THE ALKALOIDS OF *STEPHANIA ELEGANS*[1]

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ABSTRACT.—Nine alkaloids have been isolated from the leaves, stems and roots of *Stephania elegans* and characterized by physicochemical data and chemical transformation as epihernandolinol (**3**), *N*-methylcorydalmine (**5**), hasubanonin (**1**), aknadinin (**2**), cyclanoline (**6**), magnoflorine (**7**), isotetrandrine (**8**), isochondodendrine (**9**) and cycleanine (**10**).

Although a number of *Stephania* species (Menispermaceae) have been investigated and a variety of protoberberine, aporphine, bisbenzylisoquinoline and hasubanan types of alkaloids (1 to 10) have been isolated, *S. elegans* appears to have escaped the attention of chemists and pharmacologists. It was, therefore, thought desirable to investigate, in detail, the alkaloidal constituents of *S. elegans*.

In the present investigation, six tertiary and three quaternary alkaloids have been isolated from the stem, leaves, and roots of *S. elegans* by extensive column chromatography on neutral alumina and by preparative thin layer chromatography on silica gel-G plates. The alkaloids obtained are given in the order of elution. The first and second alkaloids isolated were hasubanonin (**1**) and aknadinin (**2**), well characterized known hasubanan alkaloids isolated earlier from *S. hernandifolia* (11, 12). The identity of the alkaloids was established by comparison of spectral data and physical constants with those reported for authentic samples.

Epihernandolinol (**3**) was assigned the molecular formula $C_{20}H_{27}O_5N$ on the basis of high resolution mass spectrometry. The uv spectrum of the base had bands at 237 and 284 nm which shifted to 288 nm on the addition of alkali, indicating the phenolic nature of the base. The ir spectrum had an hydroxyl absorption band at 3360 cm^{-1}. In the nmr spectrum of the base there were signals for one *N*-methyl and three methoxy groups. The mass spectrum of the base was characteristic and resembled that of hasubanan alkaloids possessing an oxygen function at C–6. The molecular ion peak appeared at *m/e* 361. Loss of the ethanamine chain from the molecular ion gave the base peak at *m/e* 301. The other characteristic peaks in the spectrum were at *m/e* 230 and 199. The ions at *m/e* 231 and 230 were formed by breakage of the C_5–C_{13} bond and by loss of the ring C, respectively. Treatment of the base with acetic anhydride/pyridine afforded a diacetyl derivative (**4**). The structure of hernandolinol (**3** without stereochemistry) (13) emerged for the base from the spectral data presented above. Comparison of the physical constants revealed that there was a marked difference in their specific rotation. The specific rotation of the base was $[\alpha]^{27}D-16°$ (c, 1.08 EtOH) and that of hernandolinol was $[\alpha]D-97.9°$ (EtOH) (13). The base, however, was characterized as an epimer of hernandolinol as follows. Reduction of aknadinin (**2**) (12) with $NaBH_4$ gave a mixture of epimeric alcohols, alcohol A and alcohol B which were separated by careful preparative thin layer chromatography. Alcohol A was found to be identical (co-tlc, ir, ms and nmr) with epihernandolinol (**3**).

The other tertiary alkaloids isolated from the plant were isotetrandrine (**8**), (14) cycleanine (**10**) (14), and isochondodendrine (**9**) (14), all well characterized bisbenzylisoquinoline alkaloids.

N-Methylcorydalmine (**5**) isolated from the quaternary alkaloidal fraction had uv absorption maxima at 220 and 280 nm which shifted to 297 nm on addition of alkali. In the nmr spectrum of the base there were signals for one *N*-methyl and three aromatic methoxy groups. The four protons in the aromatic region

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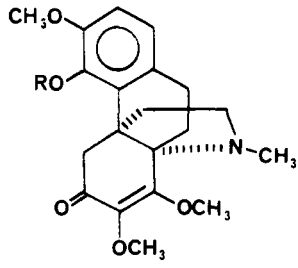

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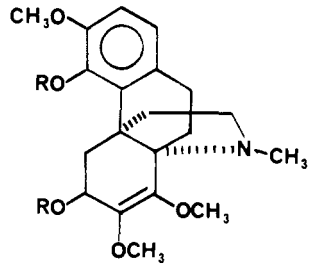
2 R=H



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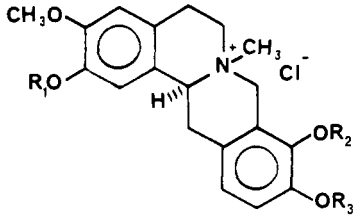
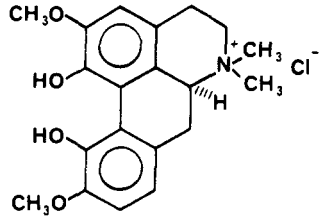
4 R=$COCH_3$



5 R_1=R_2=CH_3, R_3=H
6 R_1=R_2=H, R_3=CH_3



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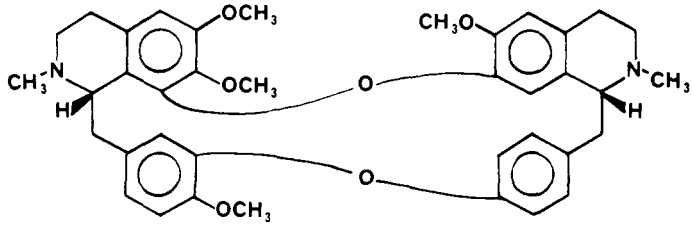

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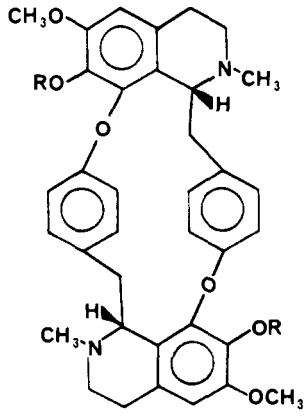

9 R=H
10 R=CH_3

resonated at τ 3.34 (s, 2H, C-$_1$, and C-$_4$, -H), 2.90 (d, 1H, J 10 Hz, C-11=H) and 3.00 (d, J 10 Hz, 1H, C-12-H). The mass fragmentation pattern of the base was in agreement with the structure (**5**). Direct comparison (m.p., co-tlc, ir, ms, nmr) of base chloride with *N*-methylcorydalmine chloride prepared from corydalmine (15) established the identity.

The two other quaternary alkaloids isolated from the plant were characterized as cyclanoline (**6**) (16) and magnoflorine (**7**) (17).

EXPERIMENTAL

SOURCE OF PLANT MATERIAL.—The leaves, stems, and roots of *Stephania elegans* (Menispermaceae) for the present investigation were collected from Dehra Dun, Uttar Pradesh, India.

EXTRACTION.—Air-dried powdered plant material (3 kg) was percolated with ethanol (5 x 4 liters). When the solvent from the percolate was removed under 40° under reduced pressure, a greenish viscous mass resulted. It was extracted with 5% HCl (10 x 120 ml). The aqueous acidic extract was defatted with petroleum ether (4 x 200 ml), basified (pH9 with Na_2CO_3, and the liberated bases were extracted with chloroform (5 x 200 ml). The chloroform extract was washed with water and dried (anhyd. Na_2SO_4); when the solvent was removed a mixture of tertiary alkaloids (5 g) was obtained. The remaining aqueous alkaline solution, when extracted with *n*-butanol (4 x 100 ml), furnished a mixture of quaternary alkaloids.

ISOLATION OF BASES.—The mixture of tertiary bases (5 g) was chromatographed on a column of neutral alumina (200 g). The column was successively eluted (tlc control) with benzene, benzene-chloroform (3:1, 1:1, 1:3) chloroform:methanol, with an increasing proportion of methanol. A total of 130 fractions of 100 ml each were collected.

HASUBANONINE (**1**) AND AKNADININ (**2**).—The residue from fractions 1 to 25 (eluted with benzene), a mixture of mainly two compounds, was subjected to preparative tlc (plates: silica gel G; solvent: chloroform:methanol, 98:2). The two major bands, so resolved, were cut. Elution of the less polar band with chloroform:methanol (80:20) gave a nonphenolic base (**1**) (40 mg) mp 114°; $[\alpha]^{27}$°D −214° (c, 2.0 MeOH); λ max (MeOH) 228 and 270 nm; ν max (KBr) 3350, 2900, 1660, 1600 cm^{-1}; nmr ($CDCl_3$): τ 7.58 (N CH_3), 6.05, 6.18, 6.30 and 6.48 (4 x OCH_3), 3.50 (bs, 2H, ArH), 6.88 (d, J=15 Hz, 1H); ms: 373 (M^+), 315 (M^+-58), 245 (M^+-128), 244 (M^+-129), 230 and 213.

The physical constants and spectral data of the nonphenolic base were almost identical with the reported data for hasubanonin (**11**) (12) mp 116.5°; $[\alpha]$D −218° (MeOH).

Elution of the more polar band with chloroform:methanol (80:20) gave the phenolic base (**2**) (110 mg), mp 66-70°; $[\alpha]^{27}$°D −289° (c, 1.80 MeOH): λ max (MeOH) 223 and 266 nm; λ max (MeOH)-NaOH, 270 nm; ν max ($CHCl_3$) 3550, 1640, 1600, 1480 and 1430 cm^{-1}; nmr ($CDCl_3$): τ 7.46 (N CH_3), 6.00, 6.25, 6.42 (3 x OCH_3); ms: m/e 359 (M^+), 301 (M^+-58), 231 (base peak) (M^+-128) and 230.

The physical constants and spectral data of the phenolic base were found to be identical with the reported data for aknadinin (**2**) (12).

EPIHERNANDOLINOL (**3**).—Fractions 26 to 40 (elution with benzene:chloroform, 3:1) were mixed, and the solvent was removed. The residue showing one major spot on tlc was subjected to preparative tlc on silica gel G plates (solvent: chloroform:methanol, 96:4). The main band on the plates was cut and eluted with chloroform:methanol (80:20) to give a phenolic base (**3**) (90 mg) as an oil; λ max (MeOH) 237 and 284 nm; λ max (MeOH-NaOH) 289 nm; ν max ($CHCl_3$) 3360, 2910, 1480, 1436, 1270 and 1210 cm^{-1}; nmr ($CDCl_3$): τ 7.60 (1 x N CH_3), 6.50 (1 x OCH_3) and 6.30 (2 x OCH_3), 3.52 (d, 1H, J=7.5 Hz, Ar-*H*), 3.50 (d, 1H, J=7.5 Hz, Ar-*H*); ms: *m/e* 361 (M^-), 231 (base peak) (M^--130), 230 (M^+-131) and 191.

ISOTETRANDRINE (**8**).—Fractions 40 to 55 (elution with benzene-chloroform, 1:1) were mixed, and the solvent was removed. The residue was subjected to preparative tlc on silica gel G (solvent: chloroform:methanol, 92:8). The major band on the plates was cut and eluted with chloroform:methanol (90:10) to give a nonphenolic base (**8**) (70 mg) mp 182-84°; $[\alpha]^{27}$°D +158° (c, 1.00 $CHCl_3$); λ max (MeOH) 238 and 283 nm, no change in MeOH-NaOH; nmr ($CDCl_3$): τ 7.52, 7.82 (2N CH_3), 6.18, 6.34, 6.48 and 6.90 (4 x OCH_3); ms: *m/e* 622 (M^+), 621, 485, 431, 396, 395, 381, 198 (base peak), 175.

The base was found to be identical (mp, mmp, co-tlc, ir, nmr and ms) with an authentic sample of isotetrandrine (**8**) (14) mp 180-82°, $[\alpha]$D +151° ($CHCl_3$).

CYCLEANINE (**10**).—Fractions 56 to 68 (elution with benzene:chloroform; 1:1) were mixed, and the solvent was removed. The residue was subjected to preparative tlc on silica gel G plates (solvent: chloroform:methanol, 92:8). The major band on the plates was cut and eluted with chloroform:methanol (9:1) to give a nonphenolic base (**10**) (120 mg), mp 280°; $[\alpha]^{27}$°D −134.4° (c, 1.00 MeOH): λ max (MeOH) 232 and 276 nm; no change in MeOH-NaOH; ν max (KBr) 2920, 1600, 1575, 1480, 1410, 1370, 1330, 1290, and 1220 cm^{-1}; nmr ($CDCl_3$): τ 7.70, 7.62 (2 x N CH_3), 6.32 (2 x OCH_3), 6.26 (2 x OCH_3); ms: *m/e* 622 (M^-), 621, 313, 312 (base peak), 311, 204, 190 and 174.

The base was found to be identical (mp, mmp, co-tlc, nmr and ms) with cycleanine (**10**) (14) mp 280°.

ISOCHONDRODENDRINE (**9**).—Fractions 90 to 110 (elution with chloroform:methanol, 95:5) were mixed, and the solvent was removed. The residue was subjected to preparative tlc of silica gel G (solvent: methanol-chloroform, 90:10). The major band on the plates was cut and eluted with chloroform:methanol (80:20) to give the phenolic base (**9**) (60 mg), mp 310–11°; $[\alpha]^{27}D+59°$ (c, 1.0 pyridine); λ max (MeOH) 283 nm; ν max (KBr) 3350, 1560, 1500 and 1380 cm^{-1}; nmr ($CDCl_3$): τ 7.70, 7.68 (2 x N CH_3), 6.25 (2 x OCH_3); ms: 594 (M^+), 298 (base peak), 297, 207, 206 and 191.

The base was found to be identical (mp, mmp, co-tlc, nmr and ms) with isochondrodendrine (**9**) (16), mp 309–10°.

THE QUATERNARY BASES.—The mixture of quaternary bases (1 g) was subjected to preparative tlc (plate: silica gel G; solvent: methanol; ammonium hydroxide solution:water, 8:1:1). The plates were run again 2 to 3 times for maximum separation of the bases. The major bands on the plates were cut and eluted with methanol. The following quaternary alkaloids were isolated and characterized:

N-Methylcorydalmine Chloride (**5**), mp 190°; λ max (MeOH) 220 and 280 nm; λ max (MeOH–NaOH) 297 nm; ν max (KBr) 3450, 2910, 1610 and 1450 cm^{-1}; nmr (TFA): τ 6.95 (1 x N CH_3), 6.04 (1 x OCH_3), 6.03 (2 x OCH_3), 3.34 (s, 2H, Ar-H), 2.90 (d, 1H, $J=10$ Hz, C_{11}-H) and 3.00 (d, 1H, $J=10$ Hz, C_{12}-H); ms: *m/e* 356 (M^+), 341 (M^+–15), 325, 310, 190, 150 and 149. The compound was found identical (mp, co-tlc, ms and nmr) with *N-methylcorydalmine chloride* prepared from corydalmine (15).

Cyclanoline chloride (**6**), mp 214–18°; $[\alpha]^{27}D-133°$ (c, 1.0 MeOH); nmr (D_2O): τ 7.32 (1 x N CH_3), 6.14 (2 x OCH_3), 2.62 (s, 2H, Ar-H) and 2.55 (s, 1H, Ar-H); ms: *m/e* 242 (M^+), 327 (M^+–15), 178, 176, 150 and 135.

The base was found to be identical (mp, mmp, co-tlc, nmr) with an authentic sample of cyclanoline (16) mp 215–20°; $[\alpha]^{27}D-129°$ (MeOH).

Magnoflorine chloride (**7**), mp 202–03° (decomp.): λ max (MeOH) 225 and 273 nm; λ max (MeOH–NaCH) 277 and 312 nm; ν max (KBr) 3380, 2280, 1605, 1550, 1450, 1403, 1110 and 920 cm^{-1}; nmr (D_2O): τ 6.75 (bs, 6H, $N^+(CH_3)_2$, 6.23 C_2-OMe and C_{11}-OMe), 3.5 (s, 1H, C_8-H), 2.92 (bs, 2H, C_5 and C_9; H).

The base was found to be identical (mp, co-tlc, nmr) with an authentic sample of magnoflorine chloride (7) (17).

ALCOHOL A AND ALCOHOL B.—$NaBH_4$ (250 mg) was added to a solution of aknadinin (**2**) (210 mg) in methanol (5 ml) at 0° during 1 hr. The resulting mixture was worked up after 3 hr. The mixture of epimeric alcohols (200 mg) was subjected to preparative tlc (plates—silica gel G; solvent: chloroform:methanol, 96:4). Two major bands that separated on the plates were cut. Elution of the more polar band with chloroform:methanol (80:20) gave alcohol A (72 mg), an oil, λ max (MeOH) 236 and 286 nm; λ max (MeOH–NaOH) 290 nm; ν max ($CHCl_3$) 3364, 2917, 1484, 1438, 1264 and 1234 cm^{-1}; nmr ($CDCl_3$): τ 7.62 (1 x N CH_3), 6.52 (1 x OCH_3) and 6.32 (2 x OCH_3), 3.50 (d, 1H, $J=7.5$ Hz, Ar-*H*), 3.52 (d, 1H, $J=7.5$ Hz, Ar-*H*); ms: *m/e* 361 (M^-), 231 (base peak) (M^-–130), 230 (M^+–131) and 191.

Elution of the less polar band with chloroform:methanol (80:20) gave alcohol B (37 mg), an oil, λ max (MeOH) 235 and 285 nm; λ max (MeOH–NaOH) 292 nm; ν max ($CHCl_3$) 3350, 2875, 1460 and 1260 cm^{-1}; nmr ($CDCl_3$) τ 7.58 (1 x N CH_3), 6.49 (1 x OCH_3) and 6.28 (2 x OCH_3), 3.50 (d, 1H, 7.5 Hz, Ar-*H*), 3.49 (d, 1H, $J=7.5$ Hz, Ar-*H*); ms: *m/e* 361 (M^-), 231 (base peak) (M^-–130), 230 (M^+–131) and 191.

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