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Biselyngbyaside, a Macrolide Glycoside from the Marine Cyanobacterium *Lyngbya* sp.

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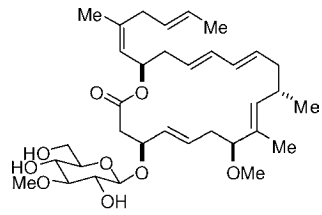
ABSTRACT



biselyngbyaside **1**

Bioassay-guided fractionation of the cytotoxic constituents of the marine cyanobacterium *Lyngbya* sp. led to the isolation of biselyngbyaside (1), a new 18-membered macrolide glycoside. The structure of 1 was established by spectroscopic analysis including 2D-NMR techniques and by synthetic studies. Biselyngbyaside (1) exhibits broad-spectrum cytotoxicity in a human tumor cell line panel.

The oceans are a good source of new biologically active substances. Over the past few decades, a considerable number of marine natural products have been reported.[1] Several compounds that are now used at the clinical trial level,[2] such as halichondrin B,[3] bryostatin 1,[4] and dolastatin 10,[5] were originally found in marine animals. Therefore, marine natural products continue to receive increasing attention. In particular, cyanobacteria are prolific producers of bioactive secondary metabolites.[6]

In our ongoing efforts to identify novel marine cyanobacterial metabolites with antitumor activity, we found biselyngbyaside (**1**). Over the past few years, several classes of macrolides have been isolated from marine sponges. These include 18-membered macrolides, tedanolide[9] and 13-deoxytedanolide[9] from *Tedania ignis* and *Mycale adhaerens*, respectively, laulimalide,[9] a 20-membered ring from *Caco-spongia mycofijiensis*, *Hyatella* sp., *Fasciospongia* sp., *Dactylospongia* sp., and a chromodorid nudibranch, and peloruside A,[10] a 16-membered ring macrolide from *Mycale hentscheli*. Biselyngbyaside (**1**) has the same macrolide ring-size as tedanolide and 13-deoxytedanolide. We report here the isolation, structure determination, and biological activity of **1**.

The marine cyanobacterium *Lyngbya* sp. (a voucher specimen was deposited at Keio University), collected in Okinawa Prefecture, was extracted with methanol. The concentrated extract was partitioned between ethyl acetate and water, and the ethyl acetate layer was concentrated and partitioned between *n*-hexane and 90% aqueous methanol. The concentrated 90% aqueous methanol layer was subjected to fractionation guided by cytotoxicity against HeLa S_3 cells

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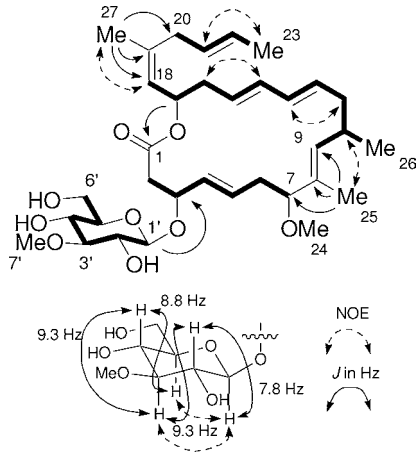
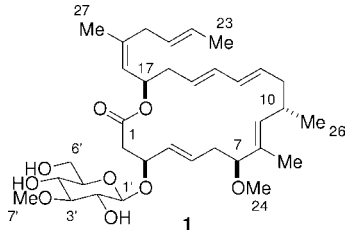


Figure 1. Gross structure of **1** determined by 2D-NMR spectroscopy (bold lines, ^{1}H−^{1}H COSY; arrows, HMBC correlations).

with column chromatography (ODS silica gel, methanol−water) and reversed-phase HPLC to give biselyngbyaside (**1**) as a colorless oil. Biselyngbyaside (**1**) exhibited cytotoxicity against HeLa S_3 cells (IC_{50} = 0.1 μg/mL).



1

The molecular formula of **1** was determined to be $C_{34}H_{52}O_9$ by ESIMS [$(M + Na)^+$, *m/z* 627.3487, Δ−2.2 mmu]. The IR spectrum contained absorption bands for hydroxyl and ester groups [3589, 3502 (br), 1725 cm^{-1}]. The NMR data for **1** are summarized in Table 1. The ^{1}H NMR spectrum of **1** showed the presence of six methyl groups (δ 3.62, 3.15, 1.67, 1.64, 1.55, and 1.02). In the ^{13}C NMR spectrum, 34 carbon signals were observed, including one carbonyl carbon (δ 172.1), 12 olefinic carbons (δ 140.1, 138.4, 135.3, 133.3, 133.0, 132.7, 132.1, 131.9, 129.3, 127.9, 127.4, and 124.9), and six methyl carbons (δ 61.0, 55.6, 23.6, 22.5, 18.1, and 10.2). The remaining carbon signals were assigned to six methylenes and nine methines, based on the results of an HMQC experiment. The presence of a 3-*O*-methylglucoside moiety in **1** was readily revealed based on the coupling constants in the ^{1}H NMR spectra (Figure 1) and correlations in the ^{1}H−^{1}H COSY spectra, and their β-anomeric structures were determined by correlations in the NOESY spectrum (H1′/H3′ and H1′/H5′) and the $^1J_{CH}$ value at the anomeric carbons ($\delta_{C1'}$ 160 Hz).[11] This result was also supported by the ^{1}H NMR spectrum, where the magnitude of $^3J_{H1'-2'}$ was 7.8 Hz as measured in CD_3OD. Regarding the macrolide moiety, three sets of proton spin systems, C2−C7, C9−C18, and C20−C23, were determined based on the ^{1}H−^{1}H COSY spectrum. The connectivities between these partial structures

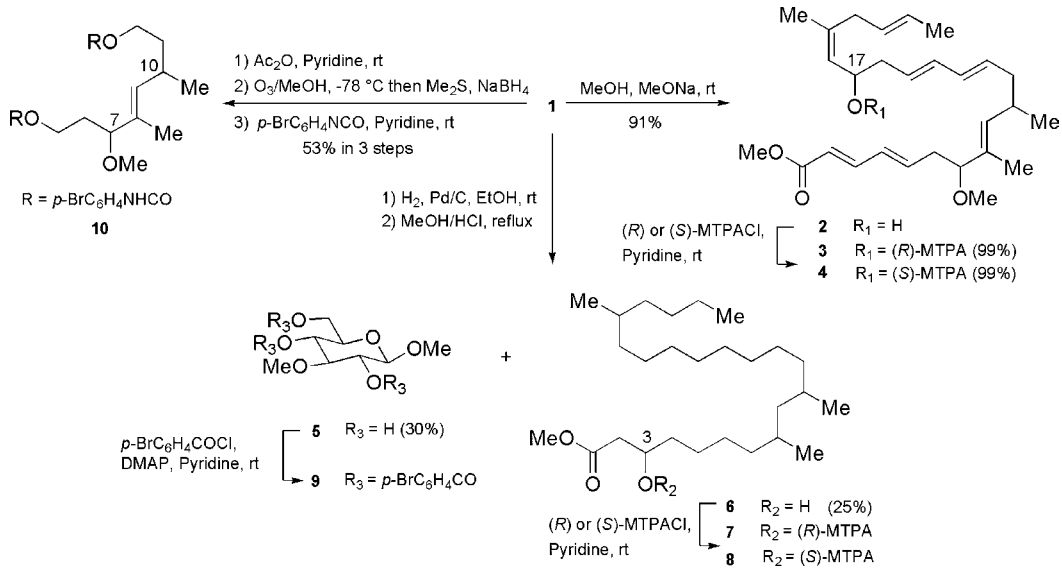
Table 1. NMR Spectral Data of **1** in CD_3OD

position	^{1}H (ppm)[a]	^{13}C (ppm)[b]	HMBC[c]
1		172.1	H-2, 3, 17
2a	2.32 m	42.9	
2b	2.57 dd (14.7, 7.8)		
3	4.50 m	77.4	H-4, 1′, 2a, 2b
4	5.42 m	131.9	H-3, 5
5	5.44 m	129.3	H-4, 6a, 6b
6a	2.31 m	36.8	H-7, 5
6b	2.32 m		
7	3.47 m	89.0	H-6a, 6b, 9, 24, 25
8		133.0	H-6, 7, 25
9	5.10 d (8.8)	138.4	H-7, 25, 26
10	2.68 m	34.0	H-9, 11a, 26
11a	1.94 m	41.5	H-9, 13, 26
11b	2.30 m		
12	5.55 m	133.3	H-11a
13	6.05 dd (17.6, 10.3)	135.3	
14	6.00 dd (17.6, 10.3)	132.1	
15	5.52 m	132.7	H-16a, 16b
16a	2.22 m	39.6	H-14, 15
16b	2.39 m		
17	5.51 m	72.0	H-1’
18	5.13 d (8.8)	124.9	H-20a, 20b, 27
19		140.1	H-20a, 20b
20a	2.72 dd (14.7, 5.9)	36.6	H-27, 18, 21
20b	2.92 dd (14.7, 6.8)		
21	5.40 m	127.9	H-20a, 20b, 23
22	5.50 m	127.4	H-20a, 20b, 23
23	1.64 d (5.9)	18.1	H-21, 22
24	3.15 s	55.6	H-7
25	1.55 s	10.2	H-7, 9
26	1.02 d (6.3)	22.5	
27	1.67 s	23.6	H-18, 20a, 20b
1′	4.25 d (7.8)	100.9	H-2’
2′	3.22 dd (9.3, 7.8)	74.5	H-1′, 3′, 4’
3′	3.04 dd (9.3, 9.3)	87.7	H-1′, 2′, 4′, 7’
4′	3.44 dd (9.3, 8.8)	70.7	H-3′
5′	3.18 m	77.6	H-4’
6’a	3.73 dd (11.7, 4.4)	62.4	H-4’
6’b	3.85 dd (11.7, 2.0)		
7′	3.62 s	61.0	H-3′

[a] Recorded at 400 MHz. Coupling constants (Hz) are in parentheses. [b] Recorded at 100 MHz. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for J_{CH} = 6 Hz.

and the 3-*O*-methylglucoside moiety in **1** were clarified by HMBC correlations: H1′/C2, H25/C7, H25/C8, H25/C9, H27/C18, H27/C19, and H27/C20. HMBC correlations H2/C1 suggested the connectivity of C1−C2. Consequently, the entire carbon chain was assembled, and all protons and carbons were assigned, as shown in Figure 1 and Table 1, respectively. The HMBC correlations H24/C7, H7/C24, H7′/C3′, and H3′/C7′ determined that the two methoxy groups were located at C7 and C3′. On the basis of its molecular formula and degree of unsaturation, **1** should contain a lactone ring. Considering the chemical shift of H17 (δ_H 5.51) and the HMBC correlation H17/C1, the lactone ring must be formed between C1 and C17. The double bond geometries in **1** were determined from proton−proton coupling constants and the NOE correlation from NOESY data as measured in

Scheme 1. Degradation Experiment of Biselyngbyaside (**1**)



C_6D_6 (see Supporting Information). The geometries of the two trisubstituted olefins in **1** were clarified to be 8*E* and 18*Z*, respectively, based on the NOESY correlation of H25/H10, and H27/H18. The 4*E*, 12*E*, and 14*E* geometries were determined from the H4−H5, H12−H13, and H14−H15 *trans*-coupling constants (15.1, 16.6, and 16.6 Hz, respectively). In addition to these results, NOESY correlations were observed between H13 and H11, and between H14 and H16. A careful analysis of NOESY correlation observed between H21 and H23 revealed a 21*E* geometry. Thus, the gross structure of biselyngbyaside (**1**) was determined to be as shown in Figure 1.

The absolute stereochemistries of C3, C17, and the 3-*O*-methylglucoside moiety in **1** were determined by the modified Mosher's method[12] and synthetic means. Methanolysis of **1** gave methyl ester **2** along with elimination of the sugar (Scheme 1). Since **1** is unstable under acidic and basic conditions, hydrogenation and subsequent acid hydrolysis provided 3-*O*-methylglucoside **5** and β-hydroxy methyl ester **6** accompanied by hydrogenolysis at C7 and C17 (Scheme 1). Derived products **2** and **6** were transformed into the (*R*)- and (*S*)-MTPA esters, **3** and **4**, and **7** and **8**, respectively (Scheme 1). The ^{1}H NMR signals of these esters were assigned based on the 2D-NMR spectra, and the $\Delta\delta$ values (δ_S-δ_R, Hz) were then calculated. These results established

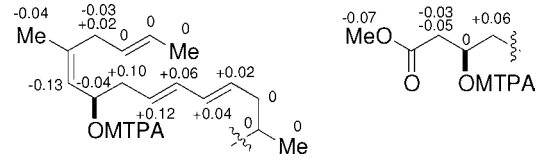


Figure 2. $\Delta\delta$ values ($\Delta\delta_{S-R}$) in ppm for bis-*S*- and bis-*R*-MTPA esters (**3** and **4**, **7** and **8**).

that the absolute stereochemistries of C3 and C17 in **1** were 3*R* and 17*R*, respectively (Figure 2). Furthermore, tribromobenzoylation of **5** afforded methyl 2,4,6-tri-*O*-(4-bromobenzoyl)-3-*O*-methyl-α-D-glucose **9**, which was identified by ^{1}H NMR data and CD data (see Supporting Information). An authentic sample of **9** was prepared from D-glucose.

The absolute stereochemistries of C7 and C10 in **1** were determined by enantioselective synthesis of the degradation product from natural **1**. The oxidative degradation of **1** into biscarbamates **10** was effected by a three-step sequence.

The synthesis of degradation product **10** started from known aldehyde **11a**.[13] The (2*R*)-aldehyde **11a** was converted into aldehyde **12** in three steps. Treatment of **12** with

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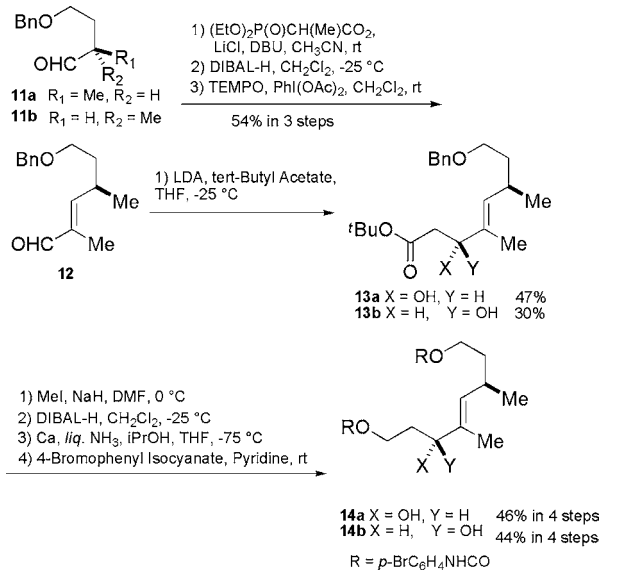
$LiCH_2COO^tBu$ gave a mixture of diastereomeric alcohols **13a** and **13b**, which was separated by silica gel column chromatography. The stereochemistries of the hydroxyl groups in **13a** and **13b** were determined by the modified Mosher's method.[12] Conversion of **13a** and **13b** into the respective biscarbamates **14a** and **14b** was achieved by a four-step sequence (Scheme 2). The ^1H NMR data of **14a**

Scheme 2. Synthesis of Degradation Product of Natural Biselyngbyaside (**1**)



fully agreed with those of degradation product **10** from natural biselyngbyaside (**1**). To determine the absolute stereochemistry of **10**, the enantiomeric (3*R*, 6*R*)-biscarbamate *ent*-**14a** was prepared from (2*S*)-aldehyde **11b** in the same manner as described above, and both enantiomers were analyzed by chiral HPLC. Of the two synthetic enantiomers **14a** and *ent*-**14a**, the retention time of *ent*-**14a** was identical to that for **10** from the natural product, which established the absolute stereochemistry of **10**. Based on these findings, the complete stereostructure of biselyngbyaside (**1**) was determined as shown in **1**.

Biselyngbyaside (**1**) exhibited cytotoxicity against HeLa S_3 cells with an IC_{50} value of 0.1 μg/mL. Biselyngbyaside (**1**) was evaluated against a disease-oriented panel composed of 39 human cancer cell lines (HCC panel) at the Japanese Foundation for Cancer Research (see Supporting Information).[14] The average GI_{50} value across all of the cell lines tested was 0.60 μM, and **1** exhibited differential cytotoxicities: the central nervous system cancer SNB-78 (GI_{50} 0.036 μM) and lung cancer NCI H522 (GI_{50} 0.067 μM) were especially sensitive. Biselyngbyaside (**1**) was COMPARE-negative,[14] indicating that it likely inhibits cancer cell proliferation through a novel mechanism.

In summary, biselyngbyaside (**1**), with a potentially new mechanism of action, was isolated from the marine cyanobacterium *Lyngbya* sp. The structure of biselyngbyaside (**1**) was determined based on 2D-NMR spectra and synthetic procedures. Biselyngbyaside (**1**) exhibits broad-spectrum cytotoxicity in a human tumor cell line panel.

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Supporting Information Available: Detailed experimental procedures, spectroscopic data and HCC panel data for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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