16-epi-Panarine, a New Betaine-Type Alkaloid from Stemmadenia minima

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16-*EPI*-PANARINE, A NEW BETAINE-TYPE ALKALOID FROM *STEMMADENIA MINIMA*[1,2]

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ABSTRACT.—A novel betaine-type indole alkaloid, 16-*epi*-panarine [**1**], was isolated from *Stemmadenia minima*. Its structure was determined by spectral studies and chemical interconversion to panarine [**2**].

We recently reported the alkaloid screening of different parts of the Central American plant *Stemmadenia minima* A. Gentry (Apocynaceae) (1). From polar fractions of the stem bark we have now isolated a new alkaloid, 16-*epi*-panarine [**1**], whose structure was established by spectroscopic investigation and chemical interconversion.

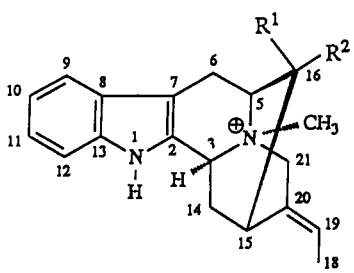
Compound **1** was precipitated from the H_2O-soluble part of the MeOH extract, using Mayer's reagent (2), and subsequently purified by chromatography on Amberlite IRA 400 and Si gel.

The highly polar alkaloid on eims exhibited ions in the high mass range at *m/z* 322 and *m/z* 336. The latter signal is considered to be derived from thermal methylation, which can easily occur with *N*-methylammonium compounds under ms conditions (3).

The elemental composition of **1** was established by hrms together with the number of carbon atoms from ^{13}C nmr analysis. Compound **1** was subjected to extensive ^{1}H- and ^{13}C-nmr studies; homonuclear and heteronuclear 2D measurements demonstrated the ring system to be of the sarpagine type (4) and allowed the assignment of all signals.

The *E* configuration of the ethylidene side chain as well as other configurational information were supported by nOe results (Figure 1).

A structure of this type has recently been described for panarine [**2**] from a Venezuelan curare (5). The spectral data published for **2** are very similar to those of **1**, but some significant differences were also evident. Direct comparison with an authentic sample of **2** demonstrated the non-identity.



1 $R^1=CO_2^{\ominus}$, $R^2=H$
2 $R^1=H$, $R^2=CO_2^{\ominus}$
3 $R^1=CO_2Me$, $R^2=H$
4 $R^1=H$, $R^2=CO_2Me$

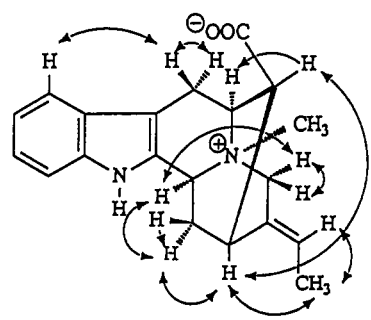


FIGURE 1. Enhancements observed in nOe studies with **1**.

[1]Dedicated to Prof. Dr. Dr.E. Mutschler, Frankfurt, on occasion of his 60th birthday.

[2]Part 41 in the series "Constituents of Tropical Medicinal Plants." For Part 40, see H. Achenbach and H. Hemrich, *Phytochemistry*, in press.

TABLE 1. ^{1}H-nmr Signals of Compounds **1–4** (in CD_3OD, if not otherwise stated).

Proton	Compound									
	1		**1**		**2**		**3**		**4**	
	δ (ppm)[a]	*J* (Hz)	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (HZ)
H-3	4.38 d	10.5	4.80 br d	10.5	4.85 d	10.5	4.99 br d	10.5	4.96 br d	10.5
H-5	3.83 dd	12, 5.5	4.26 dd	11.5, 5.5	4.35 ddm	7.5, 5	4.38 br dd	10.5, 4.5	4.41 dm	7.5, 5
H-6_A	4.04 d	17.5	4.16 br d	17.5	2.97 br d	17.5	3.72 br d	17.5	3.08 br d	17.5
H-6_B	2.73 dd	17.5, 5.5	3.11 d[b]	5.5[b]	3.35 dd	17.5, 5	3.22–3.34[b]		3.41 dd	17.5, 5
H-9	7.38 br d	8	7.47 dd	8, 1	7.50 d	8	7.48 dd	8, 1	7.52 br d	8
H-10	6.92 ddd	8, 8, 1	7.01 ddd	8, 8, 1	7.08 ddd	8, 8, 1	7.08 ddd	8, 7, 1	7.09 ddd	8, 7, 1
H-11	6.99 ddd	8, 8, 1	7.11 ddd	8, 8, 1	7.18 ddd	8, 8, 1	7.18 ddd	8, 7, 1	7.20 ddd	8, 7, 1
H-12	7.21 br d	8	7.30 dd	8, 1	7.37 d	8	7.38 dd	8, 1	7.39 br d	8
H-14_A	2.93 dm[c]	12	3.06[b]		2.20 br dd	13, 5	2.96 br dd	13, 4	2.25 br dd	13, 5
H-14_B	1.98 br dd	12, 10.5	2.26 ddm	12, 10.5	2.48 ddd	13, 10.5, 1.5	2.36 ddm	13, 10.5	2.55 ddd	13, 10.5, 1.5
H-15	3.01 br s		3.20 br s		3.55 m		3.22–3.34[b]		3.59 m	
H-16	2.85 br d	12	3.03 br dd	11.5, 2.5	2.62 dd	7.5, 2	3.22–3.34[b]		3.01 dd	7.5, 2
Me-18	1.38 ddd	7, 1, 1	1.72 ddd	7, 1.5, 1.5	1.70 br d	7	1.72 ddd	7.5, 1.5, 1.5	1.67 ddd	7, 1.5, 1.5
H-19	5.05 br q	7	5.44 br q	7	5.52 br q	7	5.56 br q	7.5	5.58 br q	7
H-21_A	3.87 dm	16.5	4.43 dm	16.5	4.35 br d	15.5	4.54 ddd	15.5, 2, 2	4.46 dm	15.5
H-21_B	3.75 br d	16.5	4.31 br d	16.5	4.21 br d	15.5	4.30 br d	15.5	4.28 br d	15.5
$\overset{\oplus}{=}$N-Me	2.50 s		3.10 s		3.13 s		3.21 s		3.17 s	
$\overline{C}OOCH_3$							3.06 s		3.74 s	

[a]In CD_3OD-C_6D_6 (2:1).
[b]Signals (partly) overlapped.
[c]Doublet-multiplet (=doublet not further resolved).

To establish the stereochemistry at C-16 of **1**, the methyl ester **3** was prepared. The ^{1}H-nmr signal of its methoxy group appeared at δ 3.06 due to the proximity of the aromatic ring system, thus demonstrating the 16*S** configuration (6). To establish the absolute configuration, cd measurements were performed with **1**. The results corroborated the same absolute configuration at C-3 for **1** and panarine [**2**]. The betaine-type alkaloid from *S. minima* is, therefore, established to be 16-*epi*-panarine. Consequently, the methyl ester **3** was treated with potassium *t*-butanolate, and this promptly caused epimerization at C-16 as shown by the characteristic downfield shift of δ_{OMe} from 3.06 to 3.74 ppm. That the epimerization product indeed was panarine methyl ester [**4**] was demonstrated by alkaline hydrolysis, which yielded panarine [**2**] as the only product.

Betaine-type quarternary alkaloids have only occasionally been found in nature.

Compound **1** has to be regarded as a genuine natural product, a status that cannot necessarily be claimed for panarine from the Venezuelan curare preparation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mp was obtained on a Kofler hot-stage apparatus and is uncorrected. Optical rotations, uv, and cd measurements were made in MeOH. Nmr spectra, if not otherwise stated, were recorded in CD_3OD with TMS as the internal standard for ^{1}H nmr at 400 MHz and for ^{13}C nmr at 100 MHz on a Jeol GX 400 instrument. Ms were run by ei at 70 eV on a Finnigan 4500 instrument and high resolution measurements on a MAT 311. If not otherwise stated, Si gel for cc was obtained from Macherey-Nagel (no. 81538). Analytical tlc was performed on precoated Si gel plates (Macherey-Nagel, no. 811023) using H_2O-saturated $CHCl_3$-MeOH (8:2) with detection by uv and ceric ammonium sulfate reagent (7).

PLANT MATERIAL.—*S. minima* was collected in December 1987 and March 1990 in Cerro Jefe, Provincia de Panama, Panama, and identified by Prof. Mireya Correa (Curator of the Herbarium of the University of Panama). A voucher specimen (no. 4968) is kept at the Herbarium of the University of Panama.

EXTRACTION AND ISOLATION.—Stem bark (156 g) was extracted at room temperature with petroleum ether and then with MeOH. The concentrated MeOH extract was suspended in MeOH-H_2O (1:1) and extracted with $CHCl_3$ to afford 3 g of $CHCl_3$ extract. The remaining aqueous solution, after evaporation, was redissolved in H_2O and precipitated at pH 4 with Mayer's reagent (2). The precipitate (130 mg), after being carefully washed with H_2O, was taken up in Me_2CO-MeOH-H_2O (6:2:1) and passed over Amberlite IRA-400® (chloride form) to yield 72 mg of crude alkaloid. Further purification by cc over SiO_2 with H_2O-saturated $CHCl_3$-MeOH (8:2) afforded **1** (21 mg).

16-*epi*-PANARINE [**1**].—Colorless needles: mp 226° (from MeOH); $[\alpha]^{20}D$ −29° (c = 0.7); tlc R_f 0.18, pale yellow with Ce-IV reagent; ir ν max (KBr) 1597 (broad) cm^{-1}; uv λ max 220 (log ε 4.74), 271 (4.00), 282 (sh), 288 nm (3.88); cd Δε (nm) −2.58 (288), −1.22 (284), −1.55 (283), +1.10 (262), −2.38 (230); ^{1}H nmr see Table 1; ^{13}C nmr (δ) 12.65 (C-18), 21.18 (C-6), 27.99 (C-15), 29.16 (C-14), 47.27 (C-16), 49.85 (C$\overset{\oplus}{=}$N-Me), 62.16 (C-3), 65.53 (C-5), 66.49 (C-21), 102.69 (C-7), 112.34 (C-12), 117.93 (C-19), 119.64 (C-9), 120.49 (C-10), 123.32 (C-11), 127.35 (C-8), 132.01 and 132.79 (C-2 and C-20), 138.86 (C-13), 175.26 (-$COO^{\ominus}$); ms *m/z* (rel. int.) 337 (2), 336.1839 (calcd for $C_{21}H_{24}N_2O_2$, 336.1838) (12), 323 (23), $[M]^+$ 322.1681 (calcd for $C_{20}H_{22}N_2O_2$, 322.1681) (100), 321 (14), 308 (16), 307 (27), 291 (7), 278 (16), 277 (40), 263 (25), 250 (25), 249 (24), 247 (22), 246 (22), 235 (21), 218 (19), 217 (17), 207 (14), 206 (27), 194 (20), 183 (35), 182 (13), 180 (27), 169 (34), 168.0688 (calcd for $C_{11}H_8N_2$, 168.0687) (64), 167 (44), 156 (22), 154 (21), 122 (35).

16-*epi*-PANARINE METHYL ESTER [**3**].—Compound **1** was treated in MeOH with thionyl chloride for 12 h at room temperature. Separation of the methylation product from unchanged **1** was achieved by cc on SiO_2. From 15 mg of **1**, 9 mg of **3** was obtained as a colorless oil: tlc R_f 0.38; violet with Ce-IV; $[\alpha]^{20}D$ +33° (c = 0.18); ir ν max ($CHCl_3$) 1730 cm^{-1} (C=O); uv λ max 218 (4.37), 270 (3.65), 282 (sh), 288 nm (3.53); ^{1}H nmr see Table 1; ms *m/z* (rel. int.) 337 (6), $[M-1]^+$ 336.1839 (calcd for $C_{21}H_{24}N_2O_2$, 336.1838) (38), 323 (19), $[M-Me]^+$ 322.1681 (calcd for $C_{20}H_{22}N_2O_2$, 322.1681) (100), 321.1604 (calcd for $C_{20}H_{21}N_2O_2$, 321.1603) (59), 307 (18), 291 (5), 278 (5), 277.1705 (calcd for $C_{19}H_{21}N_2$, 277.1705) (27), 263 (27), 249 (17), 235 (9), 221 (5), 206 (8), 194 (7), 183 (15), 182 (13), 181 (12), 180 (16), 170 (9), 169 (62), 168.0688 (calcd for $C_{11}H_8N_2$, 168.0687) (82), 167 (20), 156 (12), 154 (14), 122 (13).

PANARINE METHYL ESTER [**4**] BY EPIMERIZATION OF **3**.—Compound **3** (5 mg) was treated with

potassium *t*-butanolate in $CHCl_3$ at room temperature for 42 h. After neutralization with 0.1 N HCl, the organic layer gave panarine methyl ester [**4**] (2.5 mg) as a colorless oil: $[\alpha]^{20}D$ −23° (*c* = 0.15); tlc R_f 0.41; green with Ce-IV; iv ν max ($CHCl_3$) 1736 cm^{-1} (C=O); uv λ max 218 (4.48), 268 (3.71), 282 (sh), 288 nm (3.57); ^1H nmr see Table 1; ms *m/z* (rel. int.) 337 (1), 336 (7), 323 (7), $[M-Me]^+$ 322 (40), 321 (30), 308 (22), 307 (26), 279 (7), 278 (26), 277 (17), 264 (18), 263 (39), 249 (21), 247 (18), 235 (14), 233 (10), 232 (8), 221 (13), 220 (10), 207 (13), 206 (12), 195 (13), 194 (14), 193 (11), 184 (7), 183 (33), 182 (38), 181 (26), 180 (26), 170 (18), 169 (81), 168 (100), 167 (39), 166 (16), 158 (9), 157 (16), 156 (32), 155 (16), 154 (29), 144 (17), 143 (19), 135 (10), 134 (10), 130 (25), 129 (17), 128 (25), 122 (45).

PANARINE [**2**] BY HYDROLYSIS OF **4**.—Compound **4** (1 mg) was treated with 0.1 N NaOH in MeOH at room temperature for 8 h. Subsequent neutralization with 0.1 N HCl yielded as the only product a compound (0.8 mg) identical in every respect with an authentic sample of **2**: $[\alpha]^{20}D$ −28 ± 10° (*c* = 0.05); tlc R_f 0.16, gray-green with Ce-IV. Identity of all the nmr signals requires measurement at the same pH.

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