Suberedamines A and B, New Bromotyrosine Alkaloids from a Sponge *Suberea* Species

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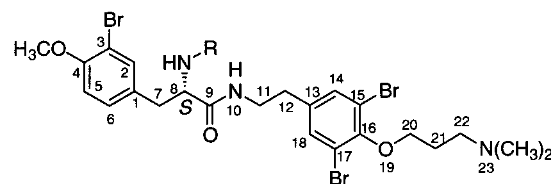
Two new cytotoxic bromotyrosine alkaloids, suberedamines A (**1**) and B (**2**), have been isolated from an Okinawan marine sponge *Suberea* sp. The structures were elucidated on the basis of spectroscopic data and chemical means.

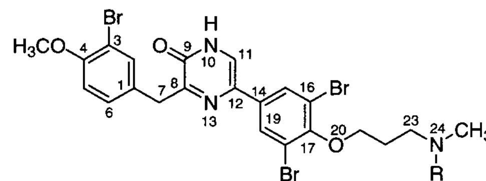
Marine sponges of the order Verongidae have been found to contain a number of bromotyrosine alkaloids.[1] In our search for bioactive substances from marine sponges,[2] a series of bromotyrosine alkaloids have been isolated from a Verongid marine sponge, *Psammaplysilla purea*.[3] Recently we have investigated extracts of an Okinawan marine sponge, *Suberea* sp., and isolated two unique bromotyrosine alkaloids, ma'edamines A (**3**) and B (**4**), with a 2(1H)pyrazinone moiety.[4] Further investigation of extracts of the same sponge *Suberea* sp. led to the isolation of two new bromotyrosine alkaloids, suberedamines A (**1**) and B (**2**). Here we describe the isolation and structure elucidation of **1** and **2**.

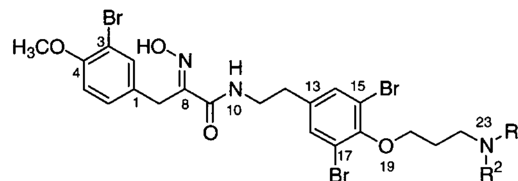
The sponge *Suberea* sp. (family, Aplysinellidae; order, Verongida) collected off Maeda Cape, Okinawa, was extracted with MeOH. The *n*-BuOH-soluble materials of the extract were subjected to a silica gel column chromatography ($CHCl_3$/*n*-BuOH/AcOH/H_2O) followed by reversed-phase HPLC (CH_3CN/H_2O/CF_3CO_2H) to yield suberedamines A (**1**, 0.04%, wet weight) and B (**2**, 0.025%) as colorless, amorphous solids together with known bromotyrosine alkaloids, ma'edamines A (**3**) and B (**4**), aplysamine-2 (**5**),[5] and purpuramines H (**6**) and I (**7**).[6]

The FABMS spectrum of suberedamine A {**1**, $[\alpha]^{23}_D$ +16° (*c* 0.1, MeOH)} showed the pseudomolecular ion peaks in the ratio of 1:3:3:1 at *m/z* 634, 636, 638, and 640, respectively, indicating the presence of three bromine atoms in the molecule. HRFABMS data of **1** revealed the molecular formula $C_{23}H_{30}N_3O_3Br_3$ [*m/z* 633.9403 $(M + H)^+$, Δ −0.2 mmu]. Suberedamine A (**1**) was positive to a ninhydrin test, indicating that **1** possessed primary amino group(s). IR absorptions suggested the presence of OH and/or NH (3420 cm^{-1}) and amide carbonyl (1685 cm^{-1}) groups, while the UV absorption [λ_{max}281 nm (ϵ 2400)] was attributable to substituted benzenoid chromophore(s). The ^{13}C NMR (Table 1) spectrum disclosed signals due to seven sp^2 quaternary carbons containing an amide (δ_C 170.30), five sp^2 methines, an sp^3 methine, six sp^3 methylenes, and three methyls. The ^1H NMR (Table 1) spectrum showed signals due to a 1,3,4-trisubstituted benzene ring (C-1−C-6) and a 1,3,4,5-tetrasubstituted ring (C-13−C-18), an aminopropanol unit (O-19−N-23), a methoxy, and a dimethylamino group. The presence of 3-bromo-4-methoxyphenyl (C-1−C-6) and 3,5-dibromo-4-(3-dimethylamino)propyloxyphenyl (C-13−N-23) moieties was deduced from comparison of the ^{13}C NMR data of **1** with those of aplysamine-2 (**5**).[5] Analyses of $^1H-^1H$ COSY and HMQC spectra revealed the connectivities from C-7 to C-8 and from C-11 to C-12. The methine carbon

Chart 1. Structures of Suberedamines A (**1**) and B (**2**), Ma'edamines A (**3**) and B (**4**), Aplysamine-2 (**5**), and Purpureamines H (**6**) and I (**7**)







signal at δ_C 56.52 was suggested to be adjacent to a primary amino and an amide carbonyl group.[7] Selected HMBC correlations of **1** are shown in Figure 1. The absolute stereochemistry at C-8 was determined as follows. Suberedamine A (**1**) was hydrolyzed with hydrobromic acid and then subjected to catalytic reduction with palladium-charcoal under H_2 atmosphere.[8] Chiral HPLC analyses of the reaction mixture revealed a peak for a L-tyrosine residue, and no peak for D-tyrosine was observed, indicating that **1** has the 8*S*-configuration. Thus, the structure of suberedamine A was concluded to be **1**.

HRFABMS data [*m/z* 647.9280 $(M + H)^+$, Δ +1.8 mmu] of suberedamine B (**2**) showed the molecular formula $C_{24}H_{32}N_3O_3Br_3$, indicating that **2** was a monomethyl form of suberedamine A (**1**). ^1H and ^{13}C NMR data (Table 1) of **2** were similar to those of **1**, although an *N*-methyl signal (δ_H 2.62, δ_C 33.26) was newly observed. The methine carbon at C-8 (δ_C 64.88) was shifted to lower field than that (δ_C

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Table 1. ^{1}H and ^{13}C NMR Data of Suberedamines A (**1**) and B (**2**) in CD_3OD

position	**1** δ_H[a]		J	δ_C[b]		**2** δ_H[a]		J	δ_C[b]	
1				129.80	s				129.35	s
2	7.47	d	1.9	136.05	d	7.44	d	1.1	136.06	d
3				113.64	s				113.58	s
4				157.90	s				157.92	s
5	7.04	d	8.5	114.46	d	7.04	d	8.4	114.40	d
6	7.20	dd	1.9, 8.5	131.71	d	7.18	dd	1.1, 8.4	131.75	d
7	3.08	dd	7.8, 14.0	38.30	t	3.12	dd	5.7, 13.6	37.37	t
	2.98	dd	7.3, 14.0			3.05	dd	8.6, 13.6		
8	4.00	t	7.3	56.52	d	3.90	m		64.88	d
9				170.30	s				168.89	s
11	3.56	m		42.28	t	3.47	m		42.10	t
	3.32	dd	6.6, 13.2			3.38	m			
12	2.74[a]	t	7.2	35.77	t	2.73	m		35.75	t
						2.63	m			
13				140.86	s				140.77	s
14	7.51	s		135.13	d	7.47	s		135.09	d
15				119.73	s				119.70	s
16				153.17	s				153.17	s
17				119.73	s				119.70	s
18	7.51	s		135.13	d	7.47	s		135.09	d
20	4.13[a]	t	5.6	71.90	t	4.12[a]	t	5.5	71.87	t
21	2.32[a]	tt	5.6, 7.9	27.17	t	2.32[a]	tt	5.5, 7.5	27.15	t
22	3.54[a]	t	7.9	57.79	t	3.54[a]	t	7.4	57.75	t
OCH_3	3.91[b]	s		57.60	q	3.90[b]	s		57.59	q
$N(CH_3)_2$	3.00[c]	s		44.49	q	3.00[c]	s		44.44	q
NCH_3						2.62[b]	s		33.26	q

[a] 2H. [b] 3H. [c] 6H.

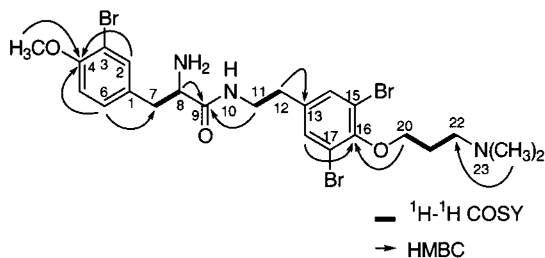

Figure 1. Selected 2D NMR correlations of suberedamine A (**1**).

56.62) of **1**, indicating that **2** was the 8-*N*-methyl form of **1**. This was supported by the HMBC correlation from NCH_3 to C-8. Suberedamine B (**2**) was subjected to the same reaction as described above to afford *N*-methyltyrosine, which was found to be the L-form by chiral HPLC analysis, thus indicating that the absolute configuration at C-8 was *S*. Therefore, the structure of suberedamine B was elucidated to be **2**.

Most of bromotyrosine alkaloids possess an oxime group for the α-carbon of tyrosine residues, while bromotyrosine alkaloids having a primary amino or an *N*-methyl amino group at the α-carbon such as suberedamines A (**1**) and B (**2**) are rare.[8-10] Biogenetically suberedamine A (**1**) may be converted into aplysamine-2 (**5**) through oxidation, followed by generation of ma'edamine A (**3**). Suberedamines A (**1**) and B (**2**) exhibited cytotoxicity against murine leukemia L1210 cells (IC_{50}, 8.0 and 8.6 μg/mL, respectively) and epidermoid carcinoma KB cells (IC_{50}, 9.0 and >10 μg/mL, respectively) in vitro. Furthermore, suberedamines A (**1**) and (**2**) showed antibacterial activity against *Micrococcus luteus* with an MIC value of 12.6 μg/mL.

Experimental Section

General Experimental Procedures. These were reported elsewhere.[2] The sponge *Suberea* sp. was described previously.[4]

Extraction and Isolation. The sponge *Suberea* sp. (0.85 kg, wet wt) was extracted with MeOH (1 L × 2), and the extract was partitioned between EtOAc (500 mL × 3) and 1 N aqueous NaCl (500 mL). Then the aqueous layer was extracted with *n*-BuOH (500 mL × 3). Parts (5 g) of the *n*-BuOH soluble materials (20 g) were subjected to a Si gel column ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5:6:1:1) and then reversed-phase HPLC [TSK-GEL ODS-80TS, TOSOH Co., Ltd., 20 × 250 mm; eluent, $CH_3CN/H_2O/CF_3CO_2H$, 30:70:0.1; flow rate, 6 mL/min; UV detection at 245 nm] to afford suberedamines A (**1**, 84 mg, 0.04%, wet wt, t_R 22 min) and B (**2**, 54 mg, 0.025%, t_R 26 min).

Suberedamine A (1): colorless, amorphous solid; mp 64–67 °C; $[\alpha]^{25}_D$ +21° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 281 nm (ε 2400); IR (KBr) ν_{max} 3425 and 1655 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); FABMS (pos.) *m/z* 634, 636, 638, and 640 (1:3:3:1, M + H)$^+$; HRFABMS *m/z* 633.9944 [calcd for $C_{23}H_{31}N_3O_3{}^{79}Br_3$, (M + H)$^+$ 633.9916].

Suberedamine B (2): colorless, amorphous solid; mp 79–81 °C; $[\alpha]^{25}_D$ +16° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 281 nm (ε 2900); IR (KBr) ν_{max} 3425 and 1655 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); FABMS (pos.) *m/z* 648, 650, 652, and 654 (1:3:3:1, M + H)$^+$; HRFABMS *m/z* 648.0092 [calcd for $C_{24}H_{33}N_3O_3{}^{79}Br_3$, (M + H)$^+$ 648.0072].

Absolute Configuration at C-8. Suberedamine A (**1**, 3.0 mg) was treated with 48% hydrobromic acid (100 μL) at 120 °C for 48 h. After evaporation in vacuo, the residue was subjected to C_{18} HPLC (TSK-GEL ODS-80TS, 4.6 × 250 mm; eluent, MeOH/H_2O, 33:67; flow rate, 0.9 mL/min; UV detection at 230 nm) to afford 3-bromotyrosine (0.4 mg, t_R 4.8 min). To a solution of 3-bromotyrosine (0.3 mg) in MeOH was added 10% Pd/C (0.1 mg), and stirring was continued under H_2 atmosphere at room temperature for 1 h. Chiral HPLC analyses of the reaction mixture were carried out using a SUMICHIRAL OA-5000 column [Sumitomo Chemical Industry; 4 × 150 mm; eluent, MeOH/H_2O (15:85) containing 2 mM $CuSO_4$, flow rate 1 mL/min; detection at 254 nm]. Retention times (min) of authentic D- and L-tyrosine were 20.4 and 14.2 min, respectively, and that of the hydrolysate of **1** was 14.2 min.

Suberedamine B (**2**, 3.1 mg) was hydrolyzed and purified under the same procedure as described above to yield *N*-methyl-3-bromotyrosine (0.3 mg, t_R 3.6 min), a part (0.2 mg) of which was reduced under the same conditions. Chiral HPLC analyses of the reaction mixture were performed using the following conditions: SUMICHIRAL OA-5000; 4 × 150 mm; eluent, MeOH/H_2O (25:75) containing 2 mM $CuSO_4$, flow rate 1 mL/

min; detection at 254 nm. Retention times (min) of authentic amino acids were as follows: *N*-methyl-L-tyrosine (9.6 min) and *N*-methyl-D-tyrosine (8.8 min), and that of the hydrolysate of **2** was 9.6 min.

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