Spongiacidins A−D, New Bromopyrrole Alkaloids from *Hymeniacidon* Sponge

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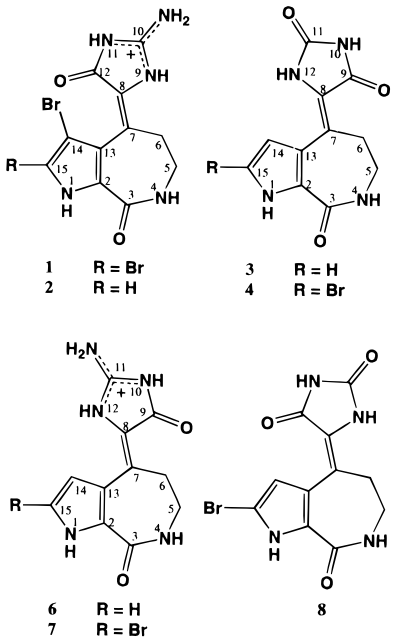
Received December 21, 1997

Four new bromopyrrole alkaloids, spongiacidins A−D (**1**−**4**), have been isolated from an Okinawan marine sponge *Hymeniacidon* sp. and the structures elucidated on the basis of spectral data and chemical means.

Bromopyrrole alkaloids are secondary metabolites characteristic of marine Porifera belonging to several genera.[1] During our search for bioactive substances from marine organisms,[2] we previously isolated several bioactive bromopyrrole alkaloids such as ageliferins,[3] oxysceptrin,[4] manzacidins A−C,[5] konbu'acidin A,[6] and tauroacidins A and B[7] from *Agelas* or *Hymeniacidon* sponges. Recently we have investigated extracts of an Okinawan sponge *Hymeniacidon* sp. and isolated four new bromopyrrole alkaloids, spongiacidins A−D (**1**−**4**). Here we describe the isolation and structure elucidation of **1**−**4**.

The sponge *Hymeniacidon* sp., collected off Ishigaki Island, Okinawa, was extracted with MeOH. The *n*-BuOH-soluble materials of the extract were subjected to Sephadex LH-20 (MeOH) and C_{18} columns (CH_3CN−H_2O−CF_3CO_2H) followed by C_{18} HPLC (MeOH−H_2O−CF_3CO_2H) to yield spongiacidins A (**1**, 2×10^{-4} %, wet wt), B (**2**, 1×10^{-4} %), C (**3**, 2×10^{-4} %), and D (**4**, 4×10^{-4} %) as colorless amorphous solids, together with the known bromopyrrole alkaloids, debromohymenialdisine[8] (**6**, 0.02%), hymenin[9] (8×10^{-4} %), and manzacidin B[5] (2×10^{-4} %).

Spongiacidin A (**1**) showed pseudomolecular ion peaks at *m*/*z* 402, 404, and 406 in the ratio of 1:2:1 in its ESIMS, and the molecular formula $C_{11}H_9N_5O_2Br_2$ was confirmed by HRESIMS. UV absorptions of **1** at 332 (ϵ 7000) and 270 nm (7000) were reminiscent of those of debromohymenialdisine (**6**)[8] and hymenialdisine (**7**).[10] ^{13}C NMR data (Table 1) of **1** contained signals due to nine sp^2 quaternary carbons and two sp^3 methylenes, and the ^1H NMR spectrum (Table 1) showed five NH and two methylene proton resonances. Because it was difficult to determine the structure of **1** from its 2D NMR data, further structure elucidation was performed by 2D NMR correlations (Figure 1) on its 1,12,14-trimethyl derivative (**5**), obtained by treatment of **1** with CH_2N_2. The HMBC spectrum of **5** showed cross peaks for H_2-5/C-7, H_2-6/C-8, 9-NMe/C-8, 9-NMe/C-10, 11-NMe/C-10, and 11-NMe/C-12, indicating that the aminoimidazolinone ring was attached at C-7. The (*E*)-geometry of the tetrasubstituted double bond at C-7 was deduced from the carbon chemical shift of C-6 (δ_C 38.8) in **1**, corresponding to that (δ_C 36.2) of axinohydantoin[11] (**8**) rather than that (δ_C 31.2) of debromohymenialdisine[8] (**6**). Thus the structure of spongiacidin A was assigned as **1**.



The molecular formula of spongiacidin B (**2**) was established to be $C_{11}H_{10}N_5O_2Br$ by HRESIMS. ^1H and ^{13}C NMR data of **2** were similar to those of spongiacidin A (**1**), except for the presence of an sp^2 methine resonance [δ_H 7.28 (s), δ_C 123.2 (d)] in **2**, which was assignable to C-15 on the basis of $^1J_{C-H}$ (187 Hz).[12] Therefore, spongiacidin B (**2**) was 2-debromo-**1**.

HRFDMS data of spongiacidin C (**3**) indicated the molecular formula to be $C_{11}H_{10}N_4O_3$. ^1H and ^{13}C NMR data of **3** were close to those of spongiacidins A (**1**) and B (**2**), implying that **3** possessed the same ring system. The proton signals at δ_H 11.77 (br s), δ_H 6.53 (br s), and δ_H 6.97 (br s) corresponded to NH-1, H-14, and H-15, respectively, on the pyrrole ring of **6**, while the carbon chemical shifts of C-8 (δ_C 122.8), C-9 (δ_C 165.4), and C-11 (δ_C 154.4) suggested the presence of a hydantoin ring. The $D^{7,8}$ double bond was assigned a (*Z*)-geometry from the chemical shift of C-6 (δ_C 30.7). Thus, spongiacidin C (**3**) was concluded to be (7*Z*)-debromoaxinohydantoin.

The molecular formula of spongiacidin D (**4**) was confirmed to be $C_{11}H_9N_4O_3Br$ by HRFDMS, suggesting

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S0163-3864(97)00565-X CCC: $15.00
Published on Web 05/01/1998

Table 1. ^{1}H and ^{13}C NMR Data of Spongiacidins A−D (**1**−**4**) in DMSO-d_6

	1		2		3		4	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C[b]
1	13.38		12.54		11.77		12.34	
2		127.2		126.1		125.5		127.0
3		163.4		163.4		163.0		162.3
4	8.04		8.00		7.88		7.94	
5	3.26	38.8	3.25	41.2	3.28	40.2	3.24	40.0
6	3.26	38.8	3.25	40.4	3.23	30.7	3.24	31.0
7		125.6		125.8		122.5		122.7
8		124.1		123.8		122.8		123.5
9	10.82		10.79			165.4		165.3
10		154.5		155.1	9.41		9.58	
11	8.42		8.39			154.4		154.4
12		163.0		164.0	11.02		11.06	
13		120.8		119.4		121.4		121.0
14		98.6		98.5	6.53	109.8	6.53	111.7
15		109.4	7.28	123.2	6.97	122.2		104.4
10-NH_2	9.21		9.31					

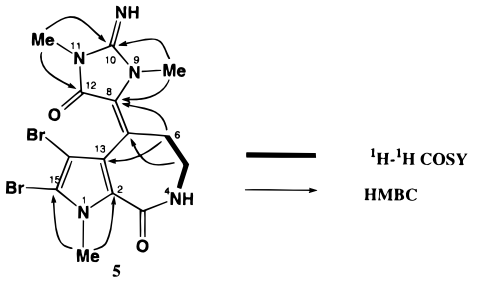

Figure 1. 2D NMR correlations for trimethyl derivative (**5**) of spongiacidin A (**1**).

a monobrominated form of spongiacidin C (**3**). Although the ^{1}H NMR spectrum of **4** was similar to that of **3**, one of two sp^2 methine signals in **3** was not observed for **4**. The carbon chemical shifts for C-14 [δ_C 111.7 (d)] and C-15 [δ_C 104.4 (s)] as well as $^1J_{C-H}$ for C-14 (163 Hz)[12] indicated that the bromine was attached at C-15. Thus, spongiacidin D (**4**) was assigned as the (7*Z*)-isomer of axinohydantoin (**8**).

Spongiacidins A (**1**) and B (**2**) are pyrrolo[2,3-*c*]-azepine-type bromopyrrole alkaloids possessing (7*E*)-geometry, while spongiacidins C (**3**) and D (**4**) have the (7*Z*)-geometry. Spongiacidins A (**1**) and B (**2**) exhibited inhibitory activity against *c-erbB*-2 kinase (IC_{50}, 8.5 and 6.0 μg/mL, respectively) and cyclin-dependent kinase 4 (IC_{50}, 32 and 12 μg/mL, respectively), while spongiacidins C (**3**) and D (**4**) showed no inhibitory activity against either kinase (IC_{50}, >50 μg/mL).

Experimental Section

General Procedures. Optical rotations were recorded on a JASCO DIP-360 polarimeter. The IR and UV spectra were taken on JASCO FT/IR-5300 and JASCO Ubest-35 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX-600 and JEOL EX-400 spectrometers, respectively. ESIMS and FDMS were obtained on a JEOL SX-102A spectrometer. EIMS were measured using a JEOL DX-303 spectrometer at 70 eV.

Sponge Materials. The brown sponge *Hymeniacidon* sp. (order Halichondrida; family Halichondriidae) was collected off Konbu, Okinawa Island, and kept frozen until used. The soft and compressible sponge has some membranous tissue around the oscules. Its mesohyl skeleton has a plumose to plumoreticulate skeleton with strong fiber development; the fibers are 180 mm across and are cored centrally by approximately seven styles. The meshes between the primary fibers are narrow centrally, and the spicules form fans at the surface. Megascleres (mean size, 502 × 9 mm) are long and smooth with some tylosis. Microsclere is not observed. The voucher specimen (SS-964) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (1.4 kg, wet wt) was extracted with MeOH (1 L × 2). The MeOH extract (53.6 g) was partitioned between EtOAc (500 mL × 3) and H_2O (500 mL), and the aqueous layer was extracted with *n*-BuOH (500 mL × 3). The *n*-BuOH-soluble material (6.92 g) was subjected to Sephadex LH-20 (MeOH), C_{18} columns (Develosil ODS-LOP, Nomura Chemical, 45 × 490 mm; $CH_3CN-H_2O-CF_3CO_2H$, 20:80:0.1), and C_{18} HPLC (Develosil ODS−HG-5, Nomura Chemical, 10 × 250 mm; $MeOH-H_2O-CF_3CO_2H$, 46:54:0.1; flow rate, 2.5 mL/min; UV detection at 260 nm) to yield spongiacidins A (**1**, 2 × 10^{-4} %, t_R 16 min), B (**2**, 1 × 10^{-4} %, t_R 14 min), C (**3**, 2 × 10^{-4} %, t_R 9 min), and D (**4**, 4 × 10^{-4} %, t_R 12 min),

Spongiacidin A (1): colorless amorphous solid; UV (MeOH) λ_{max} 206 (ϵ 10 000), 270 (7000), and 332 nm (7000); IR (film) ν_{max} 3440 (br), 1680, 1470, 1205, and 1140 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS (pos., MeOH) *m/z* 544, 546, and 548 [$(M + H)^+$, ca. 1:2:1]; HRESIMS *m/z* 401.9221 $(M + H)^+$ (calcd for $C_{11}H_{10}N_5O_2{}^{79}Br_2$, 401.9201).

Spongiacidin B (2): colorless amorphous solid; UV (MeOH) λ_{max} 204 (ϵ 10 000), 272 (10 000), and 331 nm (9000); IR (film) ν_{max} 3440 (br), 1680, 1470, 1210, and 1135 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS (pos., MeOH) *m/z* 324 and 326 [$(M + H)^+$, ca. 1:1]; HRESIMS *m/z* 324.0087 $(M + H)^+$ (calcd for $C_{11}H_{11}N_5O_2{}^{79}Br$, 324.0096).

Spongiacidin C (3): colorless amorphous solid; UV (MeOH) λ_{max} 273 (ϵ 9700) and 312 nm (3800); IR (film) ν_{max} 3420 (br), 1690, 1630, 1210, and 1040 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); FDMS (pos.) *m/z* 246 $(M)^+$; HRFDMS *m/z* 246.0729 M^+ (calcd for $C_{11}H_{10}N_4O_3$, 246.0753).

Spongiacidin D (4): colorless amorphous solid; UV (MeOH) λ_{max} 272 (ϵ 10 000) and 310 nm (4000); IR (film) ν_{max} 3420 (br), 1690, 1635, 1210, and 1040 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); FDMS (pos.) *m/z* 324 and 326 (M^+, ca. 1:1); HRFDMS *m/z* 323.9847 M^+ (calcd for $C_{11}H_9N_4O_3Br$, 323.9857).

Methylation of Spongiacidin A (1). A MeOH solution (0.5 mL) of spongiacidin A (**1**, 12.4 mg) was treated with CH_2N_2 in Et_2O (1 mL) at room temperature for 1 h. After evaporation, the residue was subjected to a Si gel column ($CHCl_3$−MeOH) to give the trimethyl derivative (**5**) as a colorless amorphous solid: UV (MeOH) λ_{max} 207 (ϵ 17 000), 248 (sh) and 339 nm (11 000); IR (film) ν_{max} 3300 (br) and 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.71 (3H, s, 12-NMe), 2.98 (1H, m, H-9), 3.16 (3H, s, 14-NMe), 3.35 (1H, m, H-8), 3.42 (1H, m, H-8), 3.73 (1H, m, H-9), 3.92 (3H, s, 1-NMe), and 5.92 (1H, t, J = 6.3 Hz, NH-7); ^{13}C NMR ($CDCl_3$) δ 25.2 (q, 14-NMe), 30.8 (q, 12-NMe), 35.4 (q, 1-NMe), 36.6 (t, C-9), 40.1 (t, C-8), 100.8 (s, C-3), 111.9 (s, C-2), 114.0 (s, C-10), 123.8 (s, C-4), 125.7 (s, C-5), 130.3 (s, C-11), 154.5 (s,

C-13), 163.3 (s, C-15), and 165.0 (s, C-6); EIMS (70 eV) *m/z* 443, 445, and 447 (M^+, ca. 1:2:1); HREIMS *m/z* 442.9580 M^+ (calcd for $C_{14}H_{15}Br_2N_5O_2$, 442.9593).

Acknowledgment. We thank Dr. K. Watanabe, NMR laboratory, Faculty of Agriculture, Hokkaido University, for measurements of ESIMS and FDMS; Mr. Z. Nagahama for his help with sponge collection; Dr. J. Fromont, Western Australian Museum, for identification of the sponge; and Banyu Pharmaceutical Co., Ltd., for kinase assays. This work was partly supported by a grant-in-aid from the Akiyama Foundation and by a grant-in-aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

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NP970565H