

## **Sporiolides A and B, New Cytotoxic Twelve-Membered Macrolides from a Marine-Derived Fungus *Cladosporium* Species**

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**Abstract:** Two new cytotoxic twelve-membered macrolides, sporiolides A (**1**) and B (**2**), were isolated from the cultured broth of a fungus *Cladosporium* sp., which was separated from an Okinawan marine brown alga *Actinotrichia fragilis*, and the structures were elucidated by spectroscopic data. Sporiolides A (**1**) and B (**2**) exhibited cytotoxicity against murine lymphoma L1210 cells. Sporiolide A (**1**) showed antifungal activity against *Cryptococcus neoformans* and *Neurospora crassa*.

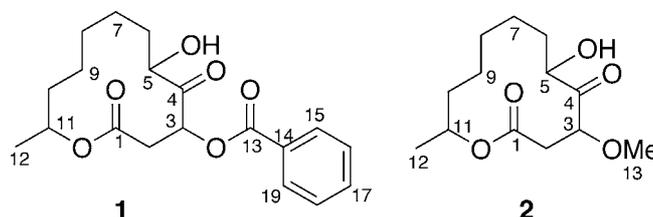
**Keywords:** marine-derived fungus, *Cladosporium* sp., macrolide; cytotoxic.

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### **Introduction**

Marine microorganisms such as bacteria, fungi, and microalgae have proven to be a rich source of structurally novel and biologically active secondary metabolites [1]. In our search for new substances from marine microorganisms [2], two new cytotoxic twelve-membered macrolides,

sporiolides A (**1**) and B (**2**), were isolated from the cultured broth of a fungus *Cladosporium* sp., which was separated from an Okinawan marine brown alga *Actinotrichia fragilis*. In this paper we describe the isolation and structure elucidation of **1** and **2**.

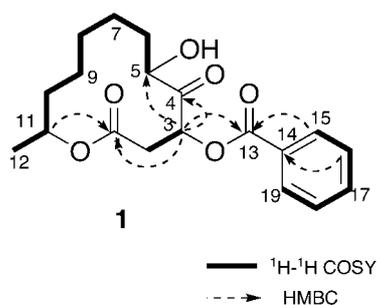


## Results and Discussion

The fungus *Cladosporium* sp. (L037) was separated from the brown alga collected off Seragaki Beach, Okinawa Island, and grown in SC broth [starch (1%) and casein (0.1%) in 50% sea water, pH 7.4] at 28°C for 14 days. The filtrate of the cultured broth (10 L) was extracted with EtOAc (1 L x 2). The EtOAc-soluble portions (58 mg) were subjected to a silica gel column (hexane/EtOAc, 70:30) followed by C<sub>18</sub> reversed-phase HPLC (Develosil ODS-5, Nomura Chemical, 1.0 x 25 cm; flow rate 2.5 mL/min; UV detection at 254 nm; eluent: MeOH/H<sub>2</sub>O, 70:30) to give sporiolides A (**1**, 2.7 mg) and B (**2**, 11.5 mg) together with a known related macrolide, cladospolide D [3] (7.0 mg). On the other hand, other known compounds, cladospolide A [4-6] (5.0 mg), iso-cladospolide B [7] (2.0 mg), and seco-patulolide C [7] (3.0 mg), were isolated from the EtOAc extract of the mycelium.

Sporiolide A (**1**) {[ $\alpha$ ]<sub>D</sub><sup>25</sup> -14° (c 0.2, MeOH)} was obtained as colorless amorphous solid. The molecular weight of **1** was elucidated to be 348 Dalton on the basis of FABMS data that showed the pseudomolecular ion at *m/z* 371 (M+Na)<sup>+</sup>. The molecular formula, C<sub>19</sub>H<sub>24</sub>O<sub>6</sub>, of **1** was established by HRFABMS data [*m/z* 371.1483, (M+Na)<sup>+</sup>,  $\Delta$  +1.2 mmu]. The IR spectrum suggested the presence of hydroxy (3426 cm<sup>-1</sup>), unsaturated ester and/or ketone carbonyl (1724 cm<sup>-1</sup>) groups. The UV absorptions at 237 (9200) and 209 (11700) nm indicated the presence of benzoyl chromophore. The <sup>1</sup>H NMR (Table 1) spectrum of **1** showed proton signals due to a benzoyl group [ $\delta$ <sub>H</sub> 8.05 (2H, m), 7.56 (1H, m), and 7.43 (2H, m)].

**Figure 1.** Selected 2D NMR correlations for sporiolide A (**1**)



**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data of Sporiolides A (**1**) and B (**2**) in  $\text{CDCl}_3$ .

position	<b>1</b>				<b>2</b>					
	$\delta_{\text{H}}^a$		$\delta_{\text{C}}^b$		$\delta_{\text{H}}^a$		$\delta_{\text{C}}^b$			
1			168.8	s			171.5	s		
2	3.52	dd	18.0, 9.8	40.5	t	3.30	m	42.2	t	
	2.95	d	18.0			2.66	m			
3	5.90	d	9.8	67.4	d	4.46	dd	9.0,2.0	74.1	d
4				207.1	s				207.8	s
5	4.40	m		76.0	d	4.36	dd	6.1,1.8	75.8	d
6	2.02	m		30.5	t	1.99	m		30.5	t
	1.77	m				1.69	m			
7	1.34	m		19.0	t	1.47	m		22.8	t
	1.17	m				1.05	m			
8	1.50	m		26.6	t	1.37 <sup>a</sup>	m		26.6	t
	1.12	m								
9	1.27	m		22.6	t	1.50	m		23.5	t
	1.21	m				1.42	m			
10	1.67	m		33.4	t	1.59	m		33.6	t
	1.40	m				1.32	m			
11	4.89	m		74.4	d	4.89	m		73.6	d
12	1.46 <sup>b</sup>	d	5.3	20.8	q	1.22 <sup>b</sup>	d	6.5	21.0	q
13				165.5	s	3.45 <sup>b</sup>	s		58.2	q
14				129.2	s					
15, 19	8.05	m		129.9	d					
16, 18	7.43	m		128.4	d					
17	7.56	m		133.5	d					

<sup>a</sup>2H <sup>b</sup>3H.

Analysis of the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (Figure 1) revealed connectivities of C-2 to C-3 and C-5 to C-12. HMBC correlations of H-3 ( $\delta_{\text{H}}$  5.90) to C-1 ( $\delta_{\text{C}}$  168.8), C-4 ( $\delta_{\text{C}}$  207.1), and C-5 ( $\delta_{\text{C}}$  76.0) and H-11 ( $\delta_{\text{H}}$  4.89) to C-1 indicated that **1** possessed a twelve-membered macrocyclic lactone with a ketone group at C-4 and a hydroxy at C-5. An HMBC correlation between H-3 to C-13 ( $\delta_{\text{C}}$  165.5) revealed that the benzoyl group was attached to C-3. Thus, the structure of sporiolide A was assigned as **1**, which corresponded to be a 3-*O*-benzoyl form of pandangolide 1 [7].

Sporiolide B (**2**)  $\{[\alpha]_{\text{D}}^{25} -33^\circ$  (*c* 0.3, MeOH) $\}$  was obtained as colorless amorphous solid. The molecular weight of **2** was elucidated by  $m/z$  281(M+Na)<sup>+</sup> in the positive mode FABMS. The molecular formula,  $\text{C}_{13}\text{H}_{22}\text{O}_5$ , of **2** was established by HRFABMS data ( $m/z$  281.1367 [M+Na]<sup>+</sup>,  $\Delta + 0.2\text{mmu}$ ). The IR spectrum suggested the presence of hydroxy ( $3429\text{ cm}^{-1}$ ), unsaturated ester and/or ketone carbonyl ( $1710\text{ cm}^{-1}$ ) groups. Detailed analysis of  $^1\text{H}$ ,  $^{13}\text{C}$ , and 2D NMR data revealed that the structure of **2** was similar to that of **1**, except for functional group at C-3. An HMBC correlation of H-3 ( $\delta_{\text{H}}$  4.46) to C-13 ( $\delta_{\text{C}}$  58.2, MeO) indicated the presence of a methoxy group at C-3. Thus, the structure of sporiolide B (**2**) was elucidated to be a 3-*O*-methoxy form of pandangolide 1 [7].

Sporiolides A (**1**) and B (**2**) were new twelve-membered macrocyclic lactones from the cultured broth of a marine-derived fungus *Cladosporium* sp. [8], although similar twelve-membered macrocyclic lactone such as cladospolide A has been obtained from a terrestrial fungus *Cladosporium* sp. and more recently, cladospolide D [3], *iso*-cladospolide B, *seco*-patulolide C, and pandangolides 1 and 2 have been isolated from an unidentified marine fungus [6,7], while pandagolides 2 and 3 were isolated from a marine-derived fungus *Cladosporium herbarum* [9]. Sporiolides A (**1**) and B (**2**) exhibited cytotoxicity against murine lymphoma L1210 cells with IC<sub>50</sub> values of 0.13 and 0.81 µg/mL, respectively. Sporiolide A (**1**) showed antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus niger*, and *Neurospora crassa* and antibacterial activity against *Micrococcus luteus*, while sporiolide B (**2**) had antibacterial activity against *Micrococcus luteus* (Table 2).

**Table 2.** Antimicrobial Activity of Sporiolides A (**1**) and B (**2**).

Test organisms	MIC (µg/ml)	
	<b>1</b>	<b>2</b>
<i>Micrococcus luteus</i>	16.7	16.7
<i>Bacillus subtilis</i>	>33.3	>33.3
<i>Escherichia coli</i>	>33.3	>33.3
<i>Candida albicans</i>	16.7	>33.3
<i>Cryptococcus neoformans</i>	8.4	>33.3
<i>Paecilomyces variotii</i>	>33.3	>33.3
<i>Aspergillus niger</i>	16.7	>33.3
<i>Neurospora crassa</i>	8.4	>33.3

Mueller Hinton broth and Sabouraud dextrose broth were used for bacteria and fungi, respectively.

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## Experimental

### General

Optical rotations were measured on a JASCO DIP-1000 polarimeter. 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. NMR spectra were recorded on a Bruker AMX-600 spectrometer. FAB mass spectrum was obtained on a JEOL HX-110 spectrometer using nitrobenzyl alcohol as a matrix.

### Fungal Material and Fermentation

The fungus *Cladosporium* sp. (L037) was separated from the brown alga *Actinotrichia fragilis*, which was collected off Seragaki Beach at Okinawa Island. Subcultures of the organism are deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University. The fungus was grown in SC broth [starch (1%) and casein (0.1%) in 50% sea water, pH 7.4] at 28°C for 14 days. The cultured broth (10 L) was filtered.

### Extraction and Separation

The filtrate of the cultured broth (10 L) was extracted with EtOAc (1 L x 2). The EtOAc-soluble portions (58 mg) were subjected to a silica gel column (hexane/EtOAc, 70:30) followed by C<sub>18</sub> reversed-phase HPLC [Develosil ODS-5, Nomura Chemical, 1.0 x 25 cm; flow rate 2.5 mL/min; UV detection at 254 nm; eluent: MeOH/H<sub>2</sub>O, 70:30] to give sporiolides A (**1**, 2.7 mg) and B (**2**, 11.5 mg) together with cladospolide D (7.0 mg). On the other hand, cladospolide A, iso-cladospolide B, and seco-patulolide C were isolated from the EtOAc extract of the mycelium.

### Spectral Data

*Sporiolide A (1)*: colorless amorphous solid;  $[\alpha]_{\text{D}}^{25} -14^{\circ}$  (*c* 0.2, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  237 ( $\epsilon$  9200) and 209 (11700) nm; IR (KBr)  $\nu_{\text{max}}$  3426, 1724, and 1633 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR (Table 1); FABMS *m/z* 371 [M+Na]<sup>+</sup>; HRFABMS *m/z* 371.1483 [M+Na]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>24</sub>O<sub>6</sub>Na, 371.1471).

*Sporiolide B (2)*: colorless amorphous solid;  $[\alpha]_{\text{D}}^{25} -33^{\circ}$  (*c* 0.3, MeOH); IR (KBr)  $\nu_{\text{max}}$  3429, 1710, and 1646 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR (Table 1); FABMS *m/z* 281 [M+Na]<sup>+</sup>; HRFABMS *m/z* 281.1367 [M+Na]<sup>+</sup> (calcd for C<sub>13</sub>H<sub>22</sub>O<sub>5</sub>Na, 281.1365).

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*Sample Availability:* Samples are available from the authors.

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