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Ptilomycalin D, a Polycyclic Guanidine Alkaloid from the Marine Sponge *Monanchora dianchora*

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A new polycyclic guanidine alkaloid, ptilomycalin D, and the known crambescidic acid were isolated from the marine sponge *Monanchora dianchora* collected in Nosy-Be, northwest of Madagascar, in the Indian Ocean. The structures were elucidated using 1- and 2-D NMR and MS data, and their biogenetic implications are discussed.

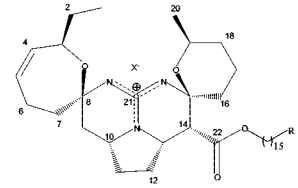
Marine sponges have proved to be a rich source of metabolites possessing novel structural features and biological activities. Among these metabolites, the crambescidin family of guanidinium alkaloids was isolated from a variety of marine sponges.[1–8] The first report of a sponge-derived polycyclic guanidine alkaloid was ptilomycalin A (**1**) (Chart 1), which was isolated from both a Caribbean *Batzella* sp., wrongly identified as *Ptilocaulis spiculifer*, by Soest et al.,[9] and a Red Sea *Hemimycale* sp. by Kashman and co-workers.[1,2] Thereafter, several analogues (hydroxylated ptilomycalins, crambescidins, and isocrambescidins) were isolated from other sponges such as *Crambe-crambe*,[3–5] *Monanchora arbuscula*,[6] and *M. unguiculata*[7] as well as the starfishes *Fromia monilis* and *Celerina heffernani*, probably due to sequestration of these alkaloids from prey sponges.[8] These compounds are unique structural types consisting of a guanidin-containing pentacyclic ring system and vary in substitution at C-14. Polycyclic guanidine alkaloids exhibit various biological activities, such as cytotoxicity toward the cancer cell line L1210[3,4] and human colon carcinoma cells (HCT-16),[3,10] antiviral activity against Herpes Simplex virus type 1 (HSV-1),[8] and antimicrobial, antifungal,[1,3] and HIV gp120-human CD4-binding inhibition, $p56^{lck}$-cd4 dissociation induction, and Ca^{2+} channel blocker activities.[2]

In our continuing studies to discover bioactive compounds from marine organisms, the new ptilomycalin D (**2**) and the known crambescidic acid[11] (**3**) were isolated and characterized from *Monanchora dianchora*. We now describe their isolation and structural elucidation.

The specimen of *M. dianchora* de Laubenfels, 1935 (phylum Porifera, class Demospongiae, order Poecilosclerida, family Crambeidae) was collected in Nosy-Be, Madagascar, Indian Ocean, in May 2003 and immediately frozen. After homogenization, the sponge (46 g, dry weight) was extracted with $CHCl_3$/MeOH (1:1) at room temperature, and the crude extract, which proved to be cytotoxic ($IC_{50} = 0.1$ μg/mL against cancer cell line P-388), was subjected to solvent partition. The $CHCl_3$ extract was fractionated on Sephadex LH-20, eluted with *n*-hexane/$CHCl_3$/MeOH (2:1:1), to afford crambescidic acid (**3**) and ptilomycalin D (**2**).

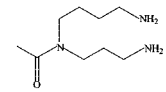
Intensive rose-violet coloring with vanillin/H_2SO_4 and strong turquoise coloring with $Co(SCN)_2$ together with characteristic proton resonances at δ 9.45 and 9.65 and a carbon resonance at δ 148.5 in the NMR spectra suggested a ptilomycalin-like skeleton, as in ptilomycalin A (**1**).

Chart 1. Structure Formulas of Compounds 1 to 3



X$^-$ = unspecified

1 Ptilomycalin A R=



2 Ptilomycalin D R= CH_3

3 Crambescidic acid R= CO_2H

Because ptilomycalin A structurally resembled a ship trailing an anchor, structure elucidation was divided in two parts, namely, the pentaheterocyclic "vessel" part and the "anchor" part,[2] i.e., the alicyclic chain ending with a spermidine group. Thus, the structural elucidation of compound **2** was carried out similarly.

1-D and 2-D NMR spectra of **2**, compared to those of ptilomycalin A,[1,2] confirmed the presence of the pentacyclic ring system. The relative configurations at positions 3, 8, 10, 13, 14, 15, and 19 were determined to be similar to those of ptilomycalin A on the basis of their comparable NMR shifts. The stereochemistry for **2** was confirmed by the NOE experiments showing, as in **1**, a correlation between CH_3-1 and H-19 (Figure 1).

The NMR data of **2** showed the absence of the spermidine moiety of ptilomycalin A. Moreover, resonances at δ_H 1.21 and δ_C 29.4 as well as 13 losses of 14 amu, from *m/z* 612 to 430 in the MS, indicated the presence of a long aliphatic chain. The molecular formula of ptilomycalin D (**2**) was established by HRESMS to be $C_{38}H_{64}N_3O_4$ $[M + H, 627]^+$, corresponding to eight degrees of unsaturation, one of which was attributable to an olefinic bond (δ 129.7, 133.2). The ^{13}C NMR spectrum also showed one carbonyl resonance at δ 168.3 and a quaternary carbon at δ 148.9, which adds two degrees of unsaturation. The remaining five degrees of unsaturation were, therefore, attributed to rings. The presence of an ester group was suggested by the HMBC correlations between the carbon resonances of C(O)-22 at δ 168.3 and CH_2O-23 at δ 65.3. The presence of this carbonyl group was confirmed in the MS. Indeed, the pentacyclic system came from *m/z* 358 ($C_{21}H_{32}N_3O_2$) and *m/z* 386 ($C_{22}H_{32}N_3O_3$) fragmentations,[2,10] on both sides of the C-22 ester carbonyl group. The downfield-shifted proton

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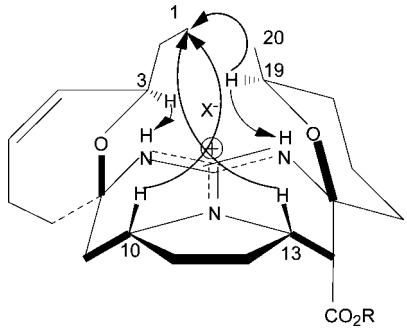

Figure 1. Key NOE for ptilomycalin D (**2**) and crambescidic acid (**3**).

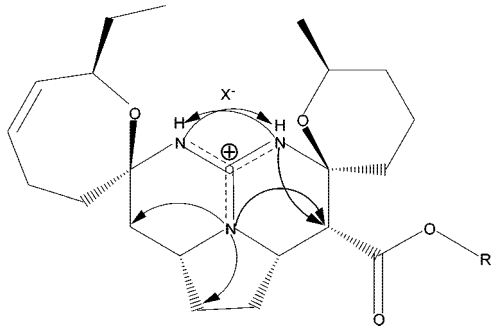

Figure 2. ^{15}NH HMBC correlations for ptilomycalin D (**2**) and crambescidic acid (**3**).

resonances at δ 3.8–4.2 revealed the presence of several protons near nitrogen- and oxygen-linked groups. The presence of nitrogen was also borne out by a ^{15}NH HMBC experiment (Figure 2), in which correlations to three carbon atoms was observed. Moreover, the downfield-shifted carbon resonance at δ 148.9 was attributed to a quaternary carbon of an imine. Therefore the observed values were similar to those of ptilomycalin A. We noted a major difference in the NMR data (Table 1) with the presence of a methyl group at δ_H 0.82 and δ_C 13.74 in addition to the two methyl groups of the pentacyclic portion, CH_3-1 at δ 0.77 and CH_3-20 at δ 1.00. Thus, compound **2**, designated as ptilomycalin D, possessed a terminal methyl group instead of the spermidine moiety in ptilomycalin A.[1]

The determination of the second guanidine alkaloid (**3**) was established by comparison of the 1- and 2-D NMR spectra of **3** with those of ptilomycalins A (**1**) and D (**2**). Thus, the "vessel" part was readily established. Moreover, the comparison of the carbon resonances of the pentacyclic part (C_1-C_{23}) suggested that **3** has the same stereochemistry as ptilomycalins A and D. Both ESMS and FABMS showed two strong peaks at *m/z* 658. The molecular formula was established to be $C_{38}H_{63}N_3O_6$ [M + H, 658.5]$^+$. The determination of the long aliphatic chain was realized by corroboration of the MS spectrum fragmentation pattern and comparison with the NMR spectra of **2**. The ^{1}H and ^{13}C NMR resonances were similar to those of ptilomycalin D (**2**). We noted differences in the NMR data: the absence of a methyl group, δ_H 0.82 and δ_C 13.74, and the presence of a carbonyl group at δ 175.4. Moreover, HMBC correlations from H-37 (δ_H 2.37) to the methylene chain and a quaternary carbon at δ 175.4 suggested the presence of a carboxylate moiety. This was confirmed by the mass spectrum, which indicated two main mass fragments at *m/z* 612 ($C_{37}H_{62}N_3O_4$) and *m/z* 640 ($C_{38}H_{62}N_3O_5$) corresponding respectively to [MH − CO − H_2O] and [MH − H_2O] and suggesting the appendix of the "vessel" to be a C_{16} ω-hydroxycarboxylic acid. Compound **3** corresponds therefore to crambescidic acid,[11] and this is the first report of this acid in the sponge *M. dianchora*.

Table 1. NMR Spectroscopic Data ($CDCl_3$) for Ptilomycalin D (**2**)[a]

position	δ_C (ppm)	δ_H (*J* in Hz)	HMBC
1	9.76, CH_3	0.77, t (6.7)	
2a	28.84, CH_2	1.50, m	1, 4
2b		1.42, m	
3	70.83, CH		1, 4, 5, 2b
4	133.20, CH	5.43, m	2a, 6b
5	129.72, CH	5.60, m	6a, 6b, 7a, 7b
6	23.20, CH_2	2.28, m	4, 5
7a	36.55, CH_2	2.40, m	5, NH
7b		1.92, m	
8	83.40, qC		6a, 6b, 7a, 7b, NH_2
9a	36.55, CH_2	2.56, m	
9b		1.46, m	
10	53.75, CH	3.94, m	9a, 9b, 11b, 12a
11a	30.30, CH_2	2.15, m	
11b		1.60, m	
12a	26.47, CH_2	2.26, m	
12b		1.72, m	
13	51.81, CH	4.24, m	11a, 12a, 12b, 14
14	49.35, CH	2.92, d (5)	
15	80.43, qC		14, NH
16	30.60, CH_2	1.64, m	
17a	18.30, CH_2	2.15, m	
17b		1.76, m	
18a	31.90, CH_2	1.60, m	20
18b		1.19, m	
19	67.05, CH	3.86, m	20
20	21.07, CH_3	1.00, d (6.7)	
21	148.90, qC		
22	168.32, qC		13, 14, 24
23	65.30, CH_2	4.04, m	24
24	26.80, CH_2	1.57, m	
25	25.54, CH_2	1.44, m	
26	29.50, CH_2	1.19, m	
27	28.19, CH_2	1.56, m	
28 to 35	29.40, CH_2	1.21, m	
36	25.20, CH_2	1.46, m	
37	32.00, CH_2	2.36, m	
38	13.74, CH_3	0.82, t (6.7)	

[a] In $CDCl_3$, 500 MHz for ^{1}H and 100 MHz for ^{13}C.

The occurrence of the new ptilomycalin D (**2**), along with crambescidic acid (**3**), may be explained by a biological ω-oxidation process of the hexadecanol moiety yielding the ω-hydroxycarboxylic acid found in crambescidic acid (**3**), and ptilomycalin D may be considered as the biogenetic precursor of **3**, which is the biogenetic precursor of ptilomycalin A.[11]

Experimental Section

General Experiment Procedures. Optical rotations were obtained with a Jasco P-1010 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker ARX-500 and Avance-400 spectrometers. ^{1}H, ^{13}C, COSY, HSQC, and HMBC were recorded using standard Bruker pulse sequences. EIMS, CIMS, and HRMS measurements were recorded on a Fisons, Autospec Q instrument.

Biological Material. The sponge, *M. dianchora* (voucher sample Museum d'Histoire Naturelle de Marseille MHNM.15615), was collected at a depth of about 18 m (site gorgones) in Nosy-Be. The living sponge is 2–3 cm thick with cylindrical oscules of 10 mm, red and white near the oscules. The identification to *M. dianchora*, from the Philippines and Central Pacific, is tentative, as our specimen, possibly an undescribed species, differs by the presence of spinose microxeas and by the shape of the small chelae (with two teeth instead of three and a slight swelling on the shaft).

Cytotoxicity Assays. Cells were maintained in logarithmic growth in EMEM/neaa, supplemented with 5% FCS, 2.0 mM L-glutamine, 10^{-2} M $NaHCO_3$, and 0.1 g/L penicillin G + 0.1 g/L streptomycin sulfate. Cytotoxic activity was screened, using an adapted form of the method described by Bergeron et al.[12] against the following cell lines: P-388 (ATCC CCL46). P-388 cells were seeded into 16 mm wells at 1×10^4 cells/well in 1- mL aliquots of EMEM 5% FCS containing different concentrations of the corresponding crude extract. A separate set of

cultures, without drug, was seeded as growth control to ensure that cells remained in the exponential phase of growth. All determinations were duplicated. After three days incubation at 37 °C, 10% CO_2, in 98% humidity, an approximate IC_{50} value (drug concentration causing a 50% reduction in cell survival) was determined by comparison of the growth in wells with drugs to growth in control wells.

Extraction and Isolation. The freeze-dried sponge (46 g) was exhaustively extracted with $CHCl_3$/MeOH (1:1). The crude extract (2.8 g) was subjected to solvent partition (*n*-hexane, CCl_4, $CHCl_3$, and *n*-butanol), and the $CHCl_3$ fraction (1.7g) subsequently was purified on Sephadex LH-20 (30 × 1.5 cm), eluted with *n*-hexane/$CHCl_3$/MeOH (2:1:1), to afford ptilomycalin D (**2**) (85 mg, 0.002%, dry weight) and crambescidic acid (**3**) (90 mg, 0.002%, dry weight).

Ptilomycalin D (2): $[\alpha]^{25}_D$ −7.2 (*c* 0.26, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$) see Table 1; HRFABMS *m/z* 627.4994 (calcd for $C_{38}H_{64}N_3O_4$, 626.4975).

Crambescidic acid (3): white, amorphous solid; $[\alpha]^{25}_D$ +4.4 (*c* 0.20, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.61 (H-5, m), 5.43 (H-4, m), 4.23 (H-13, m), 3.94 (H-10, m), 3.86 (H-19, m), 2.90 (H-14, d, J = 5 Hz), 2.51 (H-9, m), 2.37 (H-7 and H-37, m), 2.28 (H-6, m), 2.26 (H-12, m), 2.15 (H-11 and H-17, m), 1.90 (H-7, m), 1.74 (H-12, m), 1.71 (H-17, m), 1.60 (H-16 and H-18, m), 1.57 (H-24, m), 1.56 (H-27, m), 1.56 (H-11, m), 1.48 (H-2, m), 1.40 (H-2, m), 1.37 (H-9, m), 1.22 (H-25, m), 1.19 (H-18 and H-26 and H-28 to H-35, m), 1.01 (Me-20, d, J = 6.7 Hz), 0.79 (Me-1, t, J = 6.7 Hz); HRFABMS *m/z* 658.4811 (calcd for $C_{38}H_{63}N_3O_6$, 657.4795).

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