Cladionol A, a Polyketide Glycoside from Marine-Derived Fungus *Gliocladium* Species

Yuu Kasai,[†] Kazusei Komatsu,[†] Hideyuki Shigemori,[†] Masashi Tsuda,[†] Yuzuru Mikami,[‡] and Jun'ichi Kobayashi*[,†]

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, and Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-0856, Japan

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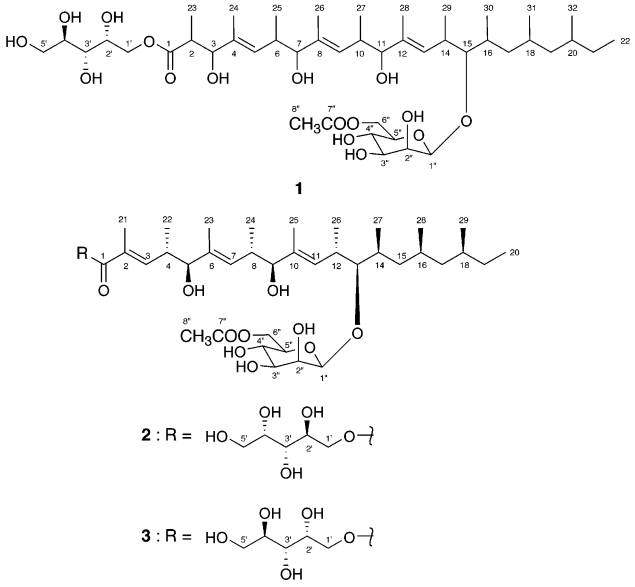
A new polyketide glycoside, cladionol A (**1**), was isolated from the cultured broth of a fungus *Gliocladium* sp., which was separated from sea grass *Syringodium isoetifolium,* and the structure was elucidated by spectroscopic data. The relative stereochemistry of C-2−C-3 was assigned by mainly *J*-based configuration analysis, while those of two sugar units were elucidated to be *β*-mannopyranoside and arabitol on the basis of NOESY data and/or ^{1}H−^{1}H couplings. Furthermore, the absolute configuration of the mannose moiety was determined as the D-form on the basis of chiral HPLC analysis of a benzoyl derivative of the acid hydrolysate of **1**. Cladionol A (**1**) exhibited modest cytotoxicity.

Marine-derived fungi have proven to be a rich source of structurally unique and biologically active secondary metabolites.[1] In our search for new metabolites from marine-derived fungi,[2] a new cytotoxic polyketide glycoside, cladionol A (**1**), was isolated from the cultured broth of a fungus *Gliocladium* sp., which was separated from a sea grass. In this paper, we describe the isolation and structure elucidation of **1**.

The fungus *Gliocladium* sp. (strain L049) was separated from a sea grass *Syringodium isoetifolium* collected at Maeda Cape, Okinawa Island, and grown in starch-casein liquid medium containing 50% seawater for 14 days at 28 °C. The supernatant of the culture broth (12 L) was extracted with EtOAc, and the EtOAc-soluble portions were subjected to Si gel column chromatography ($CHCl_3$/MeOH, 80:20) and then C_{18} HPLC (MeOH/H_2O, 82:18) to afford cladionol A (**1**, 2.1 mg) together with known related compounds, roselipins 2A (**2**) and 2B (**3**).[3,4]

Cladionol A (**1**) was obtained as an optically active colorless amorphous solid {$[\alpha]^{22}_D$ +36° (*c*, 0.2, MeOH)}. The molecular formula of **1** was revealed to be $C_{45}H_{80}O_{16}$ by HRFABMS [*m/z* 899.5305, $(M + Na)^+$, Δ −3.9 mmu]. The IR spectrum suggested the presence of OH (3347 cm^{-1}) and carbonyl group(s) (1734 cm^{-1}). ^{1}H and ^{13}C NMR (Table 1) data of **1** disclosed totally 45 carbon signals due to two ester carbonyls, three double bonds, 19 sp^3 methines including a hemiacetal and 10 oxymethines, six sp^3 methylenes including three oxymethylenes, and 12 methyls (an acetyl, three olefinic, seven doublet, and a triplet methyl).

The gross structure of cladionol A (**1**) was elucidated by spectroscopic data including 2D NMR data such as ^{1}H−^{1}H COSY, TOCSY, HMQC, and HMBC spectra. Six proton networks from H-2 to H-3 and H_3-23, from H-5 to H-7 and H_3-25, from H-9 to H-11 and H_3-27, and from H-13 to H_3-22 and four doublet methyls (H_3-29, H_3-30, H_3-31, and H_3-32), from H_2-1′ to H_2-5′ and from H-1″ to H_2-6″, were suggested by analysis of the ^{1}H−^{1}H COSY and TOCSY spectra (Figure 1). The presence of three trisubstituted double bonds at C-4−C-5, C-8−C-9, and C-12−C-13 was deduced from HMBC correlations for H_3-24 to C-3, C-4, and C-5, H_3-26 to C-7, C-8, and C-9, and H_3-28 to C-11, C-12, and C-13. Chemical shifts of the allylic methyl carbons of C-24 (*δ* 12.3), C-26 (*δ* 12.2), and C-28 (*δ* 12.1) suggested that the double bonds were all *E*-geometry.[5] HMBC correlations were observed for H-3, H_3-23, and H-1′ to an ester carbonyl carbon (*δ* 178.0), suggesting that the tetraol moiety of C-1′−C-5′ was attached to C-1. HMBC correlations for a proton of the hemiacetal (δ_C 103.4) to C-15 and C-5″ implied that a hexopyranose moiety was attached to C-15. Attachment on the acetyl group at C-6″ was deduced from the HMBC correlation for H-6″/C-7″.



The relative stereochemistry of the hexopyranose moiety was elucidated on the basis of NOESY data and ^{1}H−^{1}H couplings (Figure 2). NOESY correlations for H-1″/H-3″, H-1″/H-5″, and H-3″/H-5″ suggested that the hexopyranose took a boat form with axial orientations for H-1″, H-3″, and H-5″. *anti* orientations for H-3″/H-4″ and H-3″/H-5″ were deduced from the *J*(H-3″/H-4″) and *J*(H-4″/H-5″) values (9.1 and 9.6 Hz, respectively). The relatively small value (3.1 Hz) for *J*(H-2″/H-3″) indicated an equatorial orientation for H-2″. Thus, the hexopyranose unit was assigned as a *β*-mannopyranoside. Although stereochemistries for nine chiral centers of the polyketide chain were

* To whom correspondence should be addressed. Phone: +81 11 706 4985. Fax: +81 11 706 4989. E-mail: jkobay@pharm.hokudai.ac.jp.
† Hokkaido University.
‡ Chiba University.

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Table 1. ^{1}H and ^{13}C NMR Data of Cladionol A (**1**) in CD_3OD

positn.	δ_C		δ_H (m, Hz)		positn.	δ_C		δ_H (m, Hz)	
1	178.00	C			24	12.31	CH_3	1.66[a]	brs
2	35.02	CH	2.62	m	25	18.56	CH_3	0.78[a]	d, 6.8
3	87.99	CH	4.07	d, 10.4	26	12.23	CH_3	1.63[a]	d, 1.2
4	135.08	C			27	19.37	CH_3	0.94[a]	d, 6.4
5	135.57	CH	5.34	d, 9.2	28	12.06	CH_3	1.62[a]	brs
6	37.83	CH	2.60	m	29	22.13	CH_3	0.97[a]	d, 7.2
7	84.95	CH	3.80	m	30	16.34	CH_3	0.95[a]	d, 7.2
8	136.77	C			31	22.13	CH_3	0.90[a]	d, 6.4
9	134.87	CH	5.28	dd, 8.5, 1,2	32	19.37	CH_3	0.88[a]	d, 6.4
10	37.43	CH	2.72	m	1′	69.30	CH_2	4.25	dd, 11.2, 6.8
								4.19	d, 6.0
11	85.24	CH	3.64	m	2′	73.49	CH	4.11	m
12	136.02	C			3′	72.90	CH	3.51	dd, 2.0, 8.5
13	135.69	CH	5.52	dd, 9.4, 1.2	4′	72.06	CH	3.71	m
14	37.03	CH	2.72	m	5′	65.60	CH_2	3.80	dd, 10.5, 3.2
								3.62	dd, 10.5, 6.0
15	88.08	CH	3.43	dd, 6.7, 2.9	1″	103.44	CH	4.50	brs
16	33.71	CH	1.86	m	2″	73.87	CH	3.90	d, 2.8
17	44.74	CH_2	1.38	m	3″	76.44	CH	3.36	dd, 9.2, 3.2
			0.98	m					
18	29.61	CH	1.53	m	4″	70.00	CH	3.50	dd, 9.6, 9.1
19	46.85	CH_2	1.23	m	5″	79.04	CH	3.35	m
			0.90	m					
20	30.67	CH	1.31	m	6″	63.69	CH_2	4.41	dd, 11.7, 2.4
								4.25	dd, 11.2, 6.8
21	31.57	CH_2	1.42	m	7″	172.50	C		
			1.07	m					
22	12.31	CH_3	0.93[a]	t, 7.2	8″	21.53	CH_3	2.08[a]	s
23	18.62	CH_3	0.96[a]	d, 6.8					

[a] 3H.

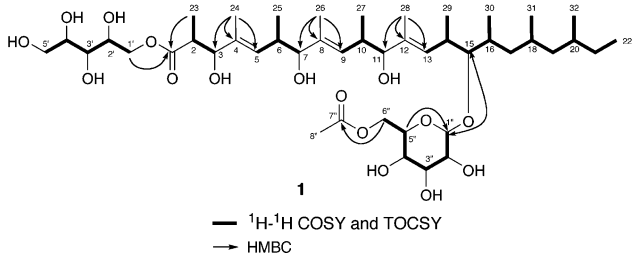

Figure 1. Selected 2D NMR correlations for cladionol A (**1**).

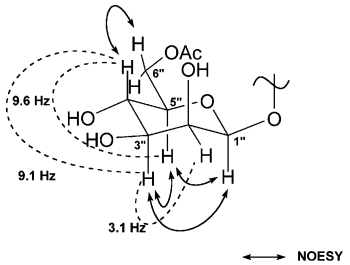

Figure 2. NOESY correlations and relative streochemistry for the hexapyranose moiety in cladionol A (**1**).

not determined unambiguously, the C-2−C-3 portion was assigned as erythro from the *J*(H-2/H-3) value (10.4 Hz) and NOESY correlations for H-2/H_3-23 and H_3-23/H_3-24 (Figure 3). Since ^{1}H and ^{13}C NMR data (Table 1) and ^{1}H−^{1}H couplings (H-1′/H-1′: 11.2 Hz; H-1′/H-2′: 6.8 and 6.0 Hz, H-2′/H-3′: 2.0 Hz, H-3′/H-4′: 8.5 Hz, H-4′/H-5′: 3.2 and 6.0 Hz, H-5′/H-5′: 10.5 Hz) for the pentitol moiety of **1** were similar to those for the corresponding part of roselipin 2B (**3**), the pentitol moiety of **1** was suggested to be arabitol like **3**. The absolute configuration of the mannose moiety was determined as the D-form on the basis of chiral HPLC analysis of a benzoyl derivative of the acid hydrolysate of **1**.

Cladionol A (**1**) is a new polyketide glycoside similar to known polyketide antibiotics such as roselipins[3,4] and

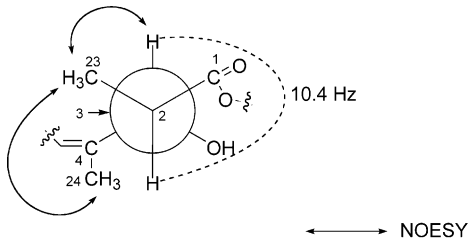

Figure 3. Rotational model for the C-2−C-3 bond in cladionol A (**1**).

TMC-151s[6] from fungi *Gliocladium* spp. Cladionol A (**1**) exhibited cytotoxicity against murine leukemia L1210 and human epidermoid carcinoma KB cells with IC_{50} values of 5 and 7 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. The IR and UV spectra were taken on a JASCO FT/IR-5300 and JASCO Ubest-35 spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a 600 MHz spectrometer using 2.5 mm micro cells for $CDCl_3$ (Shigemi Co., Ltd.). FAB mass spectra were obtained on a JEOL HX-110 spectrometer using nitrobenzyl alcohol as a matrix.

Fungal Material and Fermentation. The fungus *Gliocladium* sp. (L049) was isolated from a sea grass *Syringodium isoetifolium* collected at Maeda Cape, Okinawa Island. Subcultures of the fungus are deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University. The fungus was grown in Starch-Casein liquid medium (starch, 1%; casein, 0.1%; pH 7.5) containing 50% seawater for 14 days at 28 °C. The cultured broth (12 L) was filtered.

Extraction and Separation. The supernatant (12 L) of the cultured broth was extracted with EtOAc (1 L × 2). The EtOAc-soluble portions (115 mg, wet weight) were subjected to a Si gel column ($CHCl_3$/MeOH, 80:20) and C_{18} HPLC (Develosil ODS-5, Nomura Co., Ltd., 10 × 250 mm; eluent, MeOH/H_2O, 82:18; flow rate, 2 mL/min; UV detection at 220 nm) to afford cladionol A (**1**, 2.1 mg, t_R 25 min). Roselipins

2A (**2**, 21.8 mg) and 2B (**3**, 20.5 mg) were obtained from other fractions of the EtOAc-soluble portion.

Cladionol A (1): colorless amorphous solid; $[\alpha]^{22}_{D}$ +36° (*c* 0.2, MeOH); IR (film) ν_{max} 3347, 2924, 1734 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; FABMS (pos.) *m/z* 899 $(M + Na)^+$; HRFABMS (pos.) *m/z* 899.5305 $[(M + Na)^+$, calcd for $C_{45}H_{80}O_{16}$-Na 899.5344].

Determination of Stereochemistry of the Mannose Unit in Cladionol A (1) by Chiral HPLC. Cladionol A (**1**, 0.3 mg) was treated with 3% HCl/MeOH (300 μL) at 110 °C for 1 h. After the solvent was removed by nitrogen stream, to the residue was added $CHCl_3$ (100 μL) and then the $CHCl_3$ solution was extracted with H_2O (100 μL × 3). The aqueous fraction evaporated in vacuo was treated with pyridine (100 μL), triethylamine (15 μL), and benzoyl chloride (15 μL), at room temperature for 21 h. After addition of MeOH (100 μL), the reaction mixture was extracted with hexane (100 μL × 3). The hexane-soluble fraction was evaporated in vacuo to afford *O*-benzoyl/methyl derivatives of the sugar unit in **1**. Authentic D- and L-mannose were treated with benzoyl chloride as described above to yield *O*-benzoyl/methyl derivatives of D- and L-mannose, respectively. The *O*-benzoyl/methyl derivatives were subjected to chiral HPLC analyses using Chiralpak OP(+) (Daicel Chemical Industry, Ltd., 4.6 × 250 mm; MeOH; flow rate, 0.5 mL/min; UV detection at 254 nm). The retention time of *O*-benzoyl/methyl derivatives of the methanolysis product of **1** was found to be 24.5 min, while the retention times of *O*-benzoyl/methyl derivatives of authentic D- and L-mannose were found to be 24.5 and 26.0 min, respectively.

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