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OBOVATINE, A NEW BISINDOLE ALKALOID FROM *STEMMADENIA OBOVATA*

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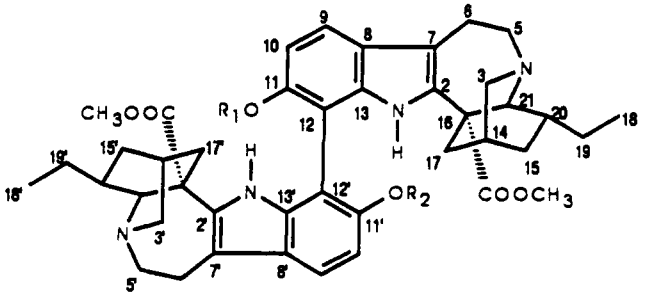
ABSTRACT.—A new bisindole alkaloid, obovatine [**1**], has been isolated from the leaves of *Stemmadenia obovata* together with the known compounds, *bis*[11-hydroxycoronaridin-12-yl] [**2**], 11-hydroxycoronaridine [**5**], and voacristine [**6**]. The structure of **1** was established by spectroscopic analysis and chemical transformations.

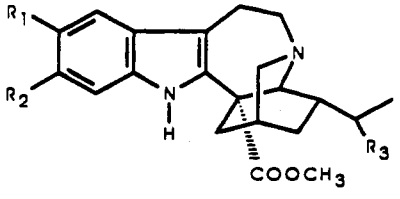
Stemmadenia obovata (Benth.) Woods. (Apocynaceae), is one of ten species (1–7) known to produce ibogaine-type indole alkaloids (8). This particular species has no place in the popular pharmacopeia, unlike, for instance, *S. donnelsmithii*, which is widely used in Central America to treat rheumatism, eye inflammation, and toothache (9). Alkaloids were first obtained from the bark, fruit and seeds of *S. obovata* by Collera *et al.* in 1962 (2) and now a new bisindole alkaloid, obovatine [**1**], has been isolated from the leaves of this species, together with the known alkaloids, *bis*[11-hydroxycoronaridin-12-yl] [**2**] (10), 11-hydroxycoronaridine [**5**] (11), and voacristine (19-hydroxyvoacangine) [**6**] (12).

The $CHCl_3$ extract of the leaves of *S. obovata* was subjected to a combination of Sephadex LH-20 and Si gel chromatography to afford four ibogaine-type indole alkaloids, **1**, **2**, **5**, and **6**. Compound **1** proved to be a new indole alkaloid dimer, obovatine.

Compound **1**, $C_{43}H_{52}N_4O_6$, amor-



1 R_1=H, R_2=Me
2 R_1=R_2=H
3 R_1=R_2=H
3 R_1=R_2=Ac
4 R_1=R_2=Me



5 R_1=R_3=H, R_2=OH
6 R_1=OMe, R_2=H, R_3=OH

phous alkaloid, $[\alpha]^{25}D$ +17.8° (c=0.13, $CHCl_3$), had a molecular ion peak at *m/z* 720 (100%) and significant fragment ions at *m/z* 360 (13%), 208 (5%), 136 (56%), and 135 (13%) characteristic of an ibogaine-type alkaloid (13). The uv spectrum showed absorptions for a substituted indole-type ring, and a bathochromic shift, after base addition, indicated the presence of a phenolic hydroxy group in the indole ring. Ir bands appeared at 3540 cm^{-1} for OH, 3340 cm^{-1} for NH, and at 1720 cm^{-1} for a carbonyl ester.

The structure of **1** was determined by comparison of its ^1H- and ^{13}C-nmr data with those of other ibogaine alkaloids, notably **2**, a symmetrical alkaloid isolated from *Bonafousia tetrastachya* (Humboldt, Bonpland et Kunth) Markgraf (Apocynaceae) (10). The 400 MHz ^1H-nmr spectrum of **1** revealed the presence of two sets of well-defined ortho-coupled aromatic protons at δ 7.52 and 6.91 (J=8.5 Hz) and δ 7.42 and 6.98 (J=8.5 Hz), in consonance with an indole nucleus. The exact chemical shifts of the aromatic moiety and the C-12/C-12′ linkage were ascertained by a long-range heteronuclear multiple bond correlation spectroscopy (HMBC) 2D nmr experiment (Table 1). The presence of two three-proton singlets at δ 3.67 and 3.64 assigned to carbomethoxy groups indicated a dimeric molecule, as did the two NH group singlets at δ 7.37 and 7.35. Proton-carbon chemical shift correlations for all the carbons directly bonded to protons could be established in an HMQC experiment (Table 2).

TABLE 1. HMBC Correlations of **1** (indole moiety) in $CDCl_3$ [δ (ppm)].

H	Correlated C
7.42 (H-9)	134.2 (C-13)/149.4 (C-11)
6.98 (H-10)	124.4 (C-8)/103.1 (C-12)
7.52 (H-9′)	134.4 (C-13′)/153.2 (C-11′)
6.91 (H-10′)	123.1 (C-8′)/102.7 (C-12′)

TABLE 2. Nmr Data of **1** [δ (ppm), $CDCl_3$].

Position	δ_C	DEPT	δ_H
2,2′[a]	137.0, 135.4	C	
3,3′[a]	52.6, 52.4	CH_2	2.90 (4H, sharp dd)
5,5′[a]	53.1 (double)	CH_2	3.40, 3.23 (2H each, m)
6,6′[a]	22.1 (double)	CH_2	3.07, 3.27 (2H each, m)
7,7′[a]	110.7 (double)	C	
8,8′	124.4, 123.1	C	
9,9′	110.1, 119.4	CH	7.42, 7.52 (1H each, d, J=8.5 Hz)
10,10′	106.2, 119.3	CH	6.98, 6.91 (1H each, d, J=8.5 Hz)
11,11′	149.4, 153.2	C	
12,12′	103.1, 102.7	C	
13,13′	134.2, 134.4	C	
14,14′[a]	27.4, 27.3	CH	1.87 (2H, m)
15,15′[a]	32.0, 31.9	CH_2	1.71, 1.13 (2H each, m)
16,16′[a]	55.1, 55.0	C	
17,17′[a]	36.5, 36.1	CH_2	1.87, 2.44 (2H each, m)
18,18′[a]	11.7, 11.6	Me	0.89 (6H, dt, J=7.1 Hz)
19,19′[a]	26.8, 26.7	CH_2	1.56, 1.43 (2H each, m)
20,20′[a]	39.0, 39.0	CH	1.29 (2H, m)
21,21′[a]	58.2, 57.2	CH	3.54 (2H, m)
C=O	175.4, 175.0	C	
OCH_3	52.3, 52.0	Me	3.67, 3.64 (3H each, s)
OCH_3 (Ar)	57.0	Me	3.79 (3H, s)
NH			7.37, 7.35 (1H each, s)

[a]The assignments for designated pairs of carbon and proton signals can be interchanged.

The ^{13}C-nmr spectrum (100 MHz) exhibited 40 signals for the 43 carbon atoms of the molecule, with three corresponding to more than one carbon. The DEPT spectrum revealed the presence of two sp^3 quaternary carbons, fourteen sp^2 quaternary carbons (one of which represented two carbons), six sp^3 methine carbons (two of two carbons apiece), four sp^2 carbon hydrogens, twelve sp^3 methylenes, and five methyl groups (Table 2). The signals for two sp^2 quaternary carbons at δ 149.4 and 153.2 indicated hydroxy and methyl groups attached to their respective aromatic rings, in good agreement with the four sp^2 CH signals. The appearance of four low-field sp^3 methylene carbons at δ 52.5, 52.4, and 53.1 (double) and two methine carbons at δ 57.2 and 58.1 suggested links with a nitrogen atom.

When compound **2** was methylated with CH_2N_2 in Et_2O, the monomethyl derivative **1** and the dimethyl derivative **4** were obtained, confirming chemically the structure of **1**. Compound **4** has not been previously found in nature.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded in $CDCl_3$ for ^{1}H nmr at 400 and 200 MHz, and for ^{13}C nmr at 100 and 50.32 MHz with TMS as internal standard. High- and low-resolution ms were run on a VG Micromass ZAB-2F spectrometer at 70 eV. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter using a Na lamp at 25°. Ir spectra were obtained on a Nicolet 5PC Ft-ir spectrometer using $CHCl_3$. The uv spectra were run on a Hewlett-Packard HP-8254-A diode array uv spectrophotometer. The gel filtration column (Sephadex LH-20) used hexane-MeOH-$CHCl_3$ (2:1:1) as solvent while the eluents for tlc and Si gel cc were *n*-hexane or EtOAc or mixtures thereof. Dragendorff or Ehrlich reagents were used as tlc spray reagents.

PLANT MATERIAL.—Leaves of *Stemmadenia obovata* (Benth.) Woods. were collected by Mrs. Carmen Galdamos in February 1993, in the area of Playa de Monagre, Provincia de Los Santos, Panama, and identified by Prof. Mireya Correa, Director of the Herbarium of the University of Panama. A voucher specimen (FLORPAN 1283) is filed with the Herbarium of the University of Panama.

EXTRACTION AND ISOLATION.—Dried, powdered leaves (678 g) were extracted with hot 90% EtOH. The solvent was evaporated *in vacuo* to a residue that was diluted in 5% HCl to precipitate a deposit. After filtration of the mixture the solution was extracted with $CHCl_3$. The aqueous layer was basified with NH_4OH to pH 10 and extracted exhaustively with $CHCl_3$ followed by drying (Na_2SO_4). Evaporation yielded a dark residue (2.9 g) that was then chromatographed on a Sephadex LH-20 column with *n*-hexane-MeOH-$CHCl_3$ (2:1:1) as eluent, affording **1** in pure form (10.2 mg); the other alkaloid fractions were combined and subjected to repeated cc on Si gel with *n*-hexane and EtOAc mixtures of increasing polarity until only EtOAc was present. The alkaloid fractions were finally purified by prep. tlc to afford three amorphous compounds, **2**, **5**, and **6** in 17.5, 6.5, and 7.8 mg yield, respectively.

Obovatine [**1**].—Amorphous, [α]D 17.8° (*c*=0.13, $CHCl_3$); hrms *m/z* found $[M]^+$ 720.3886, $C_{43}H_{52}N_4O_6$ requires 720.3886; uv (MeOH) λ max (log ε) 234 (4.76), 304 (4.38) nm; +MeONa 238 (4.76), 306 (4.31) nm; ir ($CHCl_3$) ν max 3540, 3340, 1720 cm^{-1}; ms *m/z* 720 ($[M]^+$, 100), 360 (13), 208 (5), 136 (56), 135 (13); ^{1}H- and ^{13}C-nmr data, see Table 2.

Acetylation of **2**.—Ac_2O (1 ml) was added to a solution of **2** (5.2 mg) in pyridine (1 ml) and the mixture stirred at room temperature overnight. The excess pyridine was removed under high vacuum pressure to give a residue (6.8 mg). The residue was chromatographed on Si gel to give **3** (5.0 mg) as an amorphous compound, [α]D 31.8° (*c*=0.06, $CHCl_3$); ^{1}H nmr ($CDCl_3$, 200 MHz) δ 0.87 (6H, t, *J*=7.3 Hz, H_3-18 and H_3-18′), 1.15 (2H, m, H_S-15 and H_S-15′), 1.31 (2H, m, H-20 and H-20′), 1.48 (4H, dq, *J*=7.0 Hz, H_2-19 and H_2-19′), 1.78 (2H, m, H_R-15 and H_R-15′), 1.83 (4H, m, H-14 and H-14′, and H_R-17 and H_R-17′), 1.84 (6H, s, 2×COMe), 2.48 (2H, d, *J*=13.0 Hz, H_S-17 and H_S-17′), 2.87 (4H, m, H_2-3 and H_2-3′), 3.10 (4H, m, H_2-6 and H_2-6′), 3.23 (2H, m, H_R-5 and H_R-5′), 3.38 (2H, m, H_S-5 and H_S-5′), 3.53 (2H, narrow d, *J*=1.1 Hz, H-21 and H-21′), 3.66 (6H, s, 2×COOMe), 6.93 and 7.00 (1H each, d, *J*=8.5 Hz, H-10 and H-10′), 7.40 (2H, br s, 2×NH), 7.45 and 7.55 (1H each, d, *J*=8.5 Hz, H-9, H-9′); ^{13}C nmr ($CDCl_3$, 50.32 MHz) δ 11.62 (C-18 and C-18′), 20.52 (2×COMe), 22.18 (C-6 and C-6′), 26.72 (C-19 and C-19′), 27.31 (C-14 and C-14′), 31.91 (C-15 and C-15′), 36.22 (C-17 and C-17′), 38.81 (C-20 and C-20′), 51.74 (C-3 and C-3′), 52.64 (2×COOMe), 53.05 (C-5 and C-5′), 55.06 (C-16 and C-16′), 57.75 (C-21 and C-21′), 108.62 (C-12 and C-12′), 110.72 (C-7 and C-7′), 114.55 (C-10 and C-10′), 118.95 (C-9 and C-9′), 126.90 (C-8 and C-8′), 134.36 (C-13 and C-13′), 137.63 (C-2 and C-2′), 144.32 (C-11 and C-11′), 169.83 (2×COMe), 174.77 (2×COOMe); hrms *m/z* $[M]^+$ 790.39415, calcd

for $C_{46}H_{54}N_4O_8$ [M]$^+$ 790.34416; ms *m/z* 790 [M]$^+$ (65), 395 (11), 208 (10), 136 (100), 135 (31).

Methylation of **2**.—An ethereal solution of CH_2N_2 was added to a solution of **2** (10.1 mg) in Me_2CO (3 ml) until the yellow color persisted and was then kept in the dark overnight. Removal of the solvent left a residue (12.7 mg) that showed two close running zones under tlc. These two compounds were separated by prep. tlc on Si gel using *n*-hexane and *n*-hexane-EtOAc (7:3), to obtain **1** (7.4 mg) and **4** (3.1 mg).

Dimethylobovatine [**4**].—Amorphous compound, [α]D 23.6° (*c*=0.27, $CHCl_3$); hrms *m/z* [M]$^+$ 734.4060, calcd for $C_{44}H_{54}N_4O_6$ [M]$^+$ 734.4043; ms *m/z* 734 [M]$^+$ (42), 367 (58), 208 (19), 136 (100), 135 (40); ^{1}H nmr ($CDCl_3$, 200 MHz) δ 0.89 (6H, dt, *J*=7.1 Hz, H_3-18 and H_3-18′), 1.14 (2H, m, H_S-15 and H_S-15′), 1.25 (2H, m, H-20 and H-20′), 1.60 (4H, m, H_2-19 and H_2-19′), 1.79 (2H, m, H_R-15 and H_R-15′), 1.84 (2H, m, H_R-17 and H_R-17′), 2.45 (2H, m, H_S-17 and H_S-17′), 2.90 (4H, m, H_2-3 and H_2-3′), 3.07 (2H, m, H_R-6 and H_R-6′), 3.23 (2H, m, H_R-5 and H_R-5′), 3.27 (2H, m, H_S-6 and H_S-6′), 3.40 (2H, m, H_S-5 and H_S-5′), 3.52 (2H, m, H-21 and H-21′), 3.65 (6H, s, 2×COO*Me*), 3.81 (6H, s, 2×ArO*Me*), 6.91 and 6.96 (1H each, d, *J*=8.3 Hz, H-10 and H-10′), 7.40 (2H, br s, 2×N*H*), 7.42 and 7.51 (1H each, d, *J*=8.3 Hz, H-9 and H-9′).

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