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Biselyngbyolide A, a Novel Cytotoxic Macrolide from the Marine Cyanobacterium *Lyngbya* sp.

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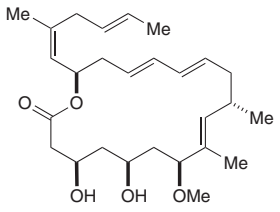
A novel cytotoxic macrolide biselyngbyolide A (**1**) was isolated from the marine cyanobacterium *Lyngbya* sp., collected on Tokunoshima Island, Japan. This macrolide was revealed to be structurally related to biselyngbyaside (**2**). The gross structure of **1** was elucidated based on the extensive application of 2D NMR techniques. The stereostructure was deduced based on analyses of the NOESY spectrum and CD data. Biselyngbyolide A (**1**) exhibited strong apoptosis-inducing activity against HeLa S_3 cells and HL60 cells.

Marine cyanobacteria produce a large number of structurally unique and biologically active secondary metabolites,[1,2] some of which have potential as therapeutic agents.[3] In our search for new bioactive substances from marine cyanobacteria,[2f,2g] we found a novel 18-membered ring macrolide, biselyngbyolide A (**1**) (Figure 1), which showed apoptosis-inducing activity. Biselyngbyolide A (**1**) was revealed to be a structural analogue of the recently reported biselyngbyaside (**2**).[2g] Biselyngbyaside (**2**) inhibits osteoclastogenesis and induces apoptosis of osteoclasts.[4] In this letter, we describe the isolation, structure determination, and biological activities of **1**.

The marine cyanobacterium *Lyngbya* sp.[5] (400 g, wet weight) was collected at the coast of Tokunoshima Island, Japan, and extracted with methanol. The extract was filtered, concentrated in vacuo, and partitioned between ethyl acetate and water. The ethyl acetate layer was concentrated and further partitioned between *n*-hexane and 90% aqueous methanol. The 90% aqueous methanol layer, which showed remarkable growth-inhibitory activity against HeLa S_3 cells, was subjected to fractionation with ODS silica gel column chromatography (MeOH–H_2O) and reversed-phase HPLC (Cosmosil 5C_{18}-MS-II, MeCN–H_2O; Cholester, MeCN–H_2O) to give **1** (30.8 mg) as a colorless oil [$[\alpha]_D^{25}$ − 115.1° (*c* 0.10, $CHCl_3$), UV (MeOH) λ_{max} 232.6 nm (ε 22742)].



biselyngbyolide A (**1**)

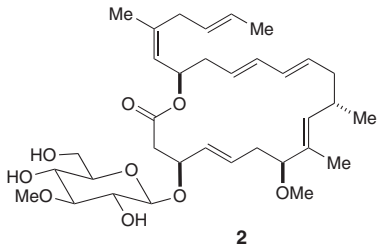


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Figure 1. Structures of biselyngbyolide A (**1**) and biselyngbyaside (**2**).

Positive-ion HR-ESIMS of **1** afforded a molecular formula of $C_{27}H_{42}O_5$ ($[M + Na]^+$, *m/z* 469.2932, calculated 469.2930, Δ +0.2 mmu). The structure of **1** was predominantly established based on NMR spectroscopy, including ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, NOESY, HMQC, and HMBC spectra. The assigned proton and carbon resonances of **1** in C_6D_6 and CD_3OD are summarized in Table 1. Analyses of ^{1}H NMR, DEPT, and HMQC spectral data revealed the presence of an aliphatic methyl (δ_H 0.92), three vinyl methyls (δ_H 1.53, 1.57, and 1.62), an *O*-methyl group (δ_H 3.04), an aliphatic methine (δ_H 2.40), four oxymethines (δ_H 3.82, 3.94, 4.14, and 5.82), and eight olefinic methines (δ_H 5.06, 5.25, 5.37, 5.41, 5.42, 5.46, 5.93, and 6.01). The remaining proton signals were assigned to six methylenes. The ^{13}C NMR spectrum of **1** showed the presence of 27 carbons including a carbonyl carbon (δ_C 171.0) and 10 olefinic carbons (δ_C 124.6, 126.6, 127.5, 128.7, 131.3, 132.6, 133.5, 134.1, 135.3, and 139.4). On the basis of the ^{1}H–^{1}H COSY spectrum, three partial structures, C2–7, C9–18, and C20–23, were constructed. Analysis of HMBC data allowed us to assemble these subunits into the 18-membered ring macrolide **1**. The lactone ring was confirmed by the HMBC correlation from H17 to C1, and the lower-field shifted chemical shift of H17 (δ_H 5.82). The connectivity of C8–C9 was revealed by the HMBC correlations of H7/C9, H9/C7, H25/C7, H25/C8, and H25/C9.

The geometries of the two trisubstituted olefins were revealed to be 8*E* and 18*Z*, based on the NOESY correlations observed for H10/H25 and H18/H27, respectively, and these assignments were strongly supported by the chemical shifts of the vinyl methyls C25 (δ_C 10.3) and C27 (δ_C 23.5). The 12*E* and 14*E* geometries of the diene portion were established by the characteristic coupling constants ($J_{12,13}$ = 15.1 Hz and $J_{14,15}$ = 15.1 Hz). The above assignments were supported by the NOESY correlations between H11/H13 and H14/H16. Although the analysis of the H21–H22 coupling constant was difficult, NOESY correlation of H21/H23 revealed 21*E*. Thus, the gross structure of **1** was assigned to be as shown in Figure 2.

The relative configuration of the macrolide moiety was determined by an analysis of the NOESY spectrum. A plausible conformation for **1** with selected NOESY correlations is shown in Figure 3. The correlation of H3/H5 and H5/H7 demonstrated that these three protons were on the same side of the 18-membered ring (Figure 3a). To confirm the relative stereochemistries of C3 and C5, an acetonide derivative of **1** was prepared (2,2-dimethoxypropane, PPTS). Prominent NOESY correlations among H3, H5, and one acetonide methyl group were observed and confirmed the relative stereochemistries of C3 and C5 (Figure 3b). Furthermore, the relative stereochemistry at C10 was determined by the NOESY correlations of

Table 1. NMR spectral data for **1** recorded in C_6D_6 and CD_3OD

Position	**1** in C_6D_6			**1** in CD_3OD	
	δ_C[a]	δ_H (mult., J in Hz)[b]	HMBC ($^1H \rightarrow {}^{13}C$)	δ_C[a]	δ_H (mult., J in Hz)[b]
1	171.0		H-2a, 2b, 3, 17	172.4	
2	44.3	**a**: 2.27 (m)	H-4a, 4b	44.4	**a**: 2.24 (m)
		b: 2.38 (m)			**b**: 2.35 (m)
3	69.6	4.14 (m)	H-2a, 2b, 5	68.5	4.07 (m)
4	44.0	**a**: 1.32 (brd, 14.6)	H-2a, 2b, 6a, 6b	45.3	**a**: 1.43 (m)
		b: 1.69 (m)			**b**: 1.64 (m)
5	70.5	3.94 (m)	H-3, 4a, 4b, 6a, 6b	69.5	3.65 (m)
6	41.3	**a**: 1.52 (m)	H-4a, 4b, 5, 7	41.3	1.59 (m, 2H)
		b: 1.87 (ddd, 13.9, 10.8, 7.2)			
7	87.3	3.82 (t, 7.2)	H-5, 6a, 6b, 9, 25	87.8	3.74 (dd, 8.0, 6.6)
8	133.5		H-6b, 7, 25	133.3	
9	135.3	5.06 (d, 10.1)	H-7, 10, 11a, 11b, 25, 26	137.9	5.17 (brd, 9.2)
10	32.8	2.40 (m)	H-9, 11a, 11b, 12, 26	34.1	2.62 (m)
11	41.6	**a**: 1.78 (m)	H-9, 10, 12, 13, 26	42.1	**a**: 1.90 (m)
		b: 2.12 (d, 14.7)			**b**: 2.26 (m)
12	132.6	5.37 (m)	H-10, 11a, 11b, 13, 14	133.5	5.47 (m)
13	131.3	5.93 (dd, 15.1, 10.9)	H-11a, 11b, 14, 15	132.1	5.97 (m)
14	134.11	6.01 (dd, 15.1, 10.9)	H-12, 13, 16	137.9	6.00 (m)
15	127.5	5.46 (m)	H-13, 14, 16, 17	127.4	5.49 (m)
16	39.6	2.31 (m, 2H)	H-14, 15, 17, 18	39.8	**a**: 2.24 (m)
					b: 2.34 (m)
17	71.4	5.82 (td, 9.1, 4.8)	H-14, 15, 16	71.6	5.56 (td, 9.2, 3.4)
18	124.6	5.25 (d, 9.1)	H-16, 17, 27	124.9	5.16 (brd, 9.2)
19	139.4		H-17, 18, 20a, 20b, 27	140.3	
20	36.2	**a**: 2.78 (dd, 14.5, 1.6)	H-18, 21, 22, 27	36.7	**a**: 2.73 (dd, 15.0, 6.5)
		b: 3.06 (d, 14.5)			**b**: 2.94 (brdd, 15.0, 6.5)
21	128.7	5.41 (m)	H-20a, 20b, 23	129.4	5.35 (m)
22	126.6	5.42 (m)	H-20a, 20b, 23	127.7	5.39 (m)
23	18.0	1.57 (m, 3H)	H-21, 22	18.0	1.64 (m, 3H)
24	55.5	3.04 (s, 3H)	H-7	55.8	3.16 (s, 3H)
25	10.3	1.53 (d, 1.2, 3H)	H-7, 9	10.2	1.51 (d, 1.2, 3H)
26	22.0	0.92 (d, 6.9, 3H)	H-9, 10, 11a, 11b	22.4	1.02 (d, 6.6, 3H)
27	23.5	1.62 (s, 3H)	H-18, 20a, 20b	23.5	1.68 (d, 1.4, 3H)

[a]Recorded at 400 MHz. [b]Recorded at 100 MHz.

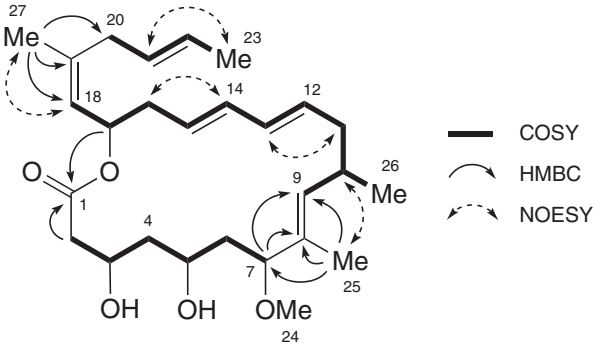

Figure 2. Selected 2D NMR correlations for **1**.

H7/H9, H9/H26, and H25/H10, and these results showed that the plane of C8–C9 olefin was perpendicular to the 18-membered ring, as shown in Figure 3. The observed NOESY cross peaks, H10/H12, H12/H14, H13/H15, and H15/H17, revealed the relative conformation of H17. The *s-trans*-conformation of the diene was deduced from these NOESY correlations and a large vicinal coupling constant ($J_{13,14} = 10.9$ Hz). Thus, the relative stereochemistry of **1** was established as depicted in Figure 3. The determined relative configuration of **1** corresponded to that of **2**.

The absolute stereostructure of **2** had been determined by degradation, the modified Mosher's method and synthetic methods.[2g] Therefore, the absolute stereochemistry of **1** could be established by comparing CD data with those of **2**. The CD spectrum of **1** in MeOH showed a pronounced negative Cotton effect (λ_{max} 231.4 nm, $\Delta\varepsilon$ −46.1). The sign was the same as that of **2** (λ_{max} 232.6 nm, $\Delta\varepsilon$ −11.3). Thus, the absolute stereostructure of **1** was determined to be as shown in Figure 1.

Biselyngbyolide A (**1**) was evaluated in terms of its growth-inhibitory activity against HeLa S_3 human cervical cancer cells and HL60 human leukemia cells using the MTT assay. After 72 h of incubation, **1** exhibited growth-inhibitory activity toward HeLa S_3 cells and HL60 cells, with IC_{50} values of 0.14 and 0.012 μM, respectively. Biselyngbyolide A showed significant cytotoxicity against HeLa S_3 cells (IC_{50} value: 0.22 μM) and HL60 cells (IC_{50} value: 0.027 μM), as determined using the trypan blue dye exclusion assay. In addition, cell death of these cells induced by **1** was suppressed in the presence of Z-VAD-FMK (Promega, Madison, WI), an irreversible and cell-

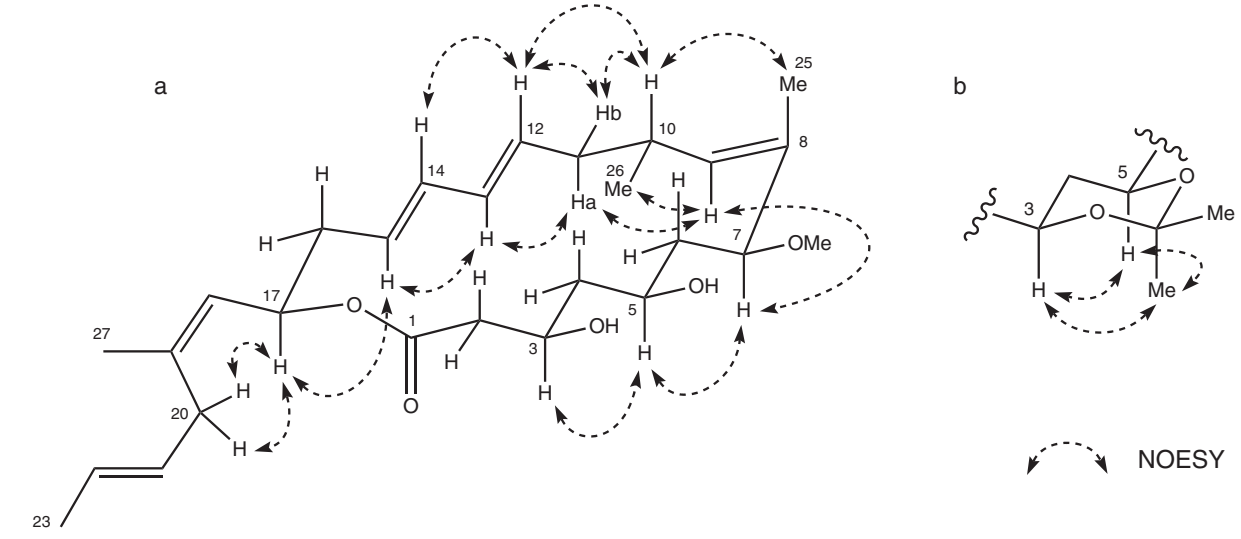

Figure 3. Plausible conformation of biselyngbyolide A (**1**) (a) and C3–C5 moiety of its acetonide (b) with selected correlations in the NOESY spectrum.

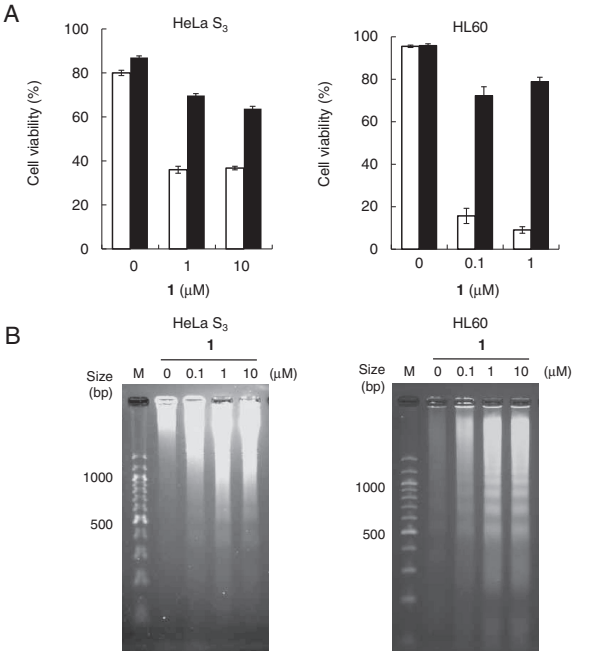

Figure 4. Induction of apoptosis in HeLa S_3 cells and HL60 cells by **1**. (A) HeLa S_3 cells and HL60 cells were preincubated (solid column) or not (open column) with Z-VAD-FMK (50 μM for HeLa S_3 cells, 25 μM for HL60 cells). They were then treated with the indicated concentrations of **1**. After further incubation for 72 (for HeLa S_3 cells) and 24 h (for HL60 cells), cell viability was determined. Values are the means ± SD of quadruplicate determinations. (B) HeLa S_3 cells and HL60 cells were incubated with the indicated concentrations of **1** for 48 and 24 h, respectively. Cellular DNA was then extracted and electrophoresed on agarose gels.

permeable inhibitor of caspases (Figure 4A). Significant DNA laddering in these cells was also observed in the presence of **1** (Figure 4B). These results indicated that **1** induced apoptosis in HeLa S_3 cells and HL60 cells. Since **1** clearly induced apoptosis in two types of cell lines, cancer cells generally might contain a specific target molecule of **1**. Further biological activities of **1** are now being investigated.

In conclusion, biselyngbyolide A (**1**), a novel 18-membered macrolide, was isolated from the marine cyanobacterium *Lyngbya* sp. The structure of biselyngbyolide A (**1**) was established on the basis of 1D and 2D NMR spectra, and CD data. Biselyngbyolide A (**1**) exhibited remarkable growth-inhibitory activity against HeLa S_3 cells and HL60 cells. Furthermore, **1** was revealed to induce apoptosis in these cells.

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5 The cyanobacterium was identified by Prof. Shoichiro Suda (University of Ryukyus).

