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Seragakinone A, a New Pentacyclic Metabolite from a Marine-Derived Fungus

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Abstract: A new anthracycline-derived pentacyclic metabolite, seragakinone A (**1**), was isolated from the mycelium of an unidentified fungus, which was separated from the Okinawan marine rhodophyta *Ceratodictyon spongiosum*, and the structure was elucidated by spectroscopic data.

Keywords: anthracyclines; antibiotics; fungi; natural products

Marine microorganisms have proven to be a good source of structurally novel and biologically active secondary metabolites, which might be useful leads in the development of new pharmaceutical agents.[1] In our search for bioactive compounds from marine microorganisms,[2] a new anthracycline-derived pentacyclic metabolite, seragakinone A (**1**), was isolated from the mycelium of an unidentified fungus, which was separated from the Okinawan marine rhodophyta *Ceratodictyon spongiosum*. In this paper we describe the isolation and structure elucidation of **1**.

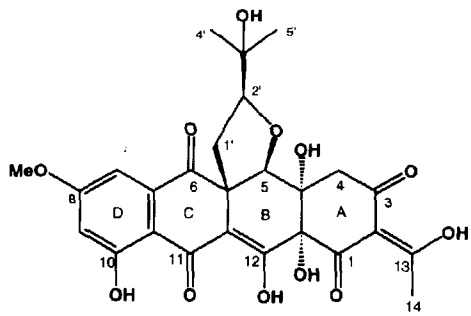

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Table 1. ^1H and ^{13}C NMR Data of Seragakinone A (**1**) in $CDCl_3$

position	^1H*		J(Hz)	^{13}C*		H coupled with C**
1				189.8	s	H-5
2				110.4	s	H_2-4,H-14
3				192.8	s	H_2-4
4	2.57	d	19.5	40.5	t	H-5, OH-4a
	2.84	d	19.5			
4a				71.4	s	H_2-4,H-5,OH-12a
4a-OH	3.56	s				
5	4.90	s		78.4	d	H_2-4
5a				56.7	s	H-5,Hα-1'
6				193.8	s	H-5,H-7,Hα-1'
6a				134.2	s	H-7
7	7.21	d	2.4	107.9	d	H-9
8				166.5	s	H-7,H-9,MeO-8
8-MeO	3.93	s		56.1	q	
9	6.71	d	2.4	106.8	d	H-7,OH-10
10				164.7	s	H-9,OH-10
10-OH	11.98	s				
10a				110.2	s	H-7,H-9,OH-10
11				189.8	s	H-5,H-7
11a				107.3	s	H-5,Hα-1',OH-12
12				170.6	s	OH-12
12-OH	14.27	s				
12a		s		77.2	s	H-4,H-5,OH-12,OH-12a
12a-OH	5.46	s				
13				202.3	s	H-14
13-OH	17.95	s				
14	2.62	s		27.7	q	
1'	(α) 2.29	dd	5.4, 13.2	44.1	t	H-5
	(β) 2.54	dd	10.7, 13.2			
2'	3.83	dd	5.4, 10.7	83.4	d	Hα-1',H-4',H-5'
3'	5.46	s		69.3	s	Hα-1',H-4',H-5',OH-3'
3'-OH	3.41	s				
4'	1.31	s		28.0	q	H-2',H-5'
5'	0.99	s		25.1	q	H-4'

*) in ppm **) in HMBC spectrum

The fungus (K063) was separated from the red alga *Ceratodictyon spongiosum* collected off Seragaki Beach at Okinawa, and grown in PYG broth [peptone (1%), yeast extract (0.5%), and glucose (2%) in sea water, pH 7.5] at 28°C for 14 days. The mycelium (82g from 10 L of culture) was extracted with $CHCl_3$/MeOH (1:1) and the extracts were partitioned with EtOAc and H_2O. The EtOAc-soluble portions were separated by a C_{18} column (MeOH/H_2O, 85:15) to give seragakinone A (**1**, 0.2 % wet weight).

The molecular formula, $C_{26}H_{26}O_{12}$, of seragakinone A (**1**) was established by HRFABMS [*m/z* 531.1495 $(M+H)^+$, Δ -0.8 mmu]. The IR spectrum suggested the presence of hydroxy (3422 cm^{-1}) and unsaturated ketone carbonyl (1738 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra were measured in $CDCl_3$.[3] The gross structure of **1** was deduced from detailed analyses of ^1H and ^{13}C NMR data (Table 1) aided with 2D NMR experiments (^1H-^1H COSY, HMQC, and HMBC). The ^{13}C NMR data indicated that the molecule possessed four ketone carbonyls and ten olefinic carbons. Since nine of fourteen unsaturations were accounted for, it was implied that **1** contained five rings. The ^1H and ^{13}C signals due

to the following functional groups were observed for **1**; a 1,2,3,5-tetrasubstituted benzenoid ring with a hydrogen-bonded hydroxy (δ_H 11.98, OH-10; δ_C 164.7, C-10), a methoxy (δ_H 3.93 and δ_C 56.1, MeO-8), two *meta*-coupled aromatic methines (δ_H 7.21, H-7; δ_C 107.9, C-7 and δ_H 6.71, H-9; δ_C 106.8, C-9), two quaternary carbinols (δ_H 3.56, OH-4a; δ_C 71.4, C-4a and δ_H 5.46, OH-12a; δ_C 77.2, C-12a), an enol carbon (δ_C 170.6, C-12), and three carbons having ketone nature (δ_C 189.8, C-1; δ_C 192.8, C-3; δ_C 202.3, C-13). The ^{1}H signals resonating in fairly low-field (δ_H 11.98, 14.27, and 17.95) were assignable to the hydroxy groups of one phenol (OH-10) and two enols (OH-12 and OH-13). HMBC correlations of H-7 to C-6a, C-8, C-9, and C-10a, and H-9 to C-7, C-8, C-10, and C-10a indicated the presence of a benzene ring, while the correlations of the phenolic hydroxy proton (δ_H 11.98) to C-9, C-10, and C-10a, the methoxy protons (δ_H 3.93) to C-8, and H-7 to C-6 (δ_C 193.8) and C-11 (δ_C 189.8) revealed the presence of a hydroxy and a methoxy groups at C-10 and C-8, respectively, and two ketones (C-6 and C-11) were connected to C-6a and C-10a, respectively, in ring D. HMBC correlations of H-5 to C-4a, C-5a, C-6, C-11, C-11a, and C-12a and OH-12 (δ_H 14.27) to C-11a, C-12, and C-12a indicated the presence of an enol (C-11a to C-12) in ring B and the connection between rings B and D through the two ketones (C-6 and C-11). The presence of an enol olefin (C-2, C-13, and OH-13) with a methyl group at C-13 was deduced from HMBC correlations of H_3-14 to C-2 and C-13 and ^{1}H and ^{13}C NMR chemical shifts of the enol moiety.[4–6] The β-diketoenol groups at C-1, C-2, C-3, and C-13 and the *E* double bond (C-2 - C-13) were suggested from comparison of the ^{13}C chemical shifts (δ_C 189.8, C-1; δ_C 110.4, C-2; δ_C 192.8, C-3; δ_C 202.3, C-13) with those of polyketomycin.[5] HMBC correlations of H_2-4 to C-2, C-3, C-4a (δ_C 71.4), and C-12a (δ_C 77.2), OH-4a (δ_H 3.56) to C-4, and OH-12a (δ_H 5.46) to C-4a, C-12, and C-12a indicated the presence of ring A with two hydroxy groups at C-4a and C-12a. HMBC correlations of Hα-1' to C-5a, C-6, C-11a, and C-2' and the NOESY correlation of H-2' to H-5 indicated that a tetrahydrofuran ring was formed by C-5, C-5a, C-1', C-2', and O-5. Two methyl proton (δ_H 1.31, H_3-4' and δ_H 0.99, H_3-5') and an oxygenated quaternary carbon (δ_C 69.3, C-3') signals indicated the presence of a hydroxyisopropyl group, which was attached to C-2' from HMBC correlations of H_3-4' and H_3-5' to C-3' and C-2', Hα-1' to C-3', and OH-3' (δ_H 3.41) to C-3'. Thus the remaining ketone group (C-1) was connected to C-12a to complete the gross structure of seragakinone A (**1**).

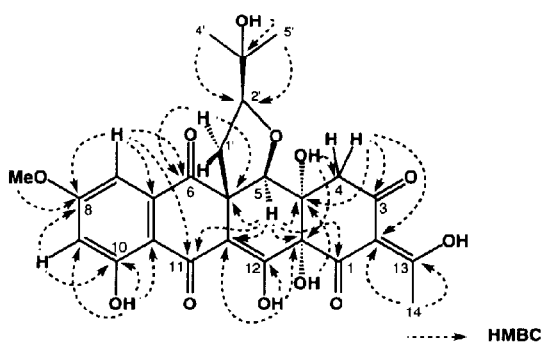

Figure 1. HMBC Correlations of Seragakinone A (**1**)

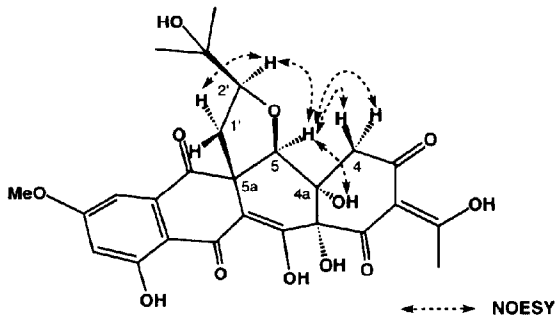

Figure 2. Relative Stereochemistry of Seragakinone A (**1**)

Relative stereochemistry of **1** was elucidated by NOESY data as follows. NOESY correlations of H-5 to H_2-4, H-2', and OH-4a and Hα-1' to H-2' indicated that C-1' was β-oriented and H-2', H-5, and OH-4a were α-oriented. The diol at C-4a and C-12a in **1** was elucidated to be *cis* from comparison of the ^{13}C chemical shifts (δ_C 71.4 and 77.2 for C-4a and C-12a, respectively) of **1** with those (δ_C 70.9 and 79.8 for C-4a and C-12a, respectively) of a related compound[7] having a *cis* diol at the corresponding ring junction.

Seragakinone A (**1**) is a new anthracycline-related pentacyclic metabolite from the mycelium of an unidentified marine-derived fungus, which should belong to ascomycetous fungi from an analysis of nuclear large subunit (26S) ribosomal DNA partial sequence.[8] The characteristic feature of **1** is the presence of a tetrahydrofuran ring with a hydroxyisopropyl group fused to ring B of the tetracyclic skeleton. Seragakinone A (**1**) exhibited weak antifungal activity against *Candida albicans* and modest antibacterial activity against *Staphylococcus aureus*, *Micrococcus luetus*, *Corynebacterium xerosis*, and *Bacillus subtilis* (Table 2). On the other hand, compound **1** did not show cytotoxicity (>10 μg/mL) against L1210 murine leukemia cells and KB human epidermoid carcinoma cells. Biosynthetically, seragakinone A (**1**) may be derived from decaketide (C-1 ~ C-14) and an isoprene-like C_5 unit (C-1' ~ C-5').

Table 2. Antimicrobial Activities of Seragakinone A (**1**)

	MIC (μg/mL)
Candida albicans ATCC90028	83
Cryptococcus neoformans ATCC90112	166
Paecilomyces variotii YM-1	166
Aspergillus niger ATCC40406	>166
Trichophyton mentagrophytes IFM40769	>166
Staphylococcus aureus 209P	10
Micrococcus luetus IFM2066	20
Corynebacterium xerosis IFM2057	20
Bacillus subtilis PCI189	41
Escherichia coli NIHJ-JC2	>166

Experimental Section

General Methods. The 7.26 ppm resonance of residual $CHCl_3$ and 77.0 ppm of $CDCl_3$ were used as internal references for ^1H and ^{13}C NMR spectra, respectively. FAB mass spectra were obtained using glycerol as a matrix.

Fungal Material. K063 strain did not show any taxonomically useful cellular morphology for the fungal identification on the various media tested. Recently new molecular methods for fungal identification using an analysis of nuclear large subunit (26S) ribosomal DNA partial sequence have been introduced.[8] Our analysis on the DNA sequences of variable D1/D2 domain of large subunit (26S) of K063 strain suggested the strain has similarity to those of *Ramichloridium cerophilum* (87.36%, AF050286), *Oosporidium margaritiferum* (84.25%, U40090), and *Baeomyces rufus* (83.9%, AF113744). These results indicate that K063 strain should belong to ascomycetous fungi although detail of further taxonomic position is necessary. Subcultures of the organism are deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

Collection and Cultivation. The fungus (K063) was separated from the marine rhodophyta *Ceratodictyon spongiosum*, which was collected off Seragaki Beach at Okinawa Island. The fungus was grown in the PYG broth [peptone (1%), yeast extract (0.5%), and glucose (2%) in sea water, pH 7.5] at 28°C for 14 days. The cultured broth (10 L) was filtered.

Extraction and Separation. The mycelium (82 g of wet weight) of the culture was extracted with $CHCl_3$/MeOH (1:1, 500 mL x 2) and evaporated under reduced pressure. The extracts were partitioned between EtOAc (100 mL x 3) and H_2O (100 mL), and the EtOAc-soluble portions were subjected to an ODS column (2.5 x 20 cm, Cosmosil 140C_{18}-PREP, Nacalai Tesque, Inc., MeOH/H_2O, 85:15) to afford seragakinone A (1, 171 mg).

Seragakinone A (1): pale yellow amorphous solid; $[\alpha]^{26}_D$ +146° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 257 (ε 25100), 279 (20800), and 383 (12800) nm; IR (film) ν_{max} 3422, 1738, and 1612 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 553 $(M+Na)^+$ and 531 $(M+H)^+$; HRFABMS *m/z* 531.1495 $(M+H)^+$ (calcd for $C_{26}H_{27}O_{12}$, 531.1503).

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References and Notes

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3. The presence of minor tautomer(s) of **1** was observed in MeOH-d_4, although the structure(s) has not been defined.
4. Comparison of NMR data of the methyl group (δ_H 2.62; δ_C 27.7) in **1** with those (δ_H 2.72; δ_C 26.68, enol methyl[5]; δ_H 2.31; δ_C 31.2, acetyl methyl[6]) of the related compounds indicated the presence of an enol methyl group (C-14) in **1**.
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