THE ALKALOIDS OF *CORYDALIS MEIFOLIA*[1]

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ABSTRACT.—Six tetrahydroprotoberberines, (+)-sinactine, apocavidine, stylopine, (+)-cavidine, cheilanthifoline, and dehydrocavidine; two spirobenzylisoquinolines, yenhusomine and yenhusomidine; one phthalideisoquinoline, corlumine; one benzophenanthridine, dihydrosanguinarine and protopine, have been isolated from the leaves and stems of *Corydalis meifolia* Wall. Of these alkaloids, dehydrocavidine was a new base. The remaining alkaloids, although known, were isolated for the first time for this plant.

(+)-Cavidine, protopine, corlumine, yenhusomine, and dehydrocavidine exhibited spasmolytic activity.

Corydalis meifolia Wall. (1) (Papaveraceae) is one of the 25 *Corydalis* species (2) that grow in the Himalayan region at an altitude of 12,000 to 15,000 ft. The extract of various *Corydalis* species is reported to be efficacious in many ailments in the Indian Ayurvedic system of medicine (3). Ochotensine (4), an alkaloid that occurs in many *Corydalis* species is reported to stimulate isolated ileum of guinea pig or rabbit's uterus and to induce a fall in blood pressure in anesthetized cats.

During a program of research at the Central Drug Research Institute, Lucknow, spasmolytic activity was confirmed in the 50% alcoholic extract of *C. meifolia* Wall. (5). In the follow-up studies, the activity was concentrated in the alkaloidal fraction of the ethanolic extractive. Careful column and preparative thin layer chromatography of the alkaloidal mixture on silica gel yielded the following 11 alkaloids: (+)-sinactine (**4**), apocavidine (**3**), stylopine (**7**), (+)-cavidine (**2**), cheilanthifoline (**10**), dehydrocavidine (**1**), yenhusomine (**8**), yenhusomidine (**9**), corlumine (**5**), dihydrosanguinarine (**11**) and protopine (**6**). Of the isolated bases, dehydrocavidine (**1**) was new. The alakloids **2-11**, although previously known, were isolated for the first time from *C. meifolia* Wall.

Dehydrocavidine (**1**) was named so because of its relationship to cavidine. The molecular formula ($C_{21}H_{20}NO_4$) for the base was confirmed by mass spectrometry (M^+ 350). In the uv spectrum of the base, the absorption maxima at 215, 270, 348, and 450 nm, which remained unchanged in the presence of alkali, suggested a berberine type of nucleus for the base. Integrated nmr spectrum of dehydrocavidine confirmed the presence of 20 protons in the molecule. The signals for two aromatic methoxy groups δ 3.84 and 3.88, respectively, and a two-proton singlet at δ 6.35 was for a methylenedioxy group. A three-proton singlet at δ 2.92 accounted for C-13 CH_3. The aromatic protons at C-11 and C-12 appeared together as a broad singlet at δ 7.74; one proton singlet each at δ 6.9 and at 7.12 were due to aromatic C-1 and C-4 protons, respectively. C-8 *H* appeared as a singlet at δ 10.4. In the mass spectrum of the base, the molecular ion peak (M^+) was at *m/e* 350. The other significant peaks in the spectrum were at *m/e* 334, 191, 177, and 145. Sodium borohydride reduction of the base gave a compound identical in all respects with (±)-cavidine (6).

The alkaloid eluted with benzene from SiO_2 column was characterized as cavidine (7), which has been isolated earlier from the tubers of *C. ambigua* Chem. and Schlecht., *C. ledebouriana* (8), and *C. thalictrifolia* (9).

The conformation of 13-methyltetrahydroprotoberberines has been discussed by Jeffs (10), and it has been concluded that in compounds in which C-13 and C-14 protons are *cis* to one another, the quinolizine system assumed a *trans*-conformation, and C-

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13 methyl adopts an axial orientation. The compounds of this stereochemistry exhibit Bohlmann's band (11) in their infrared spectrum. In the ir spectrum of (+)-cavidine, Bohlmann's bands were between 2800-2700 cm^{-1}, confirming thus *trans*-quinolizidine conformation of the B:C ring junction.

The alkaloid obtained by elution of SiO_2 column with benzene-ethyl acetate (99:1) was characterized as apocavidine (**3**) (9). The base had earlier been isolated from *C. tuberosa* DC. (9). Stereochemistry of apocavidine (**3**) has been established by converting it into (+)-cavidine (10) of known stereochemistry.

Elution of SiO_2 column with benzene-ethyl acetate (75:25) afforded a base, characterized as (+)-sinactine (**4**). (±)-And (−)-sinactine have been isolated from *Simomenium acutum* (12), *Fumaria* species (13), *Papaver rhoeas* (14), and *C. ambigua* (15). (+)-Sinactine was, however, isolated for the first time from *C. meifolia* Wall.

(+)-Sinactine (**4**) was a member of tetrahydroprotoberberine alkaloids. The members of this group which show levorotation have been assigned 'S' configuration at the asymmetric center (16). (+)-Sinactine had dextrorotation; hence, the base must have 'R' configuration at C-14.

The second alkaloid obtained from this fraction was characterized as corlumine (**5**) which had been isolated from a number of *Corydalis* species (17).

Corlumine is used as a convulsant (18). In subconvulsive doses, it had little effect on blood pressure. Subconvulsive doses of the base increase the frequency and depth of respiration. Corlumine has little effect on perfused frog heart. Its effects on isolated intestine and uterus were irregular.

Elution of the column with benzene-ethyl acetate (1:1) afforded protopine (**6**) (19). Protopine was first isolated by Hesse (20) from opium. Subsequently it has been isolated from several *Corydalis* species (21).

Protopine (22) had an inhibiting action on isolated frog heart muscle or nerve and a stimulating action on guinea pig intestine. It inhibited tumor sarcoma 180 and Ehrlish mouse carcinoma (23).

Chromatography of the alkaloidal mixture-H afforded stylopine (**7**) (24), a tetrahydroprotoberberine alkaloid that was isolated first in 1931 from *C. tuberosa* (25) and later from several *Corydalis* species (26).

Chromatography of the alkaloidal mixture E yielded a spirobenzylisoquinoline alkaloid, yenhusomine (**8**), which had been isolated in 1975 form *C. ochotensis* (27). Yenhusomine is an analgesic and hypotensive agent. Another spirobenzylisoquinoline alkaloid isolated from the alkaloidal mixture was characterized as yenhusomidine (**9**), which also had been isolated earlier from *C. ochotensis* (27).

A phenolic tetrahydroprotoberberine alkaloid obtained from the polar fractions was identified as cheilanthifoline (**10**) (28). The base had been isolated earlier from several *Corydalis* species (29). A benzophenanthridine alkaloid isolated from the alkaloidal mixture by repeated column and thin layer chromatography was characterized as dihydrosanguinarine (**11**) (30). The base was first isolated from *Argemone mexicana* (31) in 1953 and later on from many *Corydalis* species (32).

EXPERIMENTAL

Unless otherwise stated, uv absorption spectra refer to solutions in ethanol, ir absorption spectra to KBr discs, and nmr spectra to solutions in deuteriochloroform. The nmr spectra were recorded with a Varian A-60 spectrometer. Unless specified to the contrary, tlc was carried out on SiO_2 GF 254, and column chromatography over SiO_2 (BDH).

The leaves and stems of *Corydalis meifolia* Wall. (Papaveraceae) used in this investigation were collected in August 1979 from Kedar Nath, Uttar Pradesh, India, by Dr. B. N. Mehrotra of the Botany Section. A herbarium sheet of the plant is kept in the herbarium of Central Drug Research Institute, Lucknow, India.

EXTRACTION.—The air-dried plant material (10 kg) was pulverized and percolated with 95% alcohol (5 x 12 liters) at room temperature. The solvent from the percolates was removed under reduced pressure below 40° to give a concentrate (4 liters) which, when left in cold, deposited a greenish crystalline material. This was filtered and then washed with alcohol to yield alkaloidal mixture A (80 g). The solvent from the filtrate was removed under reduced pressure. The greenish viscous mass (2 kg) thus obtained was extracted with 5% aqueous hydrochloric acid (5 x 500 ml). The aqueous acidic extract was defatted with petroleum ether (5 x 500 ml) and basified with Na_2CO_3 to pH 8.5. The thick precipitate thus obtained was filtered, washed with water, and dried to give alkaloidal mixture E (38.4 g). The basic aqueous filtrate was successively extracted with chloroform and *n*-butanol to give alkaloidal mixture B (10 g) and alkaloidal mixture F (7.9 g), respectively.

FRACTIONATION OF ALKALOIDAL MIXTURE A.—Alkaloidal mixture A (80 g) was extracted with hexane (6 x 300 ml) to give the hexane soluble and hexane insoluble fractions. The hexane soluble fraction was extracted with 5% hydrochloric acid. The acidic extract was basified with aqueous Na_2CO_3 to pH 8.5. The precipitate, thus obtained was filtered off, washed with water, and dried to give alkaloidal mixture H (2.5 g). The basic filtrate was extracted with chloroform to afford the alkaloidal mixture I (370 mg).

The hexane insoluble portion of the alkaloidal mixture A was extracted with benzene to give benzene soluble and benzene insoluble fractions. The benzene soluble fraction was extracted with 5% hydrochloric acid. The benzene layer, on usual work up, gave the alkaloidal mixture J (700 mg). The acid extract was basified with aqueous Na_2CO_3 to pH 8.5; the precipitate thus obtained was filtered off to give the alkaloidal mixture K (30 g). The basic filtrate was extracted with chloroform to give the alkaloidal mixture L.

The benzene insoluble portion of alkaloidal mixture A was extracted with chloroform (8 x 250 ml) to give chloroform soluble and chloroform insoluble fractions. The chloroform soluble portion was extracted with 5% hydrochloric acid. The chloroform layer was washed with water and dried, and the solvent was removed to yield alkaloidal mixture M (2 g). The aqueous acidic extract was basified with aqueous Na_2CO_3 to pH 8.5, the precipitate thus obtained was filtered off, washed with water, and dried to give the alkaloidal mixture N (2 g). The basic filtrate was extracted with chloroform (5 x 250 ml), washed with water, dried (anhydrous Na_2SO_4), and the solvent removed to give the alkaloidal mixture O (1 g).

CHROMATOGRAPHY OF THE ALKALOIDAL MIXTURE K.—The alkaloidal mixture K (30 g) was chromatographed over a column (120 x 6 cm) of SiO_2 (1 kg). The column was successively eluted from benzene; benzene-ethyl acetate; v/v (99:1), (95:5), (90:10), (75:25), (50:50), (25:75); ethyl acetate; ethyl acetate-methanol (95:5) and methanol. Elution was monitored by tlc. A total of 180 fractions, 250 ml each, were collected and mixed on the basis of Rf values on thin layer chromatography.

Cavidine (**2**). The fractions 6-44, eluted from benzene, were mixed and the solvent removed. The residue was crystallized from benzene to afford (+)-cavidine (**2**) (19.5 g) mp 145°; $[\alpha]_D$+297° (c, 0.18 $CHCl_3$). The physical constants and spectroscopic data (mp, ms, nmr, ir, and uv) of the base were identical to those of (+)-cavidine (**2**) (9).

Apocavidine (**3**). The fractions 45-69, eluted from benzene-ethyl acetate (99:1), were mixed and the solvent removed to give a crude product (245 mg), which was further subjected to preparative tlc (*plates:* SiO_2; *solvent:* benzene-ethyl acetate, 95;5). The major band on the plates was scraped and extracted with chloroform-methanol (4:1), and the solvent removed. The product was crystallized from methanol to give apocavidine (**3**) (9) (25 mg) mp 175°. The physical constants (mp, $[\alpha]_D$) and spectroscopic data (ir, uv, nmr, and ms) of the base were identical to those of apocavidine (**3**) (9).

Sinactine (**4**). The fractions 75-83, eluted from benzene-ethyl acetate (75:25) were mixed and the solvent removed. The product was crystallized from chloroform-methanol to afford (+)-sinactine (**4**) (350 mg) mp 172°; $[\alpha]_D$+296° (c, 1.038 in $CHCl_3$). The physical constants and spectroscopic data (mp, ir, uv, nmr, and ms) of the base were identical to those of sinactine (**4**) (15).

Corlumine (**5**). The fractions 88-94, eluted from benzene-ethyl acetate (75:25), were mixed and the solvent removed. The crude product was subjected to preparative tlc (*plates:* SiO_2; *solvent:* benzene-ethyl acetate, 80:20). The major band on the plates was scraped, extracted with chloroform-methanol (3:1), and the solvent removed. The product was crystallized from methanol to give corlumine (**5**) (500 mg) mp 155°; $[\alpha]_D$+77° (c, 0.2 in $CHCl_3$). The physical constants and spectroscopic data (mp, $[\alpha]_D$, ir, uv, nmr, and ms) of the base were identical to those of corlumine (**5**) (17).

Protopine (**6**). The fractions 98-104, eluted from benzene-ethyl acetate (1:1), were mixed and the solvent removed to give a crude product, which was subjected to preparative tlc (*plates:* SiO_2; *solvent:* chloroform-methanol, 96:4). The more polar band on the plate was scraped, extracted with chloroform-methanol (3:1), and the solvent removed. The product was then crystallized from methanol to give protopine (**6**) mp 207°'. The physical constants and spectroscopic data (mp, ir, uv, nmr, and ms) of the base were identical to those of protopine (**6**) (21).

Dehydrocavidine (**1**). The fractions 133-180, eluted from methanol, were mixed and the solvent removed to give a mixture (3 g) that was rechromatographed. Elution with ethyl acetate-methanol (90:10) gave a crude product that was subjected to preparative tlc (*plates:* SiO_2; *solvent:* chloroform-methanol, 82:18). The major band on the plates was scraped, extracted with methanol, and the solvent removed.

The product thus obtained was crystallized from methanol to give dehydrocavidine (**1**) (120 mg) mp 248-52°; λ max (MeOH) nm (log ε) 215 (5.54), 270 (5.52), 348 (5.44), 450 (4.85); ν max (KBr): 2900, 1610, 1520, 1475, 1340, 1300, 1260, 1220, 1200, 1190, 1170, 1160, 1100, 1080, 1049, 980, 840 and 748 cm^{-1}; nmr (DMSO-d_6) δ: 2.92 (s, 3H, C-13 CH_3), 3.15 (m, 2H, 5C-H_2), 3,84 (s, 3H, O*Me*), 3.88 (s, 3H, O*Me*), 4.88 (m, 2H, C-6 H_2), 6,35 (s, 2H, O-CH_2-O), 6.9 (s, 1H, C-1 *H*), 7.12 (s, 1H, C-4 *H*), 7.74 (s, 2H, C-11, C-12 *H*) and 10.4 (s, 1H, C-8 *H*); ms: *m/e* (rel. int.) 350 (M^+) (63), 334 (13.7), 177 (12.8), 175 (9), 145 (8), 137 (4), 71 (11.3), 43 (100).

Stylopine (**7**). The alkaloidal mixture H (2.5 g) was chromatographed. The column was eluted with benzene and increasing percentages of ethyl acetate. The fractions 85-100, eluted from benzene, were mixed and the solvent removed. The crude product was subjected to preparative tlc (*plates:* SiO_2; *solvent:* benzene-ethyl acetate, 96:4). The major band on the plates was scraped, extracted with chloroform-methanol (4:1) to give stylopine (**6**) mp 202°, $[\alpha]_D$ −315° (c, 0.8 in $CHCl_3$). The physical constants and spectroscopic data (mp, ir, uv, nmr, and ms) of the base were identical to those of stylopine (**6**) (24).

Dihydrosanguinarine (**11**). The alkaloidal mixture E (15 g) was chromatographed. Elution with hexane-benzene (25:75) (fraction 42-61) gave a crude alkaloidal mixture which was subjected to preparative tlc (*plates:* SiO_2; *solvent:* benzene-ethyl acetate, 75:25). The major band on the plates was scraped and extracted with chloroform-methanol (75:25). The product thus obtained was crystallized from methanol to give dihydrosanguinarine (**11**) (85 mg) mp 189°. The physical constants and spectroscopic data (mp, uv, ir, nmr, and ms) of the above base were identical to those of dihydrosanguinarine (**11**) (30).

Yenhusomidine (**9**) and *cheilanthifoline* (**10**). The fractions 81-94 eluted from benzene-ethyl acetate (90:10), were mixed and the solvent removed. The mixture thus obtained was subjected to preparative tlc (*plate:* SiO_2; *solvent:* benzene-ethyl acetate, 88:12). The two major bands on the plates were scraped. Extraction of the less polar band with chloroform-methanol (70:30) afforded yenhusomidine (**9**) (35 mg) mp 175°, $[\alpha]_D$ +98.3° (c, 0.3 in $CHCl_3$). The physical constants and spectroscopic data (mp, ir, uv, nmr, and ms) of the base were identical to those of yenhusomidine (**9**).

Extraction of the more polar band with chloroform-methanol (70:30) yielded cheilanthifoline (**10**) (48 mg) mp 180°; $[\alpha]_D$ −311° (c, 0.03 in MeOH). The physical constants and spectrsocopic data (mp, ir, uv, nmr, and ms) of the base were identical to those of cheilanthifoline (**10**) (28).

Yenhusomine (**8**). The fractions 182-197, eluted from ethyl acetate-methanol (95:5), were mixed and the solvent removed. The mixture thus obtained (500 mg) was subjected to preparative tlc (*plates:* SiO_2; *solvent:* chloroform-methanol, 95;5). Two major bands on the plates were scraped. The more polar band was extracted with chloroform-methanol (70:30) and the solvent removed. The residue was crystallized from acetone to give yenhusomine (**8**) (200 mg) mp 126°; $[\alpha]_D$ +48° (c, 1,0 in MeOH). The physical constants and spectroscopic data (mp, ir, uv, nmr, and ms) of the base and its diacetate were identical to those of yenhusomine (**8**) (27).

SPASMOLYTIC ACTIVITY.—Cavidine (**2**), protopine (**6**), corlumine (**5**), yenhusomine (**8**), and dehydrocavidime (**1**) isolated from the leaves and stems of *C. meifolia* Wall. were tested for spasmolytic activity in isolated guinea pig ileum. The activity of the compounds was assessed by its ability to inhibit the contraction of the smooth muscles induced by various spasmogens, such as acetylcholine, histamine, serotonin, and barium chloride. Cavidine exhibited, at 10-μg dose 84% and, at 50-μg dose, 100% spasmolytic activity; the spasmolytic activity for protopine, at 10-μg dose, was 40% and, at 50-μg dose, 100%; corlumine, at 10-μg dose, was 0% and, at 50-μg dose, 50%; yenhusomine, at 10-μg dose, was 17% and, at 50-μg dose, 52%; and dehydrocavidine, at 10-μg dose, was 20% and, at 50-μg dose, 40%. The bases nonspecifically blocked the effect of the above, mentioned spasmogens in a dose-dependent manner. The IC_{50} (concentration causing 50% inhibition) against the spasmogen was found to be between 0.5 and 1.0 μg/ml. As evident from the results, the bases failed to block any of the spasmogens specifically, and thus had a nonspecific spasmolytic effect.

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