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Tyrokeradines A and B, new bromotyrosine alkaloids with an imidazolyl-quinolinone moiety from a Verongid sponge

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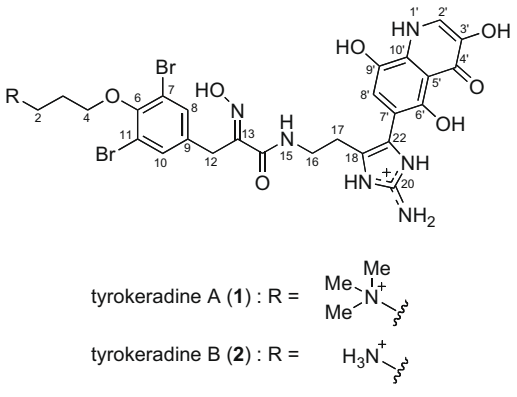
ABSTRACT

Two new bromotyrosine alkaloids, tyrokeradines A (**1**) and B (**2**), with an imidazolyl-quinolinone moiety have been isolated from an Okinawan marine sponge of the order Verongida. The structures of **1** and **2** were elucidated on the basis of spectroscopic data.

Marine sponges of the order Verongida 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 sponges such as *Pseudoceratina* (= *Psammaplysilla*) *purea*[3] and *Suberea* sp.[4] Recently, we have investigated extracts of an Okinawan Verongid sponge (SS-301) and isolated two new bromotyrosine alkaloids, tyrokeradines A (**1**) and B (**2**), with an imidazolyl-quinolinone moiety. Here we describe the isolation and structure elucidation of **1** and **2**.



The sponge of the order Verongida (SS-301) collected off Kerama Islands, Okinawa, was extracted with MeOH. BuOH-soluble materials of the extract were subjected to a C_{18} column chromatography followed by C_{18} HPLC to yield tyrokeradines A (**1**, 0.00052%, wet weight)[5] and B (**2**, 0.000016%).[6]

Tyrokeradine A (**1**) was obtained as a dark red-purple amorphous solid. The ESIMS spectrum of **1** showed the pseudomolecular ion peaks at *m/z* 750, 752, 754 [$(M-H)^+$, (1:2:1)], indicating the presence of two bromine atoms, and the molecular formula of **1** was revealed to be $C_{29}H_{35}N_7O_7{}^{79}Br_1{}^{81}Br_1$ by HRESIMS data. The UV absorption [λ_{max} 281 nm (ε 2500)] was attributed to a substituted benzenoid chromophor,[4] while IR absorptions indicated the existence of OH and/or NH (3470 cm^{-1}) and carbonyl (1690 cm^{-1}) functionalities. The ^1H and ^{13}C NMR data (Table 1) disclosed that **1** consists of two carbonyls, 14 sp^2 quaternary carbons, four sp^2 methine, six sp^3 methylenes, and three *N*-methyls.

The structure of tyrokeradine A (**1**) was elucidated by detailed analysis of 2D NMR spectra (Fig. 1). Analyses of the ^1H-^1H COSY and HMQC spectra disclosed three structural fragments, C-2 to C-4, N-15 to C-17, and N-1′ to C-2′. HMBC correlations for *N*-methyl protons to C-2 revealed that an *N*,*N*,*N*-trimethylpropyl amine moiety (N-1, C-2 to C-4) was located at a terminal in **1**. HMBC correlations for H-12 to C-9, C-8(10), H-8(10) to C-7(11) and C-6 indicated the presence of 1,3,4,5-tetrasubstituted benzene ring, which was ascribed to a 3,5-dibromo-benzyl moiety (C-6 to C-12) from a comparison of ^{13}C NMR data of **1** with those of known bromotyrosine alkaloids.[7,8] The connectivity of *N*,*N*,*N*-trimethylpropyl-amino moiety (N-1, C-2 to C-4) and a 3,5-dibromo-benzyl moiety (C-6 to C-12) at C-4 and C-6 via O-5 was implied by an HMBC cross-peak

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Table 1
^{1}H and ^{13}C NMR data of tyrokeradines A (**1**) and B (**2**) in DMSO-d_6

Tyrokeradine A (**1**)				Tyrokeradine B (**2**)			
Positn.	δ_H	δ_C		Positn.	δ_H	δ_C	
1-Me_3	3.11 (9H, s)	52.3	q	1	7.88 (3H, brs)		
2	3.59 (2H, t 7.3)	63.1	t	2	3.04 (2H, brt 6.6)	36.5	t
3	2.22 (2H, m)	23.4	t	3	2.05 (2H, m)	27.9	t
4	3.96 (2H, t 5.4)	70.0	t	4	3.96 (2H, t 5.8)	70.4	t
6		150.2	s	6		150.5	s
7(11)		117.0	s	7(11)		117.2	s
8(10)	7.42 (2H, s)	132.8	d	8(10)	7.41 (2H, s)	133.0	d
9		136.5	s	9		136.5	s
12	3.66 (2H, s)	27.8	t	12	3.66 (2H, s)	27.7	t
13		150.4	s	13		150.7	s
13-NOH	11.98 (1H, s)			13-NOH	11.98 (1H, s)		
14		163.0	s	14		163.1	s
15	8.10 (1H, brt 5.7)			15	8.07 (1H, brt 5.7)		
16	3.34 (2H, dt)	37.8	t	16	3.36 (2H, dt)	38.0	t
17	2.71 (2H, t 7.2)	24.3	t	17	2.71 (2H, t 7.1)	24.4	t
18		120.1	s	18		120.4	s
19	12.05 (1H, brs)			19	12.09 (1H, brs)		
20		146.5	s	20		146.5	s
21	12.28 (1H, brs)			21	12.36 (1H, brs)		
22		118.5	s	22		118.7	s
20-NH_2	7.30 (2H, s)			20-NH_2	7.39 (2H, s)		
1′	11.73 (1H, d 5.8)			1′	11.73 (1H, d 5.8)		
2′	7.66 (1H, d 5.8)	124.1	d	2′	7.66 (1H, d 5.8)	124.2	d
3′		139.7	s	3′		139.9	s
3′-OH	8.92 (1H, s)			3′-OH	8.92 (1H, s)		
4′		173.1	s	4′		173.3	s
5′		112.5	s	5′		112.6	s
6′		149.1	s	6′		149.5	s
6′-OH	14.32 (1H, s)			6′-OH	14.28 (1H, s)		
7′		103.1	s	7′		103.2	s
8′	6.91 (1H, s)	113.9	d	8′	6.85 (1H, s)	114.1	d
9′		137.3	s	9′		137.3	s
9′-OH	10.30 (1H, s)			9′-OH	10.30 (1H, s)		
10′		128.9	s	10′		129.1	s

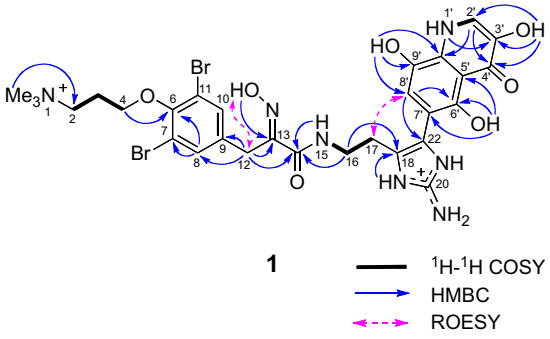

Figure 1. Selected 2D NMR correlations for tyrokeradine A (**1**).

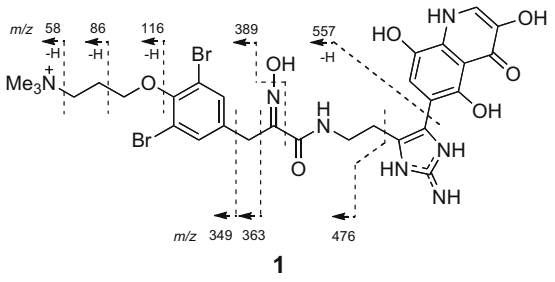

Figure. 2. Fragmentation patterns observed in positive ion ESIMS/MS spectrum of tyrokeradine A (**1**) [precursor ion, *m/z* 750 $(M\text{-}H)^+$].

of H-4 to C-6. HMBC correlations for H-12 to C-13 and C-14, 13-NOH to C-13, H-15 to C-14, and H-16 to C-14 revealed the presence of an α-ketoxime moiety (C-13, 13-NOH, and C-14) and connectivities of C-12 to C-13 and C-14 to N-15. The ROESY correlation between 13-NOH and H-12 suggested that the oxime had *E* geometry. The existence of a trihydroxy quinolinone ring (N-1′, C-2′ to C-10′) was deduced from $^{2}J_{CH}$ and $^{3}J_{CH}$ correlations from three hydroxy protons (3′-OH, 6′-OH, and 9′-OH) observed in the HMBC spectrum of **1** in addition to cross-peaks of H-1′ to C-3′, H-2′ to C-4′ and C-10′, and H-8′ to C-6′. Furthermore, HMBC correlations for H-19 to C-18 and H-8′ to C-22, and a ROESY correlation between H-17 and H-8′ suggested that C-17 and C-7′ were connected to C-18 and C-22 in an amino imidazole ring (C-18, N-19, C-20, N-21, C-22, and 20-NH_2),[7,8] respectively. The fragmentation patterns observed in the ESIMS/MS spectrum supported the structure of tyrokeradine A (**1**) elucidated from 2D NMR data (Fig. 2). Thus, the structure of tyrokeradine A was concluded to be **1**.

Tyrokeradine B (**2**) was obtained as a dark green amorphous solid. The molecular formula of **2** was established as $C_{26}H_{28}O_7N_7{}^{79}Br_1{}^{81}Br_1$ by HRESIMS data. The ^{1}H and ^{13}C NMR data (Table 1) were close to those of tyrokeradine A (**1**), except for lack of signals due to *N*-methyl groups in **1** and presence of a signal due to an amino group [δ_H 7.88 (3H, brs)]. Analysis of the 2D NMR data of **2** disclosed that a terminal of **2** is an amino group in place of trimethyl amino group of **1**. Thus, the structure of tyrokeradine B was concluded to be **2**.

Tyrokeradines A (**1**) and B (**2**) are rare bromotyrosine alkaloids possessing an imidazolyl-quinolinone moiety.[9] Tyrokeradine B (**2**) showed inhibitory activity against *Micrococcus luteus, Staphylococcus aureus,* and *Trichophyton mentagrophytes* (MIC, 25.0 μg/mL, each), and *Cryptococcus neoformans, Candida albicans,* and *Aspergillus niger* (MIC, 12.5 μg/mL, each), while tyrokeradines A (**1**) did not show such activity (MIC, >25.0 μg/mL, each).

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5. Tyrokeradine A (**1**): dark red-purple amorphous solid; UV (MeOH) λ_{max} 207 (ε 16800), 281 (2500), 369 (900), 553 (800) nm; IR (KBr) ν_{max} 3470 (br), 1690, 1540, 1460, 1200, 1140, 1020, 1000, 840, 720 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS *m/z* 750, 752, 754 [$(M-H)^+$, 1:2:1]; HRESIMS *m/z* 752.0832 [$(M-H)^+$, Δ −3.4 mmu], calcd for $C_{29}H_{34}N_7O_7{}^{79}Br_1{}^{81}Br_1$, 752.0866.
6. Tyrokeradine B (**2**): dark green amorphous solid; UV (MeOH) λ_{max} 206 (ε 27300), 279 (5600), 368 (1700), 608 (700) nm; IR (KBr) ν_{max} 3420 (br), 2920, 1680, 1540, 1460, 1200, 1140, 1000, 840, 720 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS *m/z* 708, 710, 712 [$(M-H)^+$, 1:2:1]; HRESIMS *m/z* 710.0363 [$(M-H)^+$, Δ −3.4 mmu], calcd for $C_{26}H_{28}O_7N_7{}^{79}Br_1{}^{81}Br_1$, 710.0397.
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