Headline Articles

Studies on Polyketide Metabolites of a Symbiotic Dinoflagellate, *Symbiodinium* sp.: A New C30 Marine Alkaloid, Zooxanthellamine, a Plausible Precursor for Zoanthid Alkaloids

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In studies on the biogenesis of vasocontrictive macrolides, zooxanthellatoxins isolated from a symbiotic dinoflagellate *Symbiodinium* sp., we have investigated metabolites of the dinoflagellate cultured under different conditions. Four new compounds were isolated from 70% EtOH extract of the cells cultured in f/2 medium. Two betaines (zooxanthellabetaine-A and -B) were obtained from a neutral fraction of *n*-BuOH soluble portion and the structure of zooxanthellabetaine-A was determined as 4-(4-hydroxybenzoyloxy)-3-(trimethylammonio)butyrate. The EtOAc soluble portion afforded a new C-30 alkaloid, zooxanthellamine, and a new ceramide, symbioramide-C16. The structural similarity of zooxanthellamine to zoanthid alkaloids, zoanthamines, suggested an algal origin of these zoanthamines. Zooxanthellamine might be derived biogenetically from a polyketide chain presumably started from a glycine unit, like other marine toxins such as zooxanthellatoxin and palytoxin.

Marine microalgae produce various types of compounds, including nitrogenous neurotoxins, polyether sea food toxins, sulfonium compounds of dimethyl sulfide precursors and antineoplastic macrolides.[1—3] Among them, dinoflagellate metabolites such as ciguatoxin and maitotoxin are unique and spectacular in terms of their complex structures, potent activities and intricate biogenesis.[4] Symbiotic dinoflagellates so-called zooxanthellae are distributed over a wide range of marine invertebrates[5,6] and were thought to produce bioactive metabolites which were isolated from marine invertebrates containing zooxanthella inside of the body. However, most trials failed to establish the production of bioactive substances by symbiotic organisms because of difficulties of their culture and adjustment of the culture conditions for the desired metabolite production.[7,8]

In the course of our studies on biological origins of marine toxins such as palytoxins,[9] we have isolated vasoconstrictive macrolides (zooxanthellatoxins) (Fig. 1) from one species of *Symbiodinium* (strain No. Y-6)[10,11] isolated from a flat worm *Amphiscolops* sp. (Scheme 1). Since there are structural similarities, such as polyhydroxylated polyketide back bone plausibly started from a glycine unit and the presence of a small number of tetrahydropyran rings in the large molecules, we started comparative studies of their stereostructures and the metabolic pathway.[12] Recently, palytoxin congeners were shown to be produced by a dinoflagellate *Osteropsis siamensis* by Yasumoto et al.,[13] supporting a micro-algal origin of palytoxins.

During our studies on their biosynthesis, we found that the composition of metabolites depended on the culture medium, i.e., the cells cultured in sea-water medium f/2[14] containing a relatively lower amount of organic materials gave EtOH extracts with different metabolite composition from that of the cells cultured in 1% ES (Erd-Shreiber) medium.[10a] We focused on nitrogenous components and polyketide metabolites in connection to zooxanthellatoxins; several nitrogenous substances were isolated. Here we report the structure of zooxanthellamine (ZA, **4**), a new C30 alkaloid having a similar structure to those of zoanthamines isolated from *Zoanthus*, together with three new nitrogen-containing compounds: ZB-A (**3a**), ZB-B (**3b**) (Fig. 2), and symbioramide-C16 (**5**).

Results and Discussion

Culture and Isolation. *Symbiodinium* sp. (strain Y-6) grew slowly in a medium either 1% ES or f/2. The cells

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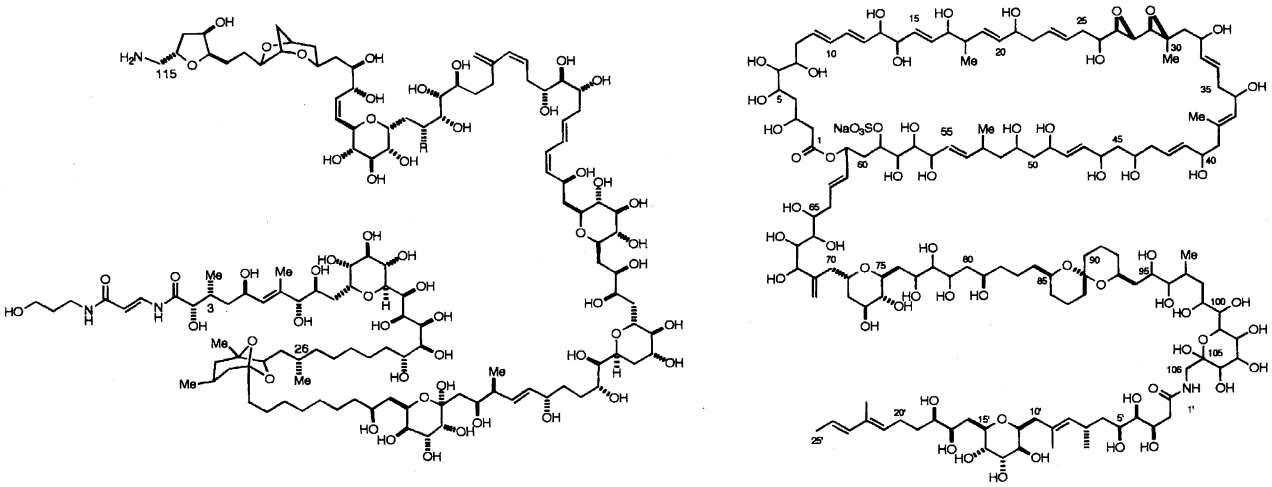
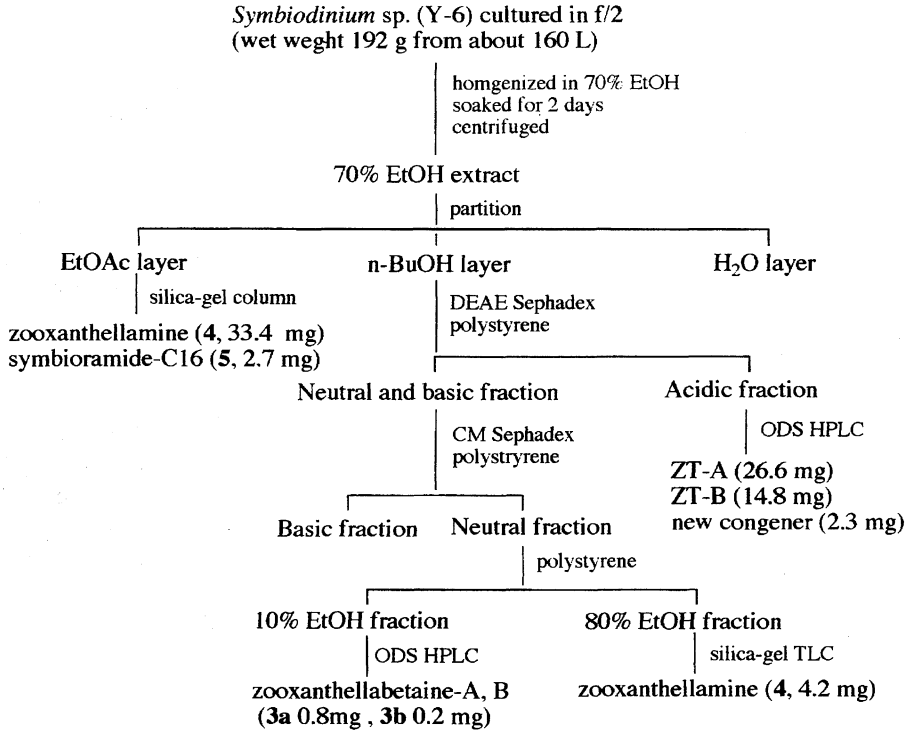


Fig. 1. Polyoxygenated long-chain amino acid derivatives, palytoxin (**1**) and zooxanthellatoxin-A (**2**).



Scheme 1. Isolation procedure for nitrogenous compounds (**3**—**5**) and zooxanthellatoxins.

reached a stationary phase at 4—5th week and were harvested at 5—9th week after inoculation of a 1/10 amount of a stationary culture, which consisted mostly of immortal cells. The amount of wet cells obtained was slightly dependent on the culture period and the medium components. The f/2 medium showed better growth than 1% ES to result in about 1.5 g wet cells/L at 9 weeks culture. Extraction effectiveness of the metabolites, especially *n*-BuOH soluble portion, was strongly dependent on the extraction process. Treatment of the cultured cells with 70% EtOH (homogenizing by an ultra-dispenser, soaking for several days, and centrifuging) gave the best result by three repetitions of the process, although the residual cells still gave a large amount of a mixture of glycerolipids by extracting with $CHCl_3$–MeOH. The viscous extracts obtained after evaporation were suspended in water and extracted with EtOAc and then with *n*-BuOH.

The *n*-BuOH soluble portion was fractionated first on ion exchange columns to acidic, basic, and neutral materials and then on a polystyrene column. The acidic fraction yielded zooxanthellatoxins (ZTs) in a lower yield than that of 1% ES (less than 1/2) and a new congener modified at the common terminal acid structure (the detailed structure has not yet been determined). The neutral portion afforded two nitrogen containing compounds, zooxanthellbetaine-A (ZB-A, **3a**) and ZB-B (the structure was not confirmed), after polystyrene column chromatography (10% EtOH) followed by

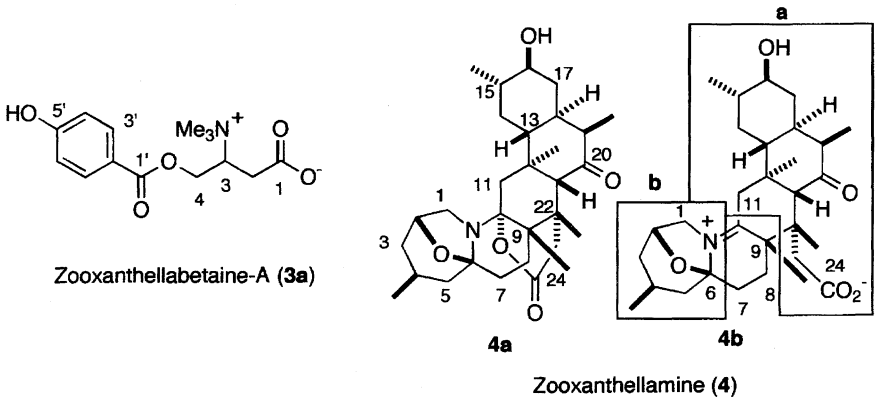


Fig. 2. Zooxanthellabetaine-A (**3a**) and zooxanthellamine (two forms, **4a** and **4b**).

HPLC separation, and 80% EtOH eluate yielded a new alkaloid, zooxanthellamine (ZA, **4**) after silica-gel TLC (95 : 5 CH_2Cl_2–MeOH).

After the structure of ZA was solved, we reinvestigated the EtOAc soluble portion. Silica-gel column chromatography afforded a large amount of ZA (33.4 mg) and a new ceramid named symbioramide-C16 (**5**) in EtOAc and 9 : 1 CH_2Cl_2–MeOH eluates, respectively.

Zooxanthellabetaine-A. ZB-A (**3a**) showed a pseudo-molecular ion at *m/z* 282.1357 in the positive HR-FAB-MS [$(M+H)^+$, $\Delta+1.6$ mmu] suggesting a molecular formula $C_{14}H_{19}NO_5$. The ^{1}H NMR spectrum in D_2O showed three sets of proton networks for three methyls [$\delta=3.14$ (9H, s)], a sequence of CH_2CHCH_2 [$\delta=2.66$ (1H, dd, $J=15$, 9 Hz), 2.86 (1H, dd, $J=15$, 3 Hz), 4.07 (1H, ddt, $J=9$, 3, 3 Hz), 4.71 (2H, d, $J=3$ Hz)], and a 1,4-disubstituted benzene ring [$\delta=6.65$ (2H, d, $J=9$ Hz), 7.76 (2H, d, $J=9$ Hz)]. The ^{13}C NMR spectrum of ZB-A gave eight detectable signals which were assigned and connected by the HSQC and HMBC spectra. Trimethylammonium group was determined on the basis of the ^{1}H and ^{13}C chemical shifts and located on C-3 carbon based on a HMBC cross peak from the methyl protons to C-3 methine carbon ($\delta_C=71.1$). The connection between C-1 carbonyl carbon ($\delta_C=175.7$) and C-2 carbon was established by 2-H_2/C-1 cross peaks. The undetermined partial structure of ZB-A was consistent with that of $C_7H_4O_3$ and a 4-hydroxybenzoate moiety was suggested by the UV spectrum (λ_{max} 259 nm). Although C-2′ and C-5′ carbon signals were missing, the ^{13}C chemical shifts for C-3′ ($\delta=132.4$) and C-4′ ($\delta=117.3$) carbons unambiguously validated the structure of 4-hydroxybenzoate portion, while the connection between C-1′ and C-4 was proved by 3′-H/C-1′ ($\delta=167.7$) and 4-H/C-1′ cross peaks. Thus, the structure of ZB-A was shown as 4-(4-hydroxybenzoyloxy)-3-(trimethylammonio)butyrate. The basic betaine structure of ZB-A is reported[15] as a synthetic compound which is a regioisomer of carnitine, an essential cofactor of fatty acid synthesis. ZT-B contained both 4-hydroxybenzoate and trimethylammonium functionalities like ZT-A but the full structure has not been determined yet.

Zooxanthellamine. The compound designated zooxanthellamine (ZA, **4**) showed a pseudo-molecular ion at *m/z* 498.3210 [$(M-H)^-$, $\Delta-0.9$ mmu] in the negative FAB-MS, confirming a molecular formula of $C_{30}H_{45}NO_5$. The ^{1}H and ^{13}C NMR spectra of ZA showed large solvent dependency (Table 1). In either D_2O or CD_3OD, only 29 carbon signals could be observed because of deuterium exchange of C-11 methylene protons. The HSQC spectrum in D_2O established the presence of three secondary and three tertiary methyls, eight methylenes including one nitrogen-bearing carbon, two oxy- and six alkyl-methines, and three sp^2 and four sp^3 quaternary carbons. The DQFCOSY and TOCSY spectra in D_2O allowed us to follow the carbon connectivities of C-1 to C-5, C-7 to C-8, and C-13 to C-19, and to locate three secondary methyl groups at C-4, C-15, and C-19 carbons.

The HMBC spectrum in D_2O was useful to connect the carbon networks. Cross peaks from 19-Me and 21-H protons to C-20 carbonyl carbon proved the connectivity from C-19 to C-21. 12-Me protons showed cross peaks to C-11, C-12, C-13, and C-21 carbons, which verified the existence of not only the cyclohexanone structure but also one missing methylene carbon (C-11, $\delta=42.45$). The sequence from C-21 to C-24 was confirmed by HMBC cross peaks, 22-Me/C-21, 22-Me/C-23, and 23-H_2/C-24 (partial structure **a**, from C-11 to C-24). Chemical shifts of C-1 ($\delta=54.93$) and C-6 ($\delta=98.41$), coupled with HMBC cross peaks, 5-H_2/C-6 and 2-H/C-6, allowed us to construct a bicyclo[3.2.1] amino acetal structure (partial structure **b**). The HMBC cross peaks, 9-Me to C-10, C-9, C-8, and C-22, and 1-H_2 to C-10 enabled us to connect all of the carbons in ZA, i.e., C-7–C-8 methylene and the partial structures **a** and **b**. Connections between C-6 and C-7, and C-10 and C-11 resulted in the stable structure **4b**. The connection between C-10 and C-11 was confirmed by HMBC cross peak from 11-H to C-10 in $CDCl_3$. The zwitter ion structure of carboxylate and iminium is consistent with their ^{13}C chemical shifts (C-10, $\delta=191.80$, and C-24 $\delta=178.95$). In either $CDCl_3$ or CD_3OD, ZA adopted a lactone structure **4a** which was established by ^{13}C chemical shifts of C-10 carbon ($\delta=102.01$ in $CDCl_3$, and 116.3 in CD_3OD). Thus the structure for ZA is shown as **4a** and **4b** in Fig. 2.

The relative stereochemistry was elucidated on the basis of the proton coupling constants ($J_{4\text{-}5}=13$ Hz, $J_{13\text{-}18}=11$ Hz,

Table 1. 400 MHz ^{1}H and 100 MHz ^{13}C NMR Data of Zooxanthellamine in D_2O, $CDCl_3$, and CD_3OD[a)]

Solvent	D_2O		$CDCl_3$		CD_3OD	
No.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.05 (dd, 13, 7)	54.93	3.24 (d, 10)	47.10	3.32 (m)	48.6
	4.15 (d, 13)		3.24 (dd, 10, 5)		3.37 (d, 8)	
2	4.86 (brd, 7)	74.22	4.54 (brd, 5)	74.20	4.54 (brd, 6)	75.33
3	1.54 (tm, 13)	35.50	1.47 (td, 12, 2)	38.84	1.40 (m)	39.31
	1.76 (m)		1.56 (m)		1.63 (m)	
4	1.81 (m)	22.68	2.27 (m)	22.90	2.20 (m)	23.73
5	1.38 (dd, 13, 14)	40.98	1.07 (m)	44.44	1.05 (m)	45.32
			2.09 (m)		2.07 (dd, 13, 5)	
	2.48 (dd, 14, 4)					
6	—	98.41	—	89.90		91.60
7	1.77 (m)	27.78	1.77 (m)	29.96	1.76 (m)	38.77
	2.19 (dt, 14, 2)		1.89 (td, 14, 5)		1.91 (td, 13, 4)	
8	1.65 (m)	24.77	1.54 (m)	23.67	1.62 (m)	24.43
	2.67 (brt, 14)		1.68 (td, 11, 4)		1.69 (td, 12, 2)	
9	—	44.35	—	39.73	—	42.06
10	—	191.80	—	102.01	—	116.3[b)]
11	—	(42.45)[b)]	1.83 (d, 14)	42.79	—	(43.20)[b)]
			2.11 (d, 14)			
12	—	45.65	—	40.04	—	40.90
13	1.98 (td, 11, 3)	47.20	1.57 (m)	50.85	1.78 (m)	51.22
14	0.91 (q, 11)	31.44	0.87 (m)	32.20	0.88 (m)	33.26
	1.59 (m)		1.61 (m)		1.76 (m)	
15	1.29 (m)	38.35	1.38 (m)	39.53	1.36 (m)	40.27
16	3.13 (td, 11, 3)	75.11	3.18 (brt, 11)	75.62	3.08 (td, 10, 4)	76.10
17	1.29 (q, 11)	36.70	1.38 (q, 11)	37.95	1.39 (m)	30.41
	1.62 (m)		1.74 (m)		1.69 (m)	
18	1.89 (tm, 12)	37.97	1.97 (m)	38.56	1.97 (m)	39.86
19	2.36 (quin, 7)	49.91	2.40 (quin, 7)	49.90	2.33 (quin, 7)	51.30
20	—	216.96	—	213.87	—	216.11
21	3.17 (s)	56.64	3.12 (s)	54.34	3.23 (s)	55.38
22	—	51.25	—	35.86	—	37.61
23	1.65 (d, 14)	42.82	2.34 (d, 20)	35.95	2.42 (d, 20)	37.25
	3.07 (d, 14)		3.69 (d, 20)		3.48 (d, 20)	
24	—	178.95	—	172.80	—	176.73
4-Me	0.87 (d, 7)	20.20	0.90 (d, 7)	21.81	0.89 (d, 7)	21.73
9-Me	1.34 (s)	23.87	1.19 (s)	18.14	1.23 (s)	19.03
12-Me	0.78 (s)	17.70	0.88 (s)	18.31	0.84 (s)	18.30
15-Me	0.88 (d, 7)	17.57	1.06 (d, 7)	18.36	1.03 (d, 7)	18.62
19-Me	1.08 (d, 7)	12.71	1.10 (d, 7)	13.55	1.13 (d, 7)	13.48
22-Me	1.16	18.70	0.95 (s)	20.79	0.98 (s)	20.90

a) Chemical shifts are expressed in ppm by using internal standard (TMS $\delta_H = \delta_C = 0$ for $CDCl_3$, HOD $\delta_H = 4.70$ and dioxin $\delta_C = 66.5$ for D_2O, CHD_2OD $\delta_H = 3.3$; $\delta_C = 49.3$ for CD_3OD) at 20 °C. Multiplicities (s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet or overlapped) and coupling constants (J) are in parenthesis. b) Determined by HMBC spectra.

$J_{15\text{-}16} = 10$ Hz, $J_{18\text{-}19} = 7$ Hz) in either D_2O or CD_3OD and NOE data obtained by NOESY and GOESY[16)] experiments in CD_3OD. The absolute configuration was established by a modified Mosher method.[17)] Positive and negative values of the difference of chemical shifts between *S*- and *R*-MTPA esters clearly suggested the absolute configuration to be 2*R*, 4*S*, 6*S*, 9*S*, 10*R*, 12*S*, 13*R*, 15*S*, 16*S*, 18*S*, 19*R*, 21*S*, 22*S* as shown in Fig. 3.

The structure of ZA was quite similar to zoanthamine alkaloids (Fig. 4). There are eleven related compounds have been isolated so far as from Indian,[18)] Arabian.[19)] and Japanese[20)] zoanthids of the genus *Zoanthus*. Zoanthamine was first isolated from Indian zoanthid *Zoanthus* sp. of unknown biosynthetic origin (probably triterpenoid origin).[18a)] Recently, Uemura et al. reported five new compounds and proposed a polyketide origin for zoanthamine alkaloids.[20a)] Further, they reported the absolute configuration of norzoanthamine (same as for zooxanthellamine) and their osteoporotic activity.[20b)] The structural similarity of zooxanthellamine to zoanthamine alkaloids including the absolute configuration might suggest that the zoanthamine alkaloids from zoanthids are symbiotic and/or dietary algal in origin.

Symbioramide-C16. Ceramide (**5**), named as symbioramide-C16, isolated from the EtOAc fraction displayed a pseudo-molecular ion at *m/z* 554.5153 $(M+H)^+$, indicating a molecular formula of $C_{34}H_{67}NO_4$ (Fig. 5). Analyses of

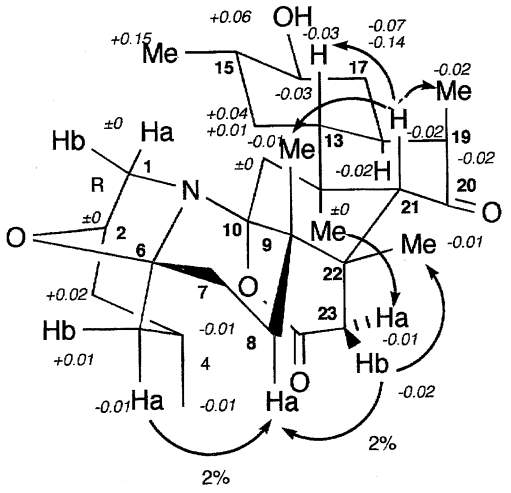
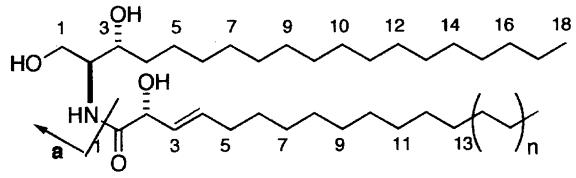


Fig. 3. Absolute configuration of zooxanthellamine (**4b**), NOE data from NOESY (↔) and GOESY (←), and $\Delta\delta$ values [$\delta_{(S\text{-MTPA ester})} - \delta_{(R\text{-MTPA ester})}$] in italic.

^{1}H and ^{13}C NMR signals by DQFCOSY, DEPT, HSQC, and HMBC experiments suggested a ceramide structure in which C_{16} carboxylic acid was assigned based on a fragment ion at *m/z* 302 in a linked-scan positive FAB-MS (cleaved at **a**).[21] The relative and absolute configuration of **5** was determined to be the same as that of symbioramide (**6**)[22] by comparing the ^{1}H NMR spectral data and optical rotation with those of symbioramide and its diastereomer.[23]

Conclusion

We have isolated four new nitrogenous compounds and zooxanthellatoxin-A and -B and a new congener from *Symbiodinium* sp. cultured in f/2 medium. Among them the minor metabolites, zooxanthellabetaines and symbioramide-C16 were difficult to be rationalized biogenetically with each others; however, it is noteworthy to point out that symbioramide-C16 is a fatty acid variant of symbioramide isolated from a different species of *Symbiodinium*. Zooxanthellamine seemed to be derived biogenetically from a polyketide precursor (a polyketide chain from C24 to C1) which presumably started from a glycine unit like other marine toxins such as zooxanthellatoxins and palytoxins; this



Symbioramide-C16 (**5**) : n=1
Symbioramide (**6**) : n=2

Fig. 5. Symbioramide-C16 (**5**) and symbioramide (6).

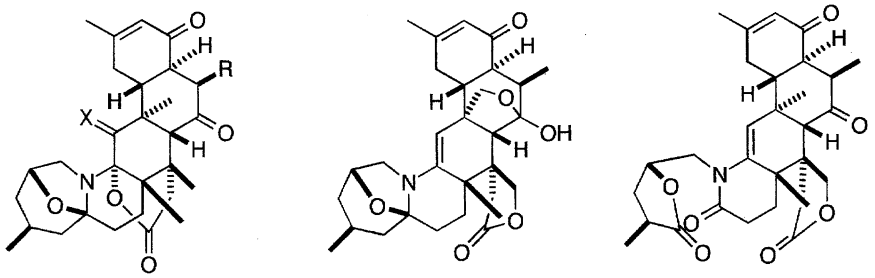
may be a common feature in dinoflagellates. Isolation yield of zooxanthellamine from f/2 cells is 5 times more than that from 1% ES cells, in contrast to a decrease in the amounts of zooxanthellatoxins.

Although no one has yet examined whether zoanthids of the genus *Zoanthus* containing zoanthamines have a symbiotic algae (so called zooxanthellae) in the body, it was reported that several *Symbiodinium* sp. were isolated from *Zoanthus* sp.[5] Relatively high content of zooxanthellamine in *Symbiodinium* (0.019% isolation yield from the wet cells) could account for algal origin of zoanthamine alkaloids via symbiotic and/or food chain mechanisms (3—15 times higher than zoanthids). Zooxanthellamine could be used as a marker to investigate symbiotic or food chain relationships which involve unknown mechanisms to accumulate specific compounds. Biosynthesis and biotransformation of zooxanthellamine using labeled compounds is now under investigation in our laboratory.

Experimental

Generals. UV spectra and optical rotations were recorded on a Shimadzu UV-160 photometer and a JASCO DIP-360 polarimeter, respectively. FAB- and EI-MS were measured on JEOL JMS-SX102A, JMS-AX500, and JMS-600H. NMR spectra were obtained on JEOL Alpha-400 (^{1}H 400 MHz, ^{13}C 100 MHz), Alpha-600 (^{1}H 600 MHz, ^{13}C 150 MHz), and AL-300 (^{1}H 300 MHz, ^{13}C 75 MHz) spectrometers. Solvents were reagent grade and used as received unless otherwise stated.

Culture. Symbiotic dinoflagellate (strain Y-6) was cultured in a 3 L flask containing 2 L medium f/2 under 12 light and 12 dark conditions at 20 °C. The medium contained the following additives in 1 L of a 9 : 1 mixture of sea water distilled water: $NaNO_3$ (75 mg), NaH_2PO_4 (5 mg), EDTA (4 mg), vitamin B_{12} (0.5 μg), biotin



Zoanthamine: X-H_2, R=Me
Zoanthaminone: X=O, R=H
Norzoanthamine: X=H_2, R=Me
Norzoanthaminone: X=O, R=H
Oxyzoanthamine: X=H_2, R=CH_2OH

Zoanthenamine

Zoanthamide

Fig. 4. Typical zoanthamine alkaloids from *Zoanthus* sp.

(0.5 μg), thiamine (100 μg), $FeCl_3 \cdot 6H_2O$ (3 μg), $ZnSO_4 \cdot 7H_2O$ (22 μg), $CoCl_2 \cdot 6H_2O$ (10 μg), $MnCl_2 \cdot 4H_2O$ (190 μg), $CuSO_4$ (9.8 μg), and soil extract (5 mL). A 1/10 amount of the cells at stationary phase was inoculated to a fresh medium. After 9 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 −20 °C until use.

Extraction and Isolation. The frozen cells (192 g) were immersed in 70% EtOH (700 mL) and then broken by an ultra-disperser, soaked for 2 d, and centrifuged. The supernatant was collected and the extraction process was applied to the pellet two more times with 70% EtOH (600 and 500 mL). The combined extracts were evaporated in vacuo with an aid of *n*-BuOH. The residue was suspended in water (150 mL) and extracted with EtOAc (150 mL×3) and *n*-BuOH (150 mL×3).

The *n*-BuOH soluble portion after evaporation was dissolved in water and applied on a DEAE Sephadex A-25 and eluted with water, 0.2 M NaCl, 0.4 M NaCl, and then 1 M NaCl (1 M = 1 mol dm^{-3}). Water eluate was passed through a CM Sephadex C-25 (Pharmacia Biotec) column and subjected to a polystyrene column (MCI CHP-20P 75—150 μm, Mitsubishi Chemical Industries Ltd.). The retained material was eluted by decreasing the solvent polarity with EtOH. 10% EtOH fraction was purified by HPLC chromatography on a Develosil ODS-5 (20 mmϕ×250 mm, Nomura Chemical) with 1 : 4 MeOH–water at a flow rate of 7.0 mL min^{-1} to give zooxanthellabetaine-A (ZB-A, **3**, t_R = 14.5 min, 0.8 mg, 0.0004%) and ZB-B (t_R = 16.7 min, 0.2 mg, 0.0001%). 80% EtOH eluate was further purified on a silica-gel TLC plate developed with 95 : 5 CH_2Cl_2–MeOH to afford zooxanthellamine (4.2 mg, R_f = 0.5, 0.0022%). 0.2 M NaCl eluate of DEAE Sephadex gave ZT-A (26.6 mg, 0.014%), ZT-B (14.8 mg, 0.0077%), and a new congener (2.3 mg, 0.0012%) by the previously reported method.[10a)]

The EtOAc soluble material (7.1 g) was separated on a silica-gel column with CH_2Cl_2 by increasing the solvent polarity to EtOAc and then with 9 : 1 CH_2Cl_2–MeOH. Zooxanthellamine (**4**, 33.4 mg, 0.017%) was obtained from AcOEt eluate and 9 : 1 CH_2Cl_2–MeOH fraction afforded symbioramide-C16 (**5**, 2.7 mg, 0.0014% yield). Zooxanthellamine was also obtained from the cells cultured in 1% ES in an isolation yield of 0.0033%.

Zooxanthellabetaine-A (3a): Colorless oil; $[\alpha]_D^{20} = -13$ (*c* 0.0035, MeOH); ^1H and ^{13}C NMR see text. HR-FAB-MS Found: *m/z* 282.1357. Calcd for $C_{14}H_{20}NO_5$: M+H, 282.1341.

Zooxanthellamine (4): Colorless solids; mp > 300 °C; $[\alpha]_D^{20}$ = +40 (*c* 0.050, MeOH); ^1H and ^{13}C NMR see Table 1. HR-FAB-MS Found: *m/z* 498.3210. Calcd for $C_{30}H_{44}NO_5$: M+H, 498.3279.

Symbioramide-C16 (5): Colorless solids; mp 106—108 °C; $[\alpha]_D^{24}$ = +6.8 (*c* 0.07, $CHCl_3$); 300 MHz ^1H NMR ($CDCl_3$) δ = 0.88 (6H, t, *J* = 7 Hz, 18-H, 16′-H), 1.26 (br, 46H), 1.39 (2H, m, 16-H), 2.08 (2H, q, *J* = 6.8 Hz, 5′-H), 2.52 (1H, br, 3-OH), 2.64 (1H, br, 1-OH), 3.17 (1H, br, 2′-OH), 3.76—3.83 (3H, m, 1-H, 2-H, 3-H), 4.03 (1H, brd, *J* = 9 Hz, H-1), 4.54 (1H, d, *J* = 7 Hz, H-2′), 5.56 (1H, dd, *J* = 15, 8 Hz, H-3′), 5.90 (1H, dt, *J* = 15, 6 Hz, H-4′), 7.03 (1H, d, *J* = 7 Hz, NH); 100 MHz ^{13}C NMR ($CDCl_3$) δ = 14.10 (C-18, C-16′), 22.67, 28.87, 29.18, 29.33, 29.46, 29.53, 29.58, 29.63, 29.66, 31.89, 32.21 (C-5′), 53.83 (C-2), 73.99 (C-3), 62.22 (C-1), 73.08 (C-2′), 127.23 (C-3′), 136.61 (C-4′), 174.80 (C-1′). HR-FAB-MS Found: *m/z* 554.5153 $(M+H)^+$. Calcd for $C_{34}H_{68}NO_4$: M+H, 554.5148.

Synthesis of Zooxanthellamine MTPA Esters. Zooxanthellamine (1.09 mg, 2.2×10^{-6} mol) was dissolved in CH_2Cl_2 (0.3 mL) and treated with Et_3N (0.01 mL, 7×10^{-5} mol), (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride (0.008 mL, 4×10^{-5} mol) and 4-dimethylaminopyridine (0.1 M in CH_2Cl_2, 0.01 mL, 1×10^{-6} mol) at 20 °C to afford 0.9 mg of a (*R*)-MTPA ester (57% yield). A (*S*)-MTPA ester was obtained by the same manner.

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