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Symbiospirols: Novel Long Carbon-Chain Compounds Isolated from Symbiotic Marine Dinoflagellate *Symbiodinium* sp.

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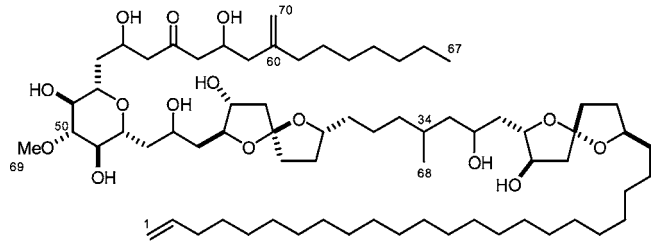
ABSTRACT



Symbiospirol A

Symbiospirols A, B, and C, long carbon-chain compounds with a molecular weight of 1207 were isolated from the cultured symbiotic dinoflagellate *Symbiodinium* sp. Their planar structures and partial relative stereochemistries were elucidated based on 1D and 2D NMR spectra and a degradation reaction. Symbiospirols consisted of a C67-linear chain with a *β*,*β*′-dihydroxyl ketone moiety, eight hydroxyl groups, one tetrahydropyran ring, and two 1,6-dioxaspiro[4,4]nonane rings. Symbiospirol A had an inhibitory effect against L-phosphatidylserine-induced PKC activation.

Dinoflagellates are widely known to be a rich source of biologically active and structurally unique secondary metabolites.[1−5] Among them, large polyhydroxyl and polyether compounds composed of a long carbon backbone that is highly functionalized by oxygen, or so-called "super-carbon-chain compounds (SCC)",[1] are some of the most unusual compounds. Several kinds of SCC have been isolated from symbiotic dinoflagellates, such as karatungiols[6] and amphidinols[7] from *Amphidinium* sp. and durinskiols[8−10] from *Durinskia* sp. These SCC have been reported to show various

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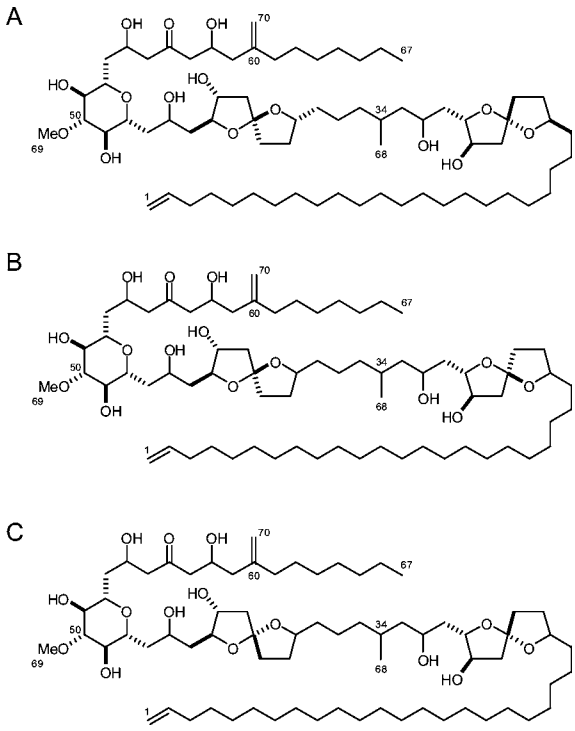

Figure 1. Structures of symbiospirol A (**1**), B (**2**), and C (**3**). The relative stereochemistries among the three ring moieties have not been clarified.

biological activities, and their functions and roles in ecosystems have been subjects of our research. In our continuing search for biologically active metabolites from marine dinoflagellates, we isolated novel spiroketal compounds, symbiospirols A (**1**), B (**2**), and C (**3**) (Figure 1), from *Symbiodinium* sp. which is the same strain that produced symbioimine, neosymbioimine, and symbiodinolide.[5,11−14] We describe here the isolation, structure elucidation, and biological activity of symbiospirols.

The symbiotic dinoflagellate *Symbiodinium* sp. was isolated from the marine acoel flatworm *Amphiscolops* sp. which was collected at Sesoko Island, Okinawa, Japan. The dinoflagellate was cultured for 60 days in 145 L of seawater medium enriched with 2% ES supplement.[15,16] After cultivation, the cells (129 g wet wt) were harvested by centrifugation and extracted with 80% aqueous EtOH for three days.

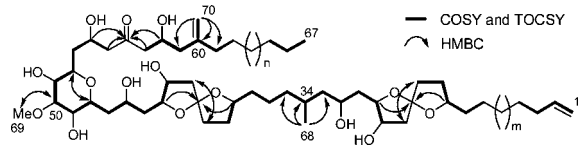

Figure 2. COSY, TOCSY, and selected HMBC correlations of **1**.

Concentrated extract was partitioned with EtOAc and H_2O, and the organic layer was chromatographed on silica gel ($CHCl_3$/MeOH). Final purification was achieved by preparative thin layer chromatography ($CHCl_3$/MeOH), which gave symbiospirols A (**1**) (117.0 mg; 0.091%), B (**2**) (9.4 mg; 0.0073%), and C (**3**) (3.4 mg; 0.0023%).

Symbiospirol A (**1**) was isolated as a yellowish amorphous gum: $[\alpha]_D^{15} = +5.55$ (*c* 3.0, MeOH). From the mass spectrometric and DEPT analyses, the molecular formula of **1** was determined to be $C_{70}H_{126}O_{15}$ (HR-ESITOFMS *m*/*z* 1229.8996 $[M + Na]^+$, $\Delta = +0.2$ mmu, calcd for $C_{70}H_{126}O_{15}Na$, 1229.8994), with 8 degrees of unsaturation. A detailed analysis of ^1H and ^{13}C NMR (Table S1 in the Supporting Information), DEPT, and HMQC spectra in CD_3OD/C_5D_5N (1/1) showed that **1** contained three methyl groups including one methoxy, 46 methylenes, 17 methines including 15 oxymethines, and four quaternary carbons. The IR (neat) spectrum showed absorption bands for a saturated ketone group (1707 cm^{-1}) and hydroxyl groups (3370 cm^{-1}, br). The number of hydroxyl groups was estimated to be 8 based on MS analysis of the acetylation product (ESITOFMS *m*/*z* 1565 $[M + Na]^+$). Based on the ^{13}C NMR spectrum, **1** contained two olefins; one was a typical terminal olefin (δ_C 114.9, 139.9) and the other was an *exo*-type olefin (δ_C 112.4, 147.8). Since all of the multiple bonds were assigned, the remaining five degrees of unsaturation were estimated to be derived from cyclic structures.

A detailed analysis of the COSY and TOCSY spectra of **1** in CD_3OD/C_5D_5N (1/1) allowed us to elucidate the following six partial structures: C1−C4, C22−26, C28−C40 including a branched methyl group, C42−C55, C57−C62 containing an *exo*-olefin, and C65−C67. HMBC correlations at H55ab/C56 and H57ab/C56 suggested that both C55 and C57 were directly connected to the C56 ketone group. Similarly, HMBC correlations at H52/C48 and H48/C52 indicated that C48 and C52 were directly connected by an ether bond, and that a C48−C52 moiety formed a tetrahydropyran ring. In addition, H69/C50 and H50/C69 HMBC correlations suggested that C50 was attached to a C69 methoxy group. Meanwhile, the connectivities of C26 to C28 and C40 to C42 were also elucidated based on HMBC correlations (H24/C27, H26ab/C27, H26ab/C28, H28ab/C26, H28ab/C27, H30/C27, H38/C41, H40ab/C41, H40ab/C42, H42ab/C40, H42ab/C41, and H44/C41). We confirmed that two partial structures, C22−C32 and C36−C46, showed the same planar structures and contained 2,8-dioxaspirononane skeletons. Thus, the center fragment of **1** could be determined (Figure 2). However, the connectivities of the C65−C67 terminal methyl, the terminal olefin, 19 methylenes, and the

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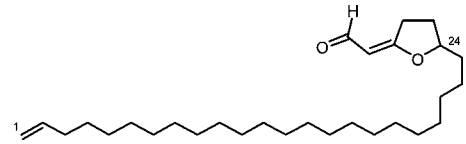

Figure 3. Structure of the degradation product **4**.

center fragment were unknown. The position of the terminal methyl and terminal olefin could not be confirmed due to the signal overlap derived from a long alkyl chain moiety in the NMR spectrum.

To confirm the connectivities at C4−C22 and C62−C65, a degradation reaction of **1** was carried out. Compound **1** was treated with HIO_4•$2H_2O$ in aqueous methanol solution for 3.5 h. The reaction mixture was then purified by silica gel column chromatography (C_6H_{14}/EtOAc) to give a degradation product **4** as a colorless amorphous gum (Figure 3). Its molecular formula was estimated to be $C_{29}H_{52}O_2$ (*m*/*z* 455.3891 $[M + Na]^+$) by HR-ESITOFMS analysis. ^1H NMR, COSY, and MS analysis suggested that **4** included a terminal olefin, an α,*β*-unsaturated aldehyde, a tetrahydrofuran ring, and a long alkyl chain which was suggested to consist of 21 methylenes (Supporting Information). This degradative fragment corresponded to the C1−C29 moiety of **1**, which was derived from 1,2-diol cleavage by periodate after the acid catalyzed acetal ring-opening reaction. Therefore, one terminal structure of **1** could be elucidated, and, consequently, the other terminal structure could be resolved. Thus, the gross structure of **1** was determined.

The relative stereochemistries of the tetrahydropyran ring (C48−C52) in **1** were determined from $^1H-^1H$ coupling constants and ROESY correlations (Figure 4A). $^1H-^1H$ coupling constants derived from the homonuclear *J*-resolve NMR spectrum indicated that the tetrahydropyran ring (C48−C52) had a chair conformation and all protons had an axial orientation ($J_{H48/H49}$ = 9.3 Hz, $J_{H49/H50}$ = 9.3 Hz, $J_{H50/H51}$ = 9.3 Hz, and $J_{H51/H52}$ = 9.3 Hz). Since all of the substituents had an equatorial orientation, it was suggested to be in the most thermodynamically stable conformation. The relative stereochemistries of the 1,6-dioxaspiro[4,4]nonane rings (C24−C30 and C38−C44) were also determined from the $^1H-^1H$ coupling constants and ROESY spectrum (Figure 4B). Since these two moieties showed the same chemical shifts and multiplicities in both the ^1H and ^{13}C NMR spectra, they were suggested to possess the same relative configurations. We elucidated that H43 and H44 had an antirelationship based on the ROE correlations of H42a/H43, H42b/H44, and H43/H45ab. The ROE correlations of H37ab/H42a and H40ab/H42b were also observed. Thus, the relative stereochemistries at the C23−C30, C37−C44, and C48−C52 moieties in **1** could be elucidated. Further studies on their relative and absolute stereostructures are now in progress.

Symbiospirols B (**2**) and C (**3**) were obtained as minor products of *Symbiodinium* sp. during the isolation of **1**. Most of the ^1H and ^{13}C NMR signals of **2** and **3** were identical to those of **1**, except for the signals in the ^{13}C NMR spectra

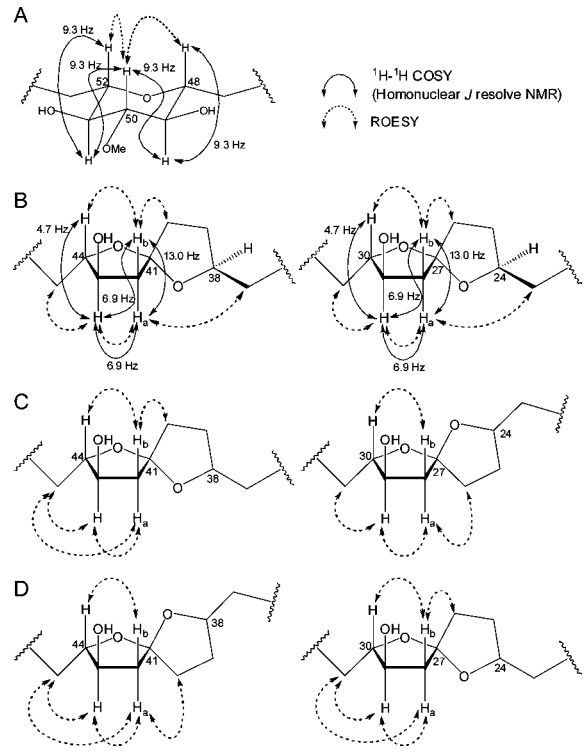

Figure 4. Relative stereochemistries of the tetrahydropyran ring (C48−C52) in **1** (A) and of the 1,6-dioxaspiro[4,4]nonane rings (C24−C30 and C38−C44) in **1** (B), **2** (C), and **3** (D).

around the 1,6-dioxaspiro[4,4]nonane rings (Table S4 in the Supporting Information). The molecular weights of **2** and **3** were also consistent with that of **1** (**2**, HR-ESITOFMS *m*/*z* 1229.9029 $[M + Na]^+$; **3**, HR-ESITOFMS *m*/*z* 1229.8985 $[M + Na]^+$). On the basis of ^1H and ^{13}C NMR, COSY, HMQC, and HMBC spectra, the planar structures of **2** and **3** were elucidated to be the same as that of **1**. Meanwhile, the relative stereochemistries of the 1,6-dioxaspiro[4,4]nonane rings (C24−C30 and C38−C44) in **2** and **3** were revealed to be different from those of **1** based on an analysis of ROESY spectra. As shown in Figure 4B and C, we elucidated that the stereochemistry at C27 in **2** was opposite that in **1** based on the ROE correlations of H28a/H26ab. On the other hand, the stereochemistry at C41 in **3** was opposite that in **1** based on the ROE correlations of H42a/H40ab (Figure 4B and D). The stereochemistries of another 1,6-dioxaspiro[4,4]nonane moiety (C38−C44 for **2** and C24−C30 for **3**) and the tetrahydropyran ring (C48−C52) in **2** and **3** were suggested to be the same as those in **1** due to the identical spectroscopic data for the three compounds. Thus, **2** was determined to be 27-*epi*-symbiospirol A and **3** was 41-*epi*-symbiospirol A.

Next, **1** was subjected to various bioassays and was not found to show a significant cytotoxicity or antifungal activities. However, **1** showed an inhibitory effect on classical protein kinase C (PKC, mixture of α, *β*, and *γ* isoforms; Promega Co., Madison, WI) that was activated by L-phosphatidylserine (PS). Figure 5 shows the effect of **1** on the activity of PKC measured using the Protein Kinase C

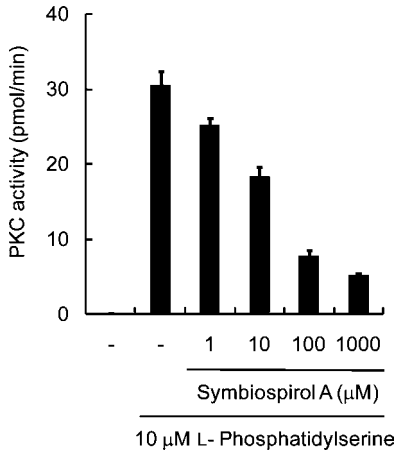

Figure 5. Inhibition of L-phosphatidylserine (PS)-stimulated PKC activity by symbiospirol A (**1**). PKC activity was determined after incubation with the indicated concentrations of **1** in the presence of 10 μM PS. Values are the mean ± SD of triplicate experiments.

Enzyme Assay System RPN77 (GE Healthcare Bioscience, Tokyo, Japan). Compound **1** significantly and dose-dependently inhibited PKC activity in the presence of 10 μM PS. The IC_{50} value was determined to be 19.7 μM. Since **1** inhibited PS-induced PKC activation, **1** was thought to bind to the phospholipid binding site of classical PKC as an antagonist-like compound. Signal transduction with classical PKC plays an important role in inflammatory processes[17,18] thus, **1** may have a potential as a reagent to suppress inflammation-related diseases.

In summary, we isolated symbiospirols A (**1**), B (**2**), and C (**3**) from the cultured marine dinoflagellate *Symbiodinium* sp. Based on the results of spectroscopic analyses and a degradation reaction, **1** was determined to be a novel compound that has eight hydroxyl groups and two 1,6-dioxaspiro[4,4]nonane moieties. The structures of **2** and **3** were elucidated based on a comparison of their spectroscopic data to those of **1**. The structural features of each symbiospirol congener included a branched methyl, an *exo*-olefin, a tetrahydropyran ring, and a β,β'-dihydroxyketone, which are well-known components of secondary metabolites from symbiotic marine dinoflagellates, as seen in amphidinols, karatungiols, durinskiols and symbiodinolide. Meanwhile, the 1,6-dioxaspiro[4,4]nonane rings are characteristic and remarkable moieties. Therefore, symbiospirols are likely to be synthesized via unusual polyketide biosynthetic routes. In addition, **1** inhibited the PS-stimulated activation of PKC. Thus, **1** may have a therapeutic potential for PKC-related inflammation.

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Supporting Information Available: Experimental details and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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