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SUNABEDINE, A NOVEL TOXIC BROMOTYROSINE-DERIVATIVE ALKALOID FROM OKINAWAN SPONGE, ORDER *VERONGIDA*

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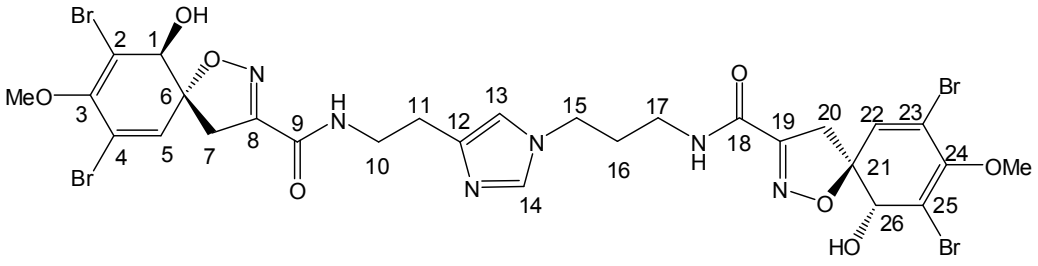
Abstract – Sunabedine (**1**), a new bromotyrosine-derivative alkaloid, was isolated from the Okinawan sponge, order *Verongida*. The structure of **1** was determined by spectroscopic analyses. **1** showed the cytotoxicity against B16 mouse melanoma cells and toxicity against brine shrimp.

Bromotyrosine-derivative alkaloids are characteristic secondly metabolites from marine sponges of the order *Verongida*. The structures of these metabolites are typically based on one or two spirocyclohexadienyl isoxazole moieties connected to diverse side chains, as seen in aerothinin[1] and aerophobin-1.[2] Various biological activities have been reported, such as cytotoxic,[3] antibacterial,[4] antihistaminic,[5] and enzyme-inhibitory[6,7] activities. We report here the isolation, structural determination and biological activities of the novel bromotyrosine-derivative alkaloid, sunabedine (**1**).



1

Dedicated to Professor Dr. Albert Eschenmoser on the occasion of his 85th birthday.

Table 1. NMR data for **1** in CD_3OD

Position	^{13}C	^{1}H	Multi (J_H)	HMBC (H → C)
1, 26	75.5 d[a, b]	4.07[c]	d (0.9) [d]	C-2, 3, 4, 5, 22, 23, 24, 25
2, 25	122.8 s			
3, 24	149.3 s			
4, 23	114.1 s			
5, 22	132.3 s	6.40 6.41	d (0.9) d (0.9)	C-1, 2, 3, 4, 6, 7, 20, 21, 23, 24, 25, 26
6, 21	92.4 s			
7a, 20a	40.2 t	3.06 3.07	d (18.2) d (18.2)	C-1, 5, 6, 8, 19, 21, 22, 26
7b, 20b		3.74 3.75	d (18.2) d (18.2)	C-1, 5, 6, 8, 19, 21, 22, 26
8	155.2 s			
19	155.3 s			
9	161.4 s			
18	161.7 s			
10	40.4 t	3.50	t (7.7)	C-9. 11, 12
11	28.7 t	2.76	t (7.7)	C-10, 12, 13
12	140.0 s			
13	117.7 d	6.96	d (1.3)	C-12, 14
14	138.2 d	7.59	d (1.3)	C-12, 13
15	45.7 t	3.91	t (6.7)	C-13, 14, 16, 17
16	31.6 t	2.01	q (6.7)	C-15, 17
17	37.6 t	3.27	t (6.7)	C-15, 16, 18
OCH_3-3, OCH_3-24	60.4 q	3.72	s	C-3, 24
OH		(not detected)		
NH – 9[e]		7.54	t (5.5)	
NH – 9[e]		7.06	t (5.9)	

[a]Recorded at 75 MHz. [b]Multiplicity was based on the HMQC spectrum. [c]Recorded at 300 MHz. [d]Coupling constants (Hz) are in parentheses. [e]Recorded in CD_3CN.

The ESIMS of **1** showed a $[M+H]^+$ ion 1:4:6:4:1 quintet at *m/z* 894.9, 896.9, 898.9, 900.9 and 902.9, which indicated the presence of four bromine atoms in its structure. **1** has a molecular formula of $C_{28}H_{30}Br_4N_6O_8$, as suggested by HRFABMS at *m/z* 896.8943 $[M+H]^+$ (calcd for $C_{28}H_{31}{}^{79}Br_3{}^{81}Br\ N_6O_8$, 896.8917). The ^1H and ^{13}C NMR data are summarized in Table 1. The absorption bands in the IR spectrum showed amino and hydroxyl groups (3339 cm^{-1}) and α-iminoamide functions (1662, 1597 and 1541 cm^{-1}), and the UV spectrum showed cyclohexadienyl moieties (284 nm). The ^1H NMR signals displayed the characteristic signals of two 2,4-dibromo-1-hydroxyl-3-methoxy-8-carbamoyl spirocyclohexadienyl isoxazole moieties at 3.06/3.07, 3.72, 3.74/3.75, 4.07 and 6.40/6.41, and corroborating ^{13}C NMR signals at 40.2, 60.4, 75.5, 92.4, 114.1, 122.8, 132.3, and 149.3. The COSY spectral analysis allowed us to connect two spin systems of methylene chains of C10-C11 and C15-C17 (Figure 1). The HMBC correlations of H7/C9, H10/C9, H17/C18 and H20/C18 indicated the connectivity between C7-C10 and C17-C20 via an amide bond. The remaining proton signals at 6.96 and 7.59, and carbon signals at 117.7, 138.2 and 140.0 were thought to represent an aromatic moiety. The HMBC correlations of H10/C12, H11/C12, H11/C13, H13/C12, H13/C14, H14/C13, H15/C13 and H15/C14 revealed the structure of a 1,4-disubstituted imidazole ring that was linked to methylene chains.

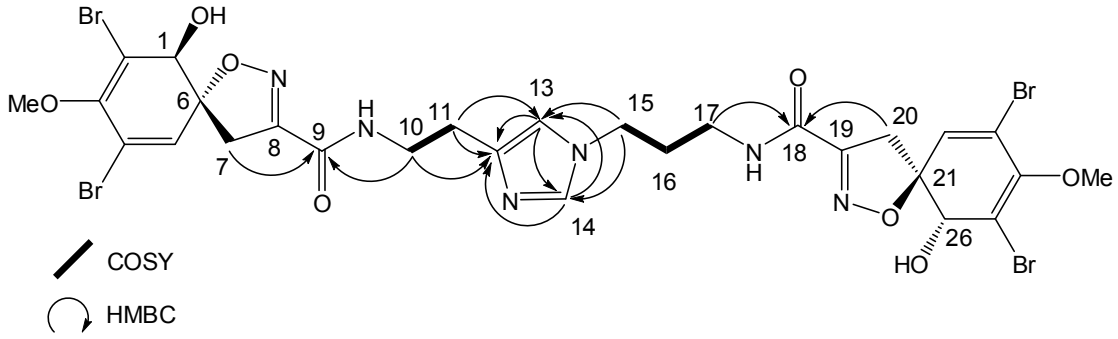


Figure1. Selected COSY and HMBC Correlations of **1**

The geometries of the vicinal oxygen atoms at C1 (C26) and C6 (C21) were established by comparison of the chemical shifts value with the synthetic *trans* and *cis* spiroisoxazoline derivertives.[8] The ^1H NMR signals of H1, H6 and H7 in *trans* compound at 4.08, 6.40, and 3.05/3.78 and *cis* compound at 4.40, 6.55, and 3.3-3.4 reveals *trans* relationship of these in **1**. The absolute configurations of spirocyclohexadienyl isoxazole moieties were thought to be determined based on the CD spectrum by comparison with the reported spectrum of aerothionin, the configuration of which was established by X-ray analysis.[9] However, **1** showed no absorption at 240-300 nm in its CD spectrum. These data, supported by the large active optical rotation $\{[\alpha]_D^{20}$ –137.1 (*c* 0.30, $CHCl_3)\}$, suggest that **1** is not a racemic mixture and the absolute configurations of C1, C6, C21 and C26 might be 1*R*, 6*S*, 21*R* and 26*S* or 1*S*, 6*R*, 21*S* and 26*R*.

The biological activities of compound **1** were examined with regard to cytotoxicity against B16 mouse melanoma cells and toxicity against brine shrimp (genus *Artemia*). After incubation, **1** showed potency with an IC_{50} of 39 μM against B16 cells and a LD_{50} of 110 μM against brine shrimp. To the best of our knowledge, this is the first report of a bromotyrosine-derivative alkaloid that is toxic toward brine shrimp.

EXPERIMENTAL

General Experimental Procedures. The ^1H, ^{13}C, and 2D NMR spectra were recorded on a JEOL A-300 spectrometer, and the ^1H and ^{13}C chemical shifts were referenced to the solvent peaks [δ_H 3.30 and δ_C 49.0 in CD_3OD and δ_H 1.93 in CD_3CN]. The IR spectrum was measured using a JASCO FT/IR-6100 spectrometer, the UV spectrum was measured using a JASCO V-570 spectrometer, the optical rotation was measured on a JASCO DIP-1000 polarimeter, and the CD spectrum was obtained in MeOH using a JASCO J-720WN spectrophotometer. The low-resolution mass spectrum (ESIMS) was determined on a Bruker HCTultra mass spectrometer, and the high-resolution mass spectrum (HRFABMS) was determined on a JEOL JMS-700 mass spectrometer. Column chromatography was performed on Silicagel BW-820MH (Fuji Silysia Chemical). Preparative TLC was performed using Silicagel 60 F_{254} (Merck). All solvents used were reagent grade.

Extraction and Isolation. The sponge order *Verongia* (270 g wet wt.), collected at Okinawa Island, Okinawa Prefecture, Japan, was crushed and extracted with 80% aqueous EtOH (1 L) for 12 days. The extract was filtered, concentrated, and partitioned between EtOAc and water. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation using a silica gel ($MeOH/CHCl_3$) and a preparative TLC ($MeOH/CHCl_3$) to give 5 mg of **1** as a colorless solid.

Sunabedine (**1**): $[\alpha]_D^{20}$ –137.1 (*c* 0.30, $CHCl_3$); IR (neat): 3339, 1662, 1597, 1541, 1437, 1308, 1268, 1219, 989 cm^{-1}; UV (MeOH): λ_{max} 227, 284 nm; ^1H NMR (CD_3OD, 300 MHz), see Table 1; ^{13}C NMR (CD_3OD, 75 MHz), see Table 1; HRFABMS: [*m / z* $(M + H)^+$] found 896.8943, calcd for $C_{28}H_{31}{}^{79}Br_3{}^{81}Br\ N_6O_8$ (*Δ* +2.6 mmu).

Cytotoxic Activity. Cultured cells of B16 mouse melanoma were seeded at 4 x 10^4 cells/mL in each well of a 96-well plate, and samples dissolved in MeOH were added. The mixture was incubated at 37 °C for 96 h in a CO_2 incubator with a humidified atmosphere containing 5% CO_2. The cell number was counted by the MTT method.

Brine Shrimp Toxicity. Brine shrimp eggs ware hatched in artificial seawater with oxygenation by an aquarium pump at 24 ˚C for 24-48 h. Ten hatched brine shrimp in 100 μL seawater were placed in each well of a 96-well microplate, and samples dissolved in 100 μL of 2.5% MeOH/seawater were added. After 24 h, the numbers of dead and live brine shrimp were counted under a microscope.

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