Nakirodin A, a Bromotyrosine Alkaloid from a Verongid Sponge

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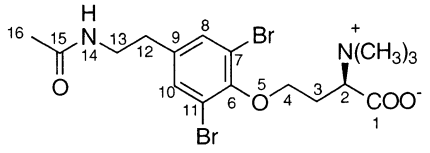
A new bromotyrosine alkaloid, nakirodin A (**1**), has been isolated from an Okinawan marine Verongid sponge. The structure was elucidated on the basis of spectroscopic data.

Bromotyrosine alkaloids are commonly encountered in marine sponges of the order Verongidae.[1] In our search for bioactive substances from marine sponges,[2] a series of bromotyrosine alkaloids have been isolated from Verongid marine sponges *Psammaplysilla purea*[3] and *Suberea* sp.[2,4] Recently we have investigated extracts of an Okinawan marine sponge (SS-995) belonging to the order Verongidae and isolated a new bromotyrosine alkaloid, nakirodin A (**1**). Here we describe the isolation and structure elucidation of **1**.

An unidentified sponge (SS-995) of the order Verongidae collected off Nakijin, Okinawa, was extracted with MeOH. The *n*-BuOH-soluble materials of the extract were subjected to silica gel column chromatography ($CHCl_3$/*n*-BuOH/AcOH/H_2O) followed by C_{18} HPLC (CH_3CN/H_2O/CF_3CO_2H) to yield nakirodin A (**1**, 0.0007%, wet weight) as a colorless oil together with a known bromotyrosine derivative, **2**.[5,6]



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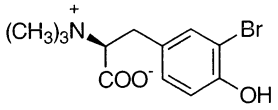

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The ESIMS spectrum of nakirodin A (**1**) showed pseudomolecular ion peaks at *m*/*z* 479, 481, and 483 in the ratio of 1:2:1, indicating the presence of two bromine atoms in the molecule. HRFABMS data of **1** revealed the molecular formula $C_{17}H_{25}N_2O_4Br_2$ [*m*/*z* 479.0176 $(M + H)^+$, Δ −0.5 mmu]. IR absorptions suggested the presence of OH and/or NH (3411 cm^{-1}) and amide carbonyl (1630 cm^{-1}) groups. The UV absorption [λ_{max}283 nm (ϵ 880)] was attributable to substituted benzenoid chromophore(s). The ^{13}C NMR (Table 1) spectrum disclosed the presence of six sp^2 quaternary carbons containing two carbonyl carbons [δ_C 174.1 and 165 (br)], two sp^2 methines, an sp^3 methine, four sp^3 methylenes, and four methyl carbons. The ^1H NMR (Table 1) spectrum showed signals due to an amide NH (δ_H 7.95, brs), a 1,3,4,5-tetrasubstituted symmetrical benzene ring (δ_H 7.50, s, 2H), an acetyl group (δ_H 1.79, s, 3H), and a trimethylammonium group (δ_H 3.25, s, 9H). The presence of a 3,5-dibromo-4-*O*-alkyltyramine (C-6−C-13 and N-14) moiety was deduced from comparison of the ^{13}C NMR data of **1** with those of aplysamine-1.[7] The methylene (C-4) at δ_C 71.1 was suggested to be attached to an oxygen atom at C-6 of the tyramine moiety. Analysis of the ^1H−^1H COSY and HMQC spectra revealed the connectivities from C-2 to C-4 and from C-12 to NH-14 (Figure 1). The NOESY correlation for NH-14/H_3-16 implied the presence of an acetamide terminus. The trimethylammonium group was attached to C-2 on the basis of the NOESY correlation for H-2/N$(CH_3)_3$. The connection of a carboxyl group (C-1) to C-2 was deduced from fragment ion peaks at *m*/*z* 360,

Table 1. ^1H and ^{13}C NMR Data of Nakirodin A (**1**)

position	δ_H[a]	m	(*J* in Hz)	δ_C[b]	m[c]
1				165[d]	s
2	4.35	dd	2.1, 2.7	77[e]	d
3	2.35	m		30.3	m
	2.70	m			
4	3.98	m		71.1	t
	4.07	m			
6				153.3	s
7				119.6	s
8	7.50	s		135.2[f]	d
9				141.2	s
10	7.50	s		135.3[f]	d
11				119.6	s
12	2.65	t	7.0	31.6	t
13	3.32[g]	m		42.8	t
14	7.95	brs			
15				174.1	s
16	1.79	s		23.8	q
2-N$(CH_3)_3$	3.25[h]	s		53.6	q

[a] In DMSO-d_6 with one drop of TFA. [b] MeOH-d_4. [c] Multiplicity was obtained by DEPT. [d] Broad signal. [e] Assigned by HMQC spectrum. [f] Interchangeable. [g] 3H. [h] 9H.

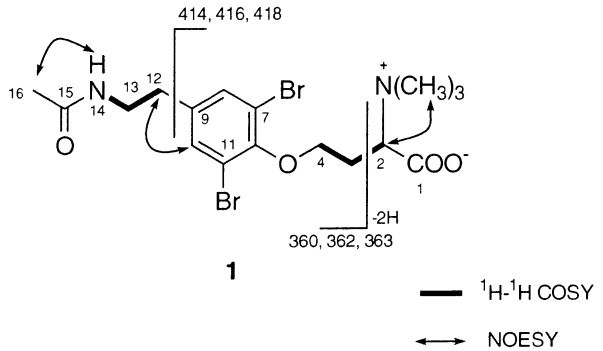

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Figure 1. Selected 2D NMR correlations for nakirodin A (**1**) and fragmentation patterns observed in the CIDMS of the sodium salt of nakirodin A (**1**).

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362, and 364 observed in the collision-induced dissociation mass spectrum (CIDMS) of ESIMS (Figure 1). Thus, the structure of nakirodin A was assigned as **1**.

Nakirodin A (**1**) was treated with 6 N HCl at 100 °C for 60 h, and then the hydrolysate was purified by C_{18} HPLC to give *N*,*N*,*N*-trimethylhomoserine. The CD spectrum of *N*,*N*,*N*-trimethylhomoserine from natural specimens showed a positive Cotton effect at 203.5 nm ($\Delta\epsilon$ +2.0), which was identical to that of the authentic sample of *N*,*N*,*N*-trimethyl-D-homoserine, while the Cotton effect (λ_{ext} 203.5 nm, $\Delta\epsilon$ −2.1) of *N*,*N*,*N*-trimethyl-L-homoserine was antipode to that of *N*,*N*,*N*-trimethylhomoserine derived from **1**. Thus, the absolute configuration at C-2 was determined to be *R*.

Although many bromotyrosine alkaloids possess one or more aminopropanol units,[1] bromotyrosine alkaloids having an *N*,*N*,*N*-trimethylhomoserine residue such as nakirodin A (**1**) are very rare.[5,6] The structure of **1** indicated that aminopropanol units found in many bromotyrosine alkaloids may be biogenetically derived from a homoserine through decarboxylation.

Experimental Section

General Experimental Procedures. The IR and UV spectra were recorded on a JASCO FT/IR-5300 and a Shimadzu UV-1600PC spectrophotometer, respectively. CD spectra were measured on a JASCO J-720 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer. ESI mass spectrum was recorded on a Shimadzu LCMS QP-8000 spectrometer. The FAB mass spectrum was obtained on a JEOL HX-110 spectrometer using glycerol as a matrix. *N*,*N*,*N*-Trimethyl-L-homoserine was prepared from L-homoserine as reported previously.[6]

Animal Material. The sponge (family Aplysinellidae, order Verongidae) was collected off Nakijin, Okinawa, and kept frozen until used. A firm, incompressible flattened piece of sponge, which has undergone an oxidation reaction, is now blackish in color. No fibers are visible in the sponge, but it is suspected that something has happened to the preservation of the sponge. The sponge is collagenous and typically Verongid in morphology. The voucher specimen (SS-995) was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (1.1 kg, wet weight) was extracted with MeOH (1 L × 2), the extract was partitioned between EtOAc (500 mL × 3) and H_2O (500 mL), and then the aqueous layer was extracted with *n*-BuOH (500 mL × 3). A portion (1 g) of the *n*-BuOH-soluble materials (3.25 g) was subjected to a silica gel column ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5:6:1:1) and then C_{18} HPLC [Wakosil-II5C18 RS, Wako Pure Chemical Ind., Ltd., 10 × 250 mm; eluent, $CH_3CN/H_2O/CF_3CO_2H$, 20:80:0.1; flow rate, 2.5 mL/min; UV detection at 220 nm] to afford nakirodin A (**1**, 2.3 mg, 0.0007%, wet weight, t_R 20 min) and **2** (5.7 mg, 0.0014%, t_R 9.6 min).

Nakirodin A (1): colorless oil; $[\alpha]^{25}_D$ +35° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 283 nm (ϵ 880); IR (KBr) ν_{max} 3411, 1630, and 1057 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS (pos) *m*/*z* 479, 481, 483 (1:2:1, $[M + H]^+$), 501, 503, and 505 (1:2:1, $[M + Na]^+$); HRFABMS *m*/*z* 479.0176 $[M + H]^+$ (calcd for $C_{17}H_{25}N_2O_4{}^{79}Br_2$, 479.0181).

Hydrolysis of Nakirodin A (1). Nakirodin A (**1**, 0.6 mg) was dissolved in 6 N HCl (2 mL) in a sealed tube and heated at 80 °C for 60 h. After evaporation of the solvent, the residue was subjected to C_{30} HPLC (Develosil RPAQUEOUS, Nomura Chemical Co., Ltd., 4.6 × 250 mm; eluent, H_2O; flow rate, 1 mL/min; UV detection at 220 nm) to afford *N*,*N*,*N*-trimethylhomoserine (0.1 mg, t_R 2.6 min): CD λ_{ext} 203.5 ($\Delta\epsilon$ +2.0), 202 ($\pm$0), and 200 nm (−7.2); ^{1}H NMR (MeOH-d_4) δ 2.6−2.8 (3H, m), 3.35 (9H, s), and 4.6−4.7 (2H).

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