Monanchorin, a Bicyclic Alkaloid from the Sponge *Monanchora ungiculata*

Karina M. Meragelman,† Tawnya C. McKee,* and James B. McMahon

Molecular Targets Development Program, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, Maryland 21702-1201

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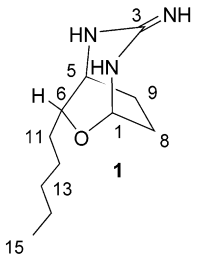
Monanchorin, a guanidine alkaloid with an unusual bicyclic skeleton, together with the known pentacyclic alkaloid crambescidin acid have been isolated from the aqueous extract of the sponge *Monanchora ungiculata*.

The sponge *Monanchora ungiculata* (Poecilosclerida, Crambeidae) is widely distributed throughout the Western and Central Pacific Ocean. It varies in color from pink to red with a "frosting" of white, gold, or yellow.[1] Several polycyclic guanidine alkaloids have been described from Monanchora, among them crambescidins 359,[2] 431,[2] and 800,[3] ptilocaulin,[4] 8β-hydroxyptilocaulin,[4] and dehydrobatzelladine C.[2] These polycyclic compounds exhibit a variety of biological activities, including cytotoxicity[5−8] and antifungal,[5] antiviral,[5,7,9] antimicrobial,[6] and Na^+/K^+- and Ca^{2+}-ATPase inhibitory[10] activities.

Two alkaloids were isolated from the aqueous extract of *M. ungiculata* collected in the Maldive Islands. The first compound was a new bicyclic guanidine alkaloid with a 6-oxa-2,4-diazabicyclo[3.2.2]nonane ring, monanchorin (**1**), and it was isolated with the pentacyclic guanidine alkaloid crambescidin acid (**2**). Crambescidin acid (**2**) has been previously described as a synthetic product,[5,11] but has never been reported as a natural product.



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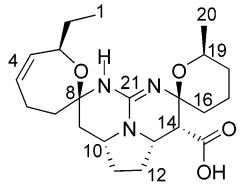

2 R = H
3 R = CH_2CH_3

A portion (7.65 g) of the aqueous extract of *M. ungiculata* was fractionated by vacuum-liquid chromatography (VLC, C_4, 40 μ) using mixtures of MeOH−H_2O and CH_2Cl_2−MeOH to give six fractions. The fraction that eluted with 2:1 MeOH−H_2O was subjected to two successive Sephadex LH-20 gel permeation columns (9:1 MeOH−H_2O, followed by 1:1 CH_2Cl_2−MeOH) to give crambescidin acid (**2**, 2.1 mg, 0.03% of extract). A second fraction from the C_4 VLC column eluted with MeOH was further purified using Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) to give monanchorin (**1**, 5.9 mg, 0.08% of extract).

The novel compound, monanchorin (**1**), gave a positive response with Sakaguchi TLC spray reagent (8-hydroxyquinolinehypobromite), which suggests the presence of a guanidine functionality.[12] The LCMS and the FABMS contained a $[M + H]^+$ ion at m/z = 212, indicating the presence of an odd number of nitrogens. The molecular formula of **1** was established as $C_{11}H_{21}N_3O$ by HRFABMS measurement of the $[M + H]^+$ peak (m/z 212.1759, calcd for $C_{11}H_{22}N_3O$ 212.1763) and requires three degrees of unsaturation. The ^{1}H NMR spectrum in DMSO-d_6 (Table 1) exhibited two broad signals for three exchangeable protons, two signals for protons (δ 4.80 and 4.11) attached to carbons bearing an oxygen atom, a signal for a proton (δ 3.28) on a carbon attached to a nitrogen atom, signals for 12 aliphatic protons (multiplets between δ 2.2 and 1.2), and a triplet integrating for three protons at δ 0.83. The ^{13}C NMR spectrum (Table 1) contained signals for 11 carbons, including a guanidine carbon signal at δ 158.2, two carbinol carbons (δ 78.9 and 75.9), one carbon bearing a nitrogen atom (δ 50.5), and seven aliphatic carbons between δ 33 and 14. DEPT experiments identified the aliphatic signals as six methylenes and one methyl group.

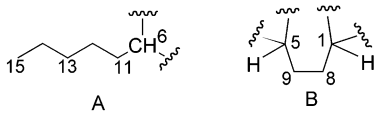

Figure 1. Structural fragments of **1** based on NMR studies.

Analysis of 2D NMR experiments, including COSY, TOCSY, HSQC, and HMBC, led to the identification of two fragments (Figure 1). Starting from the methyl group signal (δ_H 0.83, δ_C 14.0), a spin system containing four of the methylene groups was identified. The last of the methylene groups was further correlated in the COSY experiment to the methine proton at δ 4.11 (H-6). HMBC correlations between the signals for two of the methylene carbons (C-11 and C-12) on the hexanyl chain and the methine H-6 and between C-6 and the protons of C-11 supported the structure of fragment A (Figure 1). In a similar fashion, fragment B was established by COSY correlations and HMBC data (Table 1).

The presence of a signal at δ 158.2 in the ^{13}C NMR spectrum of **1** was consistent with the carbon of a guanidine group.[13] This is the only unsaturation accounted for in the NMR spectra, and therefore **1** was bicyclic. The HMBC correlation observed between the guanidine carbon and both methine protons (H-1 and H-5) was consistent with two possible structures incorporating fragment B (Figure 2). The first possibility (I) incorporated fragment B in a five-membered ring, with one of the nitrogen atoms of the guanidine as part of the ring. A second possibility (II) incorporated B into a seven-membered ring that included the carbon and two of the nitrogens of the guanidine group.

These two fragments account for all the elements from the molecular formula except an oxygen atom. The chemical shift of the methine CH-6 (δ_H 4.11, δ_C 78.9) indicated

* To whom correspondence should be addressed. Tel: 301-846-1943. Fax: 301-846-6919. E-mail: manuscripts@ncifcrf.gov.
† Current address: Avalon Pharmaceuticals, Inc., 20358 Seneca Meadows Parkway, Germantown, MD 20876.

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Table 1. ^{1}H and ^{13}C NMR Assignments of Monanchorin (**1**) at 500 and 125 MHz, Respectively, in DMSO-d_6

pos	^{13}C δ_C mult	^{1}H δ_H mult (J in Hz)	COSY	HMBC
1	75.9 d	4.80 br d (6.0)	H-8a, H-8b	H-8a, H-8b
3	158.2 s			H-1, H-5
5	50.5 d	3.28 br s	H-9a, H-9b	H-6, H-9b, H-8b, H-11a, H-11b
6	78.9 d	4.11 br t (6.0)	H-9b, H-11a, H-11b	H-1, H-9b, H-11a, H-11b
8a	28.4 t	1.85 m	H-1, H-9a, H-8b	H-1, H-5, H-9a, H-9b
8b		2.18 m	H-1, H-9b, H-8a	
9a	23.5 t	1.87 m	H-5, H-9b, H-8a	H-1, H-5, H-8a, H-8b
9b		2.01 m	H-5, H-6, H-9a, H-8b	
11a	33.0 t	1.43 m	H-6, H-11b, H-12b	H-6
11b		1.52 m	H-6, H-11a, H-12a, H-12b	
12a	24.8 t	1.20 m	H-11b, H-12b, H-13	H-6, H-11a, H-11b, H-13[a]
12b		1.34 m	H-11a, H-11b, H-12a, H-13	
13	31.2 t	1.22 br s	H-12a, H-12b	H-11a, H-11b, H-12a, H-12b, H-14, H-15
14	22.2 t	1.22 br s	H-15	H-12a, H-15
15	14.0 q	0.83 t (6.0)	H-14	H-13[a]
NH		7.38 br		
		8.67 br		

[a] HMBC correlations between this carbon and H-13 and/or H-14.

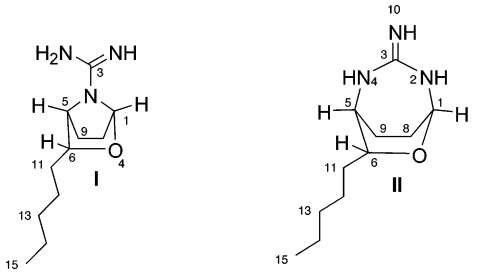

Figure 2. Possible structures incorporating fragment B in **1**.

Table 2. Carbon Chemical Shift Comparison between Monanchorin in $CDCl_3$, CS Windows ChemNMR Estimations for I and II, and Reported Values for *ent*-12,34-Oxomanzamine E in $CDCl_3$

carbon	monanchorin	I	II	*ent*-12,34-oxomanzamine E
1	75.4	88.3	72.7	101.8 [34][a]
3	159.1	157.9	163.0	
5	50.5	62.8	51.6	67.2 [26][a]
6	78.9	83.7	82.1	80.5 [12][a]
8	28.4	32.6	23.0	47.4 [35][a]
9	23.5	25.8	22.4	38.2 [25][a]

[a] Denotes numbering in manzamine system.

it was a carbinolic carbon and suggested that the oxygen was attached to C-6. A HMBC correlation from C-6 and H-1 connected C-1 and C-6 through an ether linkage. Furthermore, HMBC correlations between C-5 and H-6, H-11a and H-11b, and C-6 to H-5 and H-9b linked C-5 and C-6. A full analysis of the HMBC data, however, did not distinguish between the two possible structures of monanchorin. If the structure corresponded to I, one would expect the guanidine group to react with 2,4-pentanedione to form a 2,4-dimethylpyrimidine derivative.[14] However, repeated attempts to react **1** with 2,4-pentanedione failed to produce the pyrimidine product in amounts that could be isolated and characterized.

Calculations of the ^{13}C NMR spectrum of both possible structures (Table 2) with CS Windows ChemNMR software suggested that the NMR data we obtained for monanchorin (**1**) correlated with the calculations for II more closely than for those calculated for I. In addition, I contains a 2-oxa-7-azabicyclo[2.2.1]heptane skeleton,[15] which is also found in *ent*-12,34-oxamanzamine E (Figure 3).[16] The reported NMR chemical shift values for the corresponding skeleton in the *ent*-12,34-manzamine E structure are also listed in Table 2 and are very different from the values we observed for monanchorin. These data combined suggested that monanchorin (**1**) was structure II.

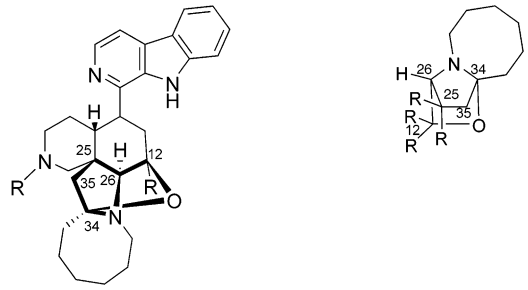

Figure 3. Simplified drawing of *ent*-12,34-oxamanzamine E with the 2-oxa-7-azabicyclo[2.2.1]heptane ring system highlighted and extracted.

The proposed structure was further supported by selective 1D ^{15}N$-^1$H HMBC experiments, which showed ^{15}N correlations to H-6, H-8a, H-8b, H-9a, and H-9b, confirming that these protons were three bonds from a nitrogen atom. A single 2D ^{15}N$-^1$H HMBC contained weak correlations between H-6 (δ 4.11) and a nitrogen at δ_N 87.1 and between H-8b (δ 2.18) and a nitrogen at δ_N 93.5. The correlations of these protons to two different nitrogen signals are only possible with II.

The relative stereochemistry for **1** was determined by a NOESY experiment. Correlations observed between H-1 and H-8b, H-8b and H-9b, H-5 and H-9b, and H-6 and H-5 suggested that they were on the same face of the molecule. This was supported by NOESY correlations between H-9a and H-11b, which is possible if the ring is in a boat configuration. In this configuration, H-6 adopts a pseudoequatorial position and H-8b is in an axial position, bringing it into close proximity to the side chain, which extends below the plane of the ring system. This relative stereochemistry is also consistent with the observed *W*-type coupling between H-6 and H-9b.

The LCMS and the FABMS of compound **2** showed a peak at $m/z = 404$. Its molecular formula was established as $C_{22}H_{34}O_4N_3$ by HRFABMS of the $[M + H]^+$ peak (m/z 404.2541, calcd 404.2563), indicating seven degrees of unsaturation. The presence of three nitrogen atoms in the molecular formula suggested that compound **2** was also a guanidine alkaloid, but polycyclic. Analysis of the chemical shifts and a standard battery of 2D NMR experiments allowed the assignment of the structure of compound **2** as a pentacyclic guanidine alkaloid closely related to crambescidins 431[2] (**3**) and 800[6−8,17,18] and ptilomycalin A.[8,10,18−20] The main differences between **2** and crambescidin 431 (**3**) were the absence of the signals for the ester chain in **2** and the shifted resonance observed for the carbonyl group (C-22, δ 172.8 in **2**, δ 168.4 in **3**). These data suggested that

compound **2** was the acid analogue of crambesidin 431 (**3**) and closely related to compounds originally reported from the sponges *Monachora*,[2] *Crambe*,[7,17] *Batzella*,[18] *Ptilocaulis*,[19] and *Hemimycale*[20] and from the starfishes *Celerina heffernani* and *Fromia monilis*.[8] The relative stereochemistries were determined by NOESY experiments to be the same as the previously reported crambescidins[2,6−8,17,18] and ptilomycalin A.[8,10,18−20] The relative stereochemistry of C-14 was further confirmed by the enhancement observed in H-13 when H-14 was irradiated in a 1D NOESY experiment.

Monanchorin (**1**) and crambescidin acid (**2**) were tested in a cytotoxicity assay utilizing IC2 murine mast cell lines;[21,22] both showed very weak inhibition [monanchorin (**1**), IC_{50} = 11.3 μg/mL, crambescidin acid (**2**), IC_{50} = 25.0 μg/mL].

Experimental Section

General Experimental Procedures. UV spectra were obtained on a Beckman DU 640 spectrophotometer, and IR spectra on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. NMR spectra were performed on a Varian Inova Unity 500 spectrometer in DMSO-d_6, MeOH-d_4, or $CDCl_3$. Selective 1D $^{15}N-^{1}H$ HMBC experiments were acquired optimized for J_{nNH} = 6.5 Hz and the number of increments set to 1 with a narrowed ^{15}N chemical shift window (85−95 ppm). The 2D $^{15}N-^{1}H$ HMBC experiment utilized the same ^{15}N window with 70 increments. Mass spectra were obtained with a JEOL SX102 mass spectrometer. LCMS data were acquired on a Hewlett-Packard HP1100 integrated LCMS system.

Collection. The sponge materials were collected at Male Atoll in the Maldive Islands by the Coral Reef Foundation under a collection contract with the National Cancer Institute and immediately frozen. The sponge was identified as *Monanchora ungiculata* (Poecilosclerida) by Michelle Kelly (National Institute of Water and Atmosphere Research). A voucher specimen (0CDN5264) has been deposited at the Smithsonian Institution.

Extraction and Isolation. The frozen sponge samples were ground in dry ice to a fine powder and extracted with H_2O at 4 °C. The sponge material was removed by centrifugation, and the aqueous extract was subsequently lyophilized to give 40 g of extract. A 7.56 g aliquot of the extract was dissolved in distilled water and placed on a C_4 chromatography column (40 μ) and eluted in a stepwise fashion with the following solvent mixtures: H_2O, 1:2 MeOH−H_2O, 1:1 MeOH−H_2O, 2:1 MeOH−H_2O, MeOH, 1:1 CH_2Cl_2−MeOH. The activity was concentrated in the fraction that eluted with 2:1 MeOH−H_2O (170 mg) and which was further purified by permeation through two Sephadex LH-20 columns (2 cm × 85 cm, 9:1 MeOH−H_2O, and 2 cm × 75 cm, 1:1 CH_2Cl_2−MeOH) to yield crambescidin acid (**2**, 2.1 mg). Additional materials that eluted from the C_4 column with MeOH (20 mg) were further purified using Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) to give monachorin (**1**, 5.9 mg).

Monanchorin (1): light yellow oil; $[\alpha]^{25}_D$ +39.0° (*c* 3.90, MeOH); IR (NaCl) ν_{max} 3330, 3249, 2926, 2860, 1668, 1606, 1462, 1202, 1052, 1026, 1005 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.99 (1H, br s, NH), 8.90 (1H, br s, NH), 7.38 (1H, br s, NH), 4.81 (1H, br s, H-1), 4.28 (1H, br s, H-6), 3.22 (1H, br s, H-5), 2.27 (1H, m, H-9b), 2.16 (1H, m, H-8b), 2.04 (1H, m, H-9a), 2.01 (1H, br d, J = 10.0 Hz, H-8a), 1.60 (1H, m, H-11b), 1.41 (2H, m, H-11a and H-12b), 1.27 (3H, br s, H-12a and H-14), 1.22 (2H, br s, H-13), 0.86 (3H, br t, J = 6.0 Hz, H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.1 (s, C-3), 79.5 (d, C-6), 76.6 (d, C-1), 51.3 (d, C-5), 33.7 (t, C-11), 31.6 (t, C-13), 28.9 (t, C-9), 25.1 (t, C-12), 23.9 (t, C-8), 22.5 (t, C-14), 13.9 (q, C-15); ^1H and ^{13}C NMR in DMSO-d_6, see Table 1; FABMS *m/z* 212 $[M + H]^+$ (100); HRFABMS *m/z* 212.1759, calcd for $C_{11}H_{22}N_3O_1$ 212.1763.

Crambescidin acid (2): $[\alpha]^{25}_D$ −19.0° (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 231.0 (3.20), 268.5 (2.84) nm; IR (NaCl) ν_{max} 3241, 2925, 2852, 1727, 1657, 1611, 1451, 1339, 1201, 1121, 1085, 1046, 1012 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 5.71 (1H, dd, J = 10.5, 8.0 Hz, H-5), 5.50 (1H, d, J = 10.5 Hz, H-4), 4.38 (1H, br d, J = 8.5 Hz, H-3), 4.29 (1H, m, H-13), 4.02 (1H, m, H-10), 3.83 (1H, dq, J = 8.5, 6.5 Hz, H-19), 2.87 (1H, br s, H-14), 2.57 (1H, dd, J = 12.5, 5.0, H-9b), 2.40 (1H, d, J = 17.0 Hz, H-6b), 2.31 (1H, m, H-12b), 2.29 (1H, m, H-7), 2.26 (1H, m, H-11b), 2.15 (1H, dt, J = 17.0, 7.0 Hz, H-6a), 1.98 (1H, m, H-8), 1.96 (1H, m, H-12a), 1.83 (2H, m, H-17), 1.79 (2H, m, H-16), 1.66 (1H, m, H-11a), 1.66 (1H, m, H-18a), 1.54 (1H, ddd, J = 14.0, 7.0, 3.5 Hz, H-2b), 1.46 (1H, dq, J = 15.0, 7.2 Hz, H-2a), 1.42 (1H, m, H-9a), 1.26 (1H, m, H-18a), 1.08 (3H, d, J = 6.5 Hz, H-20), 0.85 (3H, t, J = 7.2 Hz, H-1); ^{13}C NMR (CD_3OD, 125 MHz) δ 172.8 (s, C-22), 150.4 (s, C-21), 134.3 (d, C-4), 131.4 (d, C-5), 85.0 (s, C-8), 82.3 (s, C-15), 72.3 (d, C-3), 68.1 (d, C-19), 55.6 (d, C-10), 54.3 (d, C-13), 51.4 (d, C-14), 38.4 (t, C-7), 38.0 (t, C-9), 33.1 (t, C-18), 32.6 (t, C-16), 31.4 (t, C-11), 30.3 (t, C-2), 27.7 (t, C-12), 24.4 (t, C-6), 21.9 (q, C-20), 19.5 (t, C-17), 10.8 (q, C-1); FABMS *m/z* 426 $[M + Na]^+$ (9), 404 $[M + H]^+$ (100); HRFABMS *m/z* 404.2541, calcd for $C_{22}H_{34}O_4N_3$, 404.2563.

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