Isolation of Peridinin-Related Norcarotenoids with Cell Growth-Inhibitory Activity from the Cultured Dinoflagellate of *Symbiodinium* sp., a Symbiont of the Okinawan Soft Coral *Clavularia viridis*, and Analysis of Fatty Acids of the Dinoflagellate

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Two norcarotenoids, 1 and 2, related to peridinin (3) were isolated from the cultured dinoflagellate of the genus *Symbiodinium*, a symbiont of the Okinawan soft coral *Clavularia viridis*, which contains in abundance anti-tumor marine prostanoids such as clavulones. The structures of 1 and 2 were elucidated on the basis of spectroscopic analysis. These compounds showed significant growth-inhibitory activity *in vitro* toward cancer cells. Analysis of fatty acids of the dinoflagellate was also carried out, suggesting that the marine prostanoids are produced by the host soft coral itself.

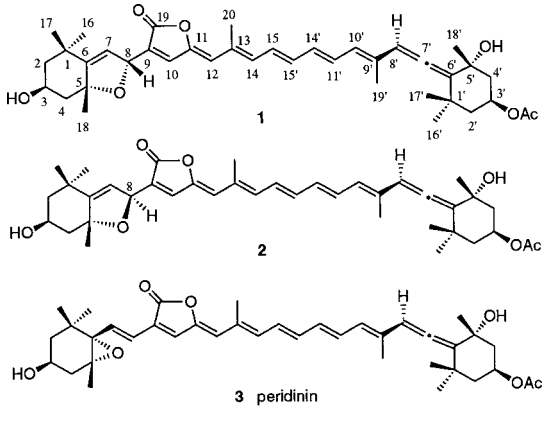
Key words dinoflagellate; *Symbiodinium* sp.; carotenoid; growth-inhibitory activity; symbiotic alga; fatty acid

The Okinawan soft coral *Clavularia viridis* has been recognized as a rich source for marine prostanoids, such as clavulones[1,2] and chlorovulones.[3,4] These marine prostanoids have received much attention owing to their unique structures and remarkable biological activities.[1,3,5,6] Biosynthesis of clavulones was also of interest, and was studied by Corey's group, which proposed a unique biosynthetic pathway,[7—10] essentially different from that for mammalian prostaglandins. However, the organism producing the marine prostanoids (the host soft coral or the symbiotic alga) has not yet been clarified. This fundamental and interesting question led us to investigate chemical constituents involving fatty acids of the symbiotic alga isolated from the host soft coral, *C. viridis*, resulting in the isolation of two peridinin-related norcarotenoids. This paper describes the isolation, structures and biological activity of these norcarotenoids, together with results of the analysis of fatty acids of the alga.

The symbiotic alga isolated from *C. viridis* was identified as a dinoflagellate of the genus *Symbiodinium* by DNA analysis, and was cultured at 23 °C for 40 d. The cultured alga was extracted with MeOH, and the MeOH extract (3.66 g) was partitioned between EtOAc and H_2O. The EtOAc-soluble portion (1.48 g) was chromatographed on a silica gel column to give five fractions (fractions 1—5). A portion of fraction 3 (116 mg) was further chromatographed repeatedly to give compounds **1** (2.4 mg) and **2** (3.8 mg), along with peridinin[11—13] (**3**, 2.2 mg). A portion of fraction 1 (78 mg) was treated with HCl–MeOH at 90 °C for 2 h to give a mixture of fatty acid methyl esters; this was analyzed by gas chromatography (GC).

The molecular formula of compound **1** {a reddish viscous oil, $[\alpha]_D^{25}$ +63.6° ($CHCl_3$)} was found to be $C_{39}H_{50}O_7$ by high-resolution electron impact mass spectra (HR-EI-MS). The degree of unsaturation (fifteen) was given from the molecular formula. All 39 carbons[14] appeared in the ^{13}C-NMR spectrum of **1** (Table 1). The distortionless enhancement by polarization transfer (DEPT) spectrum showed nine methyls, four sp^3 methylenes, three sp^3 oxymethines, ten sp^2 methines, four sp^3 quaternary carbons, eight sp^2 quaternary carbons involving two carbonyls, and one sp quaternary carbon. These spectral data, coupled with the reddish color and absorption maximum at 449 nm (ε 57800), suggested that compound **1** has a conjugated polyene system like carotenoids. The IR spectrum showed the presence of hydroxy (3458 cm^{-1}) and acetoxy (1732, 1250 cm^{-1}) groups. The presence of an allene group was indicated by the IR absorption at 1929 cm^{-1} and by the low-field ^{13}C-NMR signal at δ 202.6 (C, C-7′) ppm. The ^{1}H-NMR spectrum of **1** disclosed three oxymethine protons at δ 4.24 (1H, quint., J=3.9 Hz, H-3), 5.38 (1H, tt, J=12.1, 4.1 Hz, H-3′) and 5.61 (1H, br s, H-8) ppm, ten olefinic protons, eight methyl protons, and one acetoxy methyl proton at δ 2.04 (3H, s), as shown in Table 1. The ^{13}C-NMR signals at δ 168.9 (C) and 170.4 (C) ppm showed the presence of two ester groups, one of which was attributed to the above-mentioned acetoxy group. These spectral data resembled those of peridinin (**3**) (see Experimental), except for the NMR signals due to the 5, 6, 7 and 8 positions.

After assignments of the C–H direct bondings were made based on heteronuclear multiple-quantum coherence (HMQC), the heteronuclear multiple-bond correlation (HMBC) spectrum was measured. Analysis of C–H long-



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range correlations (two or three bonds) in the HMBC spectrum, as shown by dotted lines in Fig. 1, led to the gross structure for **1**. Checking the resultant structure of **1** through references, compounds **1** and **2** were found.[15,16] These compounds were not natural products, but products obtained from peridinin (**3**) by treating **3** with dilute HCl in MeOH (0.03 M) at room temperature. These compounds were found to be diastereomeric at C-8 on the dihydrofuran ring, and the stereochemistry at C-8 was determined based on NOE analysis.[16] The spectral data of the present **1** were identical with those reported.[16] Compound **1** was thus determined to be all-*trans*-(8*R*,6′*R*)-peridinin-5,8-furanoxide.

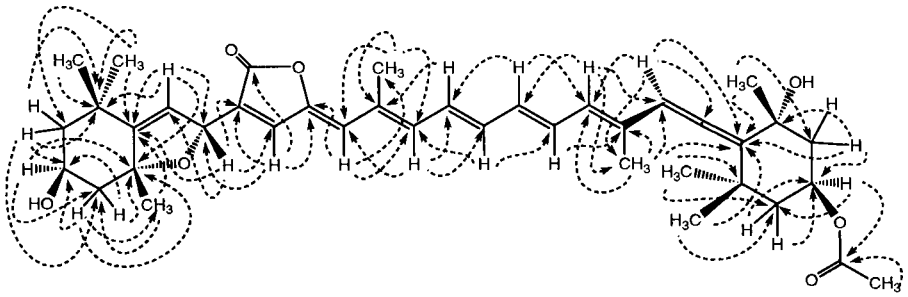

Fig. 1. HMBC Correlations for **1**

Table 1. NMR Data for **1** and **2**[a]

No.	**1**		**2**	
	^{13}C (125 MHz)[b]	^{1}H (500 MHz)[c]	^{13}C (125 MHz)[b]	^{1}H (500 MHz)[c]
1	33.9 (C)		34.2 (C)	
2	46.6 (CH_2)	1.49 (dd, 14.3, 3.9) 1.76 (ddd, 14.3, 3.9, 1.4)	47.1 (CH_2)	1.49 (dd, 14.3, 3.7) 1.79 (m)
3	67.7 (CH)	4.24 (quint, 3.9)	67.8 (CH)	4.28 (quint, 3.7)
4	47.5 (CH_2)	1.94 (dd, 13.6, 3.9) 2.18 (br dd, 13.6, 3.9)	47.0 (CH_2)	1.93 (dd, 13.8, 3.7) 2.19 (m)
5	87.8 (C)		87.9 (C)	
6	153.8 (C)		153.3 (C)	
7	117.8 (CH)	5.53 (d, 1.2)	117.0 (CH)	5.64 (d, 2.1)
8	77.2 (CH)	5.61 (br s)	78.1 (CH)	5.51 (br s)
9	132.4 (C)		133.2 (C)	
10	138.2 (CH)	7.16 (d, 1.3)	137.5 (CH)	7.19 (d, 1.4)
11	146.7 (C)		146.8 (C)	
12	118.8 (CH)	5.69 (s)	118.7 (CH)	5.72 (s)
13	133.8 (C)		133.7 (C)[d]	
14	137.8 (CH)	6.42 (br d, 11.6)	137.7 (CH)	6.43 (br d, 11.5)
15	128.8 (CH)	6.60 (dd, 11.6, 14.2)	128.8 (CH)	6.58 (m)
16	28.6 (CH_3)	1.34 (s)	28.1 (CH_3)	1.32 (s)
17	31.3 (CH_3)	1.16 (s)	31.3 (CH_3)	1.20 (s)
18	28.7 (CH_3)	1.66 (s)	31.0 (CH_3)	1.68 (s)
19	168.9 (C)		169.0 (C)	
20	15.4 (CH_3)	2.21 (s)	15.4 (CH_3)	2.21 (s)
1′	35.8 (C)		35.8 (C)	
2′	45.4 (CH_2)	1.40 (t, 12.1) 1.99 (ddd, 12.1, 4.1, 2.0)	45.4 (CH_2)	1.40 (t, 12.0) 1.99 (ddd, 12.0, 4.1, 1.9)
3′	67.9 (CH)	5.38 (tt, 12.1, 4.1)	67.9 (CH)	5.38 (tt, 12.0, 4.1)
4′	45.2 (CH_2)	1.51 (dd, 12.8, 12.1) 2.28 (ddd, 12.8, 4.1, 2.0)	45.2 (CH_2)	1.50 (m) 2.28 (ddd, 12.9, 4.1, 1.9)
5′	72.7 (C)		72.7 (C)	
6′	117.6 (C)		117.6 (C)	
7′	202.6 (C)		202.6 (C)	
8′	103.3 (CH)	6.05 (s)	103.3 (CH)	6.05 (s)
9′	133.6 (C)		133.6 (C)[d]	
10′	128.1 (CH)	6.10 (br d, 11.8)	128.1 (CH)	6.11 (br d, 11.5)
11′	131.4 (CH)	6.61 (dd, 14.5, 11.8)	131.1 (CH)	6.60 (m)
14′	133.0 (CH)	6.37 (dd, 14.5, 11.0)	133.0 (CH)	6.37 (dd, 14.4, 11.0)
15′	137.1 (CH)	6.50 (dd, 14.2, 11.0)	137.0 (CH)	6.50 (dd, 14.3, 11.0)
16′	31.3 (CH_3)	1.35 (s)	31.3 (CH_3)	1.35 (s)
17′	32.1 (CH_3)	1.07 (s)	32.0 (CH_3)	1.07 (s)
18′	29.2 (CH_3)	1.38 (s)	29.2 (CH_3)	1.38 (s)
19′	14.0 (CH_3)	1.80 (s)	14.0 (CH_3)	1.80 (s)
$CO\underline{CH_3}$	21.4 (CH_3)	2.04 (s)	21.4 (CH_3)	2.04 (s)
$\underline{C}OCH_3$	170.4 (C)		170.4 (C)	

a) Assignments of the ^{13}C and ^{1}H signals were made based on HMQC analysis. *b*) δ ppm in $CDCl_3$. *c*) δ ppm in $CDCl_3$, *J* in Hz. *d*) Values with the subscript may be interchanged.

The molecular formula of compound **2** {a reddish viscous oil, $[\alpha]_D^{25}$ −69.2° ($CHCl_3$)} was found to be $C_{39}H_{50}O_7$, the same as for **1**. NMR data of **2** were almost superimposable on those of **1** except for the signals around the chiral center at C-8, as shown in Table 1. These findings strongly suggested that compound **2** is a diastereomer of **1** at C-8. The spectral data of **2** were shown to be identical with those of **2**[16] obtained from peridinin (**3**). Compound **2** was thus determined to be all-*trans*-(8*S*,6′*R*)-peridinin-5,8-furanoxide.[17]

Peridinin and related compounds were known to exhibit anti-tumor and anti-carcinogenic activities.[18—20] Therefore, compounds **1** and **2** were examined for growth-inhibitory activities *in vitro* toward human cancer cells, as evaluated in the Japanese Foundation for Cancer Res. 39 cell line assay,[21] and the results are summarized in Table 2. Compound **2** showed moderate activities at IC_{50} 2.0—2.8 μg/ml against BSY-1 (breast cancer), SNB-75 [central nervous system (CNS) cancer], HCT-116 (colon cancer), NCI-H522 (lung cancer), DMS114 (lung cancer) and MKN7 (stomach cancer) cells. Interestingly, the activities of **2** were stronger than those of the diastereomeric compound **1**. The pattern of differential growth-inhibition for **2** was evaluated by the Compare Program, and was revealed not to be correlated with that shown by any of the other compounds, including currently used anticancer drugs; the correlation coefficient value was less than 0.5. This indicates that **2** may have a new mode of action.

The mixture of fatty acid methyl esters was analyzed by GC: methyl esters were assigned by comparison of their retention times with those of authentic samples. The main fatty acids observed were palmitic acid (16 : 0), palmitoleic acid (16 : 1), stearic acid (18 : 0), (*E*)-9-octadecenoic acid (18 : 1), γ-linolenic acid (18 : 3), octadecatetraenoic acid (18 : 4) and icosapentaenoic acid (20 : 5). Arachidonic acid (20 : 4), the biosynthetic precursor for marine prostanoids, was not detected in the present analysis, although icosapentaenoic acid as a C_{20} unsaturated fatty acid was observed. Clavulones, the main prostanoids presented in the soft coral *Clavularia viridis*, was also not detected from the EtOAc-soluble portion of the algal extracts. These findings suggested that clavulones may be produced not by the symbiotic alga, but by the host soft coral itself.

Table 2. Growth-Inhibitory Activity for **1** and **2** against 39 Human Cancer Cell Lines

Panel/Cell line	IC_{50} (μg/ml)[a]		Panel/Cell line	IC_{50} (μg/ml)	
	1	**2**		**1**	**2**
Breast cancer			Melanoma		
HBC-4	—[b]	—	LOX-IMVI	—	6.3
BSY-1	4.3	2.6	Ovarian cancer		
HBC-5	—	8.2	OVCAR-3	6.9	4.5
MCF-7	9.5	3.7	OVCAR-4	—	8.2
MDA-MB-231	9.5	8.2	OVCAR-5	—	—
CNS cancer[c]			OVCAR-8	—	3.9
U251	—	7.6	SK-OV-3	—	—
SF-268	—	7.6	Renal cancer		
SF-295	—	6.3	RXF-631L	—	—
SF-539	—	8.2	ACHN	—	—
SNB-75	—	2.2	Stomach cancer		
SNB-78	—	8.2	St-4	—	7.6
Colon cancer			MKN1	6.9	7.6
HCC2998	—	8.8	MKN7	5.0	2.8
KM-12	—	8.2	MKN28	—	—
HT-29	—	—	MKN45	—	8.2
HCT-15	—	8.2	MKN74	4.4	3.0
HCT-116	4.2	2.8	Prostate cancer		
Lung cancer			DU-145	—	—
NCI-H23	—	5.2	PC-3	9.5	—
NCI-H226	—	3.6			
NCI-H522	5.4	2.3			
NCI-H460	—	—			
A549	—	5.9			
DMS273	7.6	6.2			
DMS114	3.3	2.0			

a) Each IC_{50} value was estimated from its GI_{50} value, the concentration that yielded 50% growth. *b*) The value was more than 10 μg/ml. *c*) CNS: central nervous system.

Experimental

General Experimental Procedures Optical rotations were measured with a JASCO DIP-370 automatic polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1600 spectrophotometer, and visible (VIS) spectra with a JASCO V-520 spectrophotometer. All NMR spectra were recorded with a Bruker DRX-500 (^1H; 500 MHz, ^{13}C; 125 MHz) in $CDCl_3$. ^1H–^1H correlation spectroscopy (COSY), HMQC and HMBC spectra were measured by a Bruker DRX-500 using standard Bruker pulse sequences. Chemical shifts are given on a δ (ppm) scale with $CHCl_3$ (^1H; 7.26 ppm, ^{13}C; 77.0 ppm) as the internal standard (s; singlet, d; doublet, t; triplet, q; quartet, m; multiplet, br; broad). MS were taken with a Micromass Auto Spec spectrometer. Column chromatography was carried out on silica gel 60 (70—230 mesh, Merck) and Sephadex LH-20 (Pharmacia). HPLC was conducted with a YMC-Pack SIL-06 column (silica gel, SH-043-5-06, normal phase). GC was undertaken with a Shimadzu GC-14B instrument equipped with a flame-ionization detector (FID) and a ULBON HR-Thermon 3000B fused SIL capillary column (25 m×0.25 mm i.d., Shinwa Chemical Ind. Ltd.). The injection, oven, and detector temperatures were 250 °C, 180 °C, and 250 °C, respectively. The carrier gas was He.

Algal Material The symbiotic alga was isolated from the soft coral *Clavularia viridis* (order Stolonifera, family Clavularidae), collected from a coral reef of Ishigaki Island, Okinawa Prefecture, Japan, and was identified as an unknown species of dinoflagellate of the genus *Symbiodinium* (order Dinophyceae, family Gymnodiniales) by the analysis of 18S RNA regions.[22,23] A voucher specimen (strain CV-I1) has been on deposit at Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Culture Condition[22] *Symbiodinium* sp. was grown in 10 l culture bottles with a modified INK medium developed by our group referring to the commercially available medium DAIGO (Nihon Pharmacy) [$NaNO_3$ (2 g), Na_2HPO_4 (14 mg), K_2HPO_4 (50 mg), NH_4Cl (26.8 mg), $Na_2EDTA\cdot 2H_2O$ (372 μg), FeEDTA (2.59 mg), MnEDTA (3.32 mg), vitamin B_1 (2 mg), vitamin B_{12} (15 μg), biotin (15 μg), $MnCl_2\cdot 4H_2O$ (0.9 mg), $ZnSO_4\cdot 7H_2O$ (240 μg), $CoSO_4\cdot 7H_2O$ (120 μg), $CuSO_4\cdot 5H_2O$ (6 μg), H_2SeO_3 (3 μg), artificial seawater 10 l] at 23 °C for 40 d (a 14 : 10-h light:dark regime, *ca.* 20 μmol photons $m^{-2}\,s^{-1}$).

Extraction and Isolation Wet specimens of the cultured alga (44 g from 70 l of media) were extracted with MeOH. The MeOH extract (3.36 g) was partitioned between EtOAc and H_2O. The EtOAc soluble portion (1.48 g) was chromatographed on a silica gel column. Stepwise elution with hexane, hexane–EtOAc (3 : 1 and 1 : 1), EtOAc, and MeOH gave five fractions (fractions 1—5). A portion (5.9 mg) of fraction 1 (78 mg, eluted with hexane) was treated with 5% HCl–MeOH (2 ml) at 90 °C for 2 h to give a mixture of fatty acid methyl esters, which was analyzed by GC. A portion (36 mg) of fraction 3 [116 mg, eluted with hexane–EtOAc (2 : 3)] was chromatographed on a Sephadex LH-20 column. Elution with MeOH gave three fractions, and the second fraction (16 mg) was further subjected to HPLC [normal phase, hexane–EtOAc (2 : 3)] to give compound **1** (2.4 mg) and **2** (3.8 mg), along with peridinin (**3**, 2.2 mg).

Compound **1**: Reddish viscous oil. $[\alpha]_D^{25}$ +63.6° (*c*=0.07, $CHCl_3$). VIS λ_{max} (MeOH) nm (ε): 449 (57800). IR (dry film) cm^{-1}: 3458, 1929, 1732, 1250. ^1H- and ^{13}C-NMR, see Table 1. EI-MS *m*/*z*: 630 $[M]^+$. HR-EI-MS *m*/*z*: 630.3528 (Calcd for $C_{39}H_{50}O_7$: 630.3557 $[M]^+$).

Compound **2**: Reddish viscous oil. $[\alpha]_D^{25}$ −69.2° (*c*=0.04, $CHCl_3$). VIS λ_{max} (MeOH) nm (ε): 449 (55800). IR (dry film) cm^{-1}: 3454, 1929, 1732, 1260. ^1H- and ^{13}C-NMR, see Table 1. EI-MS *m*/*z*: 630 $[M]^+$. HR-EI-MS *m*/*z*: 630.3547 (Calcd for $C_{39}H_{50}O_7$: 630.3557 $[M]^+$).

Peridinin (**3**): Reddish viscous oil. ^1H-NMR (500 MHz, $CDCl_3$) δ: 0.97 (3H, s, H-17), 1.07 (3H, s, H-16′), 1.20 (3H, s, H-18), 1.21 (3H, s, H-16), 1.26 (1H, dd, *J*=10.8, 12.8 Hz, H-2), 1.35 (3H, s, H-18′), 1.38 (3H, s, H-

17′), 1.40 (1H, m, H-2′), 1.50 (1H, t, J=12.8 Hz, H-4′), 1.62 (1H, m, H-2), 1.63 (1H, m, H-4), 1.80 (3H, s, H-19′), 1.99 (1H, br d, J=12.8 Hz, H-2′), 2.04 (3H, s, OAc), 2.23 (3H, s, H-20), 2.28 (1H, br d, J=12.8 Hz, H-4′), 2.40 (1H, br dd, J=4.9, 14.4 Hz, H-4), 3.91 (1H, m, H-3), 5.38 (1H, m, H-3′), 5.73 (1H, s, H-12), 6.05 (1H, s, H-8′), 6.11 (1H, d, J=11.5 Hz, H-10′), 6.37 (1H, d, J=15.6 Hz, H-8), 6.38 (1H, dd, J=11.5, 14.3 Hz, H-14′), 6.45 (1H, br d, J=10.8 Hz, H-14), 6.51 (1H, dd, J=10.8, 14.3 Hz, H-15), 6.61 (2H, dd, J=11.5, 14.3 Hz, H-11′, H-15′), 7.02 (1H, s, H-10), 7.17 (1H, d, J=15.6 Hz, H-7). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 14.0 (CH_3, C-19′), 15.4 (CH_3, C-20), 19.9 (CH_3, C-18), 21.4 (CH_3, OAc), 24.9 (CH_3, C-17), 29.2 (CH_3, C-17′), 29.5 (CH_3, C-16), 31.3 (CH_3, C-18′), 32.1 (CH_3, C-16′), 35.3 (C, C-1), 35.8 (C, C-1′), 40.9 (CH_2, C-4), 45.2 (CH_2, C-4′), 45.4 (CH_2, C-2′), 47.1 (CH_2, C-2), 64.2 (CH, C-3), 67.5 (CH, C-5), 67.9 (CH, C-3′), 70.5 (C, C-6), 70.7 (C, C-5′), 103.3 (CH, C-8′), 117.6 (C, C-6′), 119.2 (CH, C-12), 121.8 (CH, C-8), 124.8 (C, C-9), 128.1 (CH, C-10′), 128.9 (CH, C-15′), 131.5 (CH, C-11′), 133.0 (CH, C-14′), 133.6 (CH, C-7), 133.9 (C, C-9′), 134.0 (CH, C-13), 136.3 (CH, C-10), 137.2 (CH, C-15), 138.0 (CH, C-14), 146.8 (C, C-11), 168.7 (C, C-19), 170.4 (C, OAc), 202.6 (C, C-7′). These NMR data coincided with those[24,25] of all-*trans*-peridinin.

Acknowledgments The authors thank Dr. Y. Shida at the Tokyo University of Pharmacy and Life Science for measurement of mass spectra.

References and Notes

1) Kikuchi H., Tsukitani Y., Iguchi K., Yamada Y., *Tetrahedron Lett.*, **23**, 5171—5174 (1982).
2) Kikuchi H., Tsukitani Y., Iguchi K., Yamada Y., *Tetrahedron Lett.*, **24**, 1549—1552 (1983).
3) Iguchi K., Kaneta S., Mori K., Yamada Y., Honda A., Mori Y., *Tetrahedron Lett.*, **27**, 5787—5790 (1985).
4) Nagaoka H., Iguchi K., Miyakoshi T., Yamada N., Yamada Y., *Tetrahedron Lett.*, **27**, 223—226 (1986).
5) Honda A., Mori Y., Iguchi K., Yamada Y., *Mol. Pharmacol.*, **32**, 530—535 (1987).
6) Bader Th., Yamada Y., Ankel H., *Antiviral Res.*, **16**, 341—355 (1991).
7) Corey E. J., Lansbury P. T., Jr., Yamada Y., *Tetrahedron Lett.*, **26**, 4171—4174 (1985).
8) Corey E. J., d'Alarcao M., Matsuda S. P. T., Lansbury P. T., Jr., *J. Am. Chem. Soc.*, **109**, 289—290 (1987).
9) Corey E. J., Matsuda S. P. T., *Tetrahedron Lett.*, **28**, 4247—4250 (1987).
10) Corey E. J., Matsuda S. P. T., Nagata R., Cleaver M. B., *Tetrahedron Lett.*, **29**, 2555—2558 (1988).
11) Strain H. H., Svec W. A., Aizetmüller K., Grandolfo M. C., Katz J. J., Kjøsen H., Norgård S., Liaaen-Jensen S., Haxo F. T., Wegfahrt P., Rapoport H., *J. Am. Chem. Soc.*, **93**, 1823—1825 (1971).
12) Strain H. H., Svec W. A., Wegfahrt P., Rapoport H., Haxo F. T., Norgård S., Kjøsen H., Liaaen-Jensen S., *Acta Chem. Scand. B*, **30**, 109—120 (1976).
13) Johansen J. E., Borch G., Liaaen-Jensen S., *Phytochemistry*, **19**, 441—444 (1980).
14) The ^{13}C signal at δ 31.3 (CH_3) for **1** (and **2**) was found to be overlapped with two methyl signals by measurement of the signal intensity.
15) Kjøsen H., Norgård S., Liaaen-Jensen S., Svec W. A., Strain H. H., Wegfahrt P., Rapoport H., Haxo F. T., *Acta Chem. Scand. B*, **30**, 157—164 (1976).
16) Haugen J. A., Englert G., Aakerman T., Glinz E., Liaaen-Jensen S., *Acta Chem. Scand.*, **48**, 769—779 (1994). The NMR data of **1** and **2** cited in this reference were only ^{1}H-NMR chemical shifts, and ^{1}H coupling constants and ^{13}C-NMR data were not described. Although the ^{1}H-NMR data in the reference were identical with those of **1** and **2** obtained from the alga, respectively, we conducted a similar acid-catalyzed conversion of peridinin (**3**) and confirmed that the ^{1}H- and ^{13}C-NMR data of **1** and **2** from the alga are identical with those of the diastereomeric products from **3**, respectively.
17) In order to confirm compounds **1** and **2** to be natural products or artificial products from peridinin (**3**) through the isolation process, the following experiments were conducted. A solution of peridinin (**3**) was kept at room temperature for 3 d in the presence of Sephadex LH-20 in MeOH or silica gel in EtOAc. The formation of **1** and **2** was not observed. Therefore, compounds **1** and **2** may be the first fully characterized natural products. A compound having the same gross structure of peridinin-5,8-furanoxide as that of **1** and **2** was described to be obtained from the dinoflagellate *Procentrum lima* in the preceding papers for 7th Int. Symp. on Marine Natural Products (Capri, 1992) by Gonzalez and coworkers. However, the stereochemistry as well as the spectral data for the compound were not shown in the preceding paper, and subsequent publication regarding the compound has not been found so far.
18) Nishino H., Satomi Y., Tokuda H., Nishino A., Iwashima A., Tanaka Y., Yamano Y., Shibata Y., Torihara M., Tamai Y., Ito M., *J. Kyoto Pref. Univ. Med.*, **100**, 831—835 (1991).
19) Nishino H., *Mutation Res.*, **402**, 159—163 (1998).
20) Maoka T., Tsushima M., Nishino H., *Chem. Pharm. Bull.*, **50**, 1630—1633 (2002).
21) Yamori T., Matsunaga A., Sato S., Yamazaki K., Komi A., Ishizu K., Mita I., Edatsugi H., Matsuba Y., Takezawa K., Nakanishi O., Kohno H., Nakajima Y., Komatsu H., Andoh T., Tsuruo T., *Cancer Res.*, **59**, 4042—4049 (1999).
22) Fujiwara S., Yasui K., Watanabe K., Wakabayashi T., Iguchi K., Tsuzuki M., *Mar. Biotechnol.*, **5**, 1—7 (2003).
23) Rowan R., Powers D. A., *Mar. Ecol. Prog. Ser.*, **71**, 65—73 (1991).
24) McLean S., Reynolds W. F., John L. M. D., Tinto W. F., *Magn. Reson. Chem.*, **30**, 362—363 (1992).
25) Krane J., Aakermann T., Liaaen-Jensen S., *Magn. Reson. Chem.*, **30**, 1169—1177 (1992).