Two New Alkaloids from Xestospongia sp., a New Caledonian Sponge

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TWO NEW ALKALOIDS FROM *XESTOSPONGIA* SP., A NEW CALEDONIAN SPONGE[1]

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ABSTRACT.—Five alkaloids have been isolated from a New Caledonian sponge *Xestospongia* sp. These include three known xestospongin derivatives, the new demethylxestospongin B [**1**] and a tetrahydrocarboline derivative **5**. The structures of the new compounds **1** and **5** have been established by nmr studies and comparison with previously described products.

As part of a study of New Caledonian marine organisms, we examined the sponge *Xestospongia* sp., which gave positive results to preliminary in vitro cytotoxicity test on KB cells. This specimen was first identified as *Amphimedon viridis* Duchassaing et Michelotti (1), but reexamination by Prof. C. Lévi (Laboratoire de Biologie des Invertébrés Marins du Museum National d'Histoire Naturelle, Paris) has assigned it to the genus *Xestospongia*. The presence of macrocyclic 1-oxaquinolizidines with vasodilatative properties from the Australian sponge *Xestospongia exigua* was reported in previous publications (2–4).

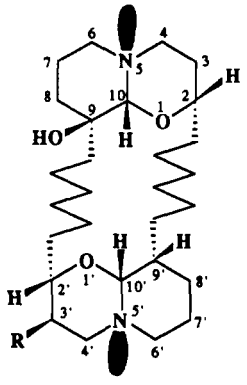
Freeze-dried specimens (100 g) were extracted at room temperature (EtOAc). The organic phase was then extracted with dilute HCl (pH 3). The aqueous phase was basified with aqueous NH_4OH (to pH 10) and extracted again with EtOAc to furnish an alkaloid fraction (3.8 g) which, after SiO_2 flash-chromatography [hexane-Et_2O-MeOH-NH_4OH (20:70:10:0.5)] afforded five alkaloids.

Four of these alkaloids belong to the xestospongin series and three of these were identified as the previously described xestospongin B [**2**] (2), xestospongin D [**3**] (2), and araguspongine F [**4**] (4). Compound **1** was isomeric with xestospongin D ($C_{28}H_{50}N_2O_3$, *m/z* 462, $[\alpha]_D+6°$). Its ir spectrum showed no Bohlman bands, suggesting the absence of a trans quinolizidine system (5,6).

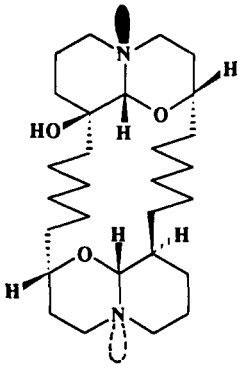
The ^{1}H-nmr spectrum of this alkaloid presented great similarities with that of xestospongin B [**2**]. Particularly diagnostic were the H-10 and H-10′ signals at δ 4.18 (s) and δ 4.41 (d, J=1.5 Hz) indicating a substitution on C-9, a cis relationship between H-10′ and H-9′, and a bis cis quinolizidine system. In ^{13}C nmr we observed the same pattern for products **1** and **2**, with the disappearance of the methyl signal at 14.6 ppm and the shielding of the C-2′ and C-4′. All these indications are in favor of the structure **1** for the new alkaloid, for which we propose the name demethylxestospongin B. The assignments of the reported ^{1}H- and ^{13}C-nmr spectral signals were made by using COSY and XHCORR experiments (Tables 1 and 2).

Comparison of rotations in this series

[1]Part of the programme "Substances Marines d'Intérêt Biologique" carried out by CNRS and ORSTOM in New Caledonia.



1 R=H
2 R=Me



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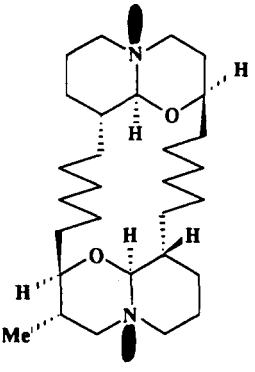

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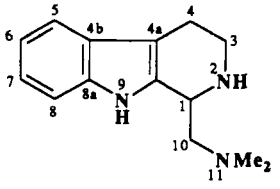
did not allow assignments of absolute configuration since the values are low. Thus we propose the relative and absolute configurations as depicted in formula **1** with reference to the parent compound **2** (2).

The last alkaloid **5**, $C_{14}H_{19}N_3$, optically inactive, presented a characteristic indole absorption pattern in the uv spectrum: λ max (EtOH) nm (log ε) 224 (4.08), 281 (3.51), and 289 (3.47). The mass spectrum gave a molecular ion peak at *m/z* 229 with the base peak at *m/z* 171 and a fragment at *m/z* 58, suggesting a 1,2,3,4-tetrahydrocarboline substituted at C-1.



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The ^{1}H-nmr spectrum showed a signal for two *N*-methyls (δ 2.38), seven aliphatic (δ 2.47–4.16), and four aromatic protons (δ 7.00–7.50) along with one NH proton (δ 9.52). ^{13}C-nmr studies of **5** revealed the presence of 14 carbons,

TABLE 1. ^{1}H-nmr Spectra of Demethylxestospongin B [**1**] and Xestospongin B [**2**] (250 MHz, C_6D_6, δ ppm, *J* in Hz).

Proton	Compound	
	1	**2**[a]
H-2	3.40 br t (10.8)	3.43 br t (11.3)
H-3α	0.65 br d (13.5)	
H-4α	2.60–2.80 m	2.68 br d (13.7)
H-4β	2.80–2.95 m	2.90 ddd (13.7, 13.7, 3.3)
H-6α	3.00–3.20 m	2.95 ddd (13.7, 13.7, 2.7)
H-6β	2.33 ddd (10.3, 2.3, 2.3)	2.09 br d (13.7)
H-10	4.18 s	4.17 s
H-2′	3.40 br t (10.8)	
H-3′α	0.65 br d (13.5)	
H-4′α	2.60–2.80 m	
H-4′β	2.80–2.95 m	
H-6′α	3.30–2.90 m	
H-6′β	2.09 br d (10.2)	2.45 br d (10.2)
H-10′	4.41 br d (1.5)	4.40 br d

[a]Data for this compound are from Nakagawa *et al.* (2).

TABLE 2. ^{13}C-nmr Spectra of Demethylxestospongin B [1] and Xestospongin B [2] (62.53 MHz, C_6D_6, ppm (δ) from TMS).

Carbon	Compound	
	1	2
C-2	76.5[a]	76.3
C-4	52.7[b]	52.7
C-6	45.6[c]	44.7
C-9	70.7	70.6
C-10	91.2	91.1
C-2′	76.2[a]	82.2
C-4′	53.0[b]	61.1
C-6′	45.5[c]	46.6
C-9′	40.7	40.8
C-10′	87.9	87.7

[a,b,c]Values may be inverted.

including two equivalent *N*-methyls (δ 46.1), three sp^3 methylenes (δ 22.5, 43.8 and 64.9), one sp^3 methine (δ 50.0), four aromatic methines, and four aromatic quaternary carbons. The assignment of the protonated carbons was made by the ^{1}H-^{13}C COSY data. The ^{1}H-^{1}H COSY spectrum showed cross peaks for H_2-3/H_2-4 and H-1/H_2-10 indicating the partial structures CH_2-CH_2 (C-3 and C-4) and CH-CH_2 (C-1 and C-10). This connectivity supported the structure **5** for this alkaloid, for which we proposed the name xestoamine. Some β-carbolines with a nitrogen-containing substituent on C-1 have been previously isolated from tunicates of the genus *Eudistoma* (7–9). However, to the best of our knowledge this is the first time that such an alkaloid has been isolated from a *Xestospongia* sponge.

The cytotoxic activity of compounds **1–3** and **5** against KB and L-1210 cells is shown in Table 3. The three macrocyclic compounds demonstrated general cytotoxic activity but did not show any in vivo activity against P-388 leukemia.

TABLE 3. Evaluation of the Cytotoxic Potential of Alkaloids **1–3** and **5**, Expressed as ED_{50} Values (μg/ml).

Compound	Cell line	
	KB	L 1210
1	2.5	0.8
2	2.5	2.0
3	2.0	0.2
5	non toxic	non toxic

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr and ^{13}C-nmr spectra were recorded on Brüker WP-200 (200 MHz), Brüker WP-250 (250 MHz) instruments in $CDCl_3$ or C_6D_6 and referenced to TMS (δ 0.0). Low and high resolution eims (70 eV) were recorded on a Kratos MS-80. Ir spectra were taken on a Infracord Perkin-Elmer.

ANIMAL COLLECTION AND EXTRACTION.—*Xestospongia* sp. was collected by scuba diving at Baie de Prony, New Caledonia, 6–10 m depth, in August 1989, and freeze-dried. Voucher specimens (N° R 602) and underwater photos are available from Zoothèque de Nouméa, New Caledonia. Freeze-dried material was submitted to classical treatment (see above) furnishing 3.8 g of alkaloids which were purified by flash chromatography. Elution with hexane Et_2O–MeOH–NH_4OH (20:70:10:0.5) gave araguspongin F (11 mg), xestospongin B (64 mg), xestospongin D (82 mg), demethylxestospongin B (28 mg), and xestoamine (32 mg).

Xestospongin D [**3**].—^{1}H nmr δ (200 MHz, $CDCl_3$) 4.06 (s, H-10), 3.56 (br t, J=10.8 Hz, H-2), 3.35 (br t, J=10.6 Hz, H-2′), 3.12–2.90 (m, H-6′α, 2×H-4, 2×H-4′, H-10′), 2.77 (br d, J=10.8 Hz, H-6α), 2.34 (ddd, J=10.7, 2.5, 1.5 Hz, H-4), 2.18 (td, J=11.7, 3.5 Hz, H-6′β), 1.99 (td, J=11.0, 3.8 Hz, H-6β).

Demethylxestospongin B [**1**].—Amorphous: $[\alpha]^{20}D$ +6° (c=0.8, $CHCl_3$); ir ν max ($CHCl_3$) cm^{-1} 3450, 2931, 1520, 1500; eims *m/z* (%) $[M]^+$ 462 (100), 444 (8), 434 (16), 419 (8), 405 (12), 390 (19), 377 (6), 152 (10), 102 (10), 96 (16); hreims *m/z* 462 ($C_{28}H_{50}N_2O_3$) (mass measured 462.3785, mass calculated 462.3820); ^{1}H nmr see Table 1; ^{13}C nmr δ (50.33 MHz, C_6D_6) 91.2 (C-10), 87.9 (C-10′), 76.5 and 76.2 (C-2 and C-2′), 70.7 (C-9), 53.0 and 52.7 (C-4 and C-4′), 45.6 and 44.5 (C-6 and C-6′), 40.7 (C-9′), 39.1, 36.8, 36.7, 33.6, 32.8, 32.3, 32.3, 30.1, 30.0, 27.8, 27.1, 26.6, 26.3, 26.0, 25.4, 23.4, 21.3.

Xestoamine **5**.—Amorphous; ir ν max ($CHCl_3$) cm^{-1} 3350, 3200, 2944, 1464, 1320; eims *m/z* (%) 229 (3), 182 (2), 172 (14), 171 (100), 154 (5), 144 (5), 58 (28); ^{1}H nmr δ (200 MHz, $CDCl_3$) 9.52 (br s, H-9), 7.49 (dd, J=7.0, 1.5 Hz, H-5), 7.34 (dd, J=7.0, 1.5 Hz, H-8), 7.14 (td, J=7.0, 1.5 Hz, H-7), 7.07 (td, J=7.0, 1.5 Hz, H-6), 4.16 (dd, J=10.4, 4.6 Hz, H-1), 3.40 (ddd, J=12.7, 4.6, 3.2 Hz, H-3eq), 3.04 (ddd, J=12.7, 8.8, 6.2 Hz,

H-3ax), 2.74 (m, 2×H-4), 2.61 (dd, J=11.7, 10.4 Hz, H-10), 2.47 (dd, J=11.7, 4.6 Hz, H-10), 2.38 (s, 2×N-Me); ^{13}C nmr δ (50.33 MHz, $CDCl_3$) 137.0 (C-9a), 135.6 (C-8a), 127.4 (C-4b), 121.3 (C-7), 119.1 (C-6), 118.0 (C-5), 111.1 (C-8), 107.7 (C-4a), 64.9 (C-10), 50.0 (C-1), 46.1 (2×N-Me), 43.8 (C-3), 22.5 (C-4).

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