



## Isolation and structure of koshikalide, a 14-membered macrolide from the marine cyanobacterium *Lyngbya* sp.

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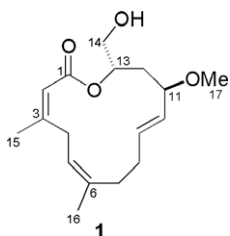
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### ABSTRACT

A 14-membered macrolide, koshikalide (**1**), was isolated from the marine cyanobacterium *Lyngbya* sp., and its planar structure was elucidated by spectroscopic analysis. The relative stereochemistry of C-11 and C-13 was elucidated by NOESY experiments and by an analysis of <sup>1</sup>H–<sup>1</sup>H coupling constants. Koshikalide (**1**) exhibited weak cytotoxicity against HeLa S<sub>3</sub> cells.

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Marine microorganisms produce a variety of chemically interesting and biologically significant secondary metabolites.<sup>1</sup> Marine cyanobacteria have been recognized as a rich source of novel bioactive substances.<sup>2</sup> In our continuing search for new substances from marine cyanobacteria,<sup>2c,d</sup> we investigated the constituents of cyanobacterium *Lyngbya* sp.<sup>3</sup> collected at Mie Prefecture, and isolated a 14-membered macrolide, koshikalide (**1**). In this Letter, we describe the isolation, structural elucidation, and biological activity of koshikalide (**1**).



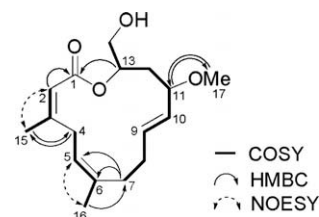
The marine cyanobacterium *Lyngbya* sp. (1.0 kg, wet weight), collected at Koshika, Shima City, Mie prefecture, was extracted with methanol. The extract was filtered, concentrated, and partitioned between EtOAc and H<sub>2</sub>O. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation with column chromatography (silica gel, CHCl<sub>3</sub>–MeOH; ODS silica gel, MeOH–H<sub>2</sub>O) and reversed-phase HPLC (Cosmosil 5C18-AR-II, MeOH–H<sub>2</sub>O–TFA; Cholesterol, MeCN–H<sub>2</sub>O–TFA) to

**Table 1**  
NMR data for koshikalide in C<sub>6</sub>D<sub>6</sub>

Position	<sup>1</sup> H NMR <sup>a</sup> δ (ppm)	<sup>13</sup> C NMR <sup>b</sup> δ (ppm)	HMBC <sup>1</sup> H→ <sup>13</sup> C
1		167.5	
2	5.58 s	115.5	C1, C3, C4, C15
3		157.8	
4a	2.08 m	31.0	C2, C3, C5, C6
4b	5.07 dd (11.7, 11.7)		C2, C3, C5, C6, C15
5	5.34 dd (11.7, 4.9)	122.7	C4, C16
6		136.8	
7a	1.76 ddd (12.7, 4.4, 4.4)	30.4	C5, C6, C8
7b	2.84 ddd (12.7, 12.7, 3.9)		C5, C6, C8, C16
8a	2.23 m	29.1	
8b	2.12 m		C7, C9, C10
9	5.44 ddd (15.1, 9.8, 3.4)	134.1	C8, C11
10	5.22 ddd (15.1, 8.8, 2.0)	129.4	C8, C11
11	3.63 ddd (9.8, 8.8, 4.4)	79.6	C10, C12, C13, C17
12a	2.22 m	35.1	C10, C11, C13, C14
12b	1.64 ddd (13.2, 9.8, 2.9)		C10, C11
13	5.12 m	70.9	C1
14a	3.40 dd (11.5, 5.7)	65.0	C12, C13
14b	3.43 dd (11.5, 4.1)		C12, C13
15	1.55 s	23.8	C2, C3, C4
16	1.58 s	22.4	C5, C6, C7
17	3.07 s	55.0	C11

<sup>a</sup> Recorded at 800 MHz. Coupling constants (Hz) are in parentheses.

<sup>b</sup> Based on HMQC and HMBC spectra.



**Figure 1.** Structure of **1** based on 2D NMR correlations.

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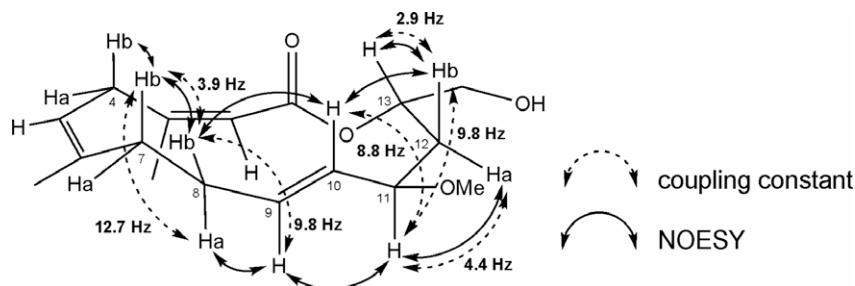


Figure 2. Relative stereochemistry of **1** based on NOESY correlations.

give koshikalide (**1**) (0.3 mg) as a colorless oil.<sup>4</sup> Koshikalide (**1**) exhibited weak cytotoxicity against HeLa cells, with an  $IC_{50}$  value of 42  $\mu\text{g/mL}$ .

The molecular formula of koshikalide (**1**) was found to be  $C_{17}H_{26}O_4$  by APCIMS ( $m/z$  317.1710, calcd for  $C_{17}H_{26}O_4Na$   $[M+Na]^+$  317.1729). The NMR data for **1** are summarized in Table 1. The  $^1\text{H}$  NMR spectrum showed the presence of two methyl groups connected to quaternary  $sp^2$  carbons ( $\delta$  1.55 and 1.58), and one methoxy group ( $\delta$  3.07). A detailed analysis of the COSY spectrum of **1** allowed for the formation of three partial structures, C2–C3–C15, C4–C6–C16, and C7–C14, as shown in Figure 1. The connectivities between these partial structures were clarified by HMBC: H4/C15, H15/C4, H7/C5, H7/C6, and H16/C7. HMBC H11/C17 and H17/C11 determined that a methoxy group was located at C11. Based on the correlations H2/C1 and H13/C1 in the HMBC spectrum and the chemical shift of C1 ( $\delta$  167.5) and H13 ( $\delta$  5.12), a lactone ring should be formed between C1 and C13. Furthermore, the presence of a hydroxyl group at C14 was suggested based on the molecular formula and the characteristic chemical shifts of H14 ( $\delta$  3.43, 3.40) and C14 ( $\delta$  65.0). The unusually low-field chemical shift of H4b ( $\delta$  5.07) is probably due to the anisotropic effect of the ester carbonyl group in the *s-cis* conformation (Fig. 2). The geometries of two trisubstituted olefins at C2 and C5 were both assigned to be *Z* by NOESY correlations, H2/H15 and H5/H16. The geometry of one disubstituted olefin at C9 was clarified to be *E* based on the magnitude of the  $^1\text{H}$ – $^1\text{H}$  coupling constant ( $J_{9-10} = 15.1$  Hz) (Fig. 1). Thus, the gross structure of koshikalide (**1**) was determined to be as shown in Figure 1.

The relative stereochemistry of **1** was determined as follows. A plausible conformation of **1** with important NOESY correlations is shown in Figure 2. The magnitudes of  $^3J_{H7b-H8a} = 12.7$  Hz,  $^3J_{H8b-H9} = 9.8$  Hz,  $^3J_{H10-H11} = 8.8$  Hz, and  $^3J_{H11-H12b} = 9.8$  Hz suggested that H7b and H8a, H8b and H9, H10 and H11, and H11 and H12b are located in *anti* arrangements, respectively. Furthermore, the magnitudes of  $^3J_{H7b-H8b} = 3.9$  Hz,  $^3J_{H11-H12a} = 4.4$  Hz, and  $^3J_{H12b-H13} = 2.9$  Hz suggested that H7b and H8b, H11 and H12a, and H12b and H13 are located in *gauche* arrangements. In addition, NOESY correlations H4b/H7b, H7b/H8b, H8b/H10, H10/H12b, H12b/H13, H8a/H9, H9/H11, and H11/H12a suggested a plausible

conformation of **1**, as shown in Figure 2. Thus, the relative stereochemistries of C11 and C13 were determined to be  $11R^*$ ,  $13S^*$ .

In conclusion, koshikalide (**1**), a 14-membered macrolide, was isolated from the cyanobacterium *Lyngbya* sp. The gross structure and relative stereochemistry of **1** were established by spectroscopic analysis including 2D NMR techniques. Koshikalide (**1**) exhibited weak cytotoxicity against HeLa  $S_3$  cells, with an  $IC_{50}$  value of 42  $\mu\text{g/mL}$ . Several other 14-membered macrolides of marine origin have been isolated including arenolide<sup>5</sup> and amphidinolides  $R^6$  and  $V$ .<sup>7</sup> In addition, some macrolides of cyanobacterial origin have been isolated including lyngbouillose,<sup>8</sup> lyngbyalose,<sup>9</sup> and laingolide.<sup>10</sup>

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- The cyanobacterium was identified by Dr. Takeshi Nakayama (Univ. of Tsukuba).
- Compound **1**:  $[\alpha]_D^{22} +156$  (c 0.03,  $\text{CH}_3\text{OH}$ ).  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR see Table 1. HRMS (APCI) exact mass calcd for  $C_{17}H_{26}O_4Na$   $[M+Na]^+$  requires  $m/z$  317.1729, found  $m/z$  317.1710.
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