Alkaloids from the Sponge *Monanchora unguifera*

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The bioassay-guided fractionation of the cytotoxic crude gum obtained from the Caribbean sponge *Monanchora unguifera* led to the isolation and characterization of the new compounds batzelladine J (**1**) and crambescidic acid (**2**) in addition to known guanidine alkaloids ptilomycalin A (**3a**), ptilocaulin (**4**), and isoptilocaulin (**5**). The structures of the compounds were elucidated by interpretation of the 1D and 2D NMR experiments. The chemotaxonomic implications of these findings are discussed.

The discovery of ptilomycalin A (**3a**), a complex pentacyclic guanidine alkaloid from the sponges *Ptilocaulis spiculifer* and *Hemimycale* sp., preceded the isolation of several analogues, namely, the crambescidins and isocrambescidin, from other sponges (*Crambe crambe*,[2−4] *Monanchora arbuscula*,[5] *M. ungiculata*[6]) as well as the starfishes *Fromia monilis* and *Celerina heffernani*.[7] Investigation of an unidentified *Monanchora* sp. resulted in the isolation of further polycyclic guanidine alkaloids.[8] Related guanidine metabolites include the crambescins (A, B, C1, C2)[9−11] obtained from *Crambe crambe*, batzelladines A−E (from Bahamanian *Batzella* sp.[12]), and batzelladines F−I (from Jamaican *Batzella* sp.[13,14]). The tricyclic guanidines ptilocaulin (**4**) and isoptilocaulin (**5**) were initially isolated from *Ptilocaulis spiculifer*,[15] while pyrroloquinoline alkaloids batzellines A−C,[16] isobatzellines A−D,[17] and, more recently, the secobatzellines A−B[18] were obtained from *Batzella* species. Many of these guanidine alkaloids display potent ichthyotoxicity[10] and antibacterial and antifungal activity.[1] Antiviral activity has been exhibited against the Herpes Simplex virus, type 1 (HSV-1),[7] and selected batzelladines (A and B) display potential in inhibiting the HIV virus that causes AIDS.[16] Cytotoxicity was exhibited against murine leukemia cell lines (L1210, IC_{50} 0.1−1.0 μg/mL) and human colon carcinoma cells (HCT-16, IC_{50} 0.24 μg/mL).[2,11] Potent Ca^{2+} antagonist activity (IC_{50} 1.5 $\times$ 10^{-4} μM)[3] and enzyme inhibition[18] have also been observed. The unique and fascinating structures of these guanidines, coupled with the wide ranging activities that they display, have made them attractive synthetic targets.[19]

In our continuing studies to provide new drug leads from compounds obtained from the marine environment, the new compounds batzelladine J (**1**) and crambescidic acid (**2**) were isolated and characterized from *Monanchora unguifera* and are the subject of this paper. The known compounds ptilomycalin A (**3a**), ptilocaulin (**4**), and isoptilocaulin (**5**) were also identified.

The freeze-dried sponge was extracted with CH_2Cl_2/2-propanol (1:1) to yield a highly cytotoxic orange-brown gum (0.1−0.25 μg/mL), which was subjected to separation utilizing Sephadex LH-20 in methanol. Further purification of the active components utilizing a solvent system described by Tavares and co-workers[5] afforded batzelladine J (**1**). High-resolution FABMS of batzelladine J (**1**) supported the molecular formula $C_{41}H_{68}N_9O_4$ [*m/z* 750.5361, M + H^+], corresponding to 13 degrees of unsaturation. Minor quantities of two higher homologues could be identified in the mass spectrum at 765 and 779 Da. The upfield portion of the ^{1}H NMR spectrum contained methylene signals (1.0−1.4 ppm) and a methyl triplet (0.89 ppm), suggesting the presence of one or more alkyl chains. The ^{1}H NMR spectrum also revealed the presence of several protons in the 3.2−4.6 ppm region typical of nitrogen- and oxygen-linked groups. The presence of nitrogen in the molecule was also borne out by an ^{15}N HMBC experiment, in which correlations to three carbon atoms was observed. The DEPT and ^{13}C NMR experiments revealed signals for nine quaternary carbons, seven of which could be unambiguously identified: one attributable to an imine carbon (158.6 ppm), two to ester carbonyls (164.9, 165.5 ppm), and four to olefinic carbons (102.7, 102.9, 149.4, 149.6 ppm).

The main spin systems (Figure 1, substructures **a**−**d**) were identified by the ^{1}H−^{1}H COSY, HMBC, and TOCSY experiments. Fragment **a** was deduced from COSY and HMBC correlations. Further HMBC couplings were observed from the signal at 3.21 ppm (H-2, 41.9 ppm) to the quaternary carbon at 158.9 ppm; these, coupled with HMBC correlations from 4.20 ppm (64.9 ppm) to the ester carbonyl of 166.2 ppm, C-6, delineated the 4-guanidino-*n*-butyl ester moiety (C-1−C-6). Fragment **b** was identified primarily by HMBC cross-peaks, while COSY and TOCSY correlations confirmed fragment **c**. The magnitude of the carbon and proton signals of C-11, -13, and -15 suggested that they were adjacent to nitrogen atoms. Observation of the ^{1}H NMR integration pattern revealed that signals in fragment **c** (3.2−4.5 ppm) corresponded to twice as many protons as was suggested by the DEPT and HMQC experiments, confirming that **1** consisted of two repeating units. This was also borne out in the ^{13}C NMR data, where almost overlapping signals were observed (e.g., C-13/31 (53.40/53.42 ppm) and C-11/29 (58.46/58.48 ppm)). When the ^{1}H NMR spectrum was run in deuterated methanol, the methyl doublet of fragment **b** (1.38 ppm, J = 6.4 Hz) was buried in the methylene envelope (1.2−1.5 ppm) but appeared as two distinct three-proton doublets when run in deuterated pyridine (H-34, 1.43 ppm, J = 6.3 Hz and H-16, 1.49 ppm, J = 6.2 Hz). Furthermore, the H-15 and H-33 signals appeared as distinct quartets in pyridine (4.64 ppm, J = 6.2 Hz and 4.56 ppm, J = 5.9 Hz). Fragments **b** and **c** were connected by HMBC couplings from 2.83 ppm (H-9a/27a) to carbon signals at 149.4/149.6 ppm (C-8/26) and 102.7/102.9 ppm (C-25/7), while fragments **a** and **b** were connected through HMBC linkages from H-15 to C-6. Fragments **d** and **b′** were similarly coupled through H-33 and C-24. The oxygen-linked carbon in fragment **d** was

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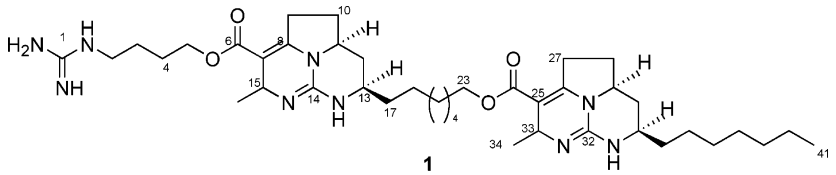
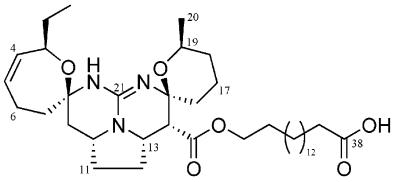
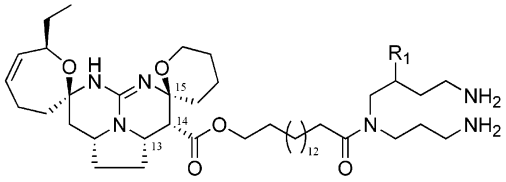
Chart 1



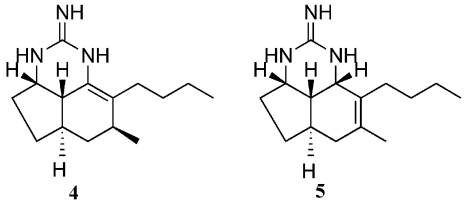
1



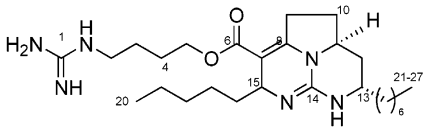
2



3a R_1 = H
3b R_1 = OH



4 **5**



6

HMBC coupled to the ester carbonyl at 166.1 ppm. The connection of these fragments, coupled with structural precedence with the batzelladines (e.g., batzelladine C (**6**),[12] Table 1), completed the assignment of the framework of **1**. In the absence of HMBC correlations to adjacent protons, the carbon assignment of the cyclic guanidine atoms (C-14/C-31) was done on the basis of literature precedence within the batzelladines.[16,17] The absence of these correlations is a commonly observed phenomenon among this group of cyclic guanidines.[13] Determination of the relative stereochemistry within the tricyclic triazadecalin system was facilitated by a ROESY experiment, which displayed cross-peaks between H-11/29, H-12b/30b, and H-13/31, suggesting that these protons all possessed a similar stereochemical orientation. No stereochemical details could be obtained on the orientation of the C-16/34 methyl substituents due to their isolated positions and being flanked by atoms bearing no protons.

Crambescidic acid, **2**, was obtained as a white amorphous solid. High-resolution FABMS established the molecular

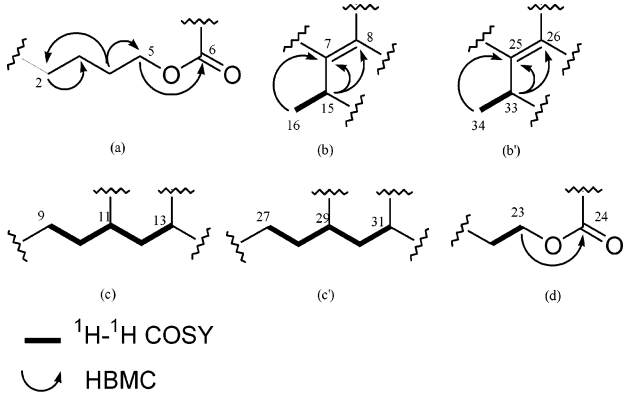


Figure 1. Substructures **a**–**d** from ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC correlations.

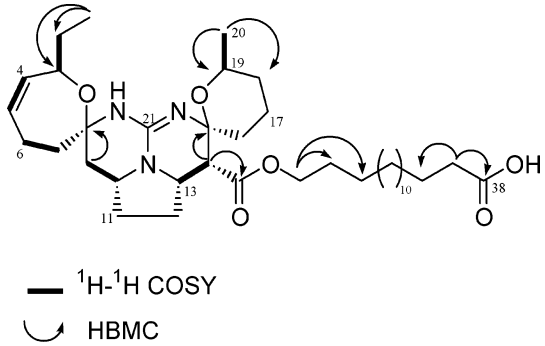


Figure 2. Key ^{1}H–^{1}H COSY and HMBC correlations for crambescidic acid (**2**).

Table 1. ^{1}H and ^{13}C NMR Data for Batzelladine J (**1**)[a] and Reported ^{13}C NMR Data for Batzelladine C (**6**) in CD_3OD[12]

	batzelladine J		batzelladine C
position	δ_H	δ_C	δ_C
1		158.6	158.7
2	3.21, t, J = 6.7 Hz	41.9	42.0
3	1.66, m	26.6	26.7
4a	1.30, m	27.1	27.0
4b	1.74, m		
5	4.20, m	64.9	65.0
6		166.2	166.3
7		102.9	101.2
8		149.4	150.4
9a	2.83, m	31.4	31.5
9b	3.36, m		
10a	1.72	30.7	30.3
10b	2.35		
11	3.86, m	58.5	58.6
12a	1.52, m	33.1	32.9
12b	2.42, m		
13	3.60, m	53.4	53.5
14		149.1	149.9
15	4.52, br s	47.5	52.2
16	1.28, m	23.5	38.2
17−21	1.30, m	30.0−30.7	24.3
22	1.68, m	29.6	32.6
23	4.16, m	65.7	23.7
24		166.1	
25		102.7	14.3
26		149.6	35.8
27a	2.83, m	31.3	25.9
27b	3.36, m		
28a	1.72, m	30.7	30.3−30.6
28b	2.35, m		
29	3.86, m	58.5	30.3−30.6
30a	1.52, m	33.1	32.6
30b	2.42, m		
31	3.60, m	53.4	23.6
32		149.1	14.4
33	4.52, m	48.5	
34	1.38, d, J = 6.4 Hz	24.6	
35a	1.59, m	35.9	
35b	1.72, m		
36−39	1.30, m	30.0−30.7	
40	1.28, m	23.6	
41	0.89	14.4	

[a] Spectra were recorded on a 500 MHz instrument.

formula as $C_{38}H_{64}N_3O_6$ $[M + H, 658.4781]^+$, corresponding to nine double-bond equivalents, one of which was attributable to an olefinic bond (131.3, 134.4 ppm). A quaternary carbon of an imine was identified further downfield at 155.1 ppm. The ^{13}C NMR spectrum also showed two carbonyl signals at 170.4 and 181.5 ppm, accounting for two degrees of unsaturation. The remaining five double-bond equivalents were therefore accounted for by rings. A pentacyclic framework was delineated by the ^{1}H−^{13}C HMBC and ^{1}H−^{1}H COSY correlations and comparison to the known compound ptilomycalin A (**3a**), which was also isolated from the sponge. The absence of resonances attributable to the spermidine unit present in ptilomycalin A suggested that this portion of the molecule was absent in **2**. HMBC correlations from H-37 (2.19 ppm) to the methylene chain and a quaternary carbon at 181.5 ppm suggested the presence of a carboxylic acid. Major mass fragments were located at *m/z* 359 and 329, indicative of cleavage at C-14/22 and further loss of the ethyl substituent at C-3, respectively. The chain length was deduced from the HRFABMS data, while the relative configurations at positions 3, 8, 15, and 19 were determined to be similar to that of the crambescidins on the basis of their closely comparable NMR shifts and coupling constants. For instance, with H-9b, the magnitude of the coupling to H-10 was consistent with that of crambescidin 800 (**3b**) (J = 4.8 and 4.5 Hz, respectively) as compared with a value of 2.3 Hz for the isomeric analogue, 13,14,15-isocrambescidin.[4] Comparable J values were also identified for H-13 (J = 5.5 and 5.6 Hz, respectively), thus precluding the 13,14,-15-isocrambescidin (J = 3.4 Hz) stereochemical orientation. This is, to the best of our knowledge, the first report of the occurrence of a crambescidin as the free carboxylic acid.

Biological evaluation of batzelladine J (**1**) against several cancer cell lines (P-388, A-549, HT-29, MEL-28, DU-145) revealed IC_{50} cytotoxicity values at levels greater than 1 μg/mL. Therefore, the initial potent bioactivity may have been due to the presence of ptilocaulin (**4**) and isoptilocaulin (**5**). There was an insufficient quantity of crambescidic acid for a biological evaluation to be conducted.

The occurrence of the cyclic guanidines ptilomycalin A (**3a**), ptilocaulin (**4**), and isoptilocaulin (**5**) along with the new compounds of the crambescidin and batzelladine type discovered in this *Monanchora* species serves as a taxonomic marker, further linking this genus with similar cyclic guanidine alkaloid-producing *Ptilocaulis*, *Batzella*, and *Crambe* species. Morphological comparison of the *Monanchora* sponge by Van Soest and collaborators with the aforementioned sponges suggests that they belong to the same genus.[20] This has been corroborated in part by chemotaxonomic evaluation of three *Monanchora* sponge genera from which crambescidins 359, 431, and 800, ptilocaulin (**2**), 8β-hydroxyptilocaulin, and dehydrobatzelladine C have been obtained.[5,6,20]

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Jasco DIP-370 polarimeter. Ultraviolet spectra were recorded on a Hewlett-Packard 8452A diode array spectrometer. Infrared spectra were obtained on a Perkin-Elmer 1600 FTIR instrument. ^{1}H, ^{13}C, and 2D spectra were recorded on either a General Electric GN Omega 500 spectrometer or a Varian Unity INOVA 400 MHz instrument. Mass spectral data were measured on a VG 70ZAB2SE mass spectrometer.

Biological Material. The sponge was collected in the shallow subtidal at a depth of about 1.5 m, on Boca del Toro Isle, on the Caribbean side of Panama, in December 1998. In life, the sponge forms a finely branched bush, branching being irregularly knobby in shape, the texture is tough, but flexible, the surface texture is velvety, and the sponge is just compressible. The color in life is bright orange crimson with an opalescent sheen. The spicules are robust styles and very fine subtylostyles forming an irregular plumose skeleton with brushes at the surface. The sponge is *Monanchora unguifera* (de Laubenfels) (order Poecilosclerida, family Crambidae). A voucher specimen has been deposited in the Natural History Museum, London (BMNH 1999.7.19.5).

Cytotoxicity Assays. Assays for IC_{50} values (recorded in μg/mL) of selected cancer cell lines were performed by Pharmamar and determined against mouse lymphoma (P-388), human lung carcinoma (A-549), human colon carcinoma (HT-29), and human lung melanoma (MEL-28 and DU145).

Extraction and Isolation. The freeze-dried sponge was exhaustively extracted with CH_2Cl_2/MeOH (1:1) to yield an orange-brown gum. Initial fractionation on a portion of the gum (5.72 g) was done using Sephadex LH-20 in methanol, from which seven main fractions, A−G, were obtained. Bioassay-guided purification of fraction B (1.599 g) was effected on Si gel using the bottom phase of the solvent system (MeOH/CH_2Cl_2/H_2O/HCOOH, 10:90:1.5:2.5). Sixteen main fractions were obtained. Fraction 16 (1.171 g), the major portion obtained from the column, was subjected to further purification on Sephadex LH-20 (MeOH/CH_2Cl_2, 1:1), yielding seven fractions, the fourth of which (496 mg) was subjected to repeated Si gel chromatography (bottom phase, $CHCl_3$/MeOH/2-pro-

Table 2. ^{1}H and ^{13}C NMR Data for Crambescidic Acid (**2**)[a]

position	δ_H	δ_C
1	0.85, t, J = 7.3 Hz	10.8
2	1.46, m	30.2−30.5
3	4.41, br d, J = 8.5 Hz	72.2
4	5.50, dt, J = 2.0, 10.7 Hz	134.4
5	5.70, br t	131.3
6	2.16, m	24.5
7a	1.94, d, J = 13.2 Hz	37.9
7b	2.43, m	
8		85.9
9a	1.41, d, J = 12.5 Hz	37.9[b]
9b	2.62, dd, J = 4.8, 13.1 Hz	
10	4.03, m	55.5
11	2.26, m	31.5
12	1.36, m	27.0[c]
13	4.33, dt, J = 5.4, 9.2 Hz	54.0
14	3.05, d, J = 5.1 Hz	51.0
15		82.2
16	1.72, m	32.8
17	1.76, m	19.5
18	1.68, m	33.0
19	3.85, m	68.3
20	1.08, d, J = 6.3 Hz	21.8
21		155.1
22		170.4
23a	4.07, dt, J = 6.4, 10.9 Hz	66.4
23b	4.18, dt, J = 6.4, 11.0 Hz	
24	1.63, m	29.5
25	1.59, m	27.6
26−35	1.29, br s	30.2−30.5
36	1.36, m	26.9[b]
37	2.19, t, J = 6.8 Hz	38.1[a]
38		181.5

[a] Spectra were recorded on a 500 MHz instrument. [b,c] Signals may be interchanged.

panol/H_2O, 9:12:1:8) to yield crambescidic acid (**2**) (2.1 mg), ptilomycalin A (**3a**) (10.0 mg), and batzelladine J (**1**) (4.9 mg). The components of fraction C were further purified on Si gel (bottom phase, MeOH/CH_2Cl_2/H_2O/HCOOH, 10:90:1.5:2.5). The main compounds from this fraction were ptilocaulin (**4**) and isoptilocaulin (**5**), which were isolated as a mixture.

Batzelladine J (1): $[\alpha]^{25}_D$ −11.8 (*c* 0.135, MeOH); UV (MeOH) λ_{max} (ϵ) 228 (8800), 295 (7200); IR (film) ν_{max} 3427, 2925, 1685, 1279, 1199, 1132, 1082 cm^{-1}; ^{1}H NMR and ^{13}C NMR (deuterated methanol), see Table 1; LRFABMS *m/z* (rel int) 592 (6), 552 (5), 498 (5), 444 (2), 391 (15), 360 (2), 332 (4), 329 (7), 305 (3), 114 (3); HRFABMS *m/z* 750.5361 (calcd for $C_{41}H_{68}N_9O_4$, 750.5394).

Crambescidic acid (2): $[\alpha]^{25}_D$ +4.4 (*c* 0.20, MeOH); IR (film) ν_{max} 3232, 1637 cm^{-1}; ^{1}H NMR and ^{13}C NMR (deuterated methanol), see Table 2; LRFABMS *m/z* (rel int) 658 (20), 359 (8), 329 (13), 285 (13), 219 (13), 176 (39), 165 (10), 155 (31), 149 (16), 123 (15); HRFABMS *m/z* 658.4781 (calcd for $C_{38}H_{64}N_3O_6$, 658.4795).

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