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STENANTHERINE AND *N*-METHYLSTENANTHERINE, NEW APORPHINES FROM *NEOSTENANTHERA GABONENSIS*[1]

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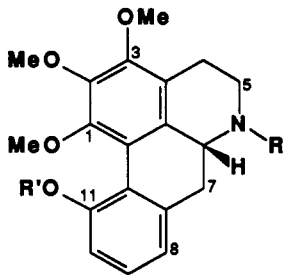
Neostenanthera gabonensis (Engl. & Diels) Exell (Annonaceae) is a forest shrub or a small tree growing in West Africa, where it is used in local folk medicine (1). From two different samples of *N. gabonensis*, both collected in Ghana, we isolated the new alkaloids (−)-stenantherine [**1**] and (−)-*N*-methylstenantherine [**2**]. To our knowledge this is the first phytochemical study on a plant from the genus *Neostenanthera* (2).

Stenantherine [**1**] was isolated as a main alkaloid from both plant samples. Its eims exhibits the molecular ion as base peak at *m/z* 327.14706 ($C_{19}H_{21}NO_4$) and intensive fragment ions at *m/z* 326 $[M-H]^+$ and *m/z* 296 $[M-OMe]^+$. In the uv spectrum (λ max 217, 274, 296 nm) a bathochromic shift upon addition of alkali indicates phenolic function(s). The ^1H nmr shows the characteristic features of a noraporphine alkaloid substituted by three "aromatic" methoxy groups (δ 3.74, 3.93, and 3.98 ppm) and one phenolic hydroxy group (δ 8.63 ppm); the chemical shifts and coupling constants of the three aromatic protons indicate that positions 1, 2, 3, and 11 of the noraporphine ring system are substituted. Since the ms of *N,O*-diacetylstenantherine [**3**] shows a significant loss of 32 mu (MeOH) from the molecular ion, one methoxy group has to be placed at C-3 (3). In the ^{13}C nmr of **1** the resonances of the three methoxy carbons appear at δ 62.6, 61.4, and 60.4 ppm, respectively. These data exclude a methoxy group at C-11 (4) and establish structure **1** for (−)-stenantherine. (−)-*N*-Methylstenantherine [**2**] was found in trace amounts only. The spectra and the color reaction on tlc show close similarity to **1**. Structure **2** was confirmed by *N*-methylation of **1**, which yields a product identical with natural **2** (cd, co-tlc, ^1H nmr). In the ^1H nmr of *O*-acetyl-*N*-methylstenantherine [**4**] significant downfield shifts (in comparison with **1** and **2**) of the three aromatic protons and a strong shielding (δ 3.44 ppm) of the methoxy group at C-1 are observed, whereas all other resonances remain practically unaffected.



1 R=R′=H
2 R=Me, R′=H
3 R=R′=Ac
4 R=Me, R′=Ac

N. gabonensis is a rare plant in Ghana[2] and, therefore, only scarce amounts of plant material were available for our investigations. The first plant collection contained stems exclusively. From this material only one further alkaloid was isolated besides **1** and **2** in trace amounts, and it was identified as the known nor-aporphine (−)-*O*-methyliso-

[1]Part 32 in the series "Constituents of Tropical Medicinal Plants." For Part 31, see H. Achenbach, M. Stöcker, and M.A. Constenla, *Phytochemistry*, **27**, 1835 (1988).

[2]A.A. Enti, Forestry Enterprises, Legon. Ghana, private communication.

piline (5). The plant material of the second collection consisted of roots (60%) and stems (40%). Compounds **1** and **2** occurred in concentrations similar to those found in the first sample, but, in addition, eight further alkaloids were isolated and identified as the known aporphines (−)-liridinine (minor alkaloid), (−)-*O*-methylisopiline (minor alkaloid), *O*-methylmoschatoline (minor alkaloid), (+)-isopiline (major alkaloid), (+)-*N*-methylisopiline (trace), (+)-lirinidine (minor alkaloid), (+)-caaverine (trace) (5), and the proaporphine (−)-*N*-methylcrotsparine (6).

Up to now the (+)-enantiomers of isopiline and *N*-methylisopiline are not reported in the literature. The co-occurrence of (−)- and (+)-aporphines in *N. gabonensis* might be of interest from a biogenetic point of view.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analytical tlc was performed on precoated plates (Nano plates Sil-20 UV, Macherey-Nagel) using the three systems: S-1 = EtOAc-MeOH (9:1), S-2 = $CHCl_3$-Et_2NH (9:1), S-3 = $CHCl_3$-MeOH (9:1); detection: uv and ceric ammonium sulfate reagent (7). Uv and cd measurements were run in MeOH unless indicated otherwise. Mass spectra were obtained by eims at 70 eV. Unless otherwise stated, ^1H nmr were recorded at 400 MHz and ^{13}C nmr at 100 MHz in $CDCl_3$ with TMS as the internal standard.

PLANT MATERIAL.—*N. gabonensis* was collected in March 1985 (stems) at Ankasa Forest Reserve (Ghana) and in July 1987 (stems and roots) at Neung Forest Reserve (Tarkwa District, Ghana) and identified by Mr. A.A. Enti (Forestry Enterprises, Legon, Ghana). Voucher specimens (no. 85-02 and no. 87-01) are deposited in our institute in Erlangen.

EXTRACTION AND ISOLATION.—Ground stems (250 g) were extracted successively with petroleum ether and then MeOH at room temperature yielding 0.85 g and 1.6 g of crude extracts, respectively. Workup of the MeOH extract with 5% HOAc in the usual manner (3) yielded 60 mg crude alkaloid mixture (extract A).

Roots (60 g) mixed with stems (45 g) were ground and extracted as described above with petroleum ether (0.5 g residue) and MeOH. The MeOH extract was concentrated to 200 ml at 200 hectoPascal, diluted with H_2O (200 ml), and then extracted successively with petroleum ether (0.4 g residue) and $CHCl_3$ to yield 0.38 g residue (extract B).

Extracts A and B were subjected to cc on Al_2O_3 (basic, activity II–III, Woelm-ICN) using $CHCl_3$ and $CHCl_3$/MeOH (increasing concentrations of MeOH). The resulting fractions were subjected to further cc over Si gel (Macherey-Nagel no. 81538) using EtOAc/MeOH or $CHCl_3$/MeOH to yield the individual alkaloids. Final purification of the alkaloids was achieved by cc over Fractogel PVA-500 (Merck) with MeOH as the eluent.

(−)-STENANTHERINE [**1**].—Colorless oil (3 mg from extract A and 6 mg from extract B); tlc R_f 0.13 (S-1), 0.59 (S-2), 0.34 (S-3), pale orange; ir ($CHCl_3$) 3240 cm^{-1} (br, OH, NH); uv λ max (EtOH) 217 (log ε 4.62), 267 (sh), 274 (4.14), 296 nm (3.82); λ max (EtOH/KOH) 267 (sh), 274, 333 nm; cd (EtOH) Δε (nm) 0 (350), +12 (292), +18 (271), 0 (246), −70 (233), 0 (223), +36 (212); $[\alpha]^{20}D$ −140° (c = 0.1, EtOH); ^1H nmr δ 2.65 (dd, $J_1 = J_2$ = 13.5 Hz, 1H), 2.76–2.94 (m, 4H), 3.39 (m, 1H), 3.69 (m, 1H), 3.74 (s, OMe-1), 3.93 (s, OMe), 3.98 (s, OMe), 6.87 (br d, J = 7.2 Hz, H-8 or H-10), 6.99 (br d, J = 8 Hz, H-10 or H-8), 7.19 (dd, J_1 = 8 Hz, J_2 = 7.2 Hz, H-9), 8.63 (s, HO-11); ^{13}C nmr δ 23.8 (C-4), 38.3 (C-7), 42.5 (C-5), 54.1 (C-6a), 60.4, 61.4, and 62.6 (3 × OMe), 118.6 (C-10), 119.7 (C-11a or C-11b), 120.1 (C-8), 120.9 (C-11b or C-11a), 124.5 (C-3a), 128.9 (C-9), 133.2 (C-11c), 137.7 (C-7a), 144.7 (C-2), 147.0 (C-1), 150.8 (C-3), 153.7 (C-11); ms *m/z* (rel. int.) $[M]^+$ 327.14706 ($C_{19}H_{21}NO_4$, 100), 326 (45), 312 (49), 310 (15), 297 (9), 296 (54), 280 (8).

N,O-DIACETYLSTENANTHERINE [**3**].—Stenantherine (0.2 mg) was treated with Ac_2O/pyridine at room temperature for 10 h yielding a homogeneous product: tlc R_f 0.50 (S-1), 0.69 (S-2), pale green; ms *m/z* $[M]^+$ 411 (15), 380 (22), 379 (100), 339 (40), 338 (30), 337 (95), 298 (10), 297 (30), 296 (11), 295 (34), 265 (13), 250 (12).

N-METHYLATION OF **1**.—Stenantherine (3 mg) dissolved in MeCN (2 ml) was stirred with 5 drops of aqueous formaldehyde (37%) and $NaB(CN)H_3$ (2 mg) for 30 min at room temperature. After evaporation of the solvent, 2 N KOH (4 ml) was added and the product extracted with Et_2O (2 × 4 ml). Purification by cc yielded *N*-methylstenantherine [**2**] (1.6 mg) as a colorless oil: $[\alpha]^{20}D$ −145° (c = 0.11, EtOH); cd, ^1H nmr, and co-tlc identical with the natural compound described below.

(−)-*N*-METHYLSTENANTHERINE [**2**].—Colorless oil (0.7 mg from extract A and 0.3 mg from extract B): tlc R_f 0.31 (S-1), 0.69 (S-2), pale

orange; uv λ max 216 (log ε 4.61), 266 (sh), 272 (4.12), 395 nm (sh) (3.85) cd Δε (nm) 0 (350), +7 (300), +8 (270), 0 (247), −55 (234), 0 (223), +16 (213); ^{1}H nmr δ 2.40 (ddd, $J_1=J_2=$ 11.8 Hz, $J_3=4$ Hz, 1H), 2.50 (dd, $J_1=J_2=$ 13.5 Hz, 1H), 2.54 (s, NMe), 2.78–2.99 (m, 3H), 3.04–3.11 (m, 2H), 3.73 (s, OMe-1), 3.92 (s, OMe), 3.97 (s, OMe), 6.89 (d, $J=7.5$ Hz, H-8 or H-10), 7.00 (d, $J=8$ Hz, H-10 or H-8), 7.20 (dd, $J_1=8$ Hz, $J_2=7.5$ Hz, H-9), 8.58 (s, HO-11); ms *m/z* [M]$^+$ 341 (100), 340 (27), 327 (15), 326 (84), 324 (29), 311 (19), 310 (99), 294 (17).

O-ACETYL-*N*-METHYLSTENANTHERINE [**4**].—Compound **2** (2 mg) was treated with Ac_2O-pyridine (1:1) for 15 h at room temperature. Purification by cc on Si gel gave **4** (2 mg) as a colorless oil: tlc R_f 0.31 (S-1), 0.69 (S-2), pale green; [α]^{20}D −60° ($c=0.10$, EtOH); uv λ max 216, 272 nm; ^{1}H nmr δ 2.25 (s, MeCOO), 2.43 (ddd, $J_1=J_2=11.8$ Hz, $J_3=4$ Hz, 1H), 2.49 (br dd, $J_1=J_2=13$ Hz, 1H), 2.53 (s, NMe), 2.77–2.97 (m, 3H), 3.03–3.13 (m, 2H), 3.44 (s, OMe-1), 3.92 (s, OMe), 3.96 (s, OMe), 7.13 (br d, $J=8$ Hz, H-8 or H-10), 7.16 (br d, $J=7.5$ Hz, H-10 or H-8), 7.27 (dd, $J_1=8$ Hz, $J_2=7.5$ Hz, H-9); ms *m/z* [M]$^+$ 383 (100), 382 (9), 369 (10), 368 (51), 352 (43), 340 (9), 326 (14), 325 (14), 324 (93), 310 (18).

(−)-*O*-METHYLISOPILINE.—Colorless oil (0.4 mg from extract A and 1.7 mg from extract B): tlc R_f 0.11 (S-1), 0.30 (S-3), pale green; [α]^{20}D −76° ($c=0.11$, MeOH); uv, ^{1}H nmr (90 MHz), and ms in accordance with published data (5); identical on co-tlc with an authentic sample (3).

O-METHYLMOSCHATOLINE.—Yellow oil (1.5 mg): tlc R_f 0.50 (S-1), 0.70 (S-2), purple-red; uv, ^{1}H nmr, and ms in accordance with published data (5).

(−)-NORLIRIDININE.—Colorless oil (2 mg): tlc R_f 0.11 (S-1), 0.09 (S-2), 0.17 (S-3), red violet; [α]^{20}D −65° ($c=0.13$, MeOH); uv, ^{1}H nmr, and ms in accordance with published data (5).

(+)-ISOPILINE.—Colorless oil (12 mg): tlc R_f 0.10 (S-1), 0.56 (S-2), gray; [α]^{20}D +55° ($c=0.15$, MeOH); uv λ max 273, 291 (sh), 311 nm, λ max (MeOH/NaOH) 256 (sh), 275, 341 nm; cd Δε (nm) 0 (320), −5 (290), −7 (270), 0 (251), +44 (235), 0 (223), −15 (216); ^{1}H nmr δ 2.71–2.99 (m, 5H), 3.42 (m, 1H), 3.85 (m, 1H), 3.88 (s, OMe), 3.95 (s, OMe), 7.17 (ddd, $J_1=J_2=7.5$ Hz, $J_3=1.5$ Hz, H-9), 7.22 (br d, $J\sim8$ Hz, H-8), 7.30 (br dd, $J_1\sim J_2\sim8$ Hz, H-10), 8.32 (d, $J\sim8$ Hz, H-11); ^{13}C nmr (22.5 MHz) and ms in accordance with published data (5).

(+)-*N*-METHYLISOPILINE.—Colorless oil (0.5 mg): tlc R_f 0.26 (S-1), 0.64 (S-2), pale gray; uv λ max 272, 291 (sh), 310 nm, λ max (MeOH/NaOH) 272 (sh), 340 nm; cd Δε (nm) 0 (320), −5 (290), −7 (267), 0 (251), +42 (235), 0 (223), −14 (217); ms *m/z* [M]$^+$ 311 (100), 310 (71), 297 (12), 296 (47), 294 (14), 280 (22), 268 (30), 253 (13); identical with the *N*-methylation product of (+)-isopiline (cd, co-tlc).

N-METHYLATION OF (+)-ISOPILINE.—Isopiline (3 mg) was subjected to the *N*-methylation procedure described above for **1**. The product was extracted from the aqueous solution at pH 8 with $CHCl_3$. Purification by cc over Si gel yielded 1 mg as a colorless oil: [α]^{20}D +53° ($c=0.05$, MeOH); ^{1}H nmr (90 MHz) in accordance with published data (5).

(+)-LIRINIDINE.—Colorless oil (1 mg): tlc R_f 0.15 (S-1), 0.63 (S-2), violet; [α]^{20}D +93° ($c=0.05$, MeOH); uv, ^{1}H nmr, and ms in accordance with published data (5).

(+)-CAAVERINE.—Colorless oil (0.3 mg): tlc R_f 0.05 (S-1), 0.50 (S-2), violet; uv λ max 231 (sh), 271, 310 nm; cd Δε (nm) 0 (295), −11 (270), 0 (252), +65 (232), 0 (217), negative tail below 217 nm; ms *m/z* [M]$^+$ 267 (61), 266 (100), 251 (6), 250 (8), 238 (7); on tlc (S-2) different from an authentic sample of asimilobine (8); *N*-methylation as described above yielded a product identical with (+)-liriniidine on tlc (solvent systems S-1 to S-3).

(−)-*N*-METHYLCROTSPARINE.—Colorless oil (2 mg): tlc R_f 0.10 (S-1), 0.56 (S-2), pale yellow; [α]^{20}D −108° ($c=0.18$, $CHCl_3$); uv λ max 233, 286 nm; ^{1}H nmr δ 2.24 (dd, $J_1=12$ Hz, $J_2=10.8$ Hz, H-7$_a$), 2.38 (dd, $J_1=12$ Hz, $J_2=6$ Hz, H-7$_b$), 2.39 (s, NMe), 2.50 (ddd, $J_1=J_2=12$ Hz, $J_3=5.5$ Hz, 1H), 2.78 (br dd, $J_1=16.5$ Hz, $J_2=5$ Hz, 1H), 2.91–3.01 (m, 1H), 3.12 (br dd, $J_1=12$ Hz, $J_2=6.5$ Hz, 1H), 3.45 (br dd, $J_1=10.8$ Hz, $J_2=6$ Hz, H-6a), 3.82 (s, OMe-2), 5.47 (br s, HO-1), 6.30 (dd, $J_1=10$ Hz, $J_2=1.8$ Hz, 1H), 6.40 (dd, $J_1=10$ Hz, $J_2=1.8$ Hz, 1H), 6.58 (s, H-3), 6.87 (dd, $J_1=10$ Hz, $J_2=3$ Hz, 1H), 7.00 (dd, $J_1=10$ Hz, $J_2=3$ Hz, 1H); ms *m/z* [M]$^+$ 297 (100), 296 (37), 268 (37), 254 (21).

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