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Ma'edamines A and B, Cytotoxic Bromotyrosine Alkaloids with a Unique 2(1H)Pyrazinone Ring from Sponge *Suberea* sp.

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Abstract—Two new cytotoxic bromotyrosine alkaloids, ma'edamines A (**1**) and B (**2**), with a unique 2(1H)pyrazinone ring have been isolated from the Okinawan marine sponge *Suberea* sp. The structures were elucidated on the basis of spectroscopic data.

Introduction

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 the Okinawan marine sponge *Suberea* sp., and isolated two unique bromotyrosine alkaloids, ma'edamines A (**1**) and B (**2**), with a 2(1H)-pyrazinone moiety. Here we describe the isolation and structure elucidation of **1** and **2**.

Results and Discussion

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 silica gel ($CHCl_3$/*n*-BuOH/AcOH/H_2O) and C_{18} (CH_3CN/H_2O/CF_3CO_2H) column chromatographies followed by reversed-phase HPLC (CH_3CN/H_2O/CF_3CO_2H) to yield ma'edamines A (**1**, 0.003%, wet weight) and B (**2**, 0.0009%) as yellow amorphous solids together with known bromotyrosine alkaloids, aplysamine-2 (**3**)[4] and purpuramines H (**4**) and I (**5**) (Chart 1).[5]

The FABMS spectrum of ma'edamine A (**1**) showed the pseudomolecular ion peaks in the ratio of 1:3:3:1 at *m/z* 528, 530, 532, and 534, respectively, indicating the presence of three bromine atoms in the molecule, and HRFABMS data of **1** revealed the molecular formula, $C_{23}H_{25}N_3O_3Br_3$ [*m/z* 631.9403 $(M+H)^+$, Δ −0.2 mmu]. IR absorptions indicated the presence of OH and/or NH (3420 cm^{-1}) and amide carbonyl (1685 cm^{-1}) groups. The UV absorption [λ_{max}285 nm (ϵ 4000)] was attributable to substituted benzenoid chromophore(s), and the absorption at 350 nm (ϵ 1000) indicated the presence of other system(s) conjugated to the benzenoid ring(s). The ^{13}C NMR (Table 1) spectrum disclosed ten sp^2 quaternary carbons containing an amide (δ_C 157.70) and an imino (δ_C 160.82) ones, six sp^2 methines, four sp^3 methylenes, and three methyl signals. The ^1H NMR spectrum showed signals due to a 1,3,4-trisubstituted benzene ring (C-1–C-6) and a 1,3,4,5-tetrasubstituted one (C-14–C-19), an aminopropanol unit (O-20–N-24), a trisubstituted olefin, a methoxy, and a dimethylamino group. Since eleven out of twelve elements of unsaturation implied by the molecular formula were accounted for, **1** was inferred to possess one more ring. The presence of 3-bromo-4-methoxyphenyl (C-1–C-6) and 3,5-dibromo-4-(3-dimethylamino)propyloxyphenyl (C-14–N-24) moieties was deduced from comparison of the ^{13}C NMR data of **1** with those of aplysamine-2 (**3**).[4] Detailed analyses of 2D NMR data (^1H–^1H COSY, HMQC, HMBC, and NOESY) and ESIMS data revealed the presence of a 3,5-disubstituted 2(1H)pyrazinone moiety in **1** (Fig. 1). A singlet methylene proton (H_2-7, δ_H 4.10) showed HMBC correlations to C-1 (δ_C 132.99), C-2 (δ_C 136.00), C-6 (δ_C 131.52), C-8 (δ_C 160.82), and C-9 (δ_C 157.70). HMBC correlations observed from a singlet olefin proton (H-11, δ_H 7.83) to C-12 (δ_C 131.52) and C-14 (δ_C 137.53) and from H-15 and H-19 to C-12 and the NOESY cross-peak for H-11/H-15(H-19) indicated the connectivity

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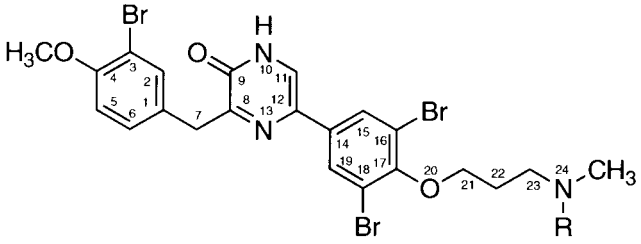
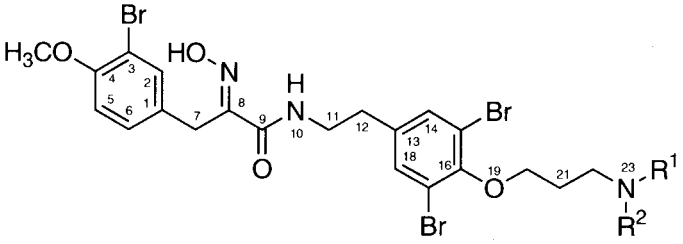


1: $R = CH_3$
2: $R = H$



3: $R^1 = R^2 = CH_3$
4: $R^1 = CH_3, R^2 = H$
5: $R^1 = R^2 = H$

Chart 1. Structures of ma'edamines A (**1**) and B (**2**), aplysamine-2 (**3**), and purpuramines H (**4**) and I (**5**).

between C-12 and C-14. The existence of the 2(1H)pyrazinone ring was supported by fragmentation ions at *m/z* 199/201, 249/251, 278/280, and 337/339/341 observed in the low-energy collision-induced dissociation (CID) mass spectrum of ESIMS. The HMBC correlation from H-11 to an amide carbon (C-9, δ_C 157.70) inferred that C-11 was attached to an amide nitrogen (N-10), which was supported by the large $^1J_{CH}$ value (185 Hz) of C-11. The ^{13}C chemical shifts of C-8, C-9, C-10, and C-11 corresponded to those of 3,5-dimethyl-2(1H)pyrazinone rather than those of 3,6-dimethyl-2(1H)pyrazinone.[6] Thus the structure of ma'edamine A was assigned as **1**.

HRFABMS data [*m/z* 615.9280 $(M+H)^+$, Δ +1.8 mmu] of ma'edamine B (**2**) showed the molecular formula, $C_{22}H_{23}N_3O_3Br_3$, indicating that **2** was the desmethyl form

Table 1. ^1H and ^{13}C NMR data of ma'edamines A (**1**) and B (**2**) in CD_3OD

Position	δ_H[a]		δ_C[b]	HMBC (H)	δ_H[a]		δ_C[b]
1			132.99 s	5, 7			133.01 s
2	7.60 d	1.7	136.00 d	6, 7	7.61 d	1.7	136.12 d
3			113.58 s	5			114.01 s
4			156.98 s	2, 6, OCH_3			157.08 s
5	7.00 d	7.0	113.98 d		7.00 d	7.0	114.09 d
6	7.33 dd	1.7, 7.0	131.52 d	6, 7	7.34 dd	1.7, 7.0	131.63 d
7	4.10[a] s		39.81 t		4.20[a] s		39.89 t
8			160.82 s	5			160.82 s
9			157.70 s	11			157.80 s
11	7.83 s		124.38 d		7.86 s		124.48 d
12			131.52 s	11			131.62 s
14			137.53 s				137.61 s
15	8.07 s		130.92 d	19	8.12 s		131.69 d
16			120.19 s	15			120.31 s
17			153.73 s	15, 19, 21			153.69 s
18			120.19 s	19			120.31 s
19	8.07 s		130.99 d	15	8.12 s		131.69 d
21	4.19[a] t	5.6	72.07 t		4.21[a] t	5.6	72.54 t
22	2.35[a] tt	5.6, 7.9	27.17 t	23	2.30[a] tt	5.6, 7.4	34.71 t
23	3.57 t	7.9	57.87 t	NCH_3	3.43[a] t	7.4	49.37 t
OCH_3	3.88[b] s		57.53 q		3.88[b] s		57.65 q
NCH_3	3.02[c] s		44.50 q	23, NCH_3	2.83[b] s		28.61 q

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

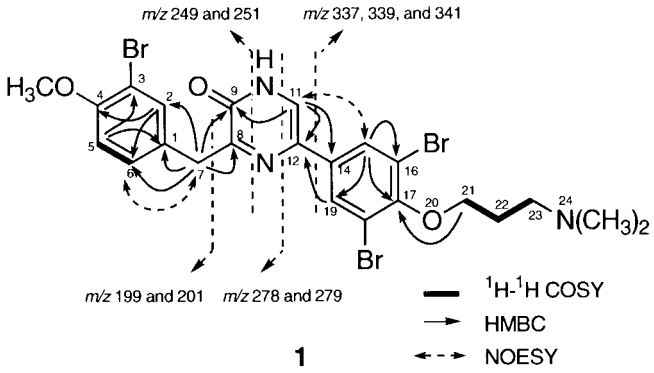
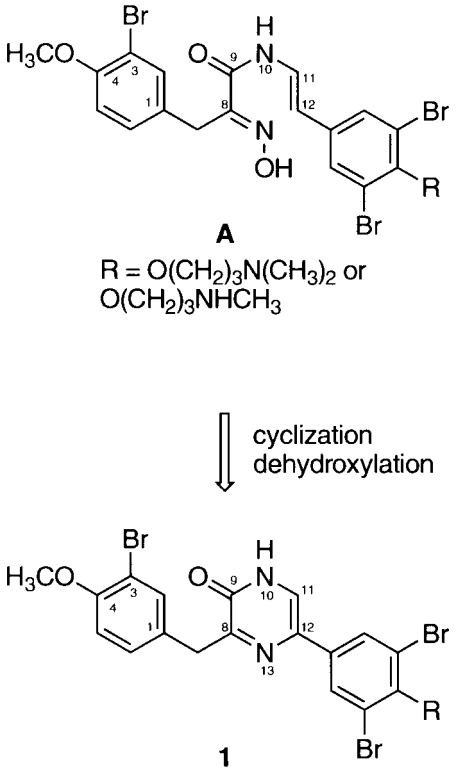


Figure 1. Selected 2D NMR correlations and fragmentation patterns observed in negative ion ESIMS spectrum of ma'edamine A (**1**).

of ma'edamine A (**1**). ^{1}H and ^{13}C NMR data of **2** were similar to those of **1** except for signals due to C-23 (δ_H 3.57, δ_C 57.87) and an *N*-methyl group (δ_H 2.83, δ_C 28.61), which were shifted to higher field than those (C-23: δ_H 3.43, δ_C 49.24; *N*-CH_3: δ_H 3.02, δ_C 44.50) of **1**. Since 3H of the *N*-methyl resonance was accounted for, **2** was deduced to possess a methylamino terminus at C-23. Therefore the structure of ma'edamine B was elucidated to be **2**.

Ma'edamines A (**1**) and B (**2**) are unique bromotyrosine alkaloids with a 2(1H)pyrazinone moiety between two bromotyrosine units. Biogenetically, ma'edamines A (**1**) and B (**2**) may be generated from 11,12-dehydro form of aplysamine-2 (**3**) or purpuramine H (**4**) through formation of a 6-membered ring and dehydroxylation (Scheme 1). Ma'edamines A (**1**) and B (**2**) exhibited cytotoxicity against murine leukemia L1210 cells (IC_{50}, 4.3 and 3.9 μg/mL, respectively) and epidermoid carcinoma KB cells (IC_{50}, 5.2 and 4.5 μg/mL, respectively) in vitro. Ma'edamine A (**1**) showed inhibitory activity against *c-erbB*-2 kinase in vitro with IC_{50} value of 6.7 μg/mL, while compound **2** did not show such activity (IC_{50}>10 μg/mL).



Scheme 1. Plausible biogenetic path of ma'edamines A (**1**) and B (**2**).

Experimental

General procedure

FAB mass spectra were obtained on a JEOL JMS HX-110 using glycerol as a matrix. ESI mass spectra were measured on a JEOL SX-102A spectrometer at 46 eV as a capillary-skimmer voltage, while CID mass spectra were obtained at 138 eV.

Sponge material

The sponge *Suberea* sp. (order Verongida, family Aplysinellidae) was collected off Maeda Cape, Okinawa, and kept frozen until used. The specimen was greenish-yellow alive and is purple in ethanol. Internally the surface has dence mesohyl with obvious pigment cells. The skeleton consists of fibres that are reticulate to fasciculate and they are grainy internally. Some foreign material occurs in the largest fibres. The pith of the fibres is wide, 170 μm, while the bark is very thin, 10 μm, and homogenous. The voucher specimen (SS-990) was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University, and Western Australian Museum.

Extraction and isolation

The sponge (0.85 kg, wet weight) was extracted with MeOH (1 L×2), and the extract was partitioned between EtOAc (500 mL×3) and 1N NaCl aq (500 mL), and 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 silica gel column ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5:6:1:1) and then a C_{18} column (Cosmosil 140C_{18} PREP, Nakalai Tesque Inc.; eluent, $CH_3CN/H_2O/CF_3CO_2H$, 45:55:0.1) to give a crude fraction of bromotyrosine alkaloids. The crude fraction was purified by reversed-phase HPLC [LUNA C5, 5 μm, Phenomenex®, 10× 250 mm; eluent, $CH_3CN/H_2O/CF_3CO_2H$, 30:70:0.1; flow

rate, 2.5 mL/min; UV detection at 230 nm] to afford ma'edamines A (**1**, 0.003%, wet weight, t_R 33 min) and B (**2**, 0.0009%, t_R 30 min).

Ma'edamine A (1). Yellow amorphous solid; UV (MeOH) λ_{max} 285 (ϵ 4000) and 350 nm (1000); IR (KBr) ν_{max} 3422 and 1680 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); FABMS (Pos.) *m/z* 628, 630, 632, and 634 (1:3:3:1, $M+H)^+$; HRFABMS *m/z* 631.9403 [calcd for $C_{23}H_{25}N_3O_3{}^{79}Br^{81}Br_2$, $(M+H)^+$: 631.9405].

Ma'edamine B (2). Yellow amorphous solid; UV (MeOH) δ_{max} 285 (ϵ 5000) and 350 nm (1200); IR (KBr) ν_{max} 3422 and 1680 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); FABMS (Pos.) *m/z* 614, 616, 618, and 620 (1:3:3:1, $M+H)^+$; HRFABMS *m/z* 615.9280 [calcd for $C_{22}H_{24}N_3O_3{}^{79}Br_2{}^{81}Br$, $(M+H)^+$: 615.9262].

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