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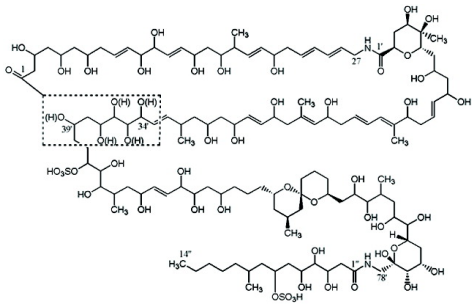

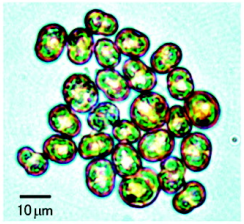

Symbiodinium sp. HA3–5

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Zooxanthellamide Cs: Vasoconstrictive Polyhydroxylated Macrolides with the Largest Lactone Ring Size from a Marine Dinoflagellate of *Symbiodinium* sp.

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Abstract: Zooxanthellamide Cs (ZAD-Cs), $C_{128}H_{220}N_2O_{53}S_2$ (ca. 2.7 kDa), was obtained from a cultured marine dinoflagellate of the genus *Symbiodinium* as an inseparable isomeric mixture of polyhydroxylated 61- to 66-membered macrolides. The chemical structures of the components were clarified by detailed 2D NMR analysis to be the macrolactonized analogues of zooxanthellamide A (ZAD-A), which had been previously isolated from the same microalgae. Chemical lability of ZAD-Cs suggests that ZAD-A is an artifact derived from ZAD-Cs during the isolation steps. Three of the components possess the largest (63-, 64-, and 66-membered) ring sizes found to date among the natural macrolides. ZAD-Cs exhibited higher vasoconstrictive activity than that of the zooxanthellatoxins, the first vasoconstrictive macrolides from *Symbiodinium* sp. The structure−activity relationship suggests that the huge macrolactone structure is important for biological activity. The relationship between the structures of the polyol metabolites and the phylogenetic systematics of *Symbiodinium* sp. is also discussed.

Introduction

Marine dinoflagellates, unicellular phytoplanktons, produce numerous bioactive and complex secondary metabolites.[1−3] Since dinoflagellate metabolites accumulate in higher organisms through the food chain or through symbiotic relationships, the identification of the true origin of bioactive marine compounds is usually difficult.[1,4,5] The genus *Symbiodinium*, belonging to the zooxanthellae, is the most representative dinoflagellate, living symbiotically in a wide range of marine invertebrates. This genus also produces unique secondary metabolites,[6−13] of which the zooxanthellatoxins (ZTs) are of interest due to their

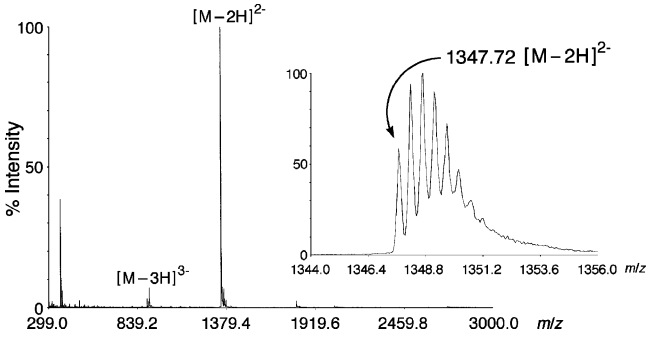

Figure 1. ESI TOF MS (negative) of ZAD-Cs.

62-membered macrolactone structure and potent vasoconstrictive activity.[10−13] In the course of studies on the function and distribution of ZTs and related metabolites in dinoflagellates, a new type of polyhydroxylated metabolites, zooxanthellamides A and B (ZAD-A and ZAD-B),[14,15] was isolated from a free-living species of *Symbiodinium* (strain HA3−5) obtained from a Hawaiian sand beach.[16,17] ZADs have a high molecular weight (ca. 2700) like ZTs, but their carbon skeletons are quite different. In a further effort to demonstrate the distribution and structural

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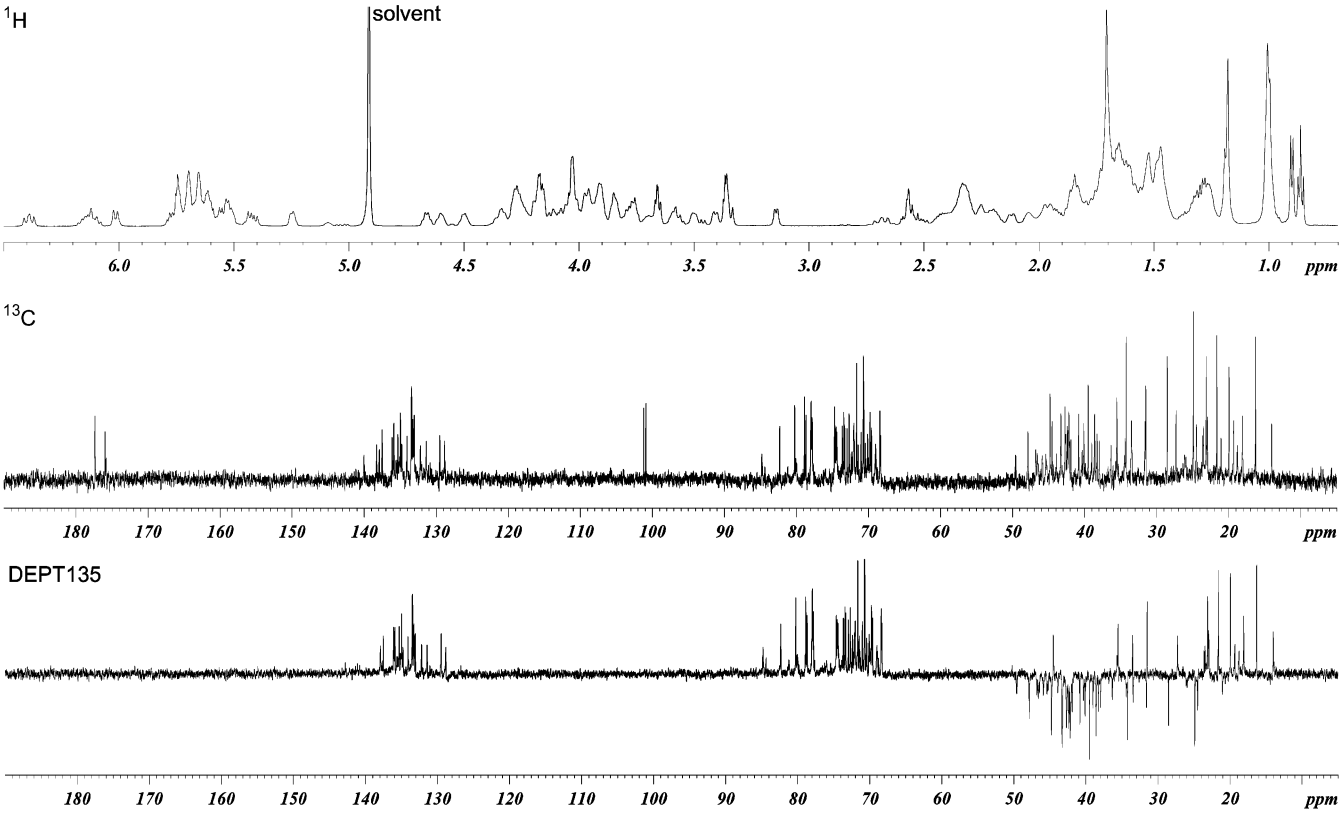


Figure 2. ^{1}H (600 MHz), ^{13}C (150 MHz), and DEPT135 spectra of ZAD-Cs in a D_2O solution.

diversity of these large polyhydroxy metabolites in the genus *Symbiodinium*, the novel ZADs, zooxanthellamide Cs (ZAD-Cs) was found. Herein, the structural elucidation of ZAD-Cs and its vasoconstrictive activity in comparison with ZT-A and other ZADs are described.

Results

Isolation. The cells of *Symbiodinium* sp. (strain HA3−5) obtained from a 132-L culture broth were extracted with 70% EtOH. The extract was defatted and extracted with BuOH. The BuOH extract was subjected to polystyrene, then to DEAE column chromatography, and finally to HPLC to yield zooxanthellamide Cs (ZAD-Cs, 0.022% based on wet cell weight), as well as the minor related products zooxanthellamide A (ZAD-A, 0.0057%) and ZAD-B (0.0097%).

Structural Elucidation. The negative ESI TOF MS showed bivalent and trivalent pseudo-molecular ions at *m*/*z* 1347.72 $[M - 2H]^{2-}$ and 898.14 $[M - 3H]^{3-}$, respectively (Figure 1). An exact monoisotopic weight was determined as 2697.4016 Da [M] by MS measurement in the presence of an internal standard followed by the data processing of the single charged conversion. A possible molecular formula was $C_{128}H_{220}N_2O_{53}S_2$ (error = −3.6 mDa), which was confirmed after the following structural analysis.

The IR spectrum suggested the presence of hydroxyl (3385 cm^{-1}), ester/lactone (1720 cm^{-1}), secondary amide (1650 cm^{-1}), and sulfate groups (1250 cm^{-1}). The structure elucidation of ZAD-Cs was performed mainly by NMR experiments consisting of ^{1}H and ^{13}C NMR, DEPT (Figure 2), COSY 90, DQF-COSY, HOHAHA, HSQC, and HMBC. The NMR experiments were performed in D_2O at 288 K due to the severe instability of ZAD-Cs in CD_3OD. When CD_3OD was used,

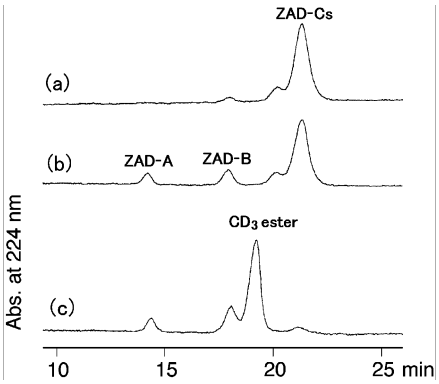


Figure 3. HPLC chromatograms of ZAD-Cs before NMR measurement (a), and after NMR measurement in D_2O (b) and CD_3OD (c). The conditions are the same as those for the preparative HPLC of ZAD-Cs described in the Experimental Section.

ZAD-Cs was gradually converted to the trideuterated methyl ester, as well as trace amounts of ZAD-A and ZAD-B in the NMR tube (Figure 3a, c). The production of the deuterated methyl ester was supported by the bivalent pseudo-molecular ion at *m*/*z* 1365.2 $[M - 2H]^{2-}$ of the ESI TOF MS and the chemical shift difference in the NMR data (Table 1). The corresponding methyl ester, which was equally produced in CH_3OH, showed the bivalent pseudo-molecular ion at *m*/*z* 1363.8 $[M - 2H]^{2-}$. In contrast to the case of CD_3OD, a solution of ZAD-Cs in D_2O scarcely promoted hydrolytic decomposition during NMR experiments (Figure 3b).

The NMR spectra of ZAD-Cs seemed to be a mixture of five similar isomers because some signals split into a maximum of five weak signals, though the HPLC analysis showed a single peak. For the structural elucidation, we focused on ZAD-C5,

Table 1. Comparison of the Partial NMR Chemical Shifts between ZAD-A and the Corresponding CD_3 Ester Generated from ZAD-Cs in CD_3OD

	ZAD-A		CD_3 ester	
position[a]	δ_C	δ_H	δ_C	δ_H
1	180.42		173.95	
2	45.56	2.36, 2.28	43.15	2.55, 2.43
3	69.01	4.08	67.87	4.20
4	45.06	1.62	41.88	1.64
5	70.08	3.97	69.79	3.96
6	44.39	1.65, 1.55	44.34	1.65, 1.55

[a] Data for other positions are superimposable on those for ZAD-A.

which is a major ZAD-C isomer. Most of the NMR signals of ZAD-C5 are superimposable on those of ZAD-A, except for the signals around the ester/lactone (C1) terminus and the oxymethine region around 5 ppm. The proton connectivities of H2 to H27, H2′ to H4′, H6′ to H14′, H16′ to H21′, H23′ to H60′, H62′ to H76′, and H2″ to H14″, including the blanched methyl groups in ZAD-C5, were established by COSY and HOHAHA experiments (Figure 4). The connectivities of the carbonyl groups to the adjacent positions, C1−C2, C1′−C2′, and C1″−C2″, were revealed by the HMBC cross-peaks of H2/C1, H3/C1, H2′/C1′, H2″/C1″, and H3″/C1″. The connectivities from C4′ to C6′, C14′ to C16′, and C21′ to C23′ were determined by the HMBC correlations of 5′-Me/C4′, 5′-Me/C6′, 15′-Me/C14′, 15′-Me/C16′, 22′-Me/C21′, and 22′-Me/C23′. The connectivities adjacent to the ketal and hemiketal carbons, C61′ (δ_c 100.21) and C77′ (δ_c 100.90), were evident from the HMBC cross-peaks of H60′/C61′, H62′/C61′, H63′/C61′, H78′/C76′, and H78′/C77′. The HMBC correlations of H27/C1′ and H78′/C1″ and the chemical shifts of C27 (δ_c 43.30) and C78′ (δ_c 47.88) suggested connectivities between C27 and C2′ and between C78′ and C2″ via an amide bond. A summary of ^1H and ^{13}C NMR data is shown in Table 2. An upper field shift of C-1 (δ_c 182.95 to 176.02) and the molecular weight loss of 18 in comparison with those of ZAD-A suggest that ZAD-Cs is a lactonized derivative of ZAD-A. To determine the position of lactonization, the ^1H NMR data for ZAD-Cs and ZAD-A were compared in detail to find the most notable differences from ZAD-A in the several signals around 5 ppm, for which integral values were all less than 1 H. The five distinguishable cross-peaks assignable to the acyloxymethine groups (COO−*CH*<: δ_H 5.01−5.53, δ_C 74.36−77.67) were finally observed by the HSQC spectrum as shown in Figure 5, suggesting that these signals are due to the lactonized oxymethines. The HSQC signal due to the position 5 of the δ-lactone derivative ZAD-B, which might be produced by interconversion during experimental handling and NMR measurement, was also observed at δ_H/δ_C 4.89/78.30. The lactonized positions were finally determined by the correlation from these oxymethine protons to C-1 in HMBC and the proton−proton connectivities around the acyloxymethine protons in COSY and HOHAHA, as shown in Table 3. These findings unambiguously determined that ZAD-Cs is an isomeric mixture of five macrolide derivatives of ZAD-A, in which the positions C34′, C35′, C36′, C37′, and C39′ are lactonized to give the constituents ZAD-C1, C2, C3, C4, and C5, respectively. An approximate ratio of these isomers was estimated by the integral values of ^1H NMR signals as ZAD-C1:C2:C3:C4:C5 = 2:1:1:3:3. Further evidence for the lactone structures of ZAD-Cs was obtained by hydrolysis in an aqueous KOH solution to give the seco-acid that was identical to ZAD-A in all respects.[14] Thus, the structure of ZAD-Cs, with the relative stereochemistry of the ring systems, is shown in Chart 1.

Table 2. NMR Data for Zooxanthellamide C5 (ZAD-C5) in D_2O at 288 K[a]

position	δ_C	δ_H (mult.[b], *J* in Hz)	position	δ_C	δ_H (mult.[b], *J* in Hz)
1	176.02		38′	39.92	1.95, 1.88
2	44.67	2.71, 2.54	39′	76.28	5.09
3	68.92	4.24	40′	32.63	1.96, 1.74
4	45.90	1.71	41′	26.06	1.72, 1.61
5	70.10	3.92	42′	84.48	4.59 (m)
6	45.31	1.72, 1.66	43′	74.40	4.12
7	71.45	3.91	44′	77.81	3.41
8	42.10	2.35, 2.18	45′	33.53	1.84
9	133.43	5.77 (dt, 14.9, 7.5)	46′	38.59	1.67, 1.46
10	134.03	5.56	47′	73.64	4.25
11	77.99	4.03	48′	137.49	5.75
12	77.67	4.05	49′	133.40	5.75
13	133.23	5.68	50′	77.90	4.03
14	137.87	5.69	51′	73.44	3.76
15	72.96	4.28	52′	41.90	1.56
16	42.41	1.67, 1.58	53′	70.71	3.84
17	74.51	3.50 (m)	54′	40.12	1.53,1.48
18	44.53	2.31	55′	24.49	1.62, 1.53
19	135.87	5.56	56′	38.28	1.47
20	135.29	5.52	57′	68.31	3.95
21	74.63	4.16	58′	39.02	1.51, 1.47
22	42.64	2.33	59′	27.30	2.04 (m)
23	133.03	5.65	60′	42.15	1.67, 1.49
24	134.94	6.11	61′	101.21	
25	134.78	6.15	62′	37.96	1.58, 1.50
26	129.52	5.61	63′	21.05	1.86, 1.62
27	43.30	3.91, 3.80	64′	33.46	1.64, 1.26
1′	175.91		65′	69.60	4.03
2′	72.00	4.19	66′	43.95	1.74
3′	34.39	2.13, 1.82	67′	69.76	4.07
4′	71.66	3.91	68′	82.33	3.14
5′	74.70		69′	35.54	1.92
6′	81.24	3.97	70′	38.61	1.97, 1.37
7′	36.35	1.99, 1.66	71′	72.68	3.85
8′	69.00	3.59	72′	78.68	3.36
9′	43.92	1.97, 1.82	73′	70.71	4.17
10′	73.33	4.27	74′	31.61	1.86, 1.62
11′	136.08	5.42 (dd, 15.3, 8.4)	75′	68.40	4.18
12′	133.03	5.69	76′	71.01	3.66 (d, 2.7)
13′	40.33	2.33, 2.21	77′	100.90	
14′	80.03	4.01	78′	47.88	3.60, 3.34 (d, 13.9)
15′	139.97		1”	177.33	
16′	128.84	6.01 (d, 10.5)	2”	42.73	2.57, 2.54
17′	131.40	6.39 (dd, 14.4, 11.4)	3”	71.66	4.17
18′	133.19	5.72	4”	78.88	3.36
19′	43.30	2.43, 2.31	5”	70.65	3.97
20′	70.44	4.50	6”	40.84	1.85, 1.72
21′	132.16	5.25	7”	80.25	4.66 (m)
22′	138.24		8”	44.80	1.65
23′	49.57	2.26	9”	31.50	1.53
24′	72.09	4.34	10”	39.51	1.33, 1.18
25′	135.87	5.70	11”	28.53	1.32, 1.28
26′	135.05	5.70	12”	34.23	1.26
27′	72.38	4.29	13”	24.90	1.29
28′	46.76	1.73, 1.61	14”	16.28	0.85 (t, 7.0)
29′	69.78	3.70	18-Me	18.10	1.00
30′	46.57	1.44	5′-Me	22.96	1.18 (s)
31′	35.54	2.39	15′-Me	14.02	1.72 (s)
32′	140.96	5.62	22′-Me	18.78	1.71 (s)
33′	130.98	5.62	31′-Me	23.46	1.00
34′	74.60	4.34	45′-Me	19.33	1.02
35′	75.98	3.60	59′-Me	23.11	1.19 (d, 8.1)
36′	74.19	3.58	69′-Me	19.95	1.00
37′	70.00	4.00	9″-Me	21.65	0.90 (d, 6.5)

[a] Measured at 600 MHz for ^1H and 150 MHz for ^{13}C. [b] Multiplicity; s: singlet, d: doublet, t: triplet, m: multiplet, unspecified: overlapping signals.

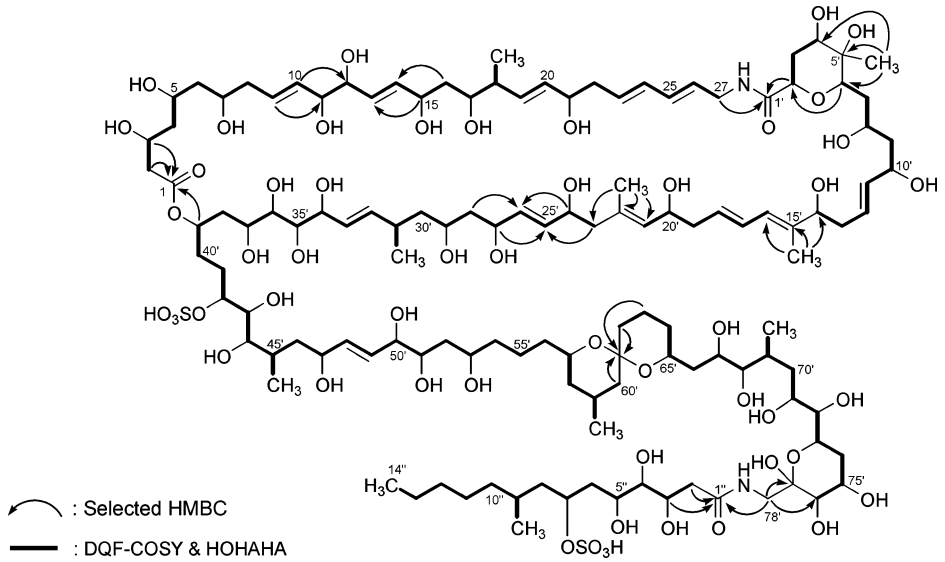


Figure 4. Two-dimensional NMR correlations in the structure of ZAD-C5.

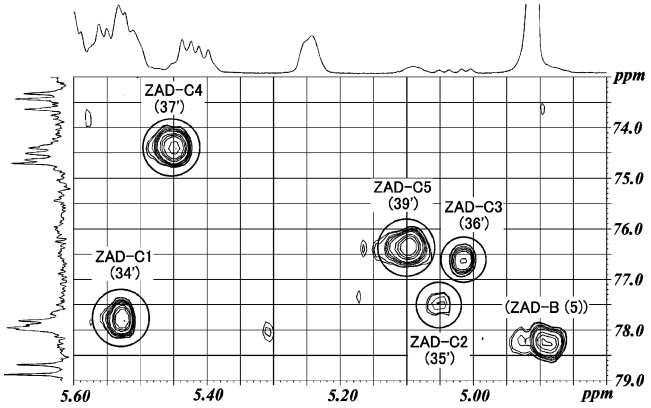


Figure 5. A partial HSQC spectrum of ZAD-Cs, indicating the presence of five macrolide isomers.

Additional support for the complex structures of ZADs was obtained from the degradation of ZAD-A. Basic hydrolysis of the amide groups of ZAD-A with aqueous LiOH yielded a product corresponding to the C1−C27 segment of ZADs (Chart 1). Isolation of the other parts was unsuccessful. The structural analysis of this 1−27 segment was much easier than that of the intact compounds. Thus, the molecular formula was determined by HR MS as $C_{28}H_{47}O_{10}N$, and all the proton connectivities were revealed by DQF-COSY and HOHAHA analysis.

Biological Activity. Vasoconstrictive activity was evaluated, using rat blood vessels, against a positive control, zooxanthellatoxin A (ZT-A), which showed an EC_{50} value of 1.2 μM in this assay.[10] The EC_{50} values for ZAD-A, ZAD-B, and ZAD-Cs are >30 μM, >3 μM, and 0.39 μM, respectively. The activity of ZAD-Cs was three times that of ZT-A, suggesting that the presence of a huge lactone ring is important for vasoconstrictive activity.

Discussion

Among the isomers in ZAD-Cs, ZAD-C3, -C4, and -C5 possess 63-, 64-, and 66-membered macrolide rings, respectively, which are the largest sizes among the secondary metabolites to date. ZAD-Cs is so labile that it is easily converted to ZAD-A, ZAD-B, or a methyl ester even under neutral conditions. Interconversion of ZAD-C isomers may also occur because the ratio of the isomers varied depending on extracted sample. Therefore, the previously isolated compounds ZAD-A and ZAD-B must be artifacts derived from ZAD-Cs during the isolation steps.

This study first revealed the diversity of the giant polyol metabolites produced by the symbiotic marine dinoflagellate *Symbiodinium* sp. ZADs resemble ZTs in molecular size (approximately 3 kDa) but are clearly distinguishable, with attention focusing on the arrangement of their continuous carbon skeletons separated by amide groups. Thus, these large polyols can be divided into two categories using the continuous carbon numbers and their combination: (1) $C_x + C_y$ ($x = 104$ or 106, $y = 25$) for ZTs and (2) $C_x + C_y + C_z$ ($x = 27$, $y = 78$, and $z = 14$) for ZADs. It is interesting to note that morphologically indistinguishable *Symbiodinium* species produce biosynthetically different and physiologically similar polyol metabolites such as ZTs and ZADs. The biodiversity of the *Symbiodinium* sp. of monomorphic microalgae has gradually been revealed by recent ribosomal DNA analysis, which demonstrated that *Symbiodinium* could be classified into several clades.[17] The giant polyols ZADs and ZTs are produced by the different strains, HA3−5 and Y-6, respectively, and not vice versa (analytical data not shown). These strains HA3−5 and Y-6 belong to the same phylogenetic class "clade A" but are identified into the different subclasses "clade A1" and "clade A2", respectively.[17] This shows signs of relevancy between the biodiversity of *Symbiodinium* and the chemical diversity of the *Symbiodinium*-specific polyols, prompting us to a further comprehensive analysis of giant polyols such as ZADs and ZTs.

Experimental Section

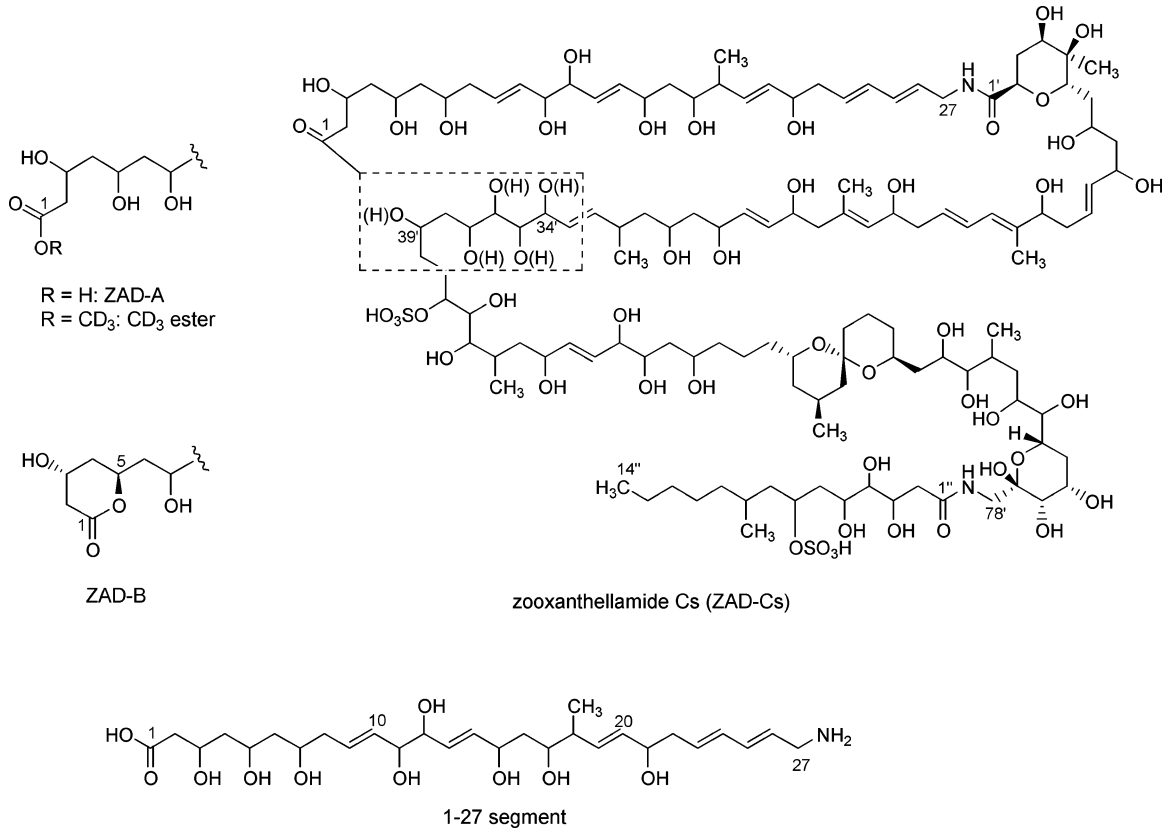
General Procedures. UV and IR spectra were recorded on V-530 and FT/IR-8300 spectrometers (Jasco, Tokyo), respectively. Optical rotations were measured on a Jasco DIP-370 polarimeter. ESI TOF MS spectra were obtained on a Mariner Biospectrometry Workstation (Applied Biosystems, Tokyo). High-resolution ESI TOF MS was performed using poly(propylene glycol) (for negative mode) or poly(ethylene glycol) (for positive mode) as an internal standard. NMR spectra were obtained by an AMX2-600 (600 MHz for ^{1}H) spectrometer (Bruker, Tsukuba). Chemical shifts (δ) of ^{1}H NMR are given in parts

Table 3. Partial ^{1}H and ^{13}C NMR Chemical Shifts of ZAD-Cs in D_2O at 288 K[a]

	ZAD-C1		ZAD-C2		ZAD-C3		ZAD-C4		ZAD-C5	
no.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	175.64		175.49		175.54		175.78		176.02	
32′	142.78	5.60	134.96	5.65	140.96	5.62	140.96	5.62	140.96	5.62
33′	126.77	5.60	130.08	5.46	130.98	5.62	130.98	5.62	130.98	5.62
34′	77.67	5.53	72.58	4.56	74.60	4.34	74.60	4.34	74.60	4.34
35′	74.23	3.76	77.37	5.05	74.50	3.82	75.98	3.60	75.98	3.60
36′	74.19	3.58	72.72	3.82	76.54	5.01	72.55	3.79	74.19	3.58
37′	70.66	4.10	70.66	4.10	69.74	4.25	74.36	5.45	70.00	4.00
38′	42.42	1.81,1.75	42.42	1.81,1.75	42.42	1.81,1.75	39.92	1.93,1.88	39.92	1.95, 1.88
39′	72.17	3.84	72.17	3.84	72.17	3.84	72.17	3.84	76.28	5.09
40′	35.53	1.85,1.51	35.53	1.85,1.51	35.53	1.85,1.51	35.53	1.85, 1.51	32.63	1.96, 1.74
41′	25.93	1.78,1.66	25.93	1.78,1.66	25.93	1.78,1.66	25.93	1.78, 1.66	26.06	1.72, 1.61
42′	84.80	4.60	84.80	4.60	84.80	4.60	84.80	4.60	84.48	4.59

[a] The signals due to the other positions were indistinguishable from the data for ZAD-C5 (Table 2).

Chart 1. Structures of ZAD-A, ZAD-B, ZAD-Cs, and 1−27 Segment[a]



[a] ZAD-C1 to C5 are the isomeric constituents lactonized at the positions of 34′, 35′, 36′, 37′, and 39′, respectively

per million (ppm) relative to the internal standard peak at δ 3.75 (dioxane) for D_2O solutions and at δ 3.30 (residual CD_2HOD) for CD_3OD solutions. Coupling constants (J) are in Hz. Chemical shifts (δ) of ^{13}C NMR are given in ppm relative to the internal standard peak at δ 69.3 (dioxane) for D_2O solutions and δ 49.0 for CD_3OD. HPLC was carried out using a Jasco PU-880 pump system equipped with a Jasco UV-875 UV/vis detector.

Culture. The dinoflagellate *Symbiodinium* sp. HA3−5 (no. CCMP2548), which is deposited at the Provasoli-Guillard National Center of Culture of Marine Phytoplankton (CCMP) (Maine, USA), was used for this study. The cells were cultured in a 3-L glass bottle containing 2 L of seawater and 20 mL of ES supplement under 12-h light and 12-h dark conditions at 25 °C. The ES supplement consists of the following in 20 mL of distilled water: $NaNO_3$ (70 mg), Na_2glycerophosphate·$5.5H_2O$ (15 mg), Fe-EDTA·$3H_2O$ (1 mg), Na_2EDTA·$2H_2O$ (5 mg), Tris (100 mg), H_3BO_3 (1 mg), thiamine·HCl (100 μg), biotin (1 μg), vitamin B_{12} (2 μg), $MnCl_2$·$4H_2O$ (314 μg), $ZnCl_2$·$2.5H_2O$ (32 μg), and $CoCl_2$·$6H_2O$ (8 μg). After 6 weeks, the culture media were removed by decantation, and the cells were torn off with a brush and collected by filtration. The cells were kept at −80 °C until used.

Extraction and Isolation. The frozen cells (103.9 g from 132 L of culture) were homogenized in 70% EtOH (200 mL) with an ULTRA-TURRAX T25 (Janke & Kunkel GmbH & Co. KG IKA-Labortechnik, Germany), soaked for 3 days at 4 °C, and centrifuged. The supernatant was collected, and the precipitates were extracted twice with 70% EtOH (200 mL each). The combined ethanolic extracts were concentrated in vacuo. The residue was suspended in water (110 mL) and extracted with EtOAc (3 × 200 mL) and then BuOH (3 × 150 mL). The BuOH soluble fraction (976 mg) was applied to a polystyrene column (40 mL of MCI CHP-20P 75−150 μm, Mitsubishi Chemical Industries Ltd., Tokyo, Japan), and the column was eluted with water (140 mL),

20% EtOH (180 mL), 40% EtOH (220 mL), 60% EtOH (140 mL), 80% EtOH (180 mL), and EtOH (180 mL) sequentially. The 40% EtOH eluate (158.4 mg) was applied on a DEAE Sephadex A-25 column (4 mL, Pharmacia Biotech Wikstroms, Sweden) and eluted with 33 mM phosphate buffer (pH 6.9, 40 mL) and then the same buffer containing 0.2 M NaCl (20 mL). Each fraction was passed through a polystyrene column to remove inorganic salts. A fraction (fr. 3, 49.3 mg) eluted with 33 mM phosphate buffer was purified by HPLC on a YMC-Pack D-ODS-5 (20 mm $\phi \times$ 250 mm, YMC Ltd, Kyoto) with 70% MeOH containing 20 mM NH_4OAc at a flow rate of 8.0 mL min^{-1} to give ZAD-A (5.6 mg, 0.0057%, $t_R = 14.1$ min), ZAD-B (9.6 mg, 0.0097%, $t_R = 17.8$ min), and ZAD-Cs (21.8 mg, 0.022%, $t_R = 21.2$ min).

Zooxanthellamide Cs (ZAD-Cs): Colorless amorphous solid; UV (MeOH) λ_{max} 233 (ϵ 39 000) nm; IR (KBr) 3385, 1720, 1645, 1250, 1215, 1065, 975 cm^{-1}; ESI TOF MS (negative) *m*/*z* 1347.72 $[M - 2H]^{2-}$, 898.14 $[M - 3H]^{3-}$; HR ESI TOF MS found *m*/*z* 1347.6912 $[M - 2H]^{2-}$, single charged conversion 2697.3980, calcd for $C_{128}H_{220}N_2O_{53}S_2$ [M] 2697.4016. For ^1H and ^{13}C NMR data, see Tables 2 and 3.

Hydrolysis Reaction of ZAD-Cs to ZAD-A. ZAD-Cs (13.5 mg) was dissolved in 1.5 mL of 0.1 M KOH in 90% aqueous MeOH and stirred at room temperature for 2 h. The solution was diluted with water (10 mL) and passed through a polystyrene column (2.6 mL of MCI CHP-20P 75−150 μm) to remove the alkali metal. The sample was eluted with 80% aqueous EtOH, and the eluate was concentrated to give a crude material (11.6 mg). The product was purified by HPLC on a YMC-Pack D-ODS-5 (20 mm $\phi \times$ 250 mm) with 70% MeOH containing 20 mM NH_4OAc at a flow rate of 8.0 mL min^{-1} to give ZAD-A (7.7 mg) as an amorphous solid: $[\alpha]^{24}_D$ +3.4° (*c* 0.30, MeOH) (lit.: $[\alpha]^{29}_D$ +1.9° (*c* 0.36, MeOH).[14]

Hydrolysis Reaction of ZAD-A to the 1−27 Segment. A 2 M solution of LiOH (0.4 mL) was added to a cooled solution of ZAD-A (4 mg) in MeOH (0.2 mL), and the mixture was stirred at room temperature for 21 h. The reaction mixture was neutralized with 2 M HCl, diluted with water (15 mL), and then applied to a CHP-20 column (5 mm $\phi \times$ 40 mm). The column was washed with water (3 mL) and eluted with 20% EtOH (4 mL). The eluate was concentrated and subjected to an ODS column (COSMOSIL 75C18-OPN, 8 mm $\phi \times$ 60 mm, Nacalai Tesque Inc., Kyoto), which was eluted with 40% MeOH in 20 mM NH_4OAc. The first 6-mL fraction (1 mg) was then purified by HPLC [CAPCELL PAK C18 SG120 (10 mm $\phi \times$ 250 mm, Shiseido, Tokyo), 40% MeOH in 20 mM NH_4OAc, flow rate 3.0 mL/min, UV 230 nm) to afford the 1−27 segment (0.4 mg): amorphous solid, $[\alpha]^{20}_D$ −8° (*c* 0.02, MeOH); UV (MeOH) 230 nm (ϵ 20 000) nm; ^1H NMR (CD_3OD) δ 6.34 (1H, dd, $J = 15.1$, 10.3 Hz, H-25), 6.12 (1H, dd, $J = 15.1$, 10.3 Hz, H-24), 5.78 (1H, dt, $J = 15.1$, 7.7 Hz, H-23), 5.75 (1H, dt, $J = 15.5$, 7.6 Hz, H-9), 5.68 (2H, m, H-13,14), 5.65 (1H, m, H-26), 5.60 (1H, dd, $J = 15.5$, 8.1 Hz, H-19), 5.54 (1H, dd, $J = 15.5$, 6.3 Hz, H-10), 5.47 (1H, dd, $J = 15.5$, 6.6 Hz, H-20), 4.25 (1H, dt, $J = 12.6$, 7.1 Hz, H-15), 4.09 (1H, m, H-3), 4.07 (1H, m, H-21), 3.97 (1H, m, H-5), 3.94 (1H, m, H-12), 3.92 (1H, m, H-11), 3.83 (1H, m, H-7), 3.58 (1H, m, H-17), 3.48 (2H, d, $J =$ 5.8 Hz, H-27), 2.36 (1H, m, H-2), 2.33 (1H, m, H-22), 2.29 (1H, m, H-22), 2.27 (1H, m, H-2), 2.25 (1H, m, H-8), 2.24 (1H, m, H-18), 2.20 (1H, m, H-8), 1.65 (1H, m, H-6), 1.62 (1H, m, H-16), 1.61 (2H, m, H-4), 1.55 (1H, m, H-6), 1.53 (1H, m, H-16), 1.01 (3H, d, $J =$ 6.9 Hz, 18-Me). HR ESI TOF MS (positive) *m*/*z* 558.3233, calcd for $C_{28}H_{48}O_{10}N$ [M + H] 558.3273.

Biological Activity Test. The vasoconstrictive activity of ZADs and ZT-A was performed by following the method described in the literature.[10]

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Supporting Information Available: (1) spectra (IR, COSY, DQF-COSY, HOHAHA, HSQC, and HMBC) of ZAD-Cs, (2) NMR data of ZAD-A measured in D_2O, (3) spectra (^1H NMR, DQF-COSY, and HOHAHA) of the 1−27 segment. This material is available free of charge via the Internet at http://pubs.acs.org.

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