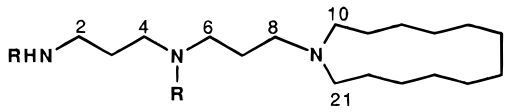
Motuporamines A−C, Cytotoxic Alkaloids Isolated from the Marine Sponge *Xestospongia exigua* (Kirkpatrick)

David E. Williams,† Peter Lassota,‡ and Raymond J. Andersen*,†

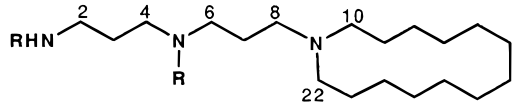
Departments of Chemistry and Oceanography, Earth & Ocean Sciences, University of British Columbia, Vancouver, British Columbia V6T 1Z1, Canada, and Oncology and Immunology Division, Wyeth Ayerst Research, 401 North Middletown Road, Pearl River, New York 10965

Received February 24, 1998

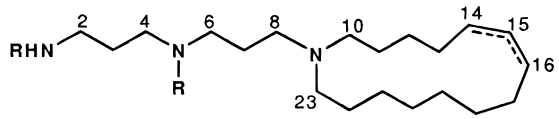
Marine sponges continue to be a rich source of structurally novel cytotoxic secondary metabolites that are of interest as potential lead compounds for the development of new anticancer drugs.[1] As part of an ongoing search for new cytotoxins from tropical sponges,[2] it was found that extracts of *Xestospongia exigua* (Kirkpatrick)[3] collected in Papua New Guinea exhibited a unique profile of selective in vitro cytotoxicities against a panel of human cancer cell lines. Bioassay-guided fractionation of the *X. exigua* extracts led to the isolation of a mixture of motuporamines A (**1**), B (**2**), and C (**3**) along with known members of the petrosin and xestospongin/araguspongine class of 3-alkylpiperidine alkaloids. The motuporamines, which contain a spermidine-like substructure, represent a new family of cytotoxic sponge alkaloids that appear to be biogenetically derived from the same basic building blocks, ammonia, acrolein, and a long-chain dialdehyde, involved in the Baldwin/Whitehead pathway to the 3-alkylpiperidine alkaloids isolated from marine sponges in the order Haplosclerida.[4,5]



1 R=H, 4 R=Ac



2 R=H, 5 R=Ac



3 R=H, 6 R=Ac

Purification of the water-soluble portion of the *X. exigua* extract via repeated Sephadex LH-20 column chromatography gave a cytotoxic fraction that contained a mixture of motuporamines A (**1**), B (**2**), and C (**3**). The mixture of **1**−**3**, obtained as a pale brown optically inactive amorphous solid that resisted all attempts at further fractionation, contained motuporamine C (**3**) as the major component (>90%). In the positive-ion HRFABMS, the mixture of **1**−**3** gave $[M + H]^+$ ions at *m*/*z* 298.3232, 312.3368, and 324.3376 consistent with the molecular formulas $C_{18}H_{39}N_3$, $C_{19}H_{41}N_3$, and $C_{20}H_{41}N_3$ for motuporamines A−C, respectively. An intense purple stain resulting from visualizing TLC plates containing the mixture of motuporamines with a standard ninhydrin spray reagent suggested that at least one of the nitrogen atoms in each of the individual compounds was present as a primary amine. The basicity of the amino functionalities in the motuporamines was thought to be a major impediment to the chromatographic separation of the three closely related alkaloids. Reaction of the mixture of motuporamines with acetic anhydride gave the less basic diacetylated derivatives that were routinely separated by reversed-phase HPLC to give samples of pure diacetylmotuporamines A (**4**), B (**5**), and C (**6**).

Diacetylmotuporamine C (**6**) was obtained as an optically inactive clear oil that gave a $[M + H]^+$ ion in the HRFABMS at *m*/*z* 408.3597, appropriate for a molecular formula of $C_{24}H_{45}N_3O_2$. Many of the carbon and some of the proton resonances in the ^{13}C and ^1H NMR spectra of **6** were doubled (see Table 1 and Supporting Material), which was attributed to a slow conformational equilibrium most likely involving acetamide rotamers. The ^1H, ^{13}C, APT, and HMQC NMR data obtained for **6** identified two methyl, 18 methylene, two methine, and two carbonyl carbons. Six of the methylene carbon resonances and their attached protons had chemical shifts appropriate for methylene groups attached to single nitrogen atoms (δ(^{13}C/^1H) 37.8/3.20; 43.7/3.42; 48.0/3.37, 52.9/3.15; 53.6/3.11; 53.9/3.11). The two methine carbon resonances were assigned to a disubsituted olefin (δ 130.6/5.32; 133.0/5.37), and the two methyl and two carbonyl carbon resonances were assigned to acetamides (δ 22.6/1.93 (s, 3H); 21.2/2.12 (s, 3H); 173.5; 174.4). All of the remaining 12 methylene carbon resonances had chemical shifts appropriate for aliphatic methylenes (Table 1).

Detailed analysis of the COSY, HMQC, and HMBC data for diacetylmotuporamine C (**6**) readily identified the spermidine-like substructure encompassing the two three carbon units C-2−C-4 and C-6−C-8 (see the Supporting Material). HMBC correlations between the H-4/H-4′ proton resonance at δ 3.37 and the C-6 resonance at δ 43.7 and between the H-6/H-6′ proton resonance at δ 3.42 and the C-4 resonance at δ 48.0 suggested that the two methylene carbons C-4 and C-6 were attached to a common nitrogen atom (N-5). HMBC correlations

* To whom corespondence should be addressed. Fax: (604) 822-6091. E-mail: randersn@unixg.ubc.ca.
† University of British Columbia.
‡ Wyeth Ayerst Research.

(1) See, for example: (a) Clark, W. D.; Corbett, T.; Valeriote, F.; Crews, P. *J. Am. Chem. Soc.* **1997**, *119*, 9285. (b) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126.

(2) For previous studies see: (a) de Silva, E. D.; Williams, D. E.; Andersen, R. J.; Klix, H.; Holmes, C. F. B.; Allen, T. M. *Tetrahedron Lett.* **1992**, *33*, 1561. (b) Kong, F.; Andersen, R. J.; Allen, T. M. *Tetrahedron Lett.* **1994**, *35*, 1643. (c) Kong, F.; Andersen, R. J.; Allen, T. M. *J. Am. Chem. Soc.* **1994**, *116*, 6007. (d) Coleman, J. E.; de Silva, E. D.; Kong, F.; Andersen, R. J.; Allen, T. M. *Tetrahedron*. **1995**, *51*, 10653.

(3) The sponge was identified by Professor Rob van Soest, University of Amsterdam, and a voucher sample has been deposited at the Zoological Museum of Amsterdam (ZMA POR 11521).

(4) Baldwin, J. E.; Whitehead, R. C. *Tetrahedron Lett.* **1992**, *33*, 2059.

(5) Andersen, R. J.; Van Soest, R. W. M.; Kong, F. In *Alkaloids, Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon: New York, 1996; Chapter 3.

S0022-3263(98)00355-7 CCC: $15.00
Published on Web 06/18/1998

Table 1. NMR Data for Diacetylmotuporamines A (4), B (5), and C (6) Recorded in MeOH-d_4 at 500 MHz

	diacetylated motuporamine A (**4**)		diacetylated motuporamine B (**5**)	diacetylated motuporamine C (**6**)	
atom	δ ^{1}H	δ ^{13}C[a]	δ ^{1}H	δ ^{1}H	δ ^{13}C[a]
2	3.20, m	37.8 (38.1)	3.20, m	3.20, m	37.8 (38.1)
3	1.80, m	29.6 (28.6)	1.81, m	1.81, m	29.5
4	3.37, m	47.7 (46.9)	3.37, m	3.37, m	48.0 (46.9)
6	3.43, m	43.7 (44.5)	3.43, m	3.42, m	43.7 (44.5)
7	1.99, m	24.2 (24.4)	2.00, m	1.99, m	24.1 (24.2)
8	3.10, m	53.6 (53.5)	3.06−3.26	3.11, m	53.9 (53.8)
10	3.13, m, 1H	53.2 (52.8)	3.06−3.26	3.11, m	53.6
	3.21, m, 1H				
11	1.72−1.78, m	22.5	1.70−1.75, 0.97 m, 1H[b]	1.71, m	23.1 (23.2)[b]
12	1.48, m, 1H, 1.53, m, 1H	25.3 (24.9)	1.25−1.60	1.49, m, 1H, 1.57, m, 1H	23.7 (23.8)[b]
13	1.41−1.44	26.8 (26.7)[b]	1.25−1.60	2.16, m	27.0
14	1.41−1.44	26.0 (26.0)[b]	1.25−1.60	5.32, m	130.6
15	1.41−1.44	25.8 (25.7)[b]	1.25−1.60	5.37, m	133.0
16	1.41−1.44	25.8 (25.7)[b]	1.25−1.60	2.12, m	26.6 (26.4)
17	1.41−1.44	26.0 (26.0)[b]	1.25−1.60	1.49, m	29.2
18	1.41−1.44	26.8 (26.7)[b]	1.25−1.60	1.35, m	27.8 (27.8)
19	1.48, m, 1H, 1.53, m, 1H	25.3 (24.9)	1.25−1.60	1.35−1.43 m	27.0[c]
20	1.72−1.78 m	22.5	1.25−1.60	1.35−1.43 m	26.6 (26.4)[c]
21	3.13, m, 1H, 3.21, m, 1H	53.2 (52.8)	1.70−1.75, 0.93 m, 1H[b]	1.43, m	27.5 (27.5)[c]
22			3.06−3.26	1.71, m	25.0 (24.8)[b]
23				3.15, m	52.9 (52.9)
N1					
CO		171.2	173.5 (173.3)		
Me	1.93 (1.92), s, 3H	22.6	1.94 (1.93), s, 3H	1.93 (1.92), s, 3H	22.6 (22.6)
N5					
CO		174.5			174.4 (174.3)
Me	2.13 (2.12), s, 3H	21.2	2.13 (2.12), s, 3H	2.12 (2.11), s, 3H	21.2 (20.7)

[a] In **4** and **6** the majority of the carbons appeared as two resonances. The δ value for the less intense resonance for each carbon is given in parentheses. [b,c] Assignments within a column are interchangeable. Assignments and some chemical shifts are based on HMQC, HMBC, and COSY data.

observed between the carbonyl resonance at δ 174.4 and the methyl singlet resonance at δ 2.12, the H-4/H-4′ proton resonance at δ 3.37, and the H-6/H-6′ proton resonance at δ 3.42 confirmed the attachment of C-4 and C-6 to N-5 and showed that the third N-5 substituent was an acetyl group. COSY correlations between the H-4/H4′ resonance at δ 3.37 and a methylene resonance at δ 1.81 (H-3/H-3′), which was in turn correlated to a methylene proton resonance at δ 3.20 (H-2/H-2′), extended the first N-5 substitutent to a linear three-carbon chain. The methylene proton resonance at δ 3.20 was correlated to a carbon at δ 37.8 in the HMQC spectrum and to the second acetamide carbonyl resonance at δ 173.5 in the HMBC spectrum, indicating that this first N-5 alkyl substituent terminated in a primary amide functionality. Additional HMBC correlations between H-2/H-2′ (δ 3.20) and C-4 (δ 48.0) and between H-4/H-4′ (δ 3.37) and C-2 (δ 37.8) confirmed the 1,3-diaminopropane nature of the N-1 to N-5 fragment. Similarly, COSY correlations between the methylene resonances at δ 3.42 (H-6/H-6′) and δ 1.99 (H-7/H-7′) and between δ 1.99 and a methylene resonance at δ 3.11 (H-8/H-8′) identified a second linear three-carbon susbtituent on N-5. The protons at δ 3.11 were correlated in the HMQC spectrum to a methylene resonance at δ 53.9, indicating that the carbon was attached to the final nitrogen atom (N-9) in the molecule. HMBC correlations between H-6/H-6′ and C-8 and between H-8/H-8′ and C-6 confirmed the presence of this second N-5−N-9 1,3-diaminopropane fragment.

Two additional methylene resonances in the ^{13}C NMR spectrum of **6** had chemical shifts (δ 53.6, C-10 and δ 52.9, C-23) appropriate for carbons attached to nitrogen. These methylene carbons (C-10 and C-23) had to be attached to the remaining two valence sites on N-9 to generate a tertiary amine, consistent with the observed lack of acetylation at this nitrogen. The two acetamide carbonyl and two olefinic methine resonances in the ^{13}C NMR spectrum identified the only three sites of unsaturation in **6** associated with functional groups. One remaining site of unsaturation required by the molecular formula had to be present as a ring that had to accommodate the disubstituted olefin and the 12 remaining aliphatic methylene carbons. Therefore, the ring had to be a 15-membered macrocyclic amine with a linear 12-carbon chain bridging the C-10 and C-23 methylene carbons attached to N-9. What remained to complete the structure of **6** was to determine the position and configuration of the disubstituted olefin in the macrocyclic amine.

The different chemical shifts observed for the two olefinic carbons and their attached protons ruled out the possibility of a symmetrical $\Delta^{16,17}$ olefin, and the COSY data unambiguously ruled out the $\Delta^{11,12}$, $\Delta^{12,13}$, and $\Delta^{13,14}$ olefin locations. However, the NMR data did not clearly distinguish between the $\Delta^{14,15}$ and $\Delta^{15,16}$ olefin positions. Simultaneous irradiation of the overlapping allylic proton resonances at δ 2.14 simplified each of the olefinic methine resonances to doublets with $J = 10.9$ Hz, demonstrating that the olefin had the *Z* configuration.

Diacetylated motuporamine A (**4**) was also obtained as an optically inactive oil with a molecular formula of $C_{22}H_{43}N_3O_2$ that differed from the molecular formula of diacetylated motuporamine C (**6**) simply by the loss of C_2H_2. As with diacetylated motuporamine C (**6**), many of the carbon as well as several of the proton resonances in the ^{13}C and ^{1}H NMR spectra of **4** were doubled. The

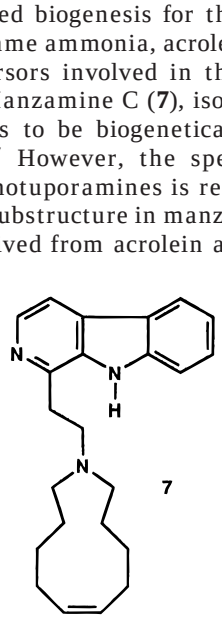
(6) Sakai, R.; Kohomoto, S.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *Tetrahedron Lett.* **1987**, *28*, 5493.

(7) While this manuscript was under review, a related compound appeared in the literature. See: Koren-Goldshlager, G.; Kashman, Y.; Schleyer, M. *J. Nat. Prod.* **1998**, *61*, 282.

^{1}H, ^{13}C, COSY, HMQC, and HMBC data obtained for **4** identified the same linear diacetylated bis-propanediamine fragment (N-1−N-9) found in diacetylmotuporamine C (**6**) (Table 1). Subtracting the atoms in this fragment ($C_{10}H_{19}N_3O_2$) from the molecular formula of **4** left $C_{12}H_{24}$ and one site of unsaturation to account for. Therefore, it was apparent that diacetylmotuporamine A contained a fully saturated 13-membered macrocyclic amine as shown in **4**. The symmetry in the macrocyclic ring of **4** was apparent in the ^{13}C NMR data, which contained only 16 resonances, six of which were assigned to the macrocyclic methylene carbons (δ 53.2, 22.5, 25.3, 26.8, 26.0, and 25.8).

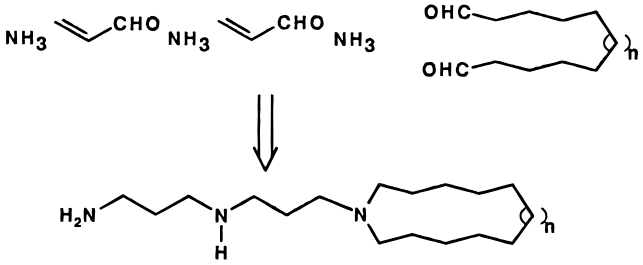
Diacetylated motuporamine B (**5**) was obtained as an optically inactive oil with a molecular formula of $C_{23}H_{45}N_3O_2$, which differed from the molecular formula of diacetylated motuporamine A (**4**) simply by the addition of CH_2. Comparison of the NMR data for **5** with the NMR data obtained for **4** and **6** showed that diacetylmotuporamine B (**5**) was simply the 14-membered macrocyclic amine homologue of **4**. The ^{1}H NMR spectrum of diacetylated motuporamine B (**5**) contained two resonances more shielded than 1 ppm (0.97 (1H) and 0.92 (1H)), whereas the ^{1}H NMR spectra of **4** and **6** had no resonances more shielded than 1.25 ppm. This difference can presumably be attributed to neighboring group shielding effects that are possible in the 14-membered ring of **5** but not in the saturated 13-membered and unsaturated 15-membered rings of diacetylmotuporamines A (**4**) and C (**6**).

Motuporamines A−C (**1**−**3**) represent the first examples of a new family of macrocyclic alkaloids. The mixture of underivatized motuporamines showed modest in vitro cytotoxicity with a mean IC_{50} of 0.6 μg/mL against a panel of human solid tumor cancer cell lines. However, they displayed none of the interesting selective cytotoxicity exhibited by the *X. exigua* crude extract. The motuporamines appear to be biogenetically related to the 3-alkylpiperidine and 3-alkylpyridine alkaloids that are useful chemotaxonomic markers of sponges in the order Haplosclerida.[5] Baldwin and Whitehead have proposed that the putative 3-alkylpiperidine building blocks of the Haploscerlida alkaloids are derived in turn from ammonia, acrolein, and a long-chain dialdehyde.[4] Scheme 1 shows a proposed biogenesis for the motuporamines that utilizes the same ammonia, acrolein, and long-chain dialdehyde precursors involved in the Baldwin/Whitehead pathway. Manzamine C (**7**), isolated from a *Haliclona* sp., appears to be biogenetically related to the motuporamines.[6,7] However, the spermidine-like substructure in the motuporamines is replaced by an alkylated β-carboline substructure in manzamine C (**7**), which appears to be derived from acrolein and tryptamine.



7

Scheme 1



Experimental Section

^{1}H chemical shifts are referenced to the residual DMSO-d_6 or MeOH-d_4 signal (δ 2.49 or 3.30 ppm, respectively), and ^{13}C chemical shifts are referenced to the DMSO-d_6 or MeOH-d_4 solvent peak (δ 39.5 or 49.5 ppm, respectively). Low- and high-resolution FABMS were recorded with xenon as the bombarding gas and a thioglycerol sample matrix.

Isolation of the Motuporamines. Specimens of *X. exigua* (Kirkpatrick) were collected by hand using scuba at a depth of 15 m from vertical walls on the outer reef off Motupore Island, Papua New Guinea, in January 1995. Freshly collected sponge was frozen on site and transported to Vancouver over dry ice. A portion of the frozen sponge (86.4 g) was cut into small pieces, immersed in MeOH, and subsequently extracted repeatedly with MeOH (3 × 150 mL). The combined methanolic extracts were concentrated in vacuo and then partitioned between EtOAc (3 × 40 mL) and H_2O (100 mL). The aqueous extract was reduced to dryness in vacuo and then suspended in 2:1 H_2O/MeOH and filtered through Celite. The filtrate was concentrated and chromatographed on Sephadex LH-20 in 3:1 H_2O/MeOH to give a 2.0 g fraction containing a baseline ninhydrin-staining spot on reversed-phase TLC. This material was suspended in MeOH and the suspension filtered and subjected to repetitive column chromatography on LH-20 with MeOH as eluent to yield 351 mg of ninhydrin-staining material as a pale brown amorphous solid. The amorphous solid consisted of a single class of spermidine-like containing compounds, motuporamines A (**1**), B (**2**), and C (**3**), with motuporamine C (**3**) making up the bulk (>90%) of the sample. In our hands, we found it impossible to separate this mixture.

Motuporamines A−C (1−3) were isolated as a single pale brown amorphous solid: ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive-ion HRFABMS $[M + H]^+$ *m/z* 298.323 24 ($C_{18}H_{40}N_3$, calcd 298.322 50), 312.336 79 ($C_{19}H_{42}N_3$, calcd 312.338 16), 324.337 59 ($C_{20}H_{42}N_3$, calcd 324.338 16) for A (**1**), B (**2**), and C (**3**), respectively.

Acetylation of Motuporamines A−C. The mixture of motuporamines (89.2 mg) was dissolved in 2 mL of 3:1 pyridine/acetic anhydride and stirred at room temperature for 16 h. Evaporation of the reagents in vacuo gave a mixture of diacetylated products that were separated by semipreparative reversed-phase HPLC, using a Whatman Magnum-9 Partisil 10 ODS-3 column, with 1:1 MeOH/(1.2% TFA/H_2O) as eluent. Pure diacetylated motuporamines A (**4**), B (**5**), and C (**6**) (1.5, 0.8, and 95.8 mg, respectively) were obtained, eluting sequentially, all as clear oils. A complex mixture of minor analogues (at least three, total 8.0 mg), eluting as a single broad peak, was also isolated.

Diacetylmotuporamine A (4): isolated as a clear oil; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive-ion HRFABMS $[M + H]^+$ *m/z* 382.3437 ($C_{22}H_{44}N_3O_2$, calcd 382.3436).

Diacetylmotuporamine B (5): isolated as a clear oil; ^{1}H NMR, see Table 1; positive-ion HRFABMS $[M + H]^+$ *m/z* 396.3592 ($C_{23}H_{46}N_3O_2$, calcd 396.3593).

Diacetylmotuporamine C (6): isolated as a clear oil; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive-ion HRFABMS $[M + H]^+$ *m/z* 408.3597 ($C_{24}H_{46}N_3O_2$, calcd 408.3593).

Acknowledgment. The authors thank Mike Leblanc for assisting the collection of *Xestospongia exigua* and Professor R. van Soest for identifying the sponge. We also express gratitude for the help and services provided by John Rewald, acting Director of the Motupore Island Research Department, University of Papua New Guinea. This work was supported by NIH Grant No. CA 67786.

Supporting Information Available: 1D and 2D NMR spectra for the mixture of motuporamines A−C (**1**−**3**) and for pure diacetylmotuporamines A (**4**), B (**5**), and C (**6**) (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO980355P