THE ALKALOIDS OF *STEPHANIA GLABRA*[1]

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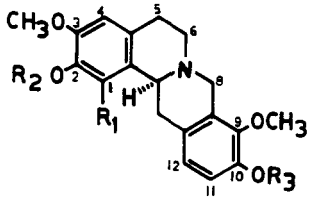
ABSTRACT.—N-Desmethylcycleanine, a new bisbenzylisoquinoline alkaloid, and capaurine and corynoxidine, the known tetrahydroprotoberberine alkaloids, have been isolated for the first time from the rhizomes of *Stephania glabra* (Roxb.) Miers. The alkaloids which have been reisolated from the plant are tetrahydroprotoberberine alkaloids tetrahydropalmatine, corydalmine, stepholidine; the proaporphine alkaloids pronuciferine and stepharine; the quaternary protoberberine salts palmatine, dehydrocorydalmine, jatrorrhizine, and stepharanine; and the bisbenzylisoquinoline alkaloid cycleanine. The alkaloids of the leaves and stems of the plant were shown to be qualitatively similar by tlc.

Pronuciferine hydrochloride has been found to exhibit spasmolytic activity. The biological activities of other alkaloids of the plant are reviewed.

Stephania glabra (Roxb.) Miers. (Menispermaceae) is a glabrous dextrous climber indigenous to the lower Himalayas (5000–6000 ft) of India. Extracts of the rhizomes of the plant have long been used by the natives as an antidysenteric, antipyretic and antiasthmatic (1, 2). To date 17 alkaloids have been isolated from the rhizomes of the plant (3–12). Some of the isolated bases possessed hypotensive

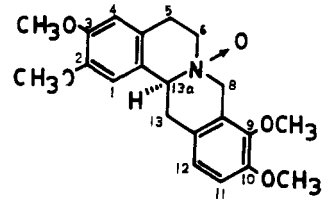


1 R_1 = OH; R_2 = R_3 = CH_3

2 R_1 = H ; R_2 = R_3 = CH_3

3 R_1 = H ; R_2 = CH_3; R_3 = H

4 R_1 = H ; R_2 = R_3 = H



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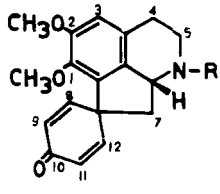
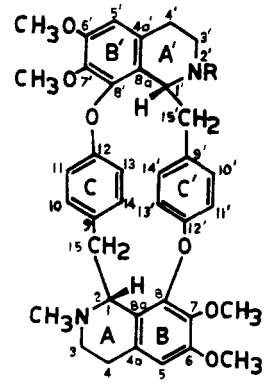


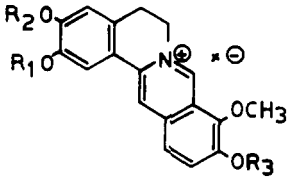
6 R = H

7 R = CH_3



8 R = H

9 R = CH_3



10 R_1 = R_2 = R_3 = CH_3; X^- = NO_3^-. 11 R_1 = R_2 = CH_3; R_3 = H; X^- = Cl^-

12 R_1 = R_3 = CH_3; R_2 = H X^- = Cl^- 13 R_1 = R_3 = H; R_2 = CH_3; X^- = Cl^-

[1]CDRI Communication No. 3018.

and antimicrobial properties (13). The ethanolic extract of the rhizomes of *S. glabra*, when put through a broad biological screen, showed hypotensive and spasmolytic activities (14). In the follow up studies the activities were found to be in the alkaloid fraction. This prompted us to reinvestigate the alkaloid constituents of the plant. The investigation resulted in the isolation of two bisbenzylisoquinoline alkaloids, cycleanine (**9**) (12) and *N*-desmethylcycleanine (**8**); five tetrahydroprotoberberine alkaloids, capaurine (**1**) (15), corynoxidine (**5**) (16), tetrahydropalmatine (**2**) (3), corydalmine (**3**) (5) and stepholidine (**4**) (5); two proaporphine alkaloids, stepharine (**6**) (10) and pronuciferine (**7**) (10); and four quaternary protoberberine salts palmatine (**10**) (3, 4), dehydrocorydalmine (**11**) (7), jatrorrhizine (**12**) (8) and stepharanine (**13**) (7). Of the isolated bases, the bisbenzylisoquinoline alkaloid, *N*-desmethylcycleanine (**8**), is a new base. The tetrahydroprotoberberine alkaloids capaurine (**1**) and corynoxidine (**5**), although known bases, were isolated for the first time from this source. The remaining alkaloids had been isolated earlier from the plant (3–12).

RESULTS

The new base (**8**) is named *N*-desmethylcycleanine because of its relationship to cycleanine (**9**). The presence of –NH grouping in the molecule was suggested by the absorption at 3350 cm^{-1} in the ir spectrum. The chemical shifts for different protons in the ^1H nmr spectrum of the base were comparable to those of cycleanine (**9**) (17, 27). The minor differences were due to the dissymmetry in the molecule produced by *N*-demethylation in one half of the molecule. In the nmr spectrum of *N*-desmethylcycleanine, the signal for the lone N-methyl group was at δ 2.51. There were also signals for four methoxyl groups. The mass spectrum of the base had a weak but observable molecular ion peak at *m/e* 608 which corresponded to formula $C_{37}H_{40}N_2O_6$ of which two of the oxygens were present as diphenyl ethers. The mass fragmentation pattern was typical of a bisbenzylisoquinoline alkaloid having two head-to-tail ether linked coclaurine units (18). That this alkaloid was not previously known was evidenced by the presence of only one N-methyl group in the nmr spectrum; all previously known examples contained two N-methyl groups (19). The ^{13}C nmr chemical shifts of the alkaloid were found to be comparable to those of cycleanine (20) except that *N*-demethylation on one half of the molecule had caused the carbons of the two halves to differ in their chemical shifts.

N-Methylation of *N*-desmethylcycleanine with formaldehyde and formic acid gave cycleanine (**9**) (27) identical by direct comparison (mp, mmp, $[\alpha]_D$ co-tlc, ms and nmr) with an authentic sample. *N*-Desmethylcycleanine, thus, has the structure and stereochemistry as shown in **8**.

Capaurine (**1**) was isolated as a minor alkaloid from the chloroform soluble alkaloid fraction of the ethanolic extract. The mass fragmentation pattern was characteristic of tetrahydroprotoberberine-type of alkaloids (28). There were two singlets for three protons in the aromatic region. Treatment of the base with an excess of ethereal diazomethane yielded O-methylcapaurine (**1**, R_1=OMe) (29). The nmr and other physical data suggested that the compound was *cis*-capaurine (1) (29). Capaurine (**1**) has previously been isolated from several *Corydalis* species (15, 25).

Corynoxidine (**5**), the other minor tetrahydroprotoberberine alkaloid isolated from the chloroform soluble fraction, was characterized by physical and spectral data as the *N*-oxide of l-tetrahydropalmatine with a *trans* B/C ring juncture. Corynoxidine has previously been isolated from *Corydalis koidzumiana* (16). Perbenzoic acid oxidation of dl-tetrahydropalmatine produced dl-corynoxidine.

(–)-Tetrahydropalmatine (**2**) (3), (–)-corydalmine (**3**) (5), (–)-stepholidine (**4**) (5), (+)-stepharine (**6**) (10), (+)-pronuciferine (**7**) (10) and (–)-cycleanine (**9**) (12) were also isolated from the chloroform soluble alkaloid fraction and were

characterized by direct comparison (mp, ir, uv, nmr, mass, sp. rotn.) with an authentic sample.

The quaternary protoberberine alkaloids palmatine (**10**) (3, 4), dehydrocorydalmine (**11**) (7), jatrorrhizine (**12**) (8) and stepharanine (**13**) (7) were isolated from the *n*-butanol soluble fraction and were characterized by physical properties, spectroscopic data and direct comparison with an authentic sample.

Several reports on the pharmacological properties of alkaloids of *S. glabra* have appeared. Tetrahydropalmatine (**2**) hydrochloride caused hyperthermia in rats (21). Palmatine (**10**) had been found to possess ACTH-like bactericidal and anticholinesterase effects, and it had been concluded that palmatine (**10**), dl-tetrahydropalmatine and ergot alkaloids have an analogous pharmacological mechanism (26). Stepharine (**13**) had been reported to have antihypertensive properties (22). Cycleanine (**9**) has been shown to exhibit an antitumor effect (23). Structure-activity relationship has been studied in capaurine (**1**) for emetine type activity (24). Pronuciferine (**7**) hydrochloride showed good spasmolytic activity. There was 75% blockage at a dose of 25 microgram when tested on the tissue ileum of a guinea-pig (30).

EXPERIMENTAL[2]

PLANT MATERIAL.—The plant material used in this study was collected from Ranikhet, Uttar Pradesh, in August 1979 and identified by Dr. K. K. Singh of Central Drug Research Institute, Lucknow. A herbarium specimen is on deposit in the herbarium of the Institute.

EXTRACTION AND FRACTIONATION.—Air-dried finely powdered rhizomes (10 kg) of *S. glabra* were exhaustively extracted with alcohol (95%) at room temperature. The percolate was concentrated under reduced pressure. A yellow crystalline solid (50 g) which precipitated out on concentration was filtered. The crystalline solid later on was found to be essentially a mixture of tetrahydropalmatine (**2**) and palmatine (**10**).

When the solvent was removed from the filtrate under reduced pressure below 40°, a brownish yellow viscous mass was obtained, which was then extracted with 5% hydrochloric acid. The aqueous acidic solution was defatted with petroleum ether (5x200 ml) and then basified with aqueous sodium carbonate (pH 9). The liberated bases were extracted with chloroform (6x150 ml), washed with water and dried. When the solvent was removed the alkaloidal mixture A (100 g) remained. The aqueous layer was extracted with *n*-butanol. The *n*-butanol extraction was washed with water, dried. When the solvent was removed the alkaloid mixture B (20 g) was obtained.

CHROMATOGRAPHY OF ALKALOID MIXTURE A.—Mixture A (30 g) was dissolved in chloroform and adsorbed on silica (80 g), and the adsorbed material was placed on a column of silica gel (1 kg) in benzene. The column was first eluted with benzene, then with chloroform and, finally, with increasing proportions of methanol in chloroform. The elution was followed by tlc. Fractions of 200 ml each were collected.

ISOLATION OF N-DESMETHYLCYCLEANINE (**8**).—Elution of the column with chloroform-methanol (90:10) (Frs. 106–110) gave a crude base which was subjected to preparative tlc (*plates*: silica gel—G, *solvent*: chloroform-methanol, 85:15). The major band on the plate was cut and extracted with chloroform-methanol (4:1). Removal of the solvent from the extract afforded a pure base which, when treated with methanol, afforded *N*-desmethylcycleanine (**8**) (35 mg), mp 102–103°; $[\alpha]^{32}D$ −165° (c, 0.29, $CHCl_3$); uv, λ max (MeOH) 237 nm (log ε 4.03) and 281 (3.26), no change in uv spectrum occurred on addition of alkali; ir, ν max (KBr) 3350 cm^{-1}, 2900, 1570, 1480, 1400, 1340, 1290, 1210, 1110, 1070 and 840; ^1H nmr ($CDCl_3$, 60 MHz): δ 2.51 (s, NCH_3), 2.65–3.40 (m, 12H), 3.40 (s, OCH_3), 3.45 (s, OCH_3), 3.80 (s, OCH_3), 3.85 (s, OCH_3), 4.23 and 4.62 (m, 1H each, H-1 and H-1'), 5.77 and 5.81 (dd, 1H each, J=2, 8 Hz, H-13, H-13'), 6.25 and 6.30 (dd, 1H each, J=2, 8 Hz, H-14, H-14'), 6.55 (s, 2H, H-5 and H-5'), 6.54 and 6.57 (dd, 1H each, J=2, 8 Hz, H-11, H-11') and 7.03 and 7.23 (dd, 1H each, J=2, 8 Hz, H-10, H-10'); ms, *m/e* 608 (M^+, $C_{37}H_{40}N_2O_6$), 312 (base peak), 311, 298, 204, 190, 176, 174, 160, 146, 145, 132 and 131; ^{13}C nmr: (20 MHz, $CDCl_3$) δ 153.69 and 154.64 (C_{12} and $C_{12'}$), 152.04 and 153.46

[2]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 polarimeter. The ^1H nmr spectra were recorded in deuterated chloroform, unless otherwise stated, on a Varian A-60D and R-32 spectrometer, and ^{13}C nmr spectra were recorded on a CFT-20 spectrometer with tetramethylsilane as internal standard and chemical shifts recorded in δ (ppm) units. The mass spectra were taken with a JMS D-300 mass spectrometer fitted with a direct inlet system. Silica gel (60–120 mesh) (BDH) and neutral alumina (BDH) were used for column chromatography and silica gel GF-254 was used for thin layer chromatography. Anhydrous sodium sulfate was routinely used for drying the organic solvents and all solvents were evaporated under reduced pressure below 40°.

(C_6 and $C_{6'}$), 143.76 and 143.97 (C_8 and $C_{8'}$), 139.14 and 140.26 (C_7 and $C_{7'}$), 129.91 and 131.42 (C_9 and $C_{9'}$), 128.98 and 128.73 (4a and 4'a), 128.64 and 128.73 (C_{14} and $C_{14'}$)$^+$, 128.41 and 128.64 (C_{10} and $C_{10'}$)$^+$, 125.36 and 125.66 (C_{8a} and $C_{8'a}$), 117.54 and 117.85 (C_{13} and $C_{13'}$)$^+$, 113.91 and 114.16 (C_{11} and $C_{11'}$)$^+$, 108.98 and 109.56 (C_5 and $C_{5'}$), 60.9 and 56.71 (C_1 and $C_{1'}$), 59.61 and 59.52 (2 x OCH_3), 56.17 (2 x OCH_3), 44.84 and 42.66 (C_3 and $C_{3'}$), 42.48 (N-CH_3), 37.52 and 38.09 (C_{15} and $C_{15'}$), 24.93 and 26.64 (C_4 and $C_{4'}$).$^+$ The assignments may be reversed.

ISOLATION OF CAPAURINE (**1**).—Elution of the column with chloroform-methanol (98:2) gave a crude mixture which was subjected to preparative tlc (*plate*: silica gel-G, *solvent*: chloroform-methanol (97:3). The major band on the plate was cut and extracted with chloroform-methanol (4:1). Removal of solvent from the extract and treatment of the residue with methanol yielded capaurine (**1**) (30 mg), mp 164–165°: $[\alpha]^{30}D-265°$ (c, 0.29, $CHCl_3$). The identity was confirmed by direct comparison (uv, ir, nmr, ms, sp. rotn., mp, mmp) with an authentic reference sample of capaurine (**1**) (15, 29).

ISOLATION OF CORYNOXIDINE (**5**).—Elution of the column with chloroform-methanol (92:8) yielded a mixture which was subjected to preparative tlc (*plate*: silica gel-G, *solvent*: chloroform-methanol, 88:12). The major band on the plates was removed and extracted with chloroform-methanol (4:1). Removal of the solvent from the extract and treatment of the residue with acetone gave corynoxidine (**5**) (26 mg), mp 181–182° (decomp.); $[\alpha]_D^{32}-58°$ (c, 0.33, $CHCl_3$). No molecular ion was observed under the ei mode of mass spectra at 70 eV, but fd and ci mass spectra showed an intense molecular ion at *m/e* 371 (M^+, $C_{21}H_{25}NO_5$). The identity was confirmed by direct comparison (uv, ir, ms, ^1H nmr, ^{13}C nmr, sp. rotn., mp) with an authentic reference sample of corynoxidine (**5**) (16).

Continued elution of the column with increasing proportions of methanol in chloroform gave tetrahydropalmatine (**2**) (Frs. 11–26, chloroform-methanol, 99:1), mp 141–142°; corydalmine (**3**) (Frs. 34–40, chloroform-methanol, 98:2), mp 174–175°; pronuciferine (**7**) (Frs. 45–48, chloroform-methanol, 97:3), mp 127–128°; stepholidine (**4**) (Frs. 58 –66, chloroform-methanol, 96:4), mp 156–157°; cycleanine (**9**) (Frs. 73–85, chloroform-methanol, 94:6), mp 272–273° and stepharine (**6**) (Frs. 95–101, chloroform-methanol, 92:8), mp 177–178°. The alkaloids were characterized by direct comparison (mp, uv, ir, ms, nmr, sp. rotn.) with an authentic sample.

CHROMATOGRAPHY OF ALKALOID MIXTURE B.—Mixture B (15 g) was dissolved in methanol, adsorbed on alumina (50 g) and the adsorbed material was placed on a column of alumina (450 g) in chloroform. The column was first eluted with chloroform, then with chloroform-ethyl acetate and, finally, with an increasing proportion of methanol in ethyl acetate. The chromatographic fractions were further subjected to preparative tlc (*plate*: silica gel-G, *solvent*: chloroform-methanol, 85:15). The major bands from the plates were removed and each was extracted with methanol. When the solvent was removed from the extracts and the residues were treated with methanol, the following compounds were obtained: palmatine (**10**), mp 239–241°; dehydrocorydalmine (**11**), mp 252–253°; jatrorrhizine (**12**), mp 205–206; and stepharanine (**13**), mp 274–275°. The identities of the isolated bases were established by direct comparison (mp, uv, ir, co-tlc) with an authentic samples.

O-METHYLCAPAURINE.—Capaurine (20 mg) (**1**) in methanol (1 ml) was treated with an excess of ethereal diazomethane. The resulting mixture, when worked up after 45 h in the usual way, gave O-methylcapaurine (**1**, R_1=OMe) (14 mg), mp 152–153°; ms *m/e* 385 (M^+, $C_{22}H_{27}NO_5$). The identity was confirmed by direct comparison (uv, mp, co-tlc, ms, nmr) with a reference sample of O-methylcapaurine (15, 29).

CYCLEANINE (**9**).—A mixture of *N*-desmethylcycleanine (**8**) (30 mg), formic acid (0.5 ml) and formaldehyde (0.5 ml) was heated on a water bath for 1 h. Excess formaldehyde and formic acid from the resulting mixture was removed. The residue so obtained was dissolved in 2% hydrochloric acid, extracted with ether and basified with an aqueous solution of sodium carbonate; the liberated base was extracted with chloroform. The solvent from the chloroform extract was removed; the residue, on treatment with methanol, gave cycleanine (**9**) (24 mg), mp 269–270°. A direct comparison (uv, ir, nmr, ms, sp. rotn, mp) with an authentic reference sample confirmed the identity of the *N*-methyl derivative as cycleanine (**9**) (27).

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