authentic sample.

It is interesting to note that the alkaloids isolated from different parts of *P. ovata* are at different stages in the biosynthetic pathway of isoquinoline alkaloids.



1 : R=Me, $R_1=R_2=H$
1a : R=Me, $R_1=H$, $R_2=D$
1b : $R=R_2=H$, $R_1=Me$



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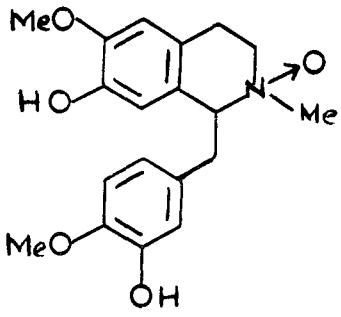
2a : R=Me



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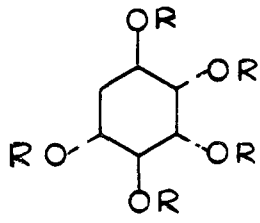
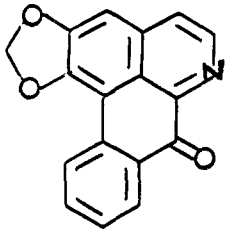


4 : R=H
4a : R=C_6H_5CO-



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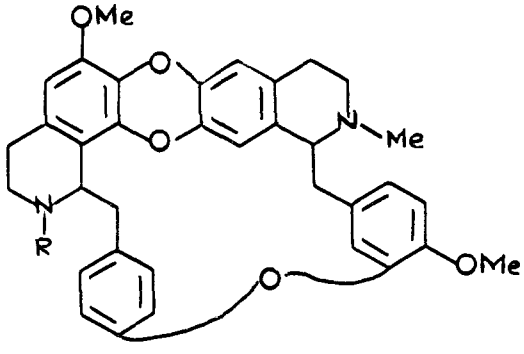
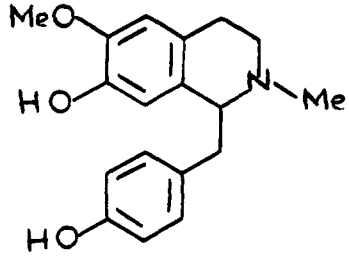


6 : R=H
6a : R=Me



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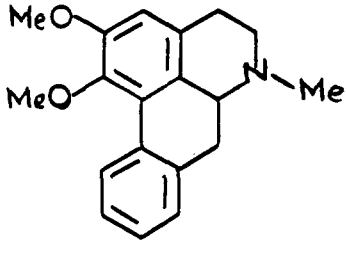

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The major leaf alkaloid reticuline was subjected to pharmacological investigation. In doses ranging between 10–20 mg/kg, i.p., reticuline produced signs of central nervous system stimulation characterized by piloerection, irritability, alertness, compulsive biting of the cage wires, and tremors. All mice exhibited Straube's tail phenomenon. When the dose was increased to 30–40 mg/kg, there were tetanic convulsions characterized by hind limb extension. The convulsions were reflex in nature. Pretreatment of the mice with mephenesin (10 mg/kg, i.p.) totally prevented reticuline-induced convulsions. In the interictal period the mice appeared to be sedated having decreased motility and ptosis.

Reticuline (5 mg/kg, i.p.) significantly ($P<0.001$) potentiated hexobarbitone induced hypnosis, the mean sleeping time (in min±S.E.M.)being 56.2±6.3 and 31.3±4.9 in the reticuline-pretreated and control hexobarbitone groups, respectively. However, this potentiation was not apparent with a higher dose of reticuline (20 mg/kg, i.p.), where the sleeping time was 23.7±3.9 ($P>0.05$).

Reticuline significantly ($P<0.001$) enhanced locomotor activity in a dose of 10 mg/kg, i.p. The 30 min cumulative actophotometer recording in control and reticuline-treated mice (5 in each group) was 286±56 and 486±92, respectively.

Reticuline produced a dose-related hyperthermic response in rats. The rise of rectal temperature with 5, 10, and 20 mg/kg, i.p., over pre-drug control level (35.5±1.2° mean±S.E.M.) was +0.36±0.12, +0.69±0.19 and +1.1±0.16, respectively. All values were statistically significant ($P<0.05$) as compared to controls.

Reticuline produced a mild to moderate degree of antinociceptive activity in doses of 10 and 20 mg/kg, i.p. The increase in the latent period of tail flick response (mean ±S.E.M.) was 7.8±1.1 and 11.9±1.6 sec, respectively. Both values were statistically significant ($P<0.001$).

Reticuline significantly ($P<0.05$) potentiated the lethal effect of amphetamine (10 mg/kg, i.p.) in aggregated rats. In the control group there was 10% death noted at 4 hours after amphetamine administration, whereas in the reticuline-treated group there was 60% mortality.

Tetanic convulsions induced in frogs after reticuline (40 mg/kg in ventral lymph sac) were present in decorticated frogs as well as in animals whose midbrain and medulla were ablated. However, the convulsions were abolished after destruction of the spinal cord.

The $L.D._{50}$ of reticuline in mice was 56±8 mg/kg, i.p.

The results suggest that reticuline has central stimulant, analgesic, hyperthermic, and convulsant actions. The convulsions were strychnine-like and were prevented by a central muscle relaxant and abolished after ablation of the spinal cord. The pharmacologic profile of activity of reticuline thus qualitatively closely resembles that of thebaine (10, 11). This finding is interesting in view of the fact that reticuline is the established precursor (12) of morphothebaine alkaloids. There is room for speculation that reticuline undergoes oxidative transformation to thebaine or a thebaine-like molecule to elicit this pharmacological activity.

EXPERIMENTAL[2]

PLANT MATERIAL.—The plant material (*Pachygone ovata* Miers ex Hook. f. & Thoms.) used in this study was procured from South India and was identified by Dr. Deb, Deputy Director, Indian Botanic Garden, Howrah, West Bengal. A herbarium specimen is preserved in the laboratory.

EXTRACTION, FRACTIONATION AND CHROMATOGRAPHY.—The powdered dried leaves (5 kg) were extracted to exhaustion by percolation with ethanol (95%, 35 liters), and the percolate

[2]Melting points were taken on a Toshniwal melting point apparatus and are uncorrected. The uv spectra were obtained on a Cary-14 recording Spectrophotometer and the ir spectra were determined on a Perkin-Elmer model 720 spectrophotometer. The pmr spectra were recorded in deuterated chloroform on a Varian A-60D spectrometer (unless otherwise mentioned) and chemical shifts reported in δ (ppm) units. Mass spectra were recorded with MS-50 mass spectrometer by Dr. B. C. Das of ICSN, Gif-sur-Yvette, France, and by National Chemical Laboratory, Poona. Optical rotations were measured on a Jasco Dip 180 photoelectric polarimeter from Regional Sophisticated Instruments Centre, CDRI, Lucknow. Silica gel (60-120 mesh BDH) and neutral alumina (Sarabhai M.) were used for column chromatography, and silica gel G (Centron Res. Lab.) was used for thin layer chromatography. Anhydrous sodium sulfate was routinely used for drying organic solvents.

was concentrated under reduced pressure to a thick syrup (0.2 liter) and mechanically stirred with aqueous citric acid (5%, 1.5 liter x 2) for 6 hours and filtered; the filtrate was washed with chloroform (1 liter x 3) to remove non-basic materials. The aqueous fraction was cooled and basified with NH_4OH to pH 9. The resulting alkaline solution was extracted successively with benzene (1 liter x 3) and chloroform (1 liter x 4). The benzene and chloroform extracts were separately dried, filtered, and concentrated to thick syrups. Thin layer chromatography of a portion of the benzene extract on silica gel G in benzene-acetone-diethylamine (49:50:1) showed essentially one Dragendorff-staining spot (R_f 0.53). The thick syrup was diluted with benzene and chromatographed over a dry-packed column of alumina (100 g) and eluted first with petroleum-ether and then with petroleum ether-benzene mixtures. Fifty ml fractions were collected. Fractions 1–10 contained no alkaloid and were discarded. Elution with petroleum ether-benzene (1:1) gave fifteen fractions (11–25) that contained a minor alkaloid which could not be crystallized or isolated in quantity for further work.

ISOLATION OF *N*-METHYLCROTSPARINE (**1**).—Elution of the column with pure benzene (Fractions 26–45) afforded a base (R_f 0.53) which crystallized from benzene as colorless plates (300 mg). It gave the following physical data: mp 223° (dec); $[\alpha]D-45.74$ (c 0.26, MeOH); uv, λ max (MeOH) 205 nm (log ϵ 4.54), 232 (4.43) and 288 (3.55); λ max (MeOH/OH) 240 and 310 nm; ir, ν max (KBr) 3400 cm^{-1}], 1660 and 1618; 90 MHz pmr, δ 2.40 (s, $3H, N-CH_3$), 2.08–3.22 (m, 6H, $3-CH_2$), 3.46 (dd, $J=10$ & 6 Hz, 1H, –CH–N), 3.82 (s, 3H, $Ar-OCH_3$), 5.77 (br, 1H, exchangeable with D_2O, Ar–OH), 6.23–6.45 and 6.80–7.08 (2 x 8 lines, 2 x 2H, $J\sim10$ & 2Hz, α and β protons of cyclohexadienone system) and 6.60 (s, 1H, isolated Ar–H); ms, M^+ *m/e* 297 (100) for $C_{18}H_{19}NO_3$, 296 (52), 268 (51) and 254 (43).

PREPARATION OF *N*-METHYL CROTSPARINE-d_2 (**1a**).—*N*-Methylcrotsparine (100 mg) was heated on a water bath with alkaline D_2O (50 ml, approx, N/50 KOH) in an atmosphere of N_2 for 2 hours. The reaction mixture was cooled and acidified with AcOH. It was then basified with NH_4OH and extracted with ether (20 ml x 3). The ether extracts were combined, dried and filtered before evaporation to afford a sticky residue (85 mg) which crystallized from benzene as plates of **1a**; R_f 0.53 in benzene-acetone-diethylamine (49:50:1); ms, M^- *m/e* 299 (100) for $C_{18}H_{17}D_2NO_3$, 298 (84), 270 (23), and 256 (26); 90 MHz pmr, δ 2.40 (s, 3H, $N-CH_3$), 2.22–3.78 (m, 7H, $3-CH_2$ & 1 methine H), 3.88 (s, 3H, $Ar-OCH_3$), 6.64 (s, 1H, isolated Ar–H) and 6.99 (dd, $J=2$Hz, 2H, 2 β protons of cyclohexadienone).

CONVERSION OF *N*-METHYLCROTSPARINE (**1**) TO NUCIFERINE (**8**).—To a solution of *N*-methylcrotsparine (100 mg) in methanol (30 ml) was added an excess of an ethereal solution of CH_2N_2 (from nitrosomethyl urea), and the mixture was kept overnight in a cool dark place. Evaporation of the solvent left a non-phenolic residue which was dissolved in methanol (25 ml) and then refluxed with KBH_4 (200 mg) for 4 hours on a water bath. The solvent was removed *in vacuo*. The residue was diluted with water then basified with NH_4OH and extracted with chloroform. The residue obtained by removal of chloroform was refluxed with 2N HCl for 2 hours. The reaction mixture was cooled then basified with NH_4OH; the liberated base was extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate then concentrated to give a solid residue (50 mg). The residue was crystallized from petroleum ether to afford colorless needles of nuciferine (**8**) (mp, co-tlc superimposable ir).

ISOLATION OF RETICULINE (**2**).—The chloroform-soluble fraction of leaf alkaloids showed one major spot (R_f 0.66) on a thin layer chromatoplate developed with benzene-acetone-diethylamine (59:40:1). The chloroform extract was adsorbed onto silica gel (10 g) and chromatographed over a column of silica gel in benzene. Elution with benzene gave fractions which on removal of the solvent yielded a trace amount of Dragendorff positive residue which was found to be a complex mixture of alkaloids by tlc analysis and was not further processed. Elution with benzene-chloroform (1:1) furnished a homogeneous oily base (750 mg). The oily base was dissolved in ethanol (20 ml), acidified with $HClO_4$ (70%) and then diluted with dry ether until a turbidity appeared. The mixture was refrigerated until a crystalline solid of reticuline perchlorate separated. This solid was collected by filtration and recrystallized from ethanol as prisms; mp 202°, $[\alpha]D+88.76$ (EtOH); uv, λ max (EtOH) 284 nm (log ϵ, 3.95), λ min 254 nm, λ max (EtOH/OH) 296 nm; ir, ν max (nujol) 3640 cm^{-1}, 3450 and 2750–2500; pmr, δ 2.43 (s, 3H, $-N-CH_3$), 2.50–3.50 (broad envelope, 6H, $3-CH_2$), 3.70 (t, partly obscured by methoxy signals, 1H, $-CH_2CH-N-$), 3.83 (s, 6H, $2-OCH_3$), 5.03 (br, exchangeable with D_2O, 2H, 2 Ar–OH) and 6.33–6.91 (m, 5H, Ar–H). The sample was found to be identical with reticuline perchlorate by direct comparison (mp, mmp, co-tlc, ir) with an authentic sample.

A methanolic solution of the free base derived from the perchlorate was treated with an ethereal solution of CH_2N_2 and then was worked up by the usual procedure; a non-phenolic base was obtained. This was crystallized from petroleum ether (60–80°) as needles, mp 87°, and is identical with (+)-laudanosine (**2a**).

ISOLATION OF RETICULINE *N*-OXIDE (**3**).—The aqueous alkaline solution left after removal of tertiary bases was acidified with dilute HCl and treated with Mayer's reagent to produce a

flocculent precipitate. The precipitate was filtered and washed free from excess of reagent. The moist precipitate was then suspended in ion-free water and stirred with IRA 400 (Cl^-) until exchange was complete. The aqueous light brown solution was separated from the resin by filtration. The filtrate was concentrated under reduced pressure to a sticky gum and finally evaporated to dryness to a pale brown foam (800 mg). The residue was dissolved in methanol, adsorbed onto silica gel (5 g) and then placed on a bed of silica gel (50 g) in chloroform. The column was eluted first with chloroform and then with chloroform-methanol mixtures containing increasing amounts of methanol; fractions of 50 ml were collected. Fractions 1–45 eluted with chloroform and chloroform-methanol (49:1) furnished non-basic materials. Fractions 46 and above were eluted with chloroform-methanol (19:1) and fractions 53–65 furnished a sticky gum which on removal of the solvent in a vacuum desiccator gave a foam (150 mg) of reticuline *N*-oxide hydrochloride. This foam could not be induced to crystallization. It gave the following data: uv, λ max (EtOH) 283 nm (log ε, 3.81), λ min 255 nm (log ε, 3.16), λ max ($EtOH/OH^-$) 301 nm; ir, ν max (nujol) 3350 cm^{-1}.

An aqueous solution of the hydrochloride was basified with NH_4OH and then extracted with chloroform in a liquid-liquid extractor. The chloroform extract on removal of solvent afforded an amorphous pale yellow solid which could not be induced to crystallize. This solid was dissolved in methanol and precipitated by addition of dry ether. The process was repeated several times to obtain a white powder of reticuline *N*-oxide (**3**). Calcd. for $C_{19}H_{20}NO_5$: C, 66.07; H, 6.67; N, 4.06%, Found: C, 65.53; H, 6.81; N, 3.92%. The compound did not show any molecular ion peak in the mass spectrum, and the highest mass range peak was a peak formed by loss of a hydroxyl from molecular ion (13): Ms, *m/e* 328 (0.37, M–OH), 327 (0.55, M–H_2O), 312 (0.45, M–H_2O–Me), 192 (100), 177 (30) and 137 (45).

REDUCTION OF RETICULINE *N*-OXIDE (**3**) TO RETICULINE (**2**).—To reticuline *N*-oxide (25 mg) was added sulfurous acid (6%, 5 ml), and the solution was allowed to stand overnight. The solution was diluted with water (50 ml), then made basic with NH_4OH and extracted with chloroform (4 x 50 ml). The chloroform extracts were combined, dried over anhydrous sodium sulfate and then filtered. The filtrate was evaported *in vacuo* to afford reticuline, which was converted to its perchlorate, mp 202°, R_f 0.66 in benzene-acetone-diethylamine (59:40:1). The identity of the reduction product with reticuline perchlorate was established by direct comparison (mp, mmp and co-tlc).

OXIDATION OF RETICULINE (**2**) TO RETICULINE *N*-OXIDE (**3**).—Reticuline (100 mg) was dissolved in chloroform (25 ml). To the solution *m*-chloroperbenzoic acid (100 mg) in chloroform (5 ml) was added slowly. The ice-cold mixture was stirred for six hours, then brought to room temperature and diluted with chloroform (25 ml). It was then washed with aqueous $NaHCO_3$ (1%, 3 x 20 ml) to remove excess acid and then washed with water. It was dried over anhydrous sodium sulfate and then filtered. The filtrate was evaporated to dryness under reduced pressure to give an amorphous powder, R_f 0.10 in methanol-aqueous ammonia (0.3%) (7:3). The compound was identical with reticuline *N*-oxide (co-tlc and ir).

ISOLATION OF (+)-QUERCITOL (**4**) FROM THE STEMS OF *P. ovata*.—The powdered dry stems of *P. ovata* (2 kg) were extracted with ethanol (95%) in a Soxhlet apparatus for 18 hours. The alcoholic extract was concentrated to a small volume (400 ml) under reduced pressure. The extract was refrigerated until a crystalline solid separated. The solid was recrystallized from aqueous alcohol as cubes (1.2 g): mp 228°, lit. (8) mp 235–37°; $[\alpha]D+25°$ (c 0.83, H_2O), R_f 0.5 (pc) in acetone-water (4:1) and the spot was visualized by spraying an aqueous alkaline $KMnO_4$ solution; pmr (D_2O), δ 1.86 (broad, 2H, $-CH_2-$) and 3.8 (m, 5H, 5–C*H*–OH); Calcd. for $C_6H_{12}O_5$: C, 43.90; H, 7.31; Found: C, 43.45; H, 7.65%.

PREPARATION OF QUERCITOL PENTABENZOATE (**4a**).—A mixture of quercitol (200 mg), benzoyl chloride (2 ml) and pyridine (5 ml) was refluxed by heating in an oil bath at 120° for 1 hour. The reaction mixture was poured over crushed ice, stirred and filtered under suction. The filtrate was rejected and the residue was washed with water and then dissolved in ether. The ether extract was first washed with aqueous $NaHCO_3$ and then with water, dried over anhydrous sulfate and then concentrated. The concentrate was chromatographed over a silica gel column (6 g). Elution of the column with a petroleum ether-benzene (1:1) mixture furnished white crystals (172 mg) which was recrystallized from petroleum ether-ether mixture to give quercitol pentabenzoate (**4a**); mp 150°, lit. (8) mp 155°; pmr, δ 2.75 (broad, 2H, $-CH_2-$), 5.60–6.40 (m, 5H, C*H*–OCOAr), 7.30–7.83 (m, 15H, Ar–H), 7.83–8.50 (m, 10H, *ortho*-aromatic protons of Ar–COOR).

ISOLATION OF LIRIODENINE (**5**).—The ethanolic extract of the stems of *P. ovata* after separation of quercitol was further concentrated to a thick syrup and then stirred with aqueous citric acid (5%, 800 ml) for 8 hours before filtration. The aqueous filtrate was basified with ammonium hydroxide and extracted with chloroform. The chloroform extract was concentrated and chromatographed over a dry packed column of alumina (25 g). Fractions of 50 ml were collected. Elution with petroleum ether and petroleum ether-ethyl acetate (3:1) gave fractions 1–15 and 16–40, respectively, which contained non-basic materials and were rejected.

Elution with petroleum ether-ethyl acetate (1:1) afforded yellow needles of liriodenine (360 mg); R_f 0.48 in benzene-acetone-diethylamine (50:49:1); uv, λ max (EtOH) 247 nm (log ε, 4.18), 268 (4.12), 310 (3.65), 400 sh (3.80), 415 (3.84), λ max ($HClO_4$) 250 nm, 280, 345, 405; ir, υ max (KBr) 1662 cm^{-1} 1480, 1420, 1360, 1120, 1015, 955, 865, 728; 90 MHz pmr (CF_3CO_2H), δ 6.71 (s, 2H, O-CH_2-O) 7.62 (s, 1H, isolated Ar-H), 7.73-8.22 (a pair of doublets of triplets, J=8 & 2 Hz, 2H, C-9 & C-11 H), 8.52 (d, J=6 Hz, 1H, C-4-H), 8.81 (d, J=6 Hz, 1H, C-5-H), 8.92 (d, J=8 Hz, C-8-H), 8.65 (dd, J=8 & 2 Hz, 1H, C-11-H); ms, M^- *m/e* 275 (100) for $C_{17}H[NO_3$, 247 (19), 219 (10), 188 (19). The compound was indistinguishable from an authentic sample of liriodenine (mp, mmp, co-tlc).

ISOLATION OF TRILOBINE (**6**) FROM THE ROOTS OF *P. ovata*.—The dried and powdered roots of *P. ovata* (2 kg) were extracted with ethanol (95%) in a Soxhlet apparatus for 18 hours. The ethanol extract was concentrated under reduced pressure to an oily liquid (50 ml) which was stirred with 5% aqueous citric acid (800 ml) for 6 hours.The acid solution was filtered, and the filtrate was extracted with chloroform (3 x 500 ml) to remove non-basic materials. The aqueous solution was basified with NH_4OH (pH=9) and extracted with chloroform (3 x 500 ml). The combined chloroform extracts, on removal of solvent, left a gummy residue (1.1 g) which was vigorously stirred with 1% aqueous NaOH (400 ml) for 1 hour to fractionate the total base into phenolic and non-phenolic fractions. The aqueous suspension was extracted with ether (3 x 75 ml). The ether solution was concentrated and then chromatographed over a dry packed column of neutral alumina (12 g). The column was first eluted with petroleum ether and then with petroleum ether-benzene mixtures of varying proportions. Fractions of 25 ml each were collected. Fractions 1-12 and 13-25, eluted respectively with petroleum ether and petroleum ether-benzene (3:1), furnished non-basic materials and were rejected. Elution with petroleum ether-benzene (1:1) afforded trilobine (**6**) as needles (120 mg); mp 232°, R_f 0.45 in benzene-acetone-diethylamine (49:50:1); λ max (EtOH) 275 (sh) nm (log ε, 3.96), 288 (3.97), 305 (3.81), λ min 260 nm (log ε, 3.82); υ max (nujol) 3450 cm^{-1}; pmr, δ 1.73 (broad, 1H, exchangeable with D_2O, -NH), 260 (s, 3H, $-NCH_3$), 2.33-3.80 (broad envelope, 14H, methylene and methine protons), 3.88 (s, 3H, Ar-OCH_3), 3.98 (s, 3H, Ar-OCH_3), 6.20-7.50 (m, 10H, Ar-H); ms, M^+ *m/e* 562 (59) for $C_{35}H_{34}N_2O_6$, 561 (59), 335 (100), 168 (45).

PREPARATION OF ISOTRILOBINE (**6a**) FROM TRILOBINE (**6**).—An ice-cold solution of trilobine (50 mg) in methanol (50 ml) was stirred with formalin (40% CH_2O, 1 ml) for 30 minutes. KBH_4 (500 mg) was added portion-wise, and the reaction mixture was stirred for 4 hours and kept over night. The residue obtained by removal of methanol from the reaction mixture was diluted with water (30 ml) and then extracted with ether (3 x 20 ml). The combined ether extract was washed with water, dried, and evaporated to a solid residue (41 mg). The residue was dissolved in a minimum volume of chloroform and chromatographed over a bed of alumina (7 g). Elution with benzene furnished isotrilobine (**6a**) as needles (29 mg); mp 212°; ms, M^- *m/e* 576 (40) for $C_{36}H_{36}N_2O_6$, 349 (100), 335 (34), 175 (72). The sample was indistinguishable from an authentic sample of isotrilobine (14) by direct comparison (mp, mmp, co-tlc, ms).

ISOLATION OF COCLAURINE (**7**).—The aqueous alkaline solution (phenolic base fraction) left after removal of non-phenolic bases by ether extraction, was acidified with dilute HCl, then basified with NH_4OH and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and then evaporated to a sticky residue (310 mg) which was then adsorbed onto silica gel (5 g) and placed over a bed of silica gel (15 g) in chloroform. The column was first eluted with chloroform and then with chloroform-methanol mixtures of varying proportions; fractions of 25 ml were collected. Fractions 1-10 eluted with chloroform and 11-25 eluted with chloroform-methanol (19:1) afforded non-basic residue. Elution with chloroform-methanol (9:1) gave nine fractions which contained a single alkaloid, R_f 0.67 in benzene-acetone-diethylamine (49:50:1). These fractions (32-40) were pooled together and crystallized from a ether-methanol mixture as needles of coclaurine (40 mg), mp 208°; uv, λ max (EtOH) 254 nm and 285; λ max (nujol) 3530 cm^{-1}, 3470, 3300; ms, M^+, *m/e* 285 (insignificant), 178 (100), 163 (20), 107 (8). The sample was proved to be coclaurine by direct comparison (mmp, co-tlc). Though a higher melting point has been reported for coclaurine the authentic sample melted in our hands at 208°. Calcd. for $C_{17}H_{19}NO_3$: C, 71.58; H, 6.66; N, 4.91%. Found: C, 71.07; H, 6.75; N, 4.83%.

PREPARATION OF *N*-METHYLCOCLAURINE (**7a**).—To an ice-cold solution of coclaurine (15 mg) in methanol (25 ml) was added formalin (40% CH_2O, 0.5 ml) dropwise with stirring. After one hour of stirring, KBH_4 (100 mg) was added slowly to the solution, and stirring was continued for four hours; the solution was left overnight. The solution was evaporated and the resulting residue was diluted with water (20 ml) then acidified with dilute HCl. It was then basified with NH_4OH and then extracted with ether (3 x 30 ml). The combined ether extract was washed with water, dried, and evaporated to a homogeneous residue (12 mg): pmr, δ 2.44 (s, 3H, N-CH_3), 3.82 (s, 3H, Ar-OCH_3), 2.52-3.78 (broad envelope, 7H, methylene and methine protons), 5.2 (s, exchangeable with D_2O, 2H, Ar-OH), 6.37 (s, 1H, Ar-H), 6.76 (A_2B_2 quartet, J=8 Hz, 4H, 2 pairs of *ortho*-aromatic protons in identical environment), 6.54 (s, 1H, merged with A_2B_2 quartet); ms, M^- *m/e* 299 (0.3), 192 (100), 177 (28), 107 (6).

PHARMACOLOGY

MATERIALS AND METHODS

The studies were conducted in Wistar strain albino rats (100–200 g), mice (20–30 g), and frogs (*Rana tigrina*, 50–100 g) of either sex at ambient temperature of 25±2°. The following methods were used:

1. Observational test for general behavior in mice.
2. Locomotor activity in mice with an actophotometer.
3. Effect on rectal temperature in rats, measured on a telethermometer.
4. Antinociceptive activity in rats produced by the rat tail-hot wire technique of Davies *et al.* (16).
5. Effect on amphetamine toxicity in aggregated mice (17).
6. Ablation studies in frogs.
7. Effect on hexobarbitone (100 mg/kg i.p.)-induced hypnosis in mice.
8. Acute toxicity in mice (18).

Each experimental group consisted of ten animals each, unless otherwise mentioned. Pretreatment time of reticuline was uniformly maintained at 30 min. Students *t* and χ^2 tests of significance were used at appropriate places.

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