Structural Assignment of Poecillastrins B and C, Macrolide Lactams from the Deep-Water Caribbean Sponge *Poecillastra* Species[#]

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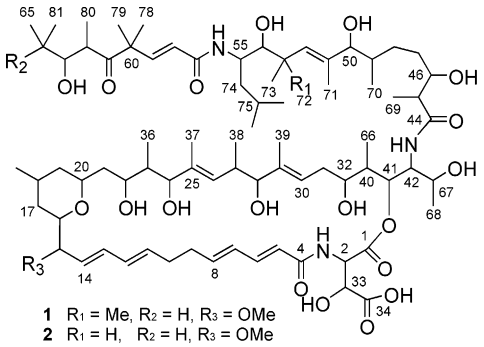
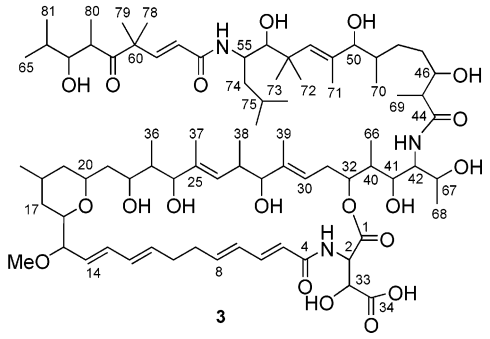
Two new chondropsin-type macrolide lactams, poecillastrins B (**1**) and C (**2**), were isolated from aqueous extracts of the marine sponge *Poecillastra* sp. These trace metabolites were isolated in low yield (400−600 μg), and their structures were determined primarily by analysis of NMR data acquired using a cyrogenically cooled probe. High-quality 1D and 2D NMR data sets allowed complete assignment of the spectroscopic data and defined the new structures as 35-membered ring analogues of poecillastrin A (**3**). Compounds **1** and **2** showed potent cytotoxic activity against a human melanoma tumor cell line (LOX) with an IC_{50} value of less than 1 μg/mL.

The chondropsin class of macrolide lactams are a series of complex, highly functionalized polyketide derivatives that have been isolated from an array of taxonomically diverse sponges. The original sponge source, *Chondropsis* sp., provided chondropsins A, B, and D,[1,2] several *Ircina* sponges yielded chondropsin C and 73-deoxychondropsin A,[3] and a collection of *Psammoclemma* sp. was reported to contain chondropsin A and its 73-deoxy analogue.[4] These compounds are all comprised of an extended acyclic portion and a core macrocyclic ring that contains both ester and amide functionalities. All of the chondropsins have 35-membered macrolide rings except for chondropsin D, which contains a 37-membered ring. We recently described the isolation of poecillastrin A (**3**), a ring-contracted member of the chondropsin family of macrolides, which was obtained from a deep-water collection (−359 m) of the Caribbean sponge *Poecillastra* sp.[5] Poecillastrin A is structurally related to the other chondropsins, but it is the first member of this class of compounds with a 33-membered macrocyclic ring. The chondropsins and poecillastrin A are all potent cytotoxins, and they produce a characteristic pattern of differential activity in the NCI 60-cell line antitumor screen. A COMPARE-algorithm analysis of their mean-graph profiles[6] with the NCI pure compound database revealed close similarity to other compounds that are known to inhibit vacuolar H^+-ATPases (V-ATPases). In particular, they showed a high COMPARE correlation coefficient with both the salicylihalamide/lobatamide[7] and bafilomycin/concanamycin[8] classes of V-ATPase inhibitors. This suggested that all of these compound classes share a common molecular target or pathway associated with their cytotoxic activity. Indeed, the chondropsins and poecillastrin A were subsequently found to be potent inhibitors of both fungal and mammalian V-ATPase with a pattern of specificity toward these enzymes that was unique from the other known inhibitors.[9] In an effort to further evaluate the structural characteristics of these bioactive macrolide lactams and establish a more complete understanding of the chemical diversity of this family of metabolites, several trace constituents of the *Poecillastra* sp. extract were further investigated. Detailed below is the isolation and structural assignment of two new members of this compound class, poecillastrins B (**1**) and C (**2**).





As described previously, the aqueous extract (20 g) of *Poecillastra* sp. was subjected to a series of multistep fractionation procedures that resulted in the isolation of the principle cytotoxic constituent, poecillastrin A (**3**).[5] Ultimately, an optimized and simplified isolation process was developed and employed to study the trace compounds that co-occurred in the extract. Crude extract and pooled chromatography fractions from the earlier separation work were partitioned between 90% aqueous MeOH and *n*-hexane. The dried 90% aqueous MeOH fraction was dissolved in water and extracted with $CHCl_3$. The $CHCl_3$ fraction, which exhibited the most potent cytotoxic activity, was subjected to gel filtration on Sephadex LH-20, followed by purification with reversed-phase

[#] Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champagne for his pioneering work on bioactive natural products.

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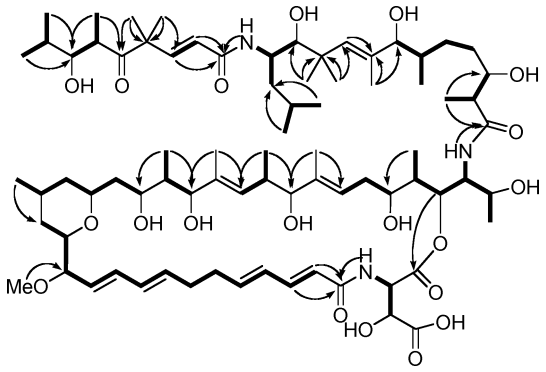

Figure 1. COSY and TOCSY correlations (bold lines) and key HMBC correlations (arrows) for poecillastrin B (**1**).

HPLC to afford two new metabolites, poecillastrins B (**1**, 600 μg) and C (**2**, 400 μg). The mass of these samples could not be reliably obtained by direct weighing but was later established by measuring their UV absorption at λ_{max} 261 nm and then employing the extinction coefficient (ϵ 10 200) previously calculated for poecillastrin A.[5] Complete NMR spectroscopic characterization of these low-mass samples (0.41 and 0.28 μmol for poecillastrins B and C, respectively) required utilization of high-sensitivity cryogenically cooled probe technologies.[10]

The molecular formula of poecillastrin B (**1**) was established as $C_{79}H_{131}N_3O_{20}$ by HRESIMS measurements (obsd $[M - H]^-$ *m*/*z* 1440.9248, calcd for $C_{79}H_{130}N_3O_{20}$, 1440.9233). An initial ^{1}H NMR spectrum of **1** showed the presence of three amide NH signals and numerous oxymethine protons, olefinic protons, and methyl groups that were very similar to those seen with poecillastrin A (**3**).[11] Partial structures comprising the various proton−proton spin systems were constructed via a combination of ^{1}H−^{1}H COSY and TOCSY cross-peaks (Figure 1) and analysis of HSQC correlations. Some cross-peaks involving oxymethine protons were partially obscured by substantial water and solvent signals in these data sets. However, all of these protons could ultimately be assigned and each adjoining partial structure in the molecule could be connected by an extensive array of HMBC correlations that were observed (Figure 1). The locations of the three amide groups and a ketone carbonyl in **1** were revealed by HMBC correlations between NH-3 (δ 7.98), H-5 (δ 6.31)/C-4 (δ 167.5), NH-43 (δ 7.65), H-69 (δ 1.13)/C-44 (δ 177.2), NH-56 (δ 7.69), H-58 (δ 6.18)/C-57 (δ 164.9), and H-78 (δ 1.23), H-79 (δ 1.29), H-80 (δ 0.86)/C-61 (δ 213.9), respectively (Figure 1).

The key spectroscopic differences observed between poecillastrin B (**1**) and poecillastrin A (**3**) centered on the C-32, C-40, C-41 region, which suggested they had different macrolide ring connections. The resonance for H-41 in **1** was shifted downfield to δ 5.30, in contrast to δ 3.66 in **3**, while H-32 appeared upfield at δ 3.51 in **1**, compared to a H-32 resonance at δ 5.26 in **3**. These data clearly indicated that the oxygen substituent on C-41 (δ 77.6) in **1** was esterified, and this was supported by a HMBC correlation between H-41 and the C-1 (δ 172.6) carbonyl group. All of the other NMR data for **1** were very similar to the NMR data previously recorded for **3**; thus poecillastrin B appeared to be the ring-expanded analogue of poecillastrin A in which the ester bridge incorporated the C-41 oxygen to give a 35-membered macrolide ring. The presence of a free carboxylic acid moiety in **1** was confirmed by treatment with trimethylsilyldiazomethane, which cleanly produced the monomethyl ester derivative as evidenced by LC-MS analysis ($[M + H]^+$ *m*/*z* 1457). Despite numerous attempts to optimize for different $^{2,3}J_{CH}$ coupling constants (3−12 Hz), no HMBC correlation was ever observed between NH-3 and C-1 that could exclude the possibility that C-34, rather than C-1, was connected to C-41 via the ester linkage. However, the C-1, C-2, C-33, C-34 fragment of **1** can be viewed as an imbedded β-hydroxyaspartic acid residue, and this residue forms an ester linked via C-1 in all of the other chondropsin/poecillastrin macrolides reported to date. Indeed, the NMR data for poecillastrin B (**1**) showed very close correspondence with the NMR data for chondropsin B (**4**),[1] a 35-membered ring analogue of **1** that differs in structure only at sights well removed from the ester bridge.[12] All of the ^{1}H and ^{13}C NMR resonances associated with the β-hydroxyaspartic acid equivalent and the point of macrocyclic ring closure were virtually identical for compounds **1** and **4** ($\Delta\delta_H$ < 0.04 ppm and $\Delta\delta_C$ < 0.5 ppm). This provided strong evidence that C-1 is the ester carbonyl and allowed us to assign the structure of poecillastrin B (**1**) as shown. Since poecillastrin A (**3**) and poecillastrin B (**1**) differ only by the point of ring closure of their respective macrocycles, interconversion from one structure to the other via a transesterification reaction is theoretically possible. While this type of rearrangement was indeed reported with chondropsin A and chondropsin D,[2] no evidence for interconversion between **1** and **3** was ever observed during the isolation or spectroscopic characterization of these compounds.

The molecular formula of poecillastrin C (**2**), as evidenced by HRESIMS measurements ($[M - H]^-$ *m*/*z* 1426.9091, calcd for $C_{78}H_{128}N_3O_{20}$, 1426.9073), was only one CH_2 group less than that of **1**, which implied the lack of a methylene or methyl group. Poecillastrin B (**1**) had four aliphatic methyl singlets and 12 methyl doublets that were discernible by analysis of its ^{1}H NMR spectrum and HSQC data; however compound **2** had only two aliphatic methyl singlets and 13 doublets. This suggested that one of the *gem*-dimethyl functionalities in **1** was replaced with a lone methyl group in **2**. 2D NMR analyses of **2** clearly indicated that there was only a single methyl substituent on C-53 in **2**. All of the other NMR data recorded for **2** were virtually identical to the NMR data obtained with **1**. Structural fragments were identified and assembled for **2** in a similar manner to that previously described for **1**. Even working with only 400 μg of **2** it was possible to assign virtually all of the NMR resonances. However, HMBC correlations that would have defined the ester and carboxylic acid carbonyl resonances in **2** were lacking in the NMR data sets, so all but these two carbons could be assigned. The close correspondence between the ^{1}H and ^{13}C NMR chemical shifts, coupling patterns, and heteronuclear correlations in both **1** and **2** revealed that poecillastrin C (**2**) is the C-53 desmethyl analogue of poecillastrin B (**1**).

Because of their close structural similarity, compounds **1** and **2** were very difficult to resolve chromatographically and purify. An advanced chromatography fraction comprised only of a mixture of **1** and **2** was obtained prior to the final HPLC resolution of the individual compounds. Due to the very limited mass of these samples, the mixture of **1** and **2** was used for cytotoxicity testing. When assayed against the LOX human melanoma cell line, these two compounds had an IC_{50} value that was less than 1 μg/mL, which is characteristic of the other chondropsin macrolides that we have tested.

Experimental Section

General Experimental Procedures. The optical rotations were measured with a Perkin-Elmer 241 polarimeter. Ultraviolet (UV) spectra were obtained on a Beckman DU-640 and IR spectra on a Perkin-Elmer 1600 FTIR spectrometer. ^{1}H NMR spectra were recorded on a Varian INOVA NMR spectrometer at 500 MHz. All 2D NMR data (^{1}H−^{1}H COSY, TOCSY, HSQC-TOCSY, HSQC, HMBC) were measured on a Varian INOVA 500 spectrometer with a 5 mm HCN inverse-detection Chili-Probe. The radio frequency coils and preamplifier of the Chili-Probe were cooled with cold helium gas to approximately 25 and 60 K, respectively. ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peak: δ_H 2.74 and δ_C 30.1 for DMF-d_7. High-resolution mass spectra were acquired on a Waters LCT Premier time-of-flight (TOF) mass spectrometer.

Animal Material. Samples of *Poecillastra* sp. were collected by a manned submersible at a depth of 359 m near Settelement Point, Grand Bahama Island, Bahamas, in November 1987. The specimen was collected by the Harbor Branch Oceanographic Institute under contract

Table 1. NMR Data of Poecillastrin B (**1**) and C (**2**) in DMF-d_7

	poecillastrin B				poecillastrin C		
pos.	^{1}H	^{13}C[a]	COSY	HMBC	^{1}H	^{13}C[a]	HMBC
1		172.6				na[b]	
2	5.04 dd (9.3, 2.1)	55.6	3, 33	C-34	5.07 dd (9.5, 2.3)	55.9	
3	7.98 d (9.6)		2	C-4	8.00[c]		C-4
4		167.5				167.8	
5	6.31 d (15.5)	123.9	6	C-4, 7	6.31[c]	124.2	C-4
6	7.19 dd (15.5, 10.9)	141.2	5, 7	C-4	7.16 dd (11.3, 15.2)	141.6	C-4
7	6.33[c]	129.5	6, 8	C-8	6.33[c]	129.8	
8	6.19[c]	142.3	7, 9	C-6	6.20 dd (15.0, 7.4)	142.8	
9	2.37[c]	34.1	8		2.34[c]	34.3	
10	2.22[c]	32.7	11		2.22[c]	32.9	
11	5.89 ddd (5.5, 9.0, 15.0)	134.9	10, 12		5.88 ddd (5.2, 9.1, 15.3)	135.2	
12	6.30[c]	131.0	11, 13		6.28[c]	131.4	
13	6.42 dd (15.0, 10.3)	134.9	12, 14	C-11, 15	6.41 dd (15.4, 10.6)	135.1	
14	5.51 dd (15.1, 9.1)	131.5	13, 15		5.52 dd (15.3, 9.2)	131.8	
15	3.98[c]	80.5	14, 16	C-16, 82	3.97[c]	80.8	
16	3.60 m	74.9	15, 17α		3.59 m	75.2	
17α	1.15 m	34.0	16		1.14 m	34.2	
17*β*	1.87 m				1.84 m		
18	1.86 m	25.9	19α, 35		1.86 m	26.0	
19α	0.84 m	41.1	18, 20		0.82 m	41.2	
19*β*	1.52 m				1.51 m		
20	3.56 m	68.1	19α, 21α		3.55 m	68.5	
21α	1.16 m	43.0	20, 22		1.15 m	43.3	
21*β*	1.42 m		22		1.40 m		
22	4.22 m	65.3	21α, 21*β*		4.21 m	65.4	
23	1.39 m	41.6	24, 36		1.37 m	41.9	
24	3.82 (10.7)	80.1	23		3.81 d (9.8)	80.3	
25		138.2				138.5	
26	5.08 d (10.6)	133.1	27, 37		5.08 d (9.2)	133.4	
27	2.51 m	36.2	26, 28, 38		2.50 m	36.3	
28	3.51 m	83.0	27		3.51 m	83.2	
29		137.0				137.3	
30	5.32[c]	126.9	31α, 31*β*, 39		5.28 dd (10.2, 6.5)	127.1	
31α	1.81 m	34.3	30, 31*β*		2.26 m	34.3	
31*β*	2.34 m		30, 31α, 32				
32	3.51 m	69.4	31*β*		3.48 m	69.0	
33	4.88 dd (5.2, 1.5)	71.6			4.88 brd (3.1)	71.5	
34		171.7				na[b]	
35	0.90[c]	22.6	18	C-17, 18, 19	0.90[c]	22.8	C-17, 18, 19
36	0.58 d (7.2)	9.2	23	C-22, 23, 24	0.57 d (7.0)	9.4	C-22, 23, 24
37	1.61 s	10.7		C-24, 25, 26	1.60 s	10.9	C-24, 25, 26
38	0.67 d (6.6)	17.6	27	C-26, 27, 28	0.65 d (6.9)	17.8	C-26, 27, 28
39	1.57 s	10.9	30	C-28, 29, 30	1.56 s	11.0	C-28, 29, 30
40	1.79 m	40.3	41, 66		1.77 m	40.4	
41	5.30 m	77.6	40, 42		5.25 m	78	
42	4.17 dd (10.0, 3.8)	53.6	41, 43, 67		4.15 dd (9.8, 3.8)	53.9	
43	7.65 d (9.7)		42	C-44	7.58 d (9.8)		
44		177.2				177	
45	2.61 m	46.5	46, 69	C-44, 49	2.58 m	47.0	C-44
46	3.53 m	73.7	45		3.51 m	74.3	
47α	1.47 m	33.2			1.46 m	33.1	
47*β*	1.51 m		48α		1.50 m		
48α	1.28 m	30.0	47*β*, 49		1.33 m	30.0	
48*β*	1.44 m				1.38 m		
49	1.58 m	36.2	48α, 50, 70		1.59 m	36.3	
50	3.60[c]	83.3	49		3.60[c]	83.0	
51		136.0				137.3	
52	5.49 s	134.8	72	C-50, 54, 71, 72, 73	5.41 d (9.9)	130.3	
53		40.7			2.64 m	35.6	
54	3.51[c]	81.5			3.38[c]	78.3	
55	4.21 m	49.9	56, 74α		4.06 m	50.4	
56	7.69 d (9.5)		54[d], 55	C-57	7.65 d (9.4)		C-57
57		164.9				165.3	
58	6.18 d (15.5)	124.4	59	C-57, 59, 60, 61	6.18 d (15.8)	124.7	C-57, 60
59	6.93 d (15.5)	146.5	58	C-57, 58, 60, 61, 78, 79	6.91 d (15.8)	147.0	C-57, 60, 78, 79
60		51.4				51.4	
61		213.9				213.7	
62	3.17 dd (9.7, 6.9)	44.4	80	C-61, 63, 80	3.13 dd (9.7, 6.8)	44.7	
63	3.53 m	77.4			3.52 m	77.6	
64	1.75 m	29.5	65, 81		1.75 m	29.6	
65	0.94[c]	20.5	64	C-63, 64, 81	0.93[c]	20.8	C-63, 64, 81
66	0.94[c]	9.1	40	C-32, 40, 41	0.93[c]	9.2	C-32, 40, 41
67	3.80 m	69.0	42, 68		3.79 m	69.2	
68	0.93[c]	15.4	67	C-42, 67	1.12 d (7.1)	21.6	C-42, 67
69	1.13 d (6.9)	21.6	45	C-44, 45, 46	1.14 d (7.1)	15.6	C-44, 45, 46
70	1.18 d (6.9)	15.4	49	C-48, 49, 50	0.94[c]	15.7	C-49, 50
71	1.68 s	13.4	52	C-50, 51, 52	1.53 s	12.0	C-50, 51, 52
72	1.12 s	25.2		C-52, 53, 54, 73	0.96 s	17.7	C-52, 53, 54
73	1.21 s	26.6		C-52, 53, 54, 72			
74α	1.48 m	40.8			1.43 m	40.6	
74*β*	1.50 m		55		1.55 m		
75	1.55 m	25.1			1.56 m	25.2	
76	0.86 d (6.8)	24.1		C-74, 75, 77	0.87 d (7.1)	24.2	C-74, 75, 77
77	0.88 d (6.8)	21.6		C-74, 75, 76	0.89 d (7.1)	21.8	C-74, 75, 76

Table 1. (Continued)

	poecillastrin B				poecillastrin C		
pos.	^{1}H	^{13}C[a]	COSY	HMBC	^{1}H	^{13}C[a]	HMBC
78	1.23 s	23.4		C-59, 60, 61, 79	1.23 s	23.7	C-59, 60, 61, 79
79	1.29 s	23.6		C-59, 60, 61, 78	1.29 s	23.6	C-59, 60, 61, 78
80	0.86 d (6.9)	15.4	62, 63[d]	C-61, 62, 63	0.86 d (7.1)	15.6	C-61, 62, 63
81	0.84 d (6.9)	14.1	64	C-63, 64, 65	0.83 d (6.9)	14.1	C-63, 64, 65
82	3.23 s	55.9		C-15	3.22 s	56.0	C-15

[a] Carbon assignments made from HSQC and HMBC data. [b] Not assigned due to lack of HMBC correlations. [c] Overlapped signals. [d] These correlations were assigned from TOCSY data.

with the NCI. Taxonomic identification was provided by Shirley A. Pomponi. The voucher number for this collection is Q66B974, and a voucher sample is maintained at the Smithsonian Institution in Washington, D.C.

Extraction and Isolation. A 2 g portion of the dried aqueous extract of *Poecillastra* species and chromatography fractions from previous fractionation efforts[5] were partitioned between 90% aqueous MeOH and hexane. The aqueous methanol fraction was dried and extracted with $CHCl_3$ after the addition of H_2O. The $CHCl_3$ fraction was subjected to a size exclusion separation on LH-20 to give four fractions. The first and second fractions were separated by C_4 wide-pore column chromatography and finally purified by repeated RP-HPLC (C_{18} Dynamax 60A 10 × 250 mm) with a linear gradient from 45% aqueous MeCN + 0.1% TFA to 100% MeCN + 0.1% TFA to afford poecillastrin B (**1**, 600 μg) and poecillastrin C (**2**, 400 μg).

Poecillastrin B (1): white gum; $[\alpha]^{25}_D$ +56 (*c* 0.02, MeOH); UV (MeOH) λ_{max} 261 nm; ^{1}H and ^{13}C NMR data, Table 1; HRFABMS [M − H]$^-$ *m*/*z* 1440.9248, calcd for $C_{79}H_{130}N_3O_{20}$, 1440.9233 (−1.5 mmu).

Poecillastrin C (2): white gum; $[\alpha]^{25}_D$ +31 (*c* 0.02, MeOH); UV (MeOH) λ_{max} 261 nm; ^{1}H and ^{13}C NMR data, Table 1; HRFABMS [M − H]$^-$ *m*/*z* 1426.9091, calcd for $C_{78}H_{128}N_3O_{20}$, 1426.9073 (−1.3 mmu).

Methylation of Poecillastrins B (1) and C (2). A 10 μg portion of **1** in 50 μL of MeOH was treated with 50 μL of trimethylsilyldiazomethane (2.0 M in hexane) for 10 min at rt. The reaction mixture was dried under a stream of N_2, which was analyzed by LC-MS to afford a molecular ion peak at *m*/*z* 1457 [M + H]$^+$. Methylation of **2** was carried out in the same manner as that of **1** to afford a molecular ion peak at *m*/*z* 1443 [M + H]$^+$.

Cytotoxicity Evaluations. DMSO solutions of the test samples were diluted 1:50 with cell culture media and assayed against the LOX cell line as previously described.[13]

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Supporting Information Available: ^{1}H NMR, COSY, TOCSY, HSQC, and HMBC spectra for compounds **1** and **2**. This information is provided free of charge via the Internet at http://pubs.acs.org.

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(11) To facilitate direct spectroscopic comparisons and to avoid confusion in the text, the numbering scheme employed with poecillastrins B (**1**) and C (**2**) was the same as that originally used for poecillastrin A (**3**).[5]
(12) Chondropsin B (**4**) is lacking the methoxyl substituent at C-15 and a methyl group at the carbon numbered C-53 in poecillastrin B (**1**), while it has an additional methyl ester functionality at its acyclic terminus.
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