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Revised Stereochemistry and Biosynthesis of Seragakinone A

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Abstract—The relative stereochemistry of seragakinone A (**1**), an anthracycline-derived pentacyclic fungal metabolite, was revised by X-ray analysis. The tetracyclic skeleton (C-1–C-14) and the C_5 moiety (C-1′–C-5′) of **1** were shown to be biosynthesized via decaketide and mevalonate pathway, respectively, on the basis of incorporation experiments.

Introduction

In our continuing search for bioactive metabolites from marine fungi,[1] a new anthracycline-derived pentacyclic metabolite, seragakinone A (**1**), was isolated from an unidentified fungus (K063) separated from the Okinawan marine rhodophyta *Ceratodictyon spongiosum*,[2] and the relative stereochemistry of **1** has been elucidated on the basis of NMR data. In this study the relative stereochemistry of seragakinone A (**1**) was revised by the X-ray analysis. On the other hand, the biosynthetic pathway of the tetracyclic skeleton (C-1–C-14) and the C_5 moiety (C-1′–C-5′) in **1** was examined on the basis of incorporation experiments. In this paper we describe the revised stereochemistry and the biosynthesis of seragakinone A (**1**) (Chart 1).

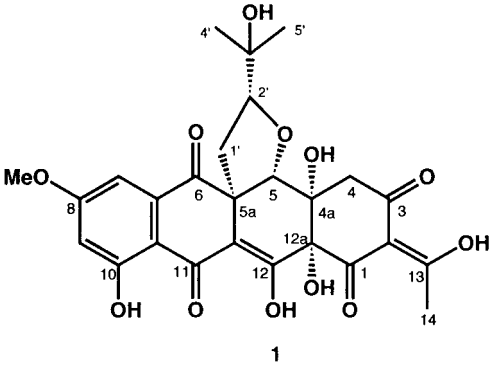

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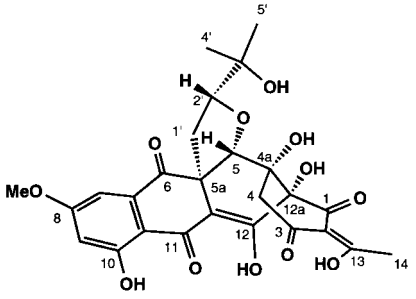
Chart 1. Structure of seragakinone A (**1**).

Results and Discussion

Seragakinone A (**1**) was crystallized from hexane/EtOH to give platelet of space group C2(#5). The determination of the lattice constants and collection of intensity data were carried out on a Rigaku RAXIS-RAPID Imaging Plate. 5669 reflections were collected for **1** and the structure was solved by direct method. Non-hydrogen atoms were refined anisotropically, while hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares method was based on 2062 observed reflections ($I>10\sigma$ (I)) with unweighed and weighed agreement factors of R=0.099 and Rw=0.152 for **1**. The ORTEP drawing of **1** was shown in Fig. 1, in which C-1′, O-5, the hydroxyisopropyl group at C-2′, and the hydroxy groups at C-4a and C-12a were all α-oriented, and intramolecular hydrogen bonds were formed between OH-12a and CO-1 (1.924 Å), between OH-10 and CO-11 (1.573 Å), between OH-12 and CO-11 (1.640 Å),



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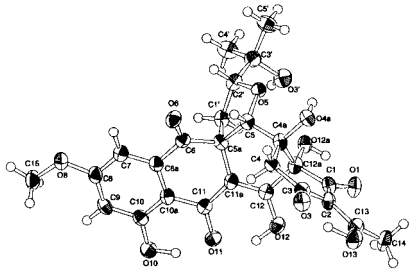

Figure 1. Perspective ORTEP drawing of the X-ray structure of **1**.

Keywords: anthracyclines; fungi; biosynthesis; mevalonate pathway.
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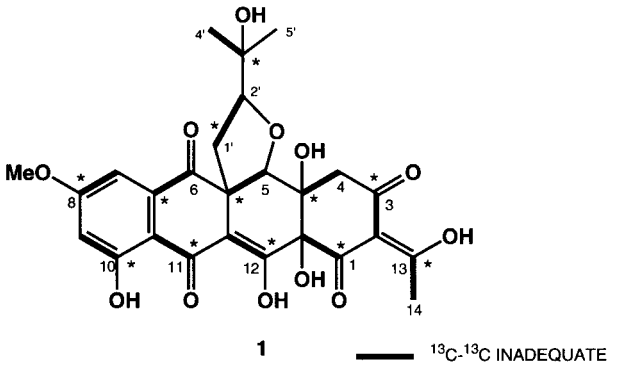

Figure 2. ^{13}C-Labelling pattern in seragakinone A (**1**) derived from [1-^{13}C]NaOAc and [1,2-^{13}C]NaOAc. Solid bars signify intact acetate units and asterisks carbon atoms deriving from C-1 carbons.

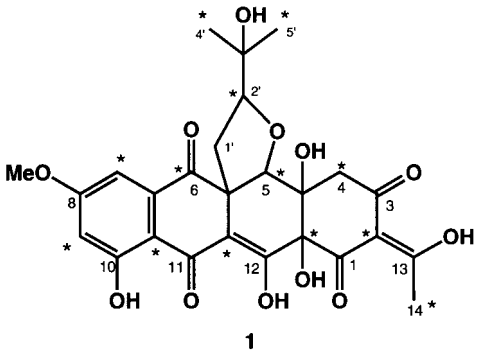

Figure 3. ^{13}C-Labelling pattern in seragakinone A (**1**) derived from [1-^{13}C]glucose. Asterisks carbon atoms deriving from C-1 carbons.

and between OH-13 and CO-3 (1.355 Å). Thus, the orientations of C-1′, O-5, and the hydroxyisopropyl group at C-2′ of seragakinone A (**1**) were revised to be all α, although these had been previously assigned as all β from NOESY data of **1**.[2]

In feeding experiments the fungus (K063) was grown in the presence of labelled precursors, [1-^{13}C]NaOAc, [1,2-^{13}C]-NaOAc, or D-[1-^{13}C]glucose. [1-^{13}C]NaOAc or [1,2-^{13}C]-NaOAc was fed to the fungus culture at 6 days, then the cultures were incubated for 8 days, while D-[1-^{13}C]glucose was added to the culture at first and then the culture was incubated for 14 days. Fractions containing seragakinone A (**1**) were separated from the filtrate of the cultures, purified by C_{18} column, and subjected to ^{13}C NMR analysis.

The ^{13}C NMR spectrum of seragakinone A (**1**) derived from [1-^{13}C]NaOAc showed clear increment of the signals of C-1, C-3, C-4a, C-5a, C-6a, C-8, C-10, C-11, C-12, C-13, C-1′, and C-3′ (Fig. 2 and Table 1), indicating that the tetracyclic skeleton (C-1–C-14) was derived from decaketide and the C_5 unit (C-1′–C-5′) was derived from isopentenyl diphosphate (IPP) via mevalonate pathway. These results were also supported by the INADEQUATE spectrum of seragakinone A (**1**) derived from [1,2-^{13}C]NaOAc (Fig. 2).

On the other hand, the ^{13}C NMR spectrum of **1** derived from D-[1-^{13}C]glucose showed clear increment of the signals of C-2, C-4, C-5, C-6, C-7, C-9, C-10a, C-11a, C-12a, C-14, C-2′, C-4′, and C-5′ (Fig. 3 and Table 1), indicating that the tetracyclic skeleton (C-1–C-14) and the C_5 unit (C-1′–C-5′) were also derived from decaketide and IPP via mevalonate, respectively.[3,4] Incorporation patterns of the ^{13}C-labelled carbons into the C_5 unit are explained by generation of [2,4,5-^{13}C]IPP from D-[1-^{13}C]glucose via mevalonate pathway (Fig. 4).

Seragakinone A (**1**) is a new anthracycline-related pentacyclic metabolite from the mycelium and the culture broth of an unidentified marine-derived fungus, probably belonging to ascomycetes. The tetracyclic skeleton (C-1–C-14) and the C_5 moiety (C-1′–C-5′) of **1** were shown to be biosynthesized via decaketide and mevalonate pathway, respectively, on the basis of incorporation experiments.

Table 1. ^{13}C-chemical shifts and normalized peak height of seragakinone A (**1**) derived from [1-^{13}C]NaOAc and D-[1-^{13}C]glucose

Carbon	Chemical shift	Peak height		Carbon	Chemical shift	Peak height	
		[1-^{13}C]NaOAc	[1-^{13}C]glucose			[1-^{13}C]NaOAc	[1-^{13}C]glucose
1	189.8	7.2	1.2	10a	110.2	0.9	3.5
2	110.4	0.6	3.8	11	189.8	4.3	1.2
3	192.8	5.9	1.5	11a	107.3	0.7	3.6
4	40.5	1.2	6.8	12	170.6	8.4	1.7
4a	71.4	5.9	1.2	12a	77.2	1.5	3.1
5	78.4	1.4	4.9	13	202.3	6.1	1.4
5a	56.7	4.0	0.9	14	27.7	0.8	3.9
6	193.8	0.7	4.2	15	56.1	1.0[a]	1.7
6a	134.2	4.9	1.0[b]	1′	44.1	9.3	2.2
7	107.9	1.3	6.3	2′	83.4	1.4	5.7
8	166.5	5.3	1.0	3′	69.3	9.5	1.5
9	106.8	1.3	5.6	4′	25.1	1.0	3.4
10	164.7	6.9	1.2	5′	28.0	1.1	3.9

[a] The signal intensities were corrected by those of unlabeled **1** and normalized to C-15.
[b] The signal intensities were corrected by those of unlabelled **1** and normalized to C-6a.

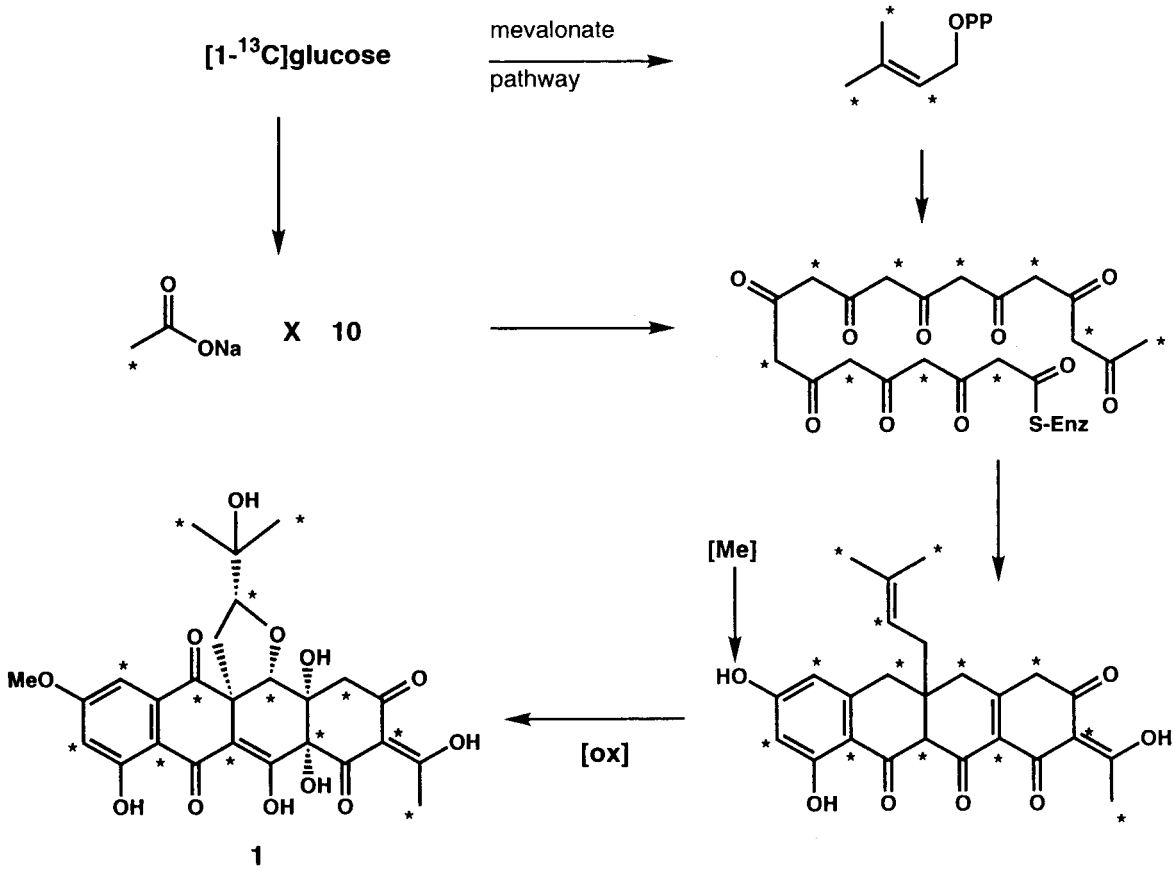

Figure 4. Proposed biosynthetic pathway of seragakinone A (**1**) derived from [1-^{13}C]glucose.

Experimental

General methods

The 7.26 ppm resonance of residual $CHCl_3$ and 77.0 ppm of $CDCl_3$ were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively.

Fungal material

Our analysis on the DNA sequences of variable D1/D2 domain of large subunit (26S) in ribosome of K063 strain suggested that the strain should belong to ascomycetous fungi.[2] Subcultures of the organism have been deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

Crystallographic analysis of 1

Empirical formula: $C_{26}H_{26}O_{12}$ (530.48); crystal color, habit: yellow, platelet; crystal dimensions: 0.40×0.08×0.03 mm; crystal system: monoclinic; lattice type: C-centered; lattice parameters: a=20.312(7) Å, b=7.727(2) Å, c=15.503(4) Å, b=95.96(1)°, V=2420(1) Å^3; space group: C2(#5); Z value: 4; D_{calc}: 1.456 g/cm^3. **1** was mounted on a Rigaku RAXIS-RAPID Imaging Plate diffractometer with graphite monochromated Mo-Ka radiation (λ=0.71069 Å) at −150.0°C. Structure of **1** was determined by the direct method using the SIR97 program and the refinement was carried out by the full-matrix least-squared method $\Sigma\omega(Fo^2-Fc^2)^2$, $\omega=1/\sigma^2(Fo^2)$. The molecular structure determined by these methods are illustrated in Fig. 1.

Collection and cultivation

The fungus (K063) was grown statically in the PYG broth [peptone (10 g), yeast extract (5 g), and glucose (20 g) in sea water, pH 7.5] (1L) at 28°C for 6 days. [1-^{13}C]NaOAc or [1,2-^{13}C]NaOAc (each 0.15 g) was added to the cultures, which was incubated statically at 28°C for 8 days. The cultured broth (1 L) was filtered.

The fungus (K063) was also grown statically in the broth [peptone (2 g), yeast extract (1 g), and glucose (1.6 g) and D-[1-^{13}C]glucose (0.4 g) in sea water, pH 7.5] (200 mL) at 28°C for 14 days. The cultured broth (100 mL) was filtered.

Extraction and separation

The filtrate of the culture was extracted with EtOAc (1 L×2 or 100 mL×2) and the EtOAc layer was evaporated under reduced pressure. The residues were subjected to an ODS column (Cosmosil 140C_{18}-PREP, Nacalai Tesque, Inc., MeOH/H_2O, 85:15) to afford seragakinone A (**1**) (84 mg from [1-^{13}C]NaOAc; 61 mg from [1,2-^{13}C]NaOAc; and 7.8 mg from D-[1-^{13}C]glucose).

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