Zooxanthellamide D, a Polyhydroxy Polyene Amide from a Marine Dinoflagellate, and Chemotaxonomic Perspective of the *Symbiodinium* Polyols[#]

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A long-chain polyhydroxy polyene amide, zooxanthellamide D (ZAD-D, **1**, $C_{54}H_{83}NO_{19}$), was isolated from a cultured marine dinoflagellate of the genus *Symbiodinium*. ZAD-D (**1**) is a polyhydroxy amide consisting of a C_{22}-acid part and a C_{32}-amine part and furnishes three tetrahydropyran rings and six isolated butadiene chromophores. The relative stereochemistry of the tetrahydropyran ring systems was elucidated by NMR techniques. This metabolite showed moderate cytotoxicity against two human tumor cell lines. A phylogenetic tree of *Symbiodinium* has been updated and compared with the structures of the hitherto isolated polyols of *Symbiodinium*, zooxanthellatoxins and zooxanthellamides, providing a promising chemotaxonomic perspective for the classification of this morphologically indistinguishable dinoflagellate.

Marine metabolites sometimes display intriguing chemical structures and biological activities. For example, long-carbon-chain polyethers/polyols are produced by microalgae and are sometimes accumulated in certain hosts or predators.[1] The marine symbiotic dinoflagellates of the genus *Symbiodinium* are among the most interesting microalgae that can produce such novel metabolites. The zooxanthellatoxins, a class of polyols from *Symbiodinium* with a long continuous carbon backbone, were isolated from the Y-6 strain and have attracted the attention of researchers due to their 62-membered macrolactone structure and vasoconstrictive activity.[2−5] In previous studies, we examined a different strain (HA3-5) of *Symbiodinium* and discovered another class of large polyols, zooxanthellamides (ZADs), which are distinguishable from the zooxanthellatoxins by three substructures connected via amide functionalities.[6−8] Zooxanthellamide C's (ZAD-C's), an isomeric mixture, contain the components with the largest lactone ring size as natural products and have higher vasoconstrictive activity than the zooxanthellatoxins.[8] A further search for the *Symbiodinium* metabolites using the strain JCUCS-1 resulted in the isolation of the third type of polyols named zooxanthellamide D (ZAD-D, **1**), which consists of two substructures connected via an amide group. We report herein the isolation, structure elucidation, and biological activity of this new metabolite. In addition, we have analyzed the hitherto unknown phylogenetic position of the zooxanthellatoxins-producing strain Y-6 to discuss the relationship between the *Symbiodinium* polyol structures and this classification.

[#] Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

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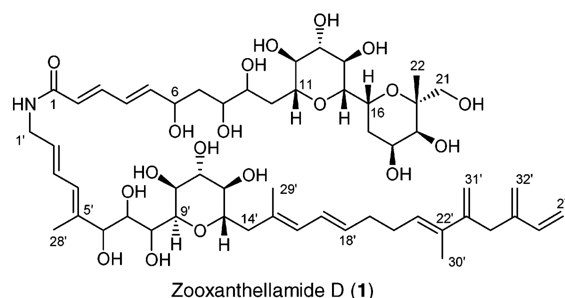

Zooxanthellamide D (**1**)

Results and Discussion

Symbiodinium strain JCUCS-1, which was originally isolated as a symbiont of a jellyfish, is classified into clade B in terms of *Symbiodinium* phylogeny.[9,10] This class is different from those producing the zooxanthellatoxins (Y-6 strain, unknown phylogenetic position)[2−5] and the zooxanthellamides (HA3-5 strain, subclade A1).[6−8] The cells obtained from a 140 L culture broth were extracted with 70% EtOH. The extract was defatted and extracted with BuOH. The BuOH extract was subjected to polystyrene column chromatography and finally to HPLC to yield ZAD-D (**1**, 0.022% based on wet cell weight).

ZAD-D (**1**) showed pseudomolecular ion peaks at *m/z* 547.8 $[M + 2Na]^{2+}$ and 1072.6 $[M + Na]^{+}$ in the ESITOFMS. The molecular formula, $C_{54}H_{83}NO_{19}$, was obtained by a high-resolution ESITOFMS measurement using the bivalent ion peak. The UV absorption at 243 nm suggested the presence of an $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl group. The IR bands at 1656, 1633, 1608, and 1597 cm^{-1} were consistent with the presence of an unsaturated secondary amide group. The main part of the structural elucidation was carried out using conventional NMR techniques, and the ^{1}H and ^{13}C NMR data (Table 1) indicated that ZAD-D (**1**) is a polyhydroxylated polyene-type metabolite. The $^{1}H-^{1}H$ COSY spectrum revealed the presence of the following connectivities: CH-(2) to CH(19), CH_2(1′) to CH(4′), CH(6′) to CH_2(14′), CH(16′) to CH(21′), and CH(26′)=CH_2(27′). A HMBC experiment (Figure 1) was used to connect these partial structures through the quaternary carbons and isolated methyl and methylene groups, providing two

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Table 1. NMR Data for Zooxanthellamide D (ZAD-D, **1**) in CD_3OD^a

no.	δ_C	δ_H, mult. (*J* in Hz)	COSY	HMBC
1	168.5			
2	125.0	6.02 d (15.2)	H-3	C-1, 3
3	141.5	7.16 dd (15.2, 11.2)	H-2, 4	C-1, 4, 5
4	129.0	6.40 dd (15.6, 11.2)	H-3, 5	C-2, 3, 6
5	144.8	6.12 dd (15.6, 6.0)	H-4, 6	C-3, 6
6	71.2	4.40 td (6.4, 6.0)	H-5, 7	C-4, 5, 7, 8
7	40.9	1.73, 1.79 m	H-6, 7, 8	C-6, 8
8	73.5	3.59 dt (9.0, 3.4)	H-7, 9	
9	71.4	3.73 ddd (10.4, 3.4, 2.4)	H-8, 10	
10a	36.4	1.55 ddd (14.4 9.6, 2.4)	H-9, 10b, 11	C-11
10b		2.07 ddd (14.4, 10.4, 2.0)	H-9, 10a, 11	C-9
11	78.7	3.40 td (9.6, 2.0)	H-10, 12	
12	75.6	3.09 t (9.6)	H-11, 13	C-11, 13
13	80.5	3.32 t (9.6)	H-12, 14	C-12, 14
14	71.0	3.56 t (9.6)	H-13, 15	C-13, 15
15	82.5	3.10 dd (9.6, 2.4)	H-14, 16	C-14
16	63.4	4.33 br d (12.0)	H-15, 17	
17a	35.1	1.66 ddd (14.0, 2.8, 2.4)	H-16, 17b, 18	
17b		1.98 ddd (14.0, 12.0, 2.8)	H-16, 17a, 18	C-16
18	69.7	4.14 dt (3.6, 2.8)	H-17, 19	
19	68.8	3.66 d (3.6)	H-18	C-20, 21, 22
20	79.2			
21	69.3	3.31, 3.47 d (11.4)	H-21	C-19, 20, 22
22	15.7	1.27 s		C-19, 20, 21
1′	42.4	3.91 d (6.0)	H-2′	C-1, 2′, 3′
2′	129.8	5.66 dt (15.2, 6.0)	H-1′, 3′	C-1′, 4′
3′	129.2	6.47 dd (15.2, 11.2)	H-2′, 4′	C-1′
4′	127.2	6.13 d (11.2)	H-3′	C-2′, 6′, 28′
5′	139.0			
6′	78.2	4.12 d (5.6)	H-7′	C-4′, 5′, 7′, 28′
7′	73.2	3.83 dd (5.6, 3.8)	H-6′, 8′	C-6′
8′	71.6	3.89 dd (6.2, 3.8)	H-7′, 9′	
9′	73.6	3.99 dd (6.2, 4.6)	H-8′, 10′	C-8′, 10′, 13′
10′	72.4	3.71 dd (7.0, 4.6)	H-9′, 11′	C-8′
11′	74.5	3.86 t (7.0)	H-10′, 12′	C−10′, 13′
12′	74.5	3.21 dd (7.0, 6.6)	H-11′, 13′	C-10′, 13′, 14′
13′	76.5	3.97 ddd (9.8, 6.6, 3.4)	H-12′, 14′	
14′a	43.0	2.23 dd (13.8, 9.8)	H-13′, 14′b	C-13′, 15′, 29′
14′b		2.54 dd (13.8, 3.4)	H-13′, 14′a	
15′	134.2			
16′	128.7	5.84 d (10.8)	H-17′	C-14′, 18′, 29′
17′	128.4	6.24 dd (15.0, 10.8)	H-16′, 18′	C-19′
18′	132.9	5.54 dt (15.0, 6.6)	H-17′, 19′	C-16′, 19′
19′	33.9	2.14 m	H-18′, 20′	C-20′
20′	29.6	2.21 m	H-19′, 21′	C-18′, 19′, 21′, 22′
21′	128.6	5.55 t (7.0)	H-20′	C-20′, 23′, 30′
22′	135.4			
23′	148.1			
24′	37.0	3.15 s		C-23′, 25′, 31′, 32′
25′	146.1			
26′	140.2	6.41 dd (17.4, 10.8)	H-27′	C-24′, 32′
27′a	113.7	5.02 d (10.8)	H-26′	C-25′
27′b		5.22 d (17.4)	H-26′	C-25′, 26′
28′	13.3	1.74 s	H-4′	C-4′, 5′, 6′
29′	17.1	1.78 s	H-16′	C-14′, 15′, 16′
30′	14.4	1.80 s	H-21′	C-21′, 22′, 23′
31′	113.0	4.89, 5.15 s	H-31′	C-22′, 24′
32′	117.9	4.94, 5.07 s	H-32′	C-24′, 26′

[a] The spectra were obtained at 600 MHz for ^1H or 150 MHz for ^{13}C.

large substructures, C-2 to C-22 and C-1′ to C-32′. The HMBC correlations of H-1′ (δ 3.91)/C-1 (δ 168.5), H-2 (δ 6.02)/C-1, and H-3 (δ 7.16)/C-1 indicated that the two large substructures were connected via an amide, which was supported by the IR data and the chemical shift of the nitrogen-bearing C-1′ (δ 42.4). Twenty-one sp^3 carbons were assigned as oxygen functionalities on the basis of their chemical shifts, δ_H 4.40−3.09 and δ_C 82.5−63.4. Among them, six oxygen functionalities were thought to be involved in the formation of three cyclic ethers on the basis of the molecular formula. The ether linkage between C-9′ and C-13′ was determined by the HMBC correlation of H-9′/C-13′, forming a tetrahydropyran ring. The other two ether functionalities were determined by the deuterium shift observed for the oxygen-bearing carbons in NMR, which was calculated as the shifts ($\Delta\delta$) of δ_C in CD_3OD from δ_C in CD_3OH (Figure 2). The results indicated the presence of the ether linkages between C-11 and C-15 and between C-16 and C-20 to form two additional tetrahydropyran rings, which were further supported by the NOE correlations of H-11/H-15 and H-16/H-22 (Figure 3). The geometries of four 1,2-disubstituted olefins (C-2−C-3, C-4−C-5, C-2′−C-3′, and C-17′−C-18′) and three trisubstituted olefins (C-4′−C-5′, C-15′−C-16′, and C-21′−C-22′) were all determined to be *E* on the basis of the large coupling constants (15.0−15.6 Hz) and NOE correlations (Figure 3), respectively. The NOE correlations, combined with the proton−proton spin coupling constants, were also helpful for elucidating the relative stereochemistry of the three tetrahydropyran ring systems. The protons on the C-11−C-15 and C-16−C-20 rings showed large (ca. 10 Hz) or small (ca. 3 Hz) vicinal coupling constants, indicating a chair confirmation with two equatorial carbon substituents for each ring system. The NOE correlations shown in Figure 3 clarified the proton orientation on these rings and, accordingly, the relative stereochemistry of C-11−C-20. On the other hand, the protons on the third ring, C-9′−C-13′, showed intermediate vicinal *J* values (4.6−7.0 Hz), suggesting a twist-boat conformation for this ring. The NOE correlations around this ring system finally led to a plausible conformation with the *trans* carbon substituents, as shown in Figure 3, revealing the relative stereochemistry of C-9′−C13′.

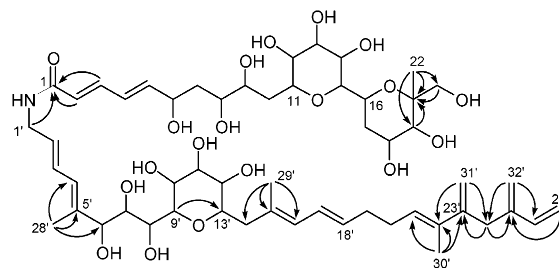

Figure 1. Key HMBC correlations of zooxanthellamide D (ZAD-D, **1**).

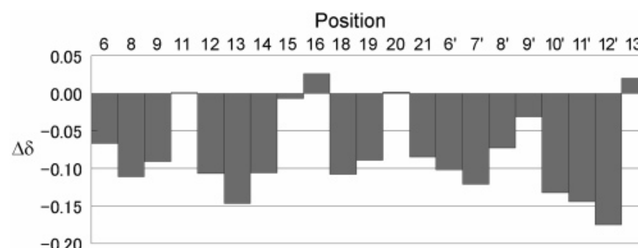

Figure 2. Deuterium shifts observed at the oxygen functionalities of ZAD-D (**1**). $\Delta\delta = \delta(\text{in } CD_3OD) - \delta(\text{in } CD_3OH)$.

ZAD-D (**1**) showed cytotoxicity against two human carcinoma cell lines, A431 and Nakata, at IC_{50} values of 4.5 and 6.6 μM, respectively (Figure 4). However, the compound did not show antifungal activity against a phytopathogenic fungus, *Phytophthora capsici* (data not shown).

ZAD-D (**1**) is a new member of the linear polyhydroxylated polyketides discovered from marine phytoplanktons and, in molecular size, is related to the amphidinols that have been isolated from the marine dinoflagellate *Amphidinium* species.[11−13] In terms of carbon-chain length, however, ZAD-D (**1**) is an amide consisting of a C_{21} acid and a C_{27} amine, whereas the amphidinols possess a

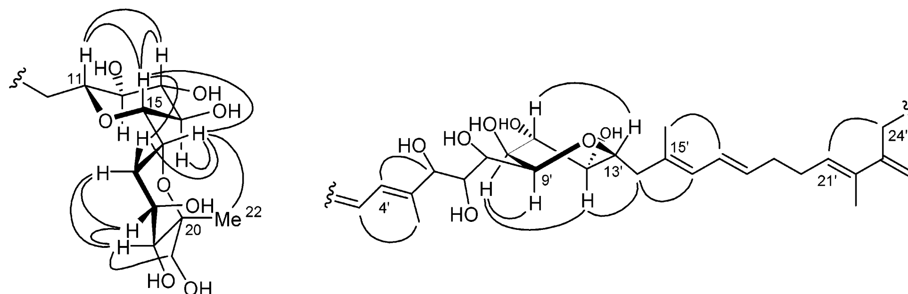

Figure 3. Key NOE correlations (ROESY) of ZAD-D (**1**).

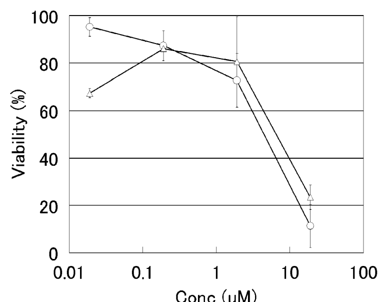

Figure 4. Cytotoxicity of ZAD-D (**1**) against human cancer cells. Circles: A431 cell line; triangles: Nakata cell line.

contiguous carbon chain (C_{55}−C_{69}) without nitrogenous functionalities. In addition to the carbon skeleton, the uncommon structural features are the presence of three tetrahydropyran rings and five butadiene chromophores. Amphidinol 2[13] has been the only example of such a tris-tetrahydropyran type among a number of the amphidinols and related members.[11−15] Zooxanthellamides (ZADs) A−C's, the larger-size polyols isolated by our group from another strain of *Symbiodinium* HA3-5, are diamides built up with three components: C_{27} ω-amino acid, C_{78} ω-amino acid, and C_{14} carboxylic acid in terms of the carbon-chain length.[6−8] Another class of the *Symbiodinium* polyols, zooxanthellatoxins, are also amides derived from a $C_{104/106}$ ω-amino acid and a C_{25} carboxylic acid.[2−5] As illustrated in Figure 5, the *Symbiodinium* polyols are characterized by long-chain amides.

The structural diversity of the *Symbiodinium* polyol metabolites prompted us to examine the chemotaxonomic classification for this morphologically homogeneous dinoflagellate. Recently, the phylogenetic study of *Symbiodinium* has been advanced by molecular genetic techniques, providing a phylogenetic tree including approximately 40 isolates.[10] The phylogenetic positions of the HA3-5 and JCUCS-1 strains producing the zooxanthellamides (ZADs) were previously analyzed, whereas that of the Y-6 strain producing the zooxanthellatoxins has not been determined. Therefore, we cloned 5.8S rDNA and internal transcribed spacer (ITS) regions from cultured cells. The nucleotide sequence obtained from the Y-6 strain was aligned with the previously reported data on other *Symbiodinium* strains.[10] Molecular phylogenetic analyses by the NJ, MP, and Bayesian methods produced a consistent topology with high statistical support. The results strongly suggested the monophyly of the Y-6 strain with the JCUSG-1 and JCUZS-2 strains in subclade A2 (Figure 6). As shown in Figure 6, the strains of clade A1 (HA3-5 strain), clade A2 (Y-6 strain), and clade B (JCUCS-1 strain) produce unique polyols, ZAD-C's, zooxanthellatoxins, and ZAD-D (**1**), respectively, indicating a certain clade-to-metabolite relationship. This hypothesis was further supported by LC-MS analyses (see Supporting Information) of the extracts from the strains P083-2 (subclade A1) and JCUSG-1 (subclade A2), showing the presence of ZAD-C's and zooxanthellatoxins A, respectively (Figure 6). Although there are still not a sufficient number of examples, the results suggest the possibility of the chemotaxonomic classification of the dinoflagellate *Symbiodinium*.

Experimental Section

General Experimental Procedures. UV and IR spectra were recorded on JASCO V-530 and FT/IR-8300 spectrometers, respectively. Optical rotations were measured on a JASCO DIP-370 polarimeter. NMR spectra were obtained by a Bruker AMX2-600 (600 MHz for ^{1}H) spectrometer. Chemical shifts (δ) of ^{1}H NMR are given in parts per million (ppm) relative to the internal standard peak at δ 3.30 (residual CD_2HOD) for CD_3OD solutions, and the coupling constants (J) are in Hz. Chemical shifts of ^{13}C NMR are given in ppm relative to the internal standard peak at δ 49.0 for CD_3OD solutions. ESITOFMS were obtained on a Mariner Biospectrometry Workstation (Applied Biosystems, Foster City, CA). High-resolution MS was performed using a peptide mixture (angiotensin I, bradykinin, and neurotensin) as an internal standard. LC-MS was measured by the same MS apparatus equipped with an Agilent 1100 HPLC system (Hewlett-Packard, Palo Alto, CA). HPLC was carried out using a system equipped with a JASCO PU-1586 pump and a JASCO UV-1570 detector.

***Symbiodinium* Strains.** JCUCS-1 is a *Symbiodinium* strain originally isolated from the jellyfish *Cassiopeia xamachana* by Dr. Robert Trench and is held by various algal culture collections; this strain was obtained (as isolate CS-153) from CSIRO Division of Marine and Atmospheric Research, Hobart, Tasmania. JCUSG-1 was isolated from the octocoral *Sarcophyton glaucum* as follows. Freshly collected specimens of *S. glaucum* were rinsed at least 10 times in Millipore-filtered seawater (MFSW), and manual pressure was applied to squeeze the zooxanthellae from their host. The resulting dinoflagellate suspension was centrifuged at 3000*g* for 15 min and the pellet resuspended and washed in MFSW 10 times. To obtain clonal cultures, the washed dinoflagellate extracts were diluted to a final concentration of 104 cells/mL and maintained in AB-F/2 medium [(F/2 medium supplemented with ampicillin (90 μg/mL), streptomycin (45 μg/mL), chloramphenicol (9 μg/mL), hygromycin (100 μg/mL), and Luria Bertani broth (5%)] for 4 days. Altogether, 103 cells were treated with SDS (0.5%)/proteinase K solution (10 μg/mL) for 30 min at room temperature and washed several times in F/2 medium. Axenic algal suspensions were plated onto sterile solid growth medium (1.0% agar in F/2 medium) and incubated at 25−28 °C. Well-isolated dinoflagellate colonies appeared after 2 months and were transferred to liquid culture medium. The strain Y-6 was previously isolated from a marine flatworm in Okinawa and kindly provided by Dr. T. Yamasu (Ryukyu University, Okinawa, Japan).[2] The strain P083-2 (MBIC10853) was previously isolated from a Palauan foraminifera and is deposited at the culture collection of the Marine Biotechnology Institute (Kamaishi, Japan).[9]

Culture and Production. *Symbiodinium* JCUCS-1 cells were cultured in a 3 L glass bottle containing 2 L of seawater and 20 mL of ES supplement under conditions of 12 h light/12 h dark at 25 °C for 43 days. The medium contents and other conditions were as previously reported.[8] The collected cells were kept at −80 °C until used. The frozen cells (98.5 g from 140 L of culture) were homogenized in 70% EtOH (150 mL) with an ULTRA-TURRAX T25 (Janke & Kunkel GmbH & Co. KG IKA-Labortechnik, Staufen, Germany) (24 000 rpm, 3 × 2 min) at 4 °C. After the mixture was centrifuged, the supernatant was collected, and the precipitates were extracted twice with 70% EtOH (150 mL each) in the same way. The combined ethanolic extracts were concentrated in vacuo. The residue was suspended in water (110 mL) and extracted with EtOAc (3 × 100 mL) and then BuOH (3 × 100 mL). The BuOH-soluble fraction (565 mg) was applied to a polystyrene column (17 mL of MCI CHP-20P 75−150 μm; Mitsubishi Chemical, Tokyo, Japan), and the column was eluted with water (50 mL), 20% EtOH (70 mL), 40% EtOH (90 mL), 60% EtOH (120 mL), and EtOH

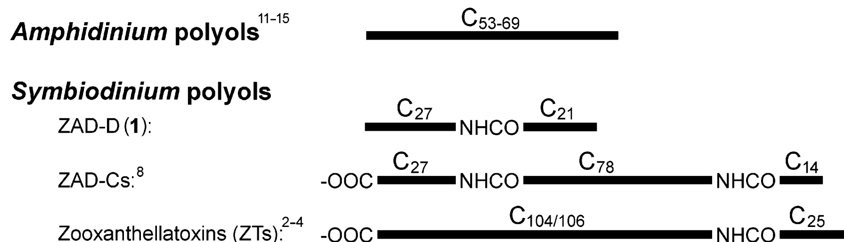

Figure 5. Comparison of the continuous linear carbon skeletons within the polyols from two dinoflagellate genera. The numbers of the branched carbons are excluded.

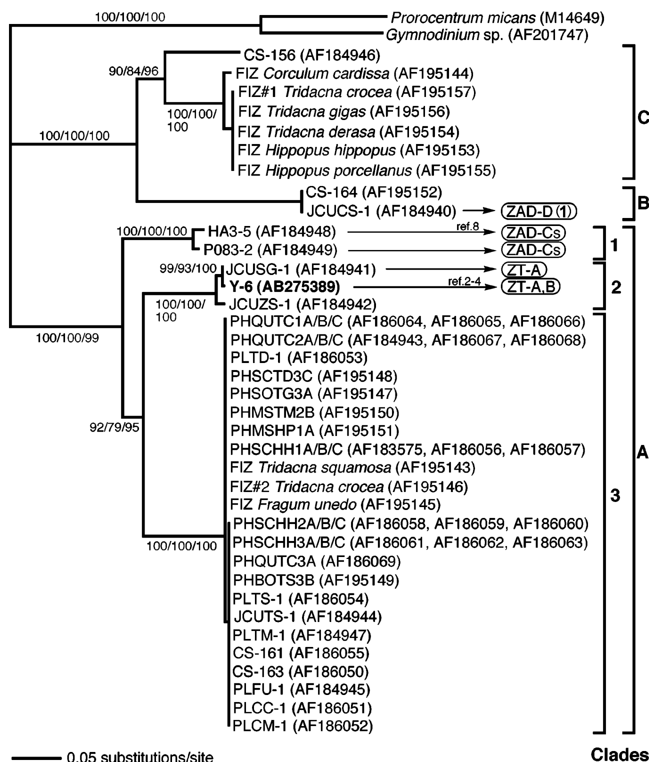

Figure 6. NJ phylogenetic tree of *Symbiodinium* isolates resulting from the analysis of the 5.8S rDNA and ITS regions, indicating the Y-6 position. Numbers at nodes refer to percent bootstrap values of NJ, MP, and posterior probabilities of Bayesian analysis, respectively (NJ/MP/Bayesian). GenBank accession numbers are shown in parentheses. ZT: zooxanthellatoxin; ZAD: zooxanthellamide.

(70 mL) sequentially. A portion (86.2 mg) of the 60% EtOH eluate (93.4 mg) was purified by HPLC on a Develosil ODS-UG-5 column (10 mm $\phi \times 250$ mm; Nomura Chemical, Aichi, Japan) with 75% MeOH containing 20 mM NH_4OAc at a flow rate of 2.5 mL min^{-1} to give ZAD-D (**1**, 2.3 mg, 0.0057%, $t_R = 28$ min detected at 220 nm): colorless powder, $[\alpha]_D^{25}$ +2 (*c* 0.20, MeOH); UV (MeOH) λ_{max} 243 nm (ϵ 48 000); IR (KBr) ν_{max} 3350, 3040, 1656, 1633, 1608 (sh), 1597, 1105, 898, 617 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESITOFMS *m/z* 1072.6 $[M + Na]^+$, 547.8 $[M + 2Na]^{2+}$; HRMS found *m/z* 547.7696, calcd for $[C_{54}H_{83}NO_{19}Na_2]^{2+}$ 547.7672.

Biological Activity Tests. A cytotoxicity test was performed using two human carcinoma cell lines, A431 vulval-derived epidermoid carcinoma and Nakata oral squamous cell carcinoma. The cell lines were cultured in a serum-free RD medium for 4 days in the presence or absence of the substances, and the cell numbers were counted with a Coulter counter. The detailed conditions were reported previously.[16] The IC_{50} values shown are the means of two replicates.

The antifungal activity was evaluated against the plant pathogen *Phytophthora capsici* using a paper disk assay, as previously reported.[17]

PCR Amplification and Phylogenetic Analysis. Total DNA was extracted from cultured Y-6 strain cells using a DNeasy plant mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The region containing the internal transcribed spacer (ITS) 1, 5.8S rRNA gene, and ITS2 was amplified by PCR using an Ex Taq polymerase (Takara, Shiga, Japan) and a GeneAmp 9700 thermal cycler (Applied Biosystems) with primers of zITSf and ITS4.[10] The amplicon was directly sequenced using a BigDye terminator kit (Applied Biosystems) and an ABI PRISM 3100 genetic analyzer (Applied Biosystems). The sequence was deposited in GenBank under accession number AB275389. The sequence data for other *Symbiodinium* taxa were collected from the GenBank database and were aligned by a Clustal X-1.83.1 multiple alignment program[18] with an IUB DNA weight matrix. Gaps and ambiguity codes were eliminated from the matrix, and a total of 537 positions remained (of which 282 were parsimony-informative). PAUP*4.0 beta 10[19] and MrBayes 3.1.2[20] were used to conduct phylogenetic analyses. For neighbor-joining (NJ) analysis,[21] Kimura's two-parameter[22] was chosen for the distance matrix. The robustness of each branch was determined by a nonparametric bootstrap test[23] with 10 000 replicates. For maximum-parsimony (MP) analysis,[24] all sites were equally weighted. Optimum trees were searched by a heuristic strategy with 10 000 random sequence addition and a tree bisection-reconnection (TBR) branch swapping algorithm. The MP heuristic search recovered a single most parsimonious tree (length = 706, CI = 0.7918, RI = 0.8306). Bootstrap values were calculated using 1000 replicates, 100 random additions per replicate, and TBR branch swapping. For Bayesian analysis, the program MrModeltest 2.2[25] was used to determine the available substitution model with the best fit to each partitioned data set. We conducted two simultaneous chains for 1 000 000 generations, sampling trees every 100 cycles, under a GTR+I+G model. The first 2500 trees were discarded, and majority rule consensus of the remaining trees was used to determine clade posterior probabilities.

LC-MS Analysis of *Symbiodinium* Polyols. *Symbiodinium* cells cultured for six weeks in a 2 L ES medium were collected by filtration and extracted with 70% EtOH (10 mL, three times). The combined extracts were concentrated, and the residual aqueous solution was washed with EtOAc (10 mL, three times) and extracted with BuOH (10 mL, three times). Each BuOH extract was pretreated with an ODS cartridge (Toyopak ODS S, Tosoh, Tokyo), and a portion (20 μg/μL in MeOH) was analyzed by LC-MS (negative ESITOF) under the following LC conditions: column, Cadenza CD-C_{18} (i.d. 2 $\times$ 75 mm, Imtakt, Kyoto, Japan); solvent, 25−40% MeCN in 20 mM NH_4OAc over 30 min linear gradient; flow rate, 0.2 mL/min (5 μL/min into MS analyzer); UV detection at 230 nm. The retention times of the polyol metabolites are 13.6 min for zooxanthellatoxins A (ZT-A) in the strains Y-6 and JCUSG-1, 11.2 min for ZAD-C's in the strains HA3-5 and P083-2, and 22.1 min for ZAD-D (**1**) in the strain JCUCS-1. See Supporting Information for the detailed results.

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Supporting Information Available: 1D and 2D NMR spectra of zooxanthellamide D (**1**); LC/MS data for the extracts of *Symbiodinium* strains. This information is available free of charge via the Internet at http://pubs.acs.org.

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