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A NEW SARPAGINE-TYPE ALKALOID, N^1-METHYL-11-HYDROXYMACUSINE A

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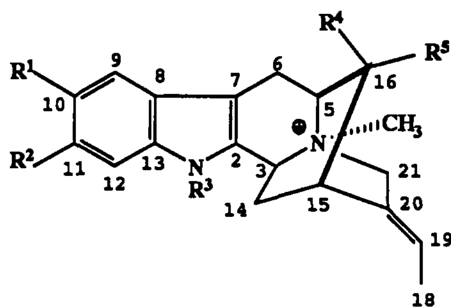
ABSTRACT.—A new quaternary sarpagan alkaloid, N^1-methyl-11-hydroxymacusine A [**1**], has been isolated from the stem bark of a Panamanian species, *Stemmadenia obovata*. Its structure was determined by spectroscopic studies.

Unlike other alkaloid skeletons, sarpagine-type alkaloids have been isolated in quaternary methosalt form from various genera of the Apocynaçeae, namely, *Aspidosperma* (1), *Pleiocarpa* (2,3), *Peschiera* (4,5), *Alstonia* (6), *Rauvolfia* (7,8), *Stemmadenia* (9) and members of the Strychnaceae-*Strychnos* group (10–13). This suggests that facile N^4-methylation occurs at some stage in the biogenesis of this type of compound. N^1-Methyl-11-hydroxymacusine A [**1**] has now been obtained from *Stemmadenia obovata* (Hook & Arn.) K. Schum. (Apocynaceae).

N^1-Methyl-11-hydroxymacusine A [**1**] was isolated in pure crystalline form as a chloride salt by means of adsorption and gel filtration cc. The ir spectrum (KBr) showed adsorption bands attributable to a carbonyl ester (1733 cm^{-1}) and hydroxy (3222 cm^{-1}) groups. The uv spectrum showed maxima at 226 (log ϵ 4.5), 276 (log ϵ 3.77), and 296 nm (log ϵ 3.77), and corresponded to an indole-substituted chromophore (14). The molecular formula ($C_{22}H_{26}O_4N_2$) was determined by hrms (*m/z* ($[M-15]^+$ 382.18715) for the demethylated alkaloid, since the molecular ion (*m/z* 397) was of very low intensity. The presence of the CH_2OH and COOMe groups in the molecule of **1** was indicated by the ms fragment peaks at *m/z* 366, $[M-31]^+$ and 351 $[M-15-31]^+$, and at *m/z* 338 $[M-59]^+$ and *m/z* 337 $[M-1-59]^+$, respectively; evidence of the sarpagan structure was provided by the observation of prominent fragment peaks at *m/z*



	R^1	R^2	R^3	R^4	R^5
1	H	OH	CH_3	$^{17}CH_2OH$	$^{22}COOCH_3$
2	OH	H	CH_3	H	CH_2OH
3	OCH_3	H	H	H	CH_2OH
4	OH	H	H	H	CH_2OH
5	H	OAc	CH_3	$^{17}CH_2OAc$	$^{22}COOCH_3$

198, 199, and 279 (15). The typical fragments at *m/z* 198 and 199 representing an indole moiety have been reported for N^1,N^4-dimethylsarpagine [**2**] and lochneram [**3**] (2); however, the 29.8 ppm shift for one of the three quaternary signals in the ^{13}C-nmr spectrum of **1** showed that an N^1-methyl group was present and pointed to the presence of a hydroxy-substituted benzene ring. The phenolic nature of compound **1** was confirmed by the bathochromic shifted uv maxima when treated with base (236 nm and 316 nm).

The fact that the uv maxima for **1** differed so greatly from those of **2** suggested C-11 functionalization, as is often the case in *Stemmadenia* alkaloids (14), with the possibility of substitution at C-9 or C-12 eliminated in view of the splitting pattern observed in the aromatic region of the ^{1}H-nmr spectrum. The C-11 position was finally determined as the site of substitution for the aromatic hydroxy group from the ^{13}C-nmr data (16) and nOe experiments (17). Thus, irradiation of the 3H singlet at 3.56 ppm enhanced the signal of the aromatic doublet at 6.75 ppm (J=1.9 Hz) by 10%, rather than the doublet signal at 7.31 ppm (J=8 Hz). Consequently, the singlet was therefore assigned as that for an N^1-methyl group.

The ^{1}H-nmr spectrum of **1** (Table 1) is very similar to those of lochneram [**3**] and spegatrine [**4**], both obtained by our group by partial synthesis from the alkaloid lochnerine (18). All ^{1}H-nmr spectral signals from the above compounds were diffused by N^4-quaternization, making it very easy to identify all the protons. In particular, the C-14 position is known to be an unsubstituted site in accordance with biogenetic theory (19), and thus, the H_2-14 protons retain the characteristic chemical shifts and splitting pattern of all the compounds with this type of structure. The normal chemical shift (3.71 ppm) for the carbomethoxy group at C-16 is in accordance with its exo-orientation (9,10), and is the same as that of the alkaloid, polyneuridine (16*R*) (20).

TABLE 1. ^{1}H-Nmr Assignments of Compounds **1** and **5** in CD_3OD (400 MHz).

Position	Compounds			
	1		**5**	
	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)
H-3	5.02 br d	9.7	5.16 br d	10.9
H-5	4.93 d	6.1	5.00 d	4.4
H_2-6	3.26 br s		3.55 br m	
H-9	7.31 d	8.0	7.45 d	8.7
H-10	6.64 dd	8.0, 2.0	6.83 dd	8.6, 2.0
H-12	6.75 d	1.9	7.21 d	1.8
H-14α	2.43 br dd	13.8, 11.5	2.52 br t	12.0
H-14β	2.03 dt	13.7, 1.0	2.15 dt	14.4, 1.1
H-15	3.33 br s		3.43 br s	
H_2-17	3.60 d AB	9.2	3.90 d AB	12.0
	3.67 d AB	9.2	4.27 d AB	12.0
18-Me	1.65 d	6.9	1.65 d	7.3
H-19	5.45 q		5.5 q	
H_2-21	4.26 br d AB	16.1	4.32 br d AB	16.8
	4.37 br d AB	16.1	4.43 br d AB	16.8
N^1-Me	3.56 s		3.64 s	
N^4-Me	3.17 s		3.19 s	
COOMe	3.71 s		3.69 s	
OAc	—		1.93 s	
OAc	—		2.24 s	

The stereochemistry of the ethylidene side-chain of **1** could also be established by nOe measurements, and was shown to have a normal *E* configuration (21). Thus, when the C-18 methyl doublet (1.65 ppm, 3H) was irradiated, the H-15 signal was enhanced by 12%.

The absolute configuration of **1** was deduced from cd measurements. Similar values to those published for 16-*epi*-panarine (9) and panarine (13) were obtained for **1**. These results point to an *S* absolute configuration at C-3.

The presence of a phenolic and aliphatic alcohol group in the molecule was further confirmed by acetylation of **1**, which yielded the diacetyl compound **5**, as was evident from the ^{1}H-nmr spectrum (Table 1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Reichert Thermovar apparatus. Nmr spectra were recorded in CD_3OD for ^{1}H-nmr at 400 MHz and for ^{13}C-nmr at 50.32 MHz on a Bruker AMX-400 spectrometer. High- and low-resolution ms were run on a VG Micromass ZAB-2F spectrometer at 70 eV. The uv spectrum was obtained on a Hewlett-Packard HP-8254-A diode array uv spectrophotometer using EtOH, and cd measurements were made on a Jasco J-600 spectropolarimeter using MeOH. The ir spectra were taken on a Nicolet 5PC Ft-ir spectrometer using a KBr disk. Analytical tlc was performed on F 1500/LS 254 (Schleicher & Schuell) Si gel plates using 20% NH_4OH-MeOH-EtOAc (1:1:5). The alkaloids were detected with an uv lamp, and Dragendorff and $FeCl_3/HOCl_4$ reagents. The stationary phases used for cc were 60 $PF_{254+366}$ [Art. 7748 (Merck)] Si gel and Sephadex LH-20 (Pharmacia).

PLANT MATERIAL.—*Stemmadenia obovata* Benth. was collected in October 1993, at Monagre, Los Santos, Panama by Mrs. C. Galdames and identified by Prof. Mireya Correa (Curator of the Herbarium of the University of Panama). A voucher specimen (No. 1283) is deposited at the Herbarium of the University of Panama.

EXTRACTION AND ISOLATION.—Dried powdered stem bark (3.21 kg) of *S. obovata* was extracted repeatedly at room temperature with 80% EtOH over a period of three months. The combined alcoholic extracts were freed of solvent under reduced pressure below 60°. A quantity of HCl (0.5 N, 500 ml) was added to the viscous residue, which was left to stand for 24 h and then filtered. The residual aqueous alkaline phase, after pH gradient extraction, was acidified (concentrated HCl) to pH 2 and Mayer's Reagent was added until no more precipitate was formed. The precipitate was dissolved in Me_2CO-MeOH-H_2O (6:2:1) and filtered. The solution was run through a column packed with Amberlite IRA-400 (chloride form) and taken to dryness (5.2 g). The crude alkaloidal chloride residue was chromatographed on a Si gel column (6×31 cm) under medium pressure with Me_2CO/MeOH 30% as eluent. Fractions 12–15 containing alkaloids (ca. 200 mg each) were then individually chromatographed on a Sephadex LH-20 column (2×42 cm) with 100% MeOH as eluent. N^1-Methyl-11-hydroxymacusine A [**1**] was crystallized (MeOH/$CHCl_3$) as a pure chloride salt (10 mg) from fraction 13.

N^1-*Methyl-11-hydroxymacusine A chloride salt* [**1**].—Colorless needles: mp>300° (MeOH/$CHCl_3$); $[\alpha]^{25}D$ −76° (*c*=0.167, MeOH); tlc R_f 0.22, orange-brown with $FeCl_3/HClO_4$ reagent; cd $\Delta\epsilon$ (nm) −10.65 (231.3), 0.67 (269.5), −1.82 (292.5); uv, see text; ir ν max (KBr) 3222, 2942, 1733, 1622, 1472, 1230, 1100 cm^{-1}; ms *m/z* 397 (3), 396 (11), 396.20476 (calcd for $C_{23}H_{28}O_4N_2$, 396.20491), 382 (10), 367 (10) 366 (37), 352 (78), 351 (72), 338 (9), 337 (23), 307 (41), 279 (23), 279.14828 (calcd for $C_{18}H_{19}ON_2$, 279.14974), 199 (100), 199.08652 (calcd for $C_{12}H_{11}ON_2$, 199.08714), 198 (95), 186 (55), 184 (55), 168 (21), 167 (18); ^{1}H- nmr data, see Table 1; ^{13}C nmr δ 12.88 (C-18), 20.23 (C-6), 29.48 (C-14), 29.83 (N^1-Me), 31.13 (C-15), 50.22 (N^4-Me), 53.42 (-COO*Me*), 56.62 (C-16), 59.46 (C-3), 63.96 (C-17), 65.75 (C-21), 65.95 (C-5), 95.99 (C-12), 103.00 (C-7), 111.45 (C-10), 119.53 (C-8), 120.61 (C-9), 120.79 (C-19), 128.70 (C-20), 131.39 (C-2), 140.91 (C-13), 155.64 (C-11), 174.15 (*C*OOMe).

N^1-*Methyl-11,17-diacetylmacusine A chloride salt* [**5**].—Compound **1** (5 mg) was treated in pyridine (1 ml) with Ac_2O (0.5 ml) for 48 h at room temperature. The pyridine was removed from the mixture using a high-vacuum pump, yielding 5.5 mg of the diacetyl compound **5**, which was not purified further: tlc R_f 0.45, deep violet with $FeCl_3/HClO_4$; ms *m/z* 481 (0.2), 467 (3), 466 (11), 465 (6), 408 (6), 407 (17), 393 (5), 362 (9), 348 (100), 347 (81), 333 (11), 321 (9), 306 (19), 305 (56), 199 (33), 198 (77), 186 (17), 185 (7), 184 (10), 155 (6); ^{1}H- nmr data, see Table 1; ^{13}C nmr δ 12.82 (C-18), 20.51 (C-6), 20.52 (*C*H_3COO-), 21.04 (*C*H_3COO-), 28.99 (C-14), 30.32 (N^1-Me), 31.25 (C-15), 50.24 (N^4-Me), 53.66 (C-16), 54.45 (COO*Me*), 59.49 (C-3), 65.32 (C-17), 65.40 (C-5), 65.72 (C-21), 102.53 (C-12), 104.37 (C-7), 115.9 (C-10), 119.96 (C-9), 121.97 (C-19), 123.83 (C-8), 127.68 (C-20), 133.88 (C-2), 139.58 (C-13), 148.97 (C-11), 170.94 (CH_3*C*OO), 171.77 (CH_3*C*OO), 172.78 (*C*OOMe).

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