Tyrokeradines C–F, New Bromotyrosine Alkaloids from the Verongid Sponges

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Four new bromotyrosine alkaloids, tyrokeradines C–F (1–4), have been isolated from Okinawan marine sponges of the order Verongidae. The structures of 1–4 were elucidated on the basis of spectroscopic data. Tyrokeradines C (1) and D (2) are new bromotyrosine alkaloids possessing an imidazole ring, while tyrokeradines E (3) and F (4) are new bromotyrosine alkaloids possessing a cyano group.

Key words marine sponge; order Verongida; bromotyrosine alkaloid; tyrokeradine

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, a series of bromotyrosine alkaloids have been isolated from Verongid marine sponges.^{2–4)} Recently, we have investigated extracts of Okinawan Verongid sponges (SS-242 and SS-301) and isolated four new bromotyrosine alkaloids, tyrokeradines C–F (1–4). Here we describe the isolation and structure elucidation of 1–4.

The sponges of the order Verongidae (SS-242 and SS-301) collected off Kerama Islands, Okinawa, were extracted with MeOH. BuOH-soluble materials of the extract of SS-301 were subjected to a C₁₈ column chromatography followed by C₁₈ HPLC to yield tyrokeradines C (**1**, 0.00006%, wet weight) and D (**2**, 0.00009%) with tyrokeradines A and B.³⁾ EtOAc-soluble materials of the extract of SS-242 were subjected to a LH-20 column followed by a SiO₂ column and C₁₈ HPLC to yield tyrokeradines E (**3**, 0.00007%) and F (**4**, 0.00002%).

Tyrokeradine C (**1**) was obtained as a colorless amorphous solid. The electrospray ionization (ESI)-MS spectrum of **1** showed the molecular ion peaks at *m/z* 531, 533, 535 [(M)⁺, (1:2:1)], indicating the presence of two bromine atoms, and the molecular formula of **1** was revealed to be C₁₈H₂₅N₆O₃Br₂ by high resolution (HR)-ESI-MS data. The UV absorption [$\lambda_{\max}$ 275 nm (ϵ 830)] was attributed to a substituted benzenoid chromophore.⁵⁾ The IR absorptions indicated the existence of OH and/or NH (3450 cm^{−1}) and carbonyl (1690 cm^{−1}) functionalities. The ¹H- and ¹³C-NMR data (Table 1) disclosed that **1** consists of one carbonyl, seven *sp*² quaternary carbons, three *sp*² methines, six *sp*³ methylenes, and one *N*-methyl. Among eighteen carbons, four *sp*² quaternary carbons [δ_C 117.1 (2C), 136.4, and 150.4] and two *sp*² methines [δ_C 132.9 (2C)] were ascribed to a 3,5-dibromo-4-oxy-benzyl moiety, while two *sp*² quaternary carbons (δ_C 124.2, 147.0) and one *sp*² methine (δ_C 109.1) were assigned to a 4-substituted 2-aminoimidazole moiety from a comparison of ¹³C-NMR data of **1** with those of known bromotyrosine alkaloids.^{2–5)} The structure of tyrokeradine C (**1**) was elucidated by detailed analysis of 2D-NMR spectra (Fig. 1). Analyses of the ¹H–¹H correlated spectroscopy (COSY) and heteronuclear multiple quantum coherence (HMQC) spectra disclosed two structural fragments, C-2 to C-4 and N-15 to C-17. Heteronuclear multiple bond

correlation (HMBC) correlations for *N*-methyl protons to C-2 revealed that an *N*-methylpropyl amine moiety (*N*-Me, N-1, C-2 to C-4) was located at a terminal in **1**. HMBC correlations for H₂-12/C-13, H₂-12/C-14, 13-NOH/C-13, H-15/C-14, and H₂-16/C-14 clarified 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 with N-15. The geometry of oxime was assigned as *E* from the chemical shift for C-12 (δ_C 27.8).⁶⁾ An HMBC correlation for H₂-12/C-8 ensured a connection of C-12 with a benzene ring. A linking of C-4 and C-6 *via* O-5 was assigned from the chemical shifts for CH₂-4 (δ_H 3.97, 2H, t, *J*=5.4 Hz, δ_C 132.9). Considering the molecular formula of **1** and ¹H and ¹³C chemical shifts, it was elucidated that C-17 was attached to C-18 in an amino imidazole ring (C-18, N-19, C-20, N-21, C-22, and 20-NH₂). Thus, the structure of tyrokeradine C was concluded to be **1** (Fig. 1).

Tyrokeradine D (**2**) was obtained as a colorless amorphous solid. The molecular formula of **2** was established as C₁₉H₂₇O₆N₃Br₂ by HR-ESI-MS data. The ¹H- and ¹³C-NMR data (Table 1) were close to those of tyrokeradine C (**1**), except for lack of a signal due to an amino proton [δ_H 8.76 (1H, brs)] in **1** and the increase of magnitude for a signal due to an *N*-methyl protons [δ_H 2.80 (6H, s)], suggesting that a terminal of **2** is an *N,N*-dimethyl amino group in place of an *N*-methyl amino group of **1**. Thus, the structure of tyrokeradine D was assigned as **2**.

Tyrokeradine E (**3**) was obtained as a colorless amorphous solid. The molecular formula of **3** was revealed to be C₁₃H₁₄O₂N₂Br₂ by HR-atmospheric pressure chemical ionization (APCI)-MS data. The UV absorption [$\lambda_{\max}$ 275 nm (ϵ 800)] indicated the existence of a substituted benzenoid chromophore and the IR absorptions suggested the existence of

Fig. 1. Selected 2D-NMR Correlations for Tyrokeradine C (**1**)

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Table 1. ^1H - and ^{13}C -NMR Data of Tyrokeradines C–F (**1–4**) in $\text{DMSO}-d_6$

Position	1		2	
	δ_{H}^a (J in Hz)	δ_{C}^b	δ_{H}^a (J in Hz)	δ_{C}^b
1	8.76, brs	—	—	—
2	3.15, ^c brs	45.7, t	3.30, ^c brs	54.2, t
3	2.10, ^c m	26.1, t	2.13, ^c m	24.7, t
4	3.97, ^c t (5.4)	70.3, t	3.96, ^c t (5.6)	70.1, t
6	—	150.4, s	—	150.3, s
7	—	117.1, s	—	117.1, s
8	7.44, ^c s	132.9, d	7.43, ^c s	132.9, d
9	—	136.4, s	—	136.5, s
10	7.44, ^c s	132.9, d	7.43, ^c s	132.9, d
11	—	117.1, s	—	117.1, s
12	3.75, ^c s	27.8, t	3.74, ^c s	27.8, t
13	—	150.8, s	—	150.8, s
14	—	163.1, s	—	163.1, s
15	8.15, brt (5.3)	—	8.13, brt (5.3)	—
16	3.35 ^{c,e}	37.2, t	3.35 ^{c,e}	37.2, t
17	2.59, ^c brs	24.3, t	2.59, ^c brs	24.3, t
18	—	124.2, s	—	124.2, s
19	11.97, brs	—	11.98, brs	—
20	—	147.0, s	—	147.0, s
21	12.33, brs	—	12.36, brs	—
22	6.56, s	109.1, d	6.55, s	109.1, d
1-Me	2.60, ^d s	32.5, q	2.80, ^d s	42.3, q
13-NOH	12.09, s	—	12.07, s	—
20-NH ₂	7.47, ^c s	—	7.44, ^c s	—

Position	3		4	
	δ_{H}^a (J in Hz)	δ_{C}^b	δ_{H}^a (J in Hz)	δ_{C}^b
1	7.89, brt (5.5)	—	7.79, brt (5.8)	—
2	3.24, ^c brq (6.7)	35.8, t	3.25, ^c brq (6.6)	35.6, t
3	1.91, ^c m	29.9, t	1.91 ^c , m	29.8, t
4	3.95, ^c t (6.3)	71.5, t	3.95, ^c t (6.3)	71.4, t
6	—	152.2, s	—	151.9, s
7	—	118.0, s	—	117.9, s
8	7.66, ^c s	132.7, d	7.66, ^c s	132.6, d
9	—	118.7, s	—	129.7, s
10	7.66, ^c s	132.7, d	7.66, ^c s	132.6, d
11	—	118.0, s	—	117.9, s
12	4.01, ^c s	21.1, t	4.02, ^c s	21.0, t
13	—	118.7, s	—	118.4, s
1'	—	169.3, s	—	172.5, s
2'	2.01, ^d s	22.7, q	2.01, ^d q (7.6)	28.6, t
3'	—	—	0.98d, t (7.6)	10.0, q

a) Recorded at 600 MHz. b) Recorded at 150 MHz. c) 2H. d) 3H. e) Due to overlapping with H_2O signal, assigned by the HMQC spectrum.

cyano (2256cm^{-1}) and amide carbonyl (1654cm^{-1}) functionalities. The ^1H - and ^{13}C -NMR data (Table 1) disclosed that **3** consists of one sp quaternary carbon, one carbonyl, four sp^2 quaternary carbons, two sp^2 methines, four sp^3 methylenes, and one methyl. Inspection of the ^1H - ^1H COSY and HMQC spectra for **3** revealed a connection of N-1 to C-4 (Fig. 2). HMBC correlations for H-1/C-1' and H_3 -2'/C-1' clarified that an *N*-acetylpropyl amine moiety (C-1' to C-2', N-1, C-2 to C-4) was located at a terminal in **3**. HMBC correlations for H-8(10)/C-6, H-8(10)/C-7(11), H_2 -12/C-8(10), and H_2 -12/C-9 indicated the presence of 3,5-dibromo-benzyl moiety (C-6 to C-12). A linking of C-4 and C-6 via O-5 was assigned from an HMBC cross-peak of H_2 -4/C-6. An HMBC correlation for H_2 -12/C-13 suggested that a cyano group was attached to

C-12. Thus, the structure of tyrokeradine E was concluded to be **3** (Fig. 2).

Tyrokeradine F (**4**) was obtained as a colorless amorphous solid. The molecular formula of **4** was established as $\text{C}_{14}\text{H}_{16}\text{O}_2\text{N}_2^{79}\text{Br}_2$ by HR-ESI-MS data. The ^1H - and ^{13}C -NMR data (Table 1) were close to those of tyrokeradine E (**3**), except for lack of a signal due to a methyl group of acetamide in **3** [δ_{H} 2.01 (3H, s)] and presence of signals due to an ethyl group [δ_{H} 2.01 (2H, q, $J=7.63\text{Hz}$) and δ_{H} 0.98 (3H, t, $J=7.63\text{Hz}$)]. Analysis of the 2D-NMR data of **4** disclosed that a terminal of **4** is a propionyl amide in place of an acetamide of **3**. Thus, the structure of tyrokeradine F was assigned as **4**.

Fig. 2. Selected 2D-NMR Correlations for Tyrokeradine E (3)

Experimental

General Methods IR and UV spectra were recorded on JASCO FT/IR-230 and Shimadzu UV-1600PC spectrophotometer, respectively. ^1H - and ^{13}C -NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm micro cells for $\text{DMSO}-d_6$. The 2.49 and 39.5 ppm resonances of residual $\text{DMSO}-d_5$ were used as internal references for ^1H - and ^{13}C -NMR spectra, respectively. ESI-MS spectra were recorded on a JEOL JMS-T100LP mass spectrometer.

Sponge Description The sponge (SS-242) is a black mound. The specimen has a dense interior and is firm and incompressible. The surface is smooth. No fibers are apparent macroscopically. The sponge (SS-301) is likely to be the same as SS-242. The voucher specimens were deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Separation The sponge (SS-301, 2.7 kg, wet weight) was extracted with MeOH (1 L $\times$ 4) and concentrated *in vacuo*, which was partitioned stepwise between organic solvents [CHCl_3 (500 mL $\times$ 3) and BuOH (500 mL $\times$ 3)] and H_2O (500 mL) to give CHCl_3 -soluble materials (3.4 g) and BuOH-soluble materials (23.0 g). The BuOH soluble materials (5.01 g) were fractionated by a C_{18} column (Cosmosil 140 C_{18} PREP, Nakalai Tesque Inc.; eluent, MeOH/ H_2O /TFA, 40:60:0.05 to 100:0:0.05) and C_{18} HPLC (YMC-pack, R&D ODS, 20 $\times$ 250 mm, YMC Co., Ltd.; eluent, MeOH/ H_2O /TFA, 70:30:0.05; flow rate, 5.0 mL/min; UV detection at 210 nm). The fraction which contained new compounds was purified by C_{18} HPLC (YMC-pack, R&D ODS, 20 $\times$ 250 mm, YMC Co., Ltd.; eluent, MeOH/ H_2O /TFA, 5:95:0.05 to 0:100:0.05; flow rate, 6.0 mL/min; UV detection at 210 nm) and C_{18} HPLC (YMC-Pack, AM-323, 10 $\times$ 250 mm, YMC Co., Ltd.; eluent, $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ /TFA, 17:83:0.05 to 20:80:0.05; flow rate, 2.0 mL/min; UV detection at 210 nm) to yield tyrokeradines C (**1**, 1.6 mg, 0.00006%, wet weight) and D (**2**, 2.4 mg, 0.00009%). Ten known related alkaloids, aplysamine-1,⁷⁾ purealin,⁸⁾ purealidins A,⁵⁾ B,⁹⁾ D,¹⁰⁾ E,¹¹⁾ F,¹¹⁾ and G,¹¹⁾ and tyrokeradines A and B³⁾ have been isolated in the purification process of **1** and **2**. The sponge (SS-242, 2.6 kg, wet weight) was extracted with MeOH (1 L $\times$ 3) and concentrated *in vacuo*, which was partitioned stepwise between organic solvents [EtOAc (300 mL $\times$ 13) and BuOH (600 mL $\times$ 5)] and H_2O (300 mL) to give EtOAc-soluble materials (2.1 g) and BuOH-soluble materials (2.7 g). The EtOAc soluble materials (0.9 g) were fractionated by gel filtration (Sephadex LH-20, GE Healthcare; eluent, MeOH). The fraction which contained new compounds was purified by a silica gel column (Wakogel C-300, Wako Pure Chemical Industries, Ltd.; eluent, *n*-hexane/EtOAc). A fraction was purified by a C_{18} column (Sep-Pak Plus C_{18} Cartridge, Waters; eluent, $\text{CH}_2\text{CN}/\text{H}_2\text{O}$,

70:30) to yield tyrokeradine E (**3**, 1.3 mg, 0.00007%). Another fraction was purified by C_{18} HPLC (YMC-pack, ODS-AM, 10 $\times$ 250 mm, YMC Co., Ltd.; eluent, MeOH/ H_2O /TFA, 60:40:0.1; flow rate, 2.5 mL/min; UV detection at 220 nm) to yield tyrokeradine F (**4**, 0.59 mg, 0.00002%).

Tyrokeradine C (1): Colorless amorphous solid; UV λ_{max} (MeOH) 275 nm (ϵ): 830; IR (KBr) ν_{max} 3450, 2920, 1690, 1540, 1460, 1390, 1210, 1140, 840, 800, 720 cm^{-1} ; ^1H - and ^{13}C -NMR, see Table 1; ESI-MS m/z 531, 533, 535 [(M)⁺, 1:2:1]; HR-ESI-MS m/z 531.03168 [(M)⁺, Δ -3.81 mmu], Calcd for $\text{C}_{18}\text{H}_{25}\text{N}_6\text{O}_3^{79}\text{Br}_2$, 531.03549.

Tyrokeradine D (2): Colorless amorphous solid; UV λ_{max} (MeOH) 274 nm (ϵ): 830; IR (KBr) ν_{max} 3440, 2920, 1690, 1540, 1460, 1390, 1210, 1140, 840, 800, 720 cm^{-1} ; ^1H - and ^{13}C -NMR, see Table 1; ESI-MS m/z 545, 547, 549 [(M)⁺, 1:2:1]; HR-ESI-MS m/z 545.04908 [(M)⁺, Δ -2.06 mmu], Calcd for $\text{C}_{19}\text{H}_{27}\text{N}_6\text{O}_3^{79}\text{Br}_2$, 545.05114.

Tyrokeradine E (3): Colorless amorphous solid; UV λ_{max} (MeOH) 275 nm (ϵ): 800; IR (KBr) 3286, 2928, 2256, 1654, 1548, 1454, 1261, 1207, 1036, 801 cm^{-1} ; ^1H - and ^{13}C -NMR, see Table 1; APCI-MS m/z 387, 389, 391 [(M-H)⁻, 1:2:1]; HR-APCI-MS m/z 386.93493 [(M-H)⁻, Δ +1.07 mmu], Calcd for $\text{C}_{13}\text{H}_{13}\text{O}_2\text{N}_2^{79}\text{Br}_2$, 386.93493.

Tyrokeradine F (4): Colorless amorphous solid; UV λ_{max} (MeOH) 275 nm (ϵ): 630; IR (KBr) 3423, 2923, 2266, 1686, 1601, 1443, 1211, 1136, 803 cm^{-1} ; ^1H - and ^{13}C -NMR, see Table 1; APCI-MS m/z 403, 405, 407 [(M+H)⁺, 1:2:1]; HR-APCI-MS m/z 402.96501 [(M+H)⁺, Δ -0.12 mmu], Calcd for $\text{C}_{14}\text{H}_{17}\text{O}_2\text{N}_2^{79}\text{Br}_2$, 402.96513.

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