New Plastoquinones Isolated from the Brown Alga, *Sargassum micracanthum*

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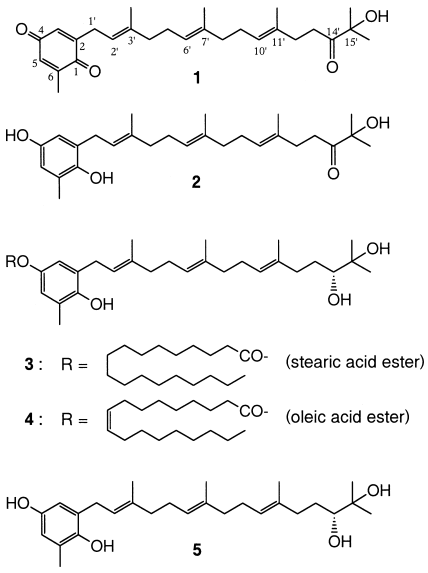
Four new plastoquinones were isolated from the methanolic extract of the brown alga, *Sargassum micracanthum*. Their structures were elucidated based on spectroscopic analysis and chemical conversions from 2-geranylgeranyl-6-methyl-1,4-benzohydroquinone. These plastoquinones exhibited significant antioxidant activities such as an inhibitory effect on lipid peroxidation and a radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH). Some of the new plastoquiones showed cytotoxic activity against cancer cell line.

Key words plastoquinone; antioxidative activity; lipid peroxidation; radical scavenger; cytotoxic activity; *Sargassum micracanthum*

From the brown algae of the genus *Sargassum* (Sargassaceae, Fucales), new secondary metabolites with various biological activities have been found. The bioactivities are attributed mainly to the unique structures, however, some of them isolated from the brown algae contain imprecise, because of the instability for ascribing to air oxidation and/or light polymerization. In addition, concerning the bioactive compounds, not only the structure–activity-relationship toward the development of new drugs or supplements, but also the biological functions in the algae themselves was still unclear. In the course of our investigations[1,2] on biologically active constituents from algae, four new plastoquinones **1**—**4** were isolated from the brown alga, *Sargassum micracanthum* (KUETZING) ENDLICHER (togemoku in Japanese). This paper describes the isolation and structural elucidation of the new plastoquinones, as well as their bioactivities focused on the antioxidant and cytotoxic activities.

The chloroform–methanol (3 : 1) soluble portion of the hot methanol extract of *S. micracanthum*, collected at the Toyama Bay coast, was fractionated by silica gel column chromatography, using chloroform–methanol (99 : 1, 9 : 1, 1 : 1) to obtain three fractions. The second fraction, eluted with chloroform–methanol (9 : 1), was chromatographed with ODS silica gel column (methanol–H_2O=3 : 1, then methanol) to remove some pigments. Further separation of the former fraction, eluted with methanol–H_2O, using silica gel flash column and reversed phase HPLC afforded plastoquinones **1** (a colorless oil, 10.8 mg), **2** (a pale yellowish oil, 8.0 mg), **3** {a colorless oil, 11.2 mg, $[\alpha]_D^{25}$ +6.2° (c=0.56, $CHCl_3$)}, and **4** {a colorless oil, 3.3 mg, $[\alpha]_D^{25}$ +6.0° (c= 0.33, $CHCl_3$)}.

The molecular formula of **1**, $C_{27}H_{38}O_4$, was determined by high-resolution EI-MS (HR-EI-MS). The IR spectrum of **1** showed a broad absorption due to a hydroxyl group (3485 cm^{-1}), and absorptions assigned as a carbonyl group (1709 cm^{-1}) and quinone (1653 cm^{-1}). The presence of a quinone moiety was demonstrated by UV absorptions at 265 ($\log \varepsilon$ 3.6) and 334 ($\log \varepsilon$ 3.4) nm. All of the carbons appeared in the ^{13}C-NMR spectrum, which was similar to that of the known plastoquinone **5** and its redox pair of 1,4-quinone derivative from the same alga,[3] however, some exceptions were observed in the ^{13}C-NMR data, such as two quinone carbonyls (δ 188.0 ppm, for two carbons) and a new carbonyl (δ 214.1 ppm) instead of a secondary hydroxyl in **5** (Table 1). The chemical shift value of a quaternary carbon (δ 76.2 ppm) and a methylene (δ 34.3 ppm) in **1** were shifted a little compared to those in **5**. The ^{1}H-NMR spectrum of **1** was also similar to that of **5**, except for the absence of the proton connecting to the carbon with the secondary hydroxyl, and for the protons assigned around the terminal of its side chain (Table 1). These observations together with the MS data suggested **1** to have a hydroxyketone group in the side chain terminal (C14′—15′). Two-dimensional (2D) NMR (COSY, HMQC, HMBC) analyses confirmed the gross structure of **1** (Fig. 1). The stereochemistry of trisubstituted carbon–carbon double bonds was demonstrated by the nuclear Overhauser effect spectroscopy (NOESY) analysis and the chemical shifts of three olefinic methyls from ^{13}C-NMR data. These spectroscopic analyses in addition to the optical rotation value ($[\alpha]_D$ 0°) established the structure of **1** to be as



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shown in Fig. 1.

Compound **2** showed the molecular formula $C_{27}H_{40}O_4$ by HR-EI-MS analysis, having two more protons compared to **1**. The IR, UV and NMR spectra of **2** were quite similar to those of **1**, except for the data assigned to the aromatic moiety. For example, ^{13}C-NMR signals corresponding to the quinone carbonyls (δ 188.0 ppm, for two carbons) in **1** disappeared, but new carbon signals in **2** were observed in the higher field area at δ 153.4 and 141.1 ppm, respectively. HMBC analysis also suggested the presence of hydroquinone in **2**. *E* stereochemistry of olefins and the terminal hydroxyketone in its side chain were elucidated by the same methodology as those of compound **1** using ^{13}C-NMR, NOESY and HMBC analyses. From these findings it was concluded that the structure of **2** had a hydroquinone group instead of the quinone in **1**.

Table 1. ^{1}H- and ^{13}C-NMR Data[a)]

No	**1**		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	188.0 (C)	—	141.1	—
2	148.5 (C)	—	131.3	—
3	132.3 (CH)	6.47 (m)	115.1	6.53 (d, 3.0)
4	188.0 (C)	—	153.4	—
5	133.1 (CH)	6.54 (m)	113.7	6.51 (d, 3.0)
6	145.9 (C)	—	134.5	—
1′	27.5 (CH_2)	3.13 (2H, d, 7.3)	28.4	3.13 (2H, d, 6.8)
2′	118.0 (CH)	5.12 (m)	121.2	5.22 (dt, 0.9, 7.3)
3′	139.9 (C)	—	137.1	—
4′	39.6* (CH_2)	1.98 (m), 2.08 (m)	39.7#	1.97 (m), 2.07 (m)
5′	26.6 (CH_2)	2.08 (m), 2.08 (m)	26.5	2.07 (m), 2.07 (m)
6′	123.9 (CH)	5.07 (m)	124.3	5.10 (m)
7′	135.2 (C)	—	134.7	—
8′	39.5* (CH_2)	1.98 (m), 2.08 (m)	39.6#	1.97 (m), 2.07 (m)
9′	26.4 (CH_2)	2.08 (m), 2.08 (m)	26.30	1.97 (m), 2.07 (m)
10′	125.2 (CH)	5.12 (m)	125.3	5.10 (m)
11′	133.3 (C)	—	133.1	—
12′	33.3 (CH_2)	2.27 (2H, t, 7.1)	33.2	2.30 (m), 2.30 (m)
13′	34.3 (CH_2)	2.63 (2H, t, 7.1)	34.1	2.63 (2H, t, 7.3)
14′	214.1 (C)	—	214.0	—
15′	76.2 (C)	—	76.4	—
6 Me	16.12 (CH_3)	2.05 (3H, s)	20.5	2.09 (3H, s)
3′ Me	16.10 (CH_3)	1.63 (3H, s)	16.4	1.63 (3H, s)
7′ Me	16.06 (CH_3)	1.61 (3H, s)	16.1	1.60 (3H, s)
11′ Me	16.01 (CH_3)	1.59 (3H, s)	15.9	1.58 (3H, s)
15′ Me	26.5 (CH_3)	1.38 (3H, s)	26.34	1.38 (3H, s)
15′ Me	26.5 (CH_3)	1.38 (3H, s)	26.34	1.38 (3H, s)

a) Measured in $CDCl_3$, ^{13}C; 125 MHz, ^{1}H; 500 MHz, δ ppm, *J* in Hz. Assignments were made based on HMQC analysis. Chemical shift values with the same superscript were each interchangeable.

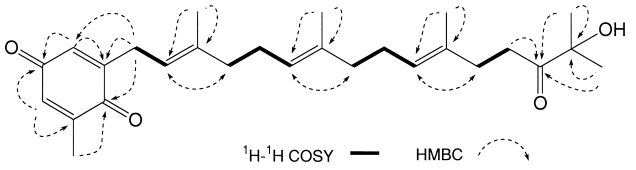


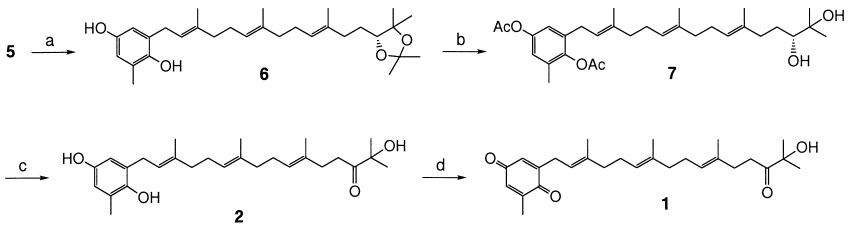
Fig. 1. The Structure of **1** and Correlations from COSY and HMBC Analyses

Chemical conversion from **5** was attempted (Chart 1). Protection of the terminal diol with acetonide gave **6** in good yield. The hydroquinone moiety was converted to diacetate, followed by hydrolysis of the acetonide to provide **7**. After oxidation of the secondary hydroxyl at C14′, deprotection of the phenolic diacetate was carried out under basic condition to afford **2**. In addition, air oxidation or treatment of **2** with ammonium cerium(IV) nitrate (CAN) yielded **1**. The physical properties of synthetic compounds **1** and **2** were identical to those of the isolated compounds from the alga.

The molecular formula of compound **3** was assigned as $C_{45}H_{74}O_5$ by HR-EI-MS analysis. The IR and UV spectra of **3** were closely similar to those of **5**, but a new IR absorption (1740 cm^{-1}) due to an ester moiety was observed. ^{1}H- and ^{13}C-NMR analyses together with the molecular formula suggested compound **3** to be a stearate derivative of **5** (Table 2). The signal at δ_H 3.33 ppm assigned as H14′ shows a normal methine proton connecting to the carbon with a hydroxyl or an alkoxyl group. No significant difference in the NMR data related to the side chain between **3** and **5** could be observed, indicating the ester function to be present in the aromatic system, especially the less hindered phenolic group at C4. Moreover, NOE effects were not found from the protons at the α position of stearate. These evidences revealed the position of the stearate ester to be connected to C4-hydroxyl. The absolute stereochemistry at C14′ was confirmed by comparison with the optical rotation value of **1**.

Physical properties of **4** were quite similar to those of **3**, except for the molecular formula, $C_{45}H_{72}O_5$, and new signals due to an olefin observed in the ^{1}H- and ^{13}C-NMR spectra (Table 2). Further spectroscopic analyses revealed the structure of **4** to be an oleate derivative of **3** instead of a stearate.

In order to confirm the structure of **3** and **4**, the following chemical conversions were tried from **6** (Chart 1). Selective esterification at the less hindered phenol was executed by treatment of **6** with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and the corresponding fatty acid (stearic acid for **3** and oleic acid for **4**, respectively) to furnish the hydroquinone mono ester **8** and **9**. After acid hydrolysis of the terminal acetonide, the desired compounds **3** and **4** were obtained. The spectral data of the synthetic compounds involving their optical rota-



Reagents and conditions: (a) 2,2-dimethoxypropane, TsOH, MeOH, rt; (b) 1. Ac_2O, pyridine, rt; 2. TsOH, MeOH, rt; (c) 1. DMSO, $(COCl)_2$, -60°C, then Et_3N, -60°C to rt; 2. K_2CO_3, MeOH, rt; (d) CAN, acetonitrile, 0°C.

Chart 1

tion value were identical to those of the corresponding natural compounds.

Compounds **2**, **3** and **4** displayed strong antioxidant activity, such as inhibitory effect on NADPH-dependent lipid peroxidation in rat liver microsomes[4] and radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH)[5] (Table 3).

The inhibitory effect on lipid peroxidation of **2**, **3** and **4** observed at IC_{50} 0.95, 44.3 and 1.15 μg/ml, respectively, was shown to be the same or stronger than that of the positive control, α-tocopherol (40.4 μg/ml), well known as vitamin E. It is interesting that the small structural difference between **3** and **4**, such as the absence or presence of an unsaturated *cis* carbon–carbon double bond in their long-chain fatty acid ester moiety, however, leads to exhibit a large difference in the inhibitory activity. On the other hand, compounds **3** and **4** were found to have moderate radical reducing effect on DPPH at a dose of each sample using 100 μg/ml. Based on these preliminary results, in the case of compound **2**, the hydroquinone moiety must participate in antioxidant activity, while in compounds **3** and **4**, hydrolysis of their ester group occurs first, and resulting **2** may owe this activity. Due to the structural similarity between them, the structure–activity relationship in detail is under investigation.

Antiproliferative activity of **2**, **3** and **4** against Colon 26-L5 cell was also evaluated (Table 3). Compounds **2** and **4** showed relatively strong cytotoxic activity with IC_{50} value of 1.51 and 1.69 μg/ml, respectively, in this experiment. Moderate activity in the case of **3** was observed at 17.5 μg/ml. In our previous report,[1] compound **5** also exhibited cytotoxicity for different cultured cells (Vero cell) at IC_{50} 8.9 μg/ml. The crucial structure for expressing this activity in these plastoquinones was unexplained from these results. Further studies are currently under investigation.

Table 2. ^{1}H- and ^{13}C-NMR Data[a)]

No	3		4	
	^{13}C	^{1}H	^{13}C	^{1}H
1	150.5 (C)	—	150.5	—
2	126.9 (C)	—	126.9	—
3	120.0 (CH)	6.65 (d, 2.6)	120.0	6.65 (d, 2.5)
4	143.4 (C)	—	143.4	—
5	121.6 (CH)	6.70 (d, 2.6)	121.6	6.71 (d, 2.5)
6	125.5 (C)	—	125.5	—
1′	31.9 (CH_2)	3.34 (2H, m)	31.9	3.33 (2H, m)
2′	121.3 (CH)	5.16 (dt, 1.0, 7.3)	121.2	5.30 (dt, 1.0, 7.3)
3′	139.1 (C)	—	139.2	—
4′	39.6* (CH_2)	2.00 (m), 2.09 (m)	39.6#	2.02 (m), 2.11 (m)
5′	29.7 (CH_2)	2.07 (m), 2.10 (m)	29.8	2.09 (m), 2.12 (m)
6′	123.7 (CH)	5.08 (dt, 1.3, 6.8)	123.7	5.08 (dt, 1.0, 6.8)
7′	135.4 (C)	—	135.4	—
8′	39.5* (CH_2)	2.00 (m), 2.08 (m)	39.5#	2.02 (m), 2.10 (m)
9′	30.3 (CH_2)	2.03 (m), 2.10 (m)	30.3	2.05 (m), 2.13 (m)
10′	125.0 (CH)	5.15 (br t, 6.8)	125.0	5.17 (dt, 1.0, 6.8)
11′	134.8 (C)	—	134.9	—
12′	36.8 (CH_2)	2.02 (m), 2.09 (m)	36.8	2.03 (m), 2.10 (m)
13′	29.7 (CH_2)	1.40 (2H, m)	29.7	1.40 (2H, m)
14′	78.3 (CH)	3.33 (m)	78.3	3.34 (m)
15′	73.0 (C)	—	73.0	—
6 Me	16.04 (CH_3)	2.20 (3H, s)	16.04	2.20 (3H, s)
3′ Me	16.19 (CH_3)	1.77 (3H, s)	16.20	1.77 (3H, s)
7′ Me	15.96 (CH_3)	1.60 (3H, s)	15.97	1.59 (3H, s)
11′ Me	15.86 (CH_3)	1.59 (3H, s)	15.87	1.60 (3H, s)
15′ Me	23.2 (CH_3)	1.14 (3H, s)	23.2	1.14 (3H, s)
15′ Me	22.7 (CH_3)	1.19 (3H, s)	22.7	1.19 (3H, s)
1″	173.0 (C)	—	173.0	—
2″	34.4 (CH_2)	2.50 (2H, t, 7.7)	34.4	2.50 (2H, t, 8.0)
3″	29.5 (CH_2)	1.72 (2H, m)	29.6	1.73 (2H, m)
4″—8″	25.0—29.7 (CH_2×5)	1.30 (10H, m)	25.0—29.6 (CH_2×5)	1.29 (10H, m)
9″	25.0—29.7 (CH_2)	1.30 (2H, m)	130.0§ (CH)	5.35 (m)
10″	25.0—29.7 (CH_2)	1.30 (2H, m)	129.7§ (CH)	5.35 (m)
11″—17″	25.0—29.7 (CH_2×7)	1.30 (14H, m)	25.0—29.6 (CH_2×7)	1.29 (14H, m)
18″	14.1 (CH_3)	0.88 (3H, t, 7.5)	14.1	0.88 (3H, t, 7.5)

a) Measured in $CDCl_3$, ^{13}C; 125 MHz, ^{1}H; 500 MHz, δ ppm, *J* in Hz. Assignments were made based on HMQC and HMBC analysis. Chemical shift values with the same superscript were each interchangeable.

Experimental

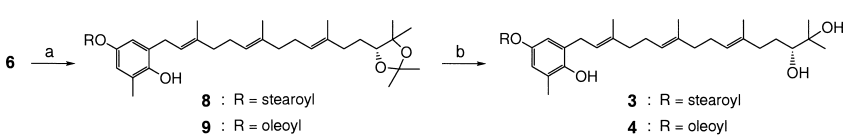
Optical rotations were measured with a JASCO DIP-1000 automatic polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1600 spectrophotometer and UV spectra with a JASCO V-530 spectrophotometer. NMR spectra were recorded with a Varian Unity-500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) in $CDCl_3$. ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{1}H–^{13}C COSY NMR spectra were measured with a Varian Unity-500 using standard Varian pulse sequences. Chemical shifts are given on a δ (ppm) scale with $CHCl_3$ (^{1}H, 7.26 ppm; ^{13}C, 77.0 ppm) as the internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Mass spectra were taken with a JEOL JMS-AX505HAD spectrometer. Column chromatography was carried out on Kanto Chemical silica gel 60 N (100—210 μm). Normal and reversed phase flash column chromatography was performed on Kanto Chemical silica gel 60 N (40—100 μm, normal phase) and Fuji Silysia Chemical ODS DM1021T (100—200 mesh, reversed phase), respectively. Medium-pressure liquid chromatography (MPLC) was carried out with a Tosoh SC-8020 apparatus using a CIG prepack column (silica gel, CPS-HS-221-05, for normal phase). HPLC with a recycling loop was conducted with a YMC-Pack SIL-06 column (silica gel, SH-043-5-06, normal phase).

Material The brown alga, *Sargassum micracanthum* (KUETZING) ENDLICHER (order Sargassaceae, family Fucales), was collected off the coast of Toyama Bay, Toyama Prefecture, Japan, in September 2001, at a depth of

Table 3. Antioxidant and Antiproliferative Activity

Samples	Inhibitory effect on lipid peroxidation[a)]	Reductive effect on DPPH[b)]	Cytotoxicity against Colon 26-L5[a)]
2	0.95	3.00	1.51
3	44.3	52.6	17.5
4	1.15	32.3	1.69
α-Tocopherol	40.4	—	—
Cisplatin	—	—	0.67

a) IC_{50} values (μg/ml), *b*) reductive effect (%) at dose (100 μg/ml) of the sample.



Reagents and conditions: (a) DCC, fatty acid (stearic acid for **8**, oleic acid for **9**), DMAP, CH_2Cl_2 rt; (b) TsOH, MeOH, rt.

Chart 2

2—5 m. A voucher specimen (No. SM-0109) has been deposited at Lead Chemical Co., Ltd., Himata, Toyama, Japan.

Extraction and Isolation Wet specimens (2.3 kg) were immersed in methanol (3×3 l). After filtration, the combined extracts were concentrated under reduced pressure. The methanol extract (442 g) was dissolved into a mixture of chloroform–methanol (3 : 1, 0.8 l), and then the precipitate was filtered with a sintered glass filter to remove highly polar compounds and salts. The precipitate on the glass filter was rinsed with a small amount of a 3 : 1 mixture of chloroform–methanol. The combined filtrate was concentrated under reduced pressure to give a chloroform–methanol (3 : 1) soluble portion (203 g). An aliquot of this portion (10.0 g) was chromatographed on a silica gel column (300 g). Stepwise elution with chloroform–methanol (99 : 1, 9 : 1 and 1 : 1, 400 ml of each) gave three fractions. The second fraction (2.2 g) eluted with chloroform–methanol (9 : 1), contained terpenoids and glycerides along with color pigments like chlorophylls from the ^{1}H-NMR analysis. Most of this fraction (2.15 g out of 2.2 g) was subjected to ODS silica gel flash column chromatography [H_2O–methanol (1 : 19) and methanol] to obtain a mixture of plastoquinones. The former fraction eluted with H_2O–methanol (1 : 19) was purified with MPLC [hexane–acetone (5 : 1)], followed by HPLC separation [hexane–ethyl acetate (4 : 1)] to afford new compounds **1** (10.7 mg), **2** (8.0 mg), and a mixture (29.8 mg) of **3** and **4**, which was separated by recycled HPLC to obtain **3** (11.2 mg) and **4** (2.3 mg). The latter fraction, obtained by the separation using ODS silica gel flash column chromatography eluted with methanol, was also separated by MPLC [hexane–ethyl acetate (5 : 2)] and HPLC [hexane–ethyl acetate (7 : 3)] to give **5** (1.28 g),[1,3] which was used for chemical conversions to determine the structures of the new compounds and their absolute configuration of the secondary alcohol on the side chain terminal in **3** and **4**.

Compound **1**: Colorless oil. UV λ_{max} (EtOH) nm (log ε): 230 (4.0), 265 (3.6), 334 (3.4). IR (dry film) cm^{-1}: 3485 (br), 1709, 1653, 1614. ^{1}H- and ^{13}C-NMR, see Table 1. HR-EI-MS *m/z*: 426.2752 [Calcd for $C_{27}H_{38}O_4$: 426.2770 (M)$^+$].

Compound **2**: Pale yellowish oil. UV λ_{max} (EtOH) nm (log ε): 234 (3.3), 285 (5.2), 310 (3.9). IR (dry film) cm^{-1}: 3445 (br), 1758, 1713, 1667. ^{1}H- and ^{13}C-NMR, see Table 1. HMBC (H→C): H-3→C-2, C-4; H-5→C-4, C-6; 6-Me→C-1; H-1′→C-1, C-2, C-3; H-2′→C-4′; 3′-Me→C-2′, C-3′; H-4′→C-2′; H-6′→C-8′; 7′-Me→C-6′, C-7′; H-8′→C-6′; H-10′→C-12′; 11′-Me→C-10′, C-11′; H-12′→C-10′; H-13′→C-14′; 15′a-Me→C-14′, C-15′; 15′b-Me→C-14′, C-15′. HR-EI-MS *m/z*: 428.2884 [Calcd for $C_{27}H_{40}O_4$: 428.2927 (M)$^+$].

Compound **3**: Colorless oil. $[\alpha]_D^{25}$ +6.2° (*c*=0.56, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε): 234 (3.3), 285 (5.2), 310 (3.9). IR (dry film) cm^{-1}: 3460 (br), 1740, 1655. ^{1}H- and ^{13}C-NMR, see Table 2. HR-EI-MS *m/z*: 694.5547 [Calcd for $C_{45}H_{74}O_5$: 694.5536 (M)$^+$].

Compound **4**: Colorless oil. $[\alpha]_D^{25}$ +6.0° (*c*=0.33, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε): 234 (3.3), 285 (5.2), 310 (3.9). IR (dry film) cm^{-1}: 3460 (br), 1739, 1655. ^{1}H- and ^{13}C-NMR, see Table 2. HR-EI-MS *m/z*: 696.5679 [Calcd for $C_{45}H_{76}O_5$: 696.5693 (M)$^+$].

Compound **5**:[1] ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 1.16 (3H, s, one of 15′-Me), 1.19 (3H, s, one of 15′-Me), 1.42 (1H, m, one of H-13′), 1.53 (1H, m, one of H-13′), 1.57 (3H, s, 7′-Me), 1.59 (3H, s, 11′-Me), 1.74 (3H, s, 3′-Me), 1.98 (1H, m, one of H-8′), 2.00 (1H, m, one of H-4′), 2.04 (1H, m, one of H-12′), 2,05 (1H, m, one of H-5′), 2.05 (1H, m, one of H-9′), 2.06 (1H, m, one of H-6′), 2.08 (1H, m, one of H-8′), 2.10 (1H, m, one of H-5′), 2.10 (1H, m, one of H-9′), 2.17 (3H, s, 6-Me), 2.23 (1H, m, one of H-12′), 3.27 (2H, br d, *J*=7.3 Hz, H-1′), 3.37 (1H, dd, *J*=1.7, 9.7 Hz, H-14′), 5.07 (1H, br t, *J*=6.9 Hz, H-6′), 5.12 (1H, br t, *J*=7.3 Hz, H-2′), 5.15 (1H, dt, *J*=0.9, 7.3 Hz, H-10′), 6.44 (1H, d, *J*=3.8 Hz, H-3), 6.52 (1H, d, *J*=3.8 Hz, H-5). ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm: 15.7 (q, 7′-Me), 15.9 (q, 11′-Me), 16.05 (q, 3′-Me), 16.09 (q, 6-Me), 23.1 (q, 15′-Me), 26.13 (q, 15′-Me), 26.19 (t, C-5′), 26.21 (t, C-9′), 29.4 (t, C-13′), 29.7 (t, C-1′), 36.8 (t, C-12′), 39.4 (t, C-8′), 39.6 (t, C-4′), 73.3 (s, C-15′), 78.2 (s, C-14′), 113.8 (d, C-3), 115.3 (d, C-5), 121.7 (s, C-2′), 123.9 (d, C-6′), 125.3 (d, C-10′), 125.5 (s, C-6), 127.6 (s, C-2), 134.7 (s, C-11′), 135.2 (s, C-7′), 138.6 (s, C-3′), 149.08 (s, C-4), 149.10 (s, C-1).

Preparation of Acetonide 6 To a solution of **5** (219 mg, 509 μmol) in MeOH (1 ml) and 2,2-dimethoxypropane (3 ml) was added *p*-toluenesulfonic acid monohydrate ($TsOH\cdot H_2O$, 10 mg) at room temperature. The reaction mixture was stirred for 30 min, triethylamine (1 drop) was added to neutralize it, and the mixture was concentrated under reduced pressure. The residue was purified by passing over a small plug of silica gel [hexane–ethyl acetate (5 : 1)] to afford **6** (236 mg, 99% yield).

Compound **6**: Colorless oil. IR (dry film) cm^{-1}: 3410 (br), 1592, 1243. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.01 (3H, s), 1.25 (3H, s), 1.34 (3H, s), 1.43 (3H, s), 1.47 (1H, m), 1.59 (3H, s), 1.60 (3H, s), 1.63 (1H, m), 1.76 (3H, s), 2.00 (3H, m), 2.08 (4H, m), 2.12 (2H, m), 2.18 (3H, br s), 2.20 (1H, m), 3.29 (2H, d, *J*=7.3 Hz), 3.68 (1H, dd, *J*=3.4, 9.0 Hz), 4.76 (1H, s), 5.08 (1H, dt, *J*=0.9, 6.8 Hz), 5.11 (1H, s), 5.14 (1H, dt, *J*=0.8, 6.8 Hz), 5.27 (1H, dt, *J*=0.8, 7.3 Hz), 6.46 (1H, d, *J*=3.0 Hz), 6.50 (1H, d, *J*=3.0 Hz). ^{13}C-NMR ($CDCl_3$) δ ppm: 15.95, 16.00, 16.07, 16.15, 22.9, 26.0, 26.30, 26.39, 26.8, 27.6, 28.5, 30.0, 36.6, 39.51, 39.66, 80.3, 82.7, 106.6, 113.9, 115.4, 121.5, 123.7, 124.8, 125.6, 127.5, 134.1, 135.4, 138.5, 146.3, 148.9. EI-MS *m/z*: 470 (M)$^+$.

Conversion to 7 Compound **6** (93 mg, 198 μmol) was treated with pyridine (2 ml) and acetic anhydride (1.5 ml) at room temperature under N_2 atmosphere. After standing over 12 h at r.t., the mixture was concentrated under reduced pressure. The residue was purified with silica gel column chromatography [hexane–acetone (3 : 1)] to provide diacetate (96 mg, 87% yield). The obtained diacetate (45 mg, 82 μmol) was dissolved into MeOH (3 ml), and then $TsOH\cdot H_2O$ (8 mg) was added to the mixture at r.t. under N_2. After stirring for 2 h, triethylamine (1 drop) was added, and the mixture was concentrated. The residue was purified with silica gel column chromatography [hexane–ethyl acetate (3 : 1)] to provide compound **7** (21 mg, 50% yield), and starting acetonide was also recovered (18 mg).

Compound **7**: Colorless oil. IR (dry film) cm^{-1}: 1739, 1585, 1210. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.14 (3H, s), 1.18 (3H, s), 1.41 (1H, m), 1.58 (1H, m), 1.59 (3H, s), 1.61 (3H, s), 1.66 (1H, m), 1.96—2.16 (9H, m), 2.14 (3H, s), 2.21 (1H, m), 2.18 (3H, br s), 2.27 (3H, s), 2.32 (3H, s), 3.18 (2H, d, *J*=7.2 Hz), 3.33 (1H, dd, *J*=2.1, 10.7 Hz), 5.11 (1H, dt, *J*=1.3, 6.9 Hz), 5.17 (1H, dt, *J*=1.2, 7.0 Hz), 5.21 (1H, dt, *J*=1.3, 7.2 Hz), 6.77 (1H, d, *J*=2.8 Hz), 6.82 (1H, d, *J*=2.8 Hz).

Conversion to 2 To a solution of oxalyl chloride (30 mg, 236 μmol) in dichloromethane (1 ml) was added dimethylsulfoxide (DMSO, 30 mg, 384 μmol) at −60 °C under N_2 atmosphere. After stirring for 15 min at this temperature, compound **7** (18 mg, 35 μmol) in dichloromethane (0.2 ml) was added, and the mixture was stirred for 20 min at −60 °C, followed by addition of triethylamine (40 mg, 395 μmol). The mixture was gradually warmed to r.t. for 1 h, diluted with a hexane–ethyl acetate (1 : 1, 30 ml) mixture, and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The oily residue was purified with silica gel column chromatography [hexane–acetone (3 : 1)] to provide hydroxyketone (14 mg, 78% yield) as a colorless oil. Basic hydrolysis of phenolic diacetate for the above compound (14 mg, 27 μmol) was carried out with potassium carbonate (14 mg, 100 μmol) in MeOH (2 ml) at r.t. under N_2. After the reaction mixture was stirred for 30 min, saturated ammonium chloride solution (3 drops) was added. Most of the solvent was removed under reduced pressure, and then the residue was diluted with ethyl acetate (20 ml). The organic phase was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified with silica gel column chromatography [hexane–acetone (2 : 1)] to provide **2** (10 mg, 86% yield) as a colorless oil. The spectral data of synthetic **2** were identical to those of the isolated compound from the alga.

Oxidation of 2 to 1 To a solution of **2** (4 mg, 10 μmol) in acetonitrile (0.6 ml) at 0 °C under N_2 was added a water solution of ammonium cerium (IV) nitrate (CAN, 10 mg, 18 μmol). The reaction mixture was stirred for 30 min at 0 °C, a $Na_2S_2O_3$ solution (3 drops) was added to quench active CAN, and the mixture was concentrated under reduced pressure. The residue was extracted twice with ethyl acetate (each 10 ml) and the combined extracts were washed with a saturated NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The oily residue was purified by passing over a small plug of silica gel [hexane–ethyl acetate (2 : 1)], followed by MPLC separation [normal phase, hexane–acetone (3 : 1)] to afford **2** (1.3 mg, 32% yield) as a pale yellowish oil. The spectral data obtained by the above procedure were identical to those of isolated **1**.

Preparation of 8 *N*,*N*′-Dicyclohexylcarbodiimide (DCC, 55 mg, 266 μmol) was added to a solution of compound **6** (33 mg, 69 μmol), stearic acid (30 mg, 105 μmol) and a catalytic amount of DMAP in chloroform (2 ml) at r.t. The mixture was stirred for 4 h at r.t., which was then diluted with hexane (2 ml), followed by filtration through a small plug of silica gel column. After eluted with hexane–ethyl acetate (3 : 1, 10 ml), the combined filtrate was concentrated under reduced pressure. The residue was purified with silica gel column chromatography [hexane–acetone (9 : 1)] to give **8** (47 mg, 92% yield).

Compound **8**: ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, *J*=7.1 Hz), 1.19 (3H, s), 1.23—1.42 (28H, m), 1.24 (3H, s), 1.32 (3H, s), 1.42 (3H, s), 1.42—1.49 (2H, m), 1.60 (3H, s), 1.61 (3H, br s), 1.73 (2H, quint, *J*=7.7 Hz), 1.78 (3H,

s), 1.95—2.14 (10H, m), 2.20 (3H, s), 2.50 (2H, t, J=7.5 Hz), 3.33 (2H, d, J=7.2 Hz), 3.66 (1H, dd, J=3.5, 9.1 Hz), 5.08 (1H, dt, J=1.3, 6.8 Hz), 5.16 (1H, dt, J=1.3, 6.8 Hz), 5.16 (1H, s), 5.31 (1H, dt, J=1.4, 7.3 Hz), 6.66 (1H, d, J=2.7 Hz), 6.71 (1H, d, J=2.7 Hz).

Preparation of 3 Compound **8** (35 mg, 48 μmol) in MeOH (4 ml) was treated with $TsOH \cdot H_2O$ (3 mg) at r.t. under N_2. After stirring for 3 h, pyridine (1 drop) was added, and the mixture was concentrated. The residue was purified with silica gel column chromatography [hexane–ethyl acetate (1 : 1)] to obtain compound **3** (10 mg, 31% yield), and the starting acetonide was also recovered (18 mg).

Compound **3**: $[\alpha]_D^{25}$ +6.5° (c=0.50, $CHCl_3$). The spectral data of the synthetic compound were identical to those of isolated **3**.

Preparation of 9 The procedure was essentially the same as that from **6** to **8**. DCC (55 mg, 266 μmol) was added to a solution of compound **6** (34 mg, 72 μmol), oleic acid (34 mg, 120 μmol) and a catalytic amount of 4-dimethyaminopyridine (DMAP) in chloroform (2 ml) at r.t. The mixture was stirred for 4 h at r.t., which was then diluted with hexane (2 ml), followed by filtration through a small plug of silica gel column. After eluted with hexane–ethyl acetate (3 : 1, 10 ml), combined filtrate was concentrated under reduced pressure. The residue was purified with silica gel column chromatography [hexane–acetone (9 : 1)] to give **9** (27 mg, 51% yield).

Compound **9**: ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=7.1 Hz), 1.19 (3H, s), 1.23—1.42 (22H, m), 1.24 (3H, s), 1.32 (3H, s), 1.42 (3H, s), 1.43—1.49 (2H, m), 1.60 (3H, s), 1.61 (3H, br s), 1.73 (2H, quint, J=7.7 Hz), 1.78 (3H, s), 1.95—2.14 (14H, m), 2.20 (3H, s), 2.50 (2H, t, J=7.5 Hz), 3.33 (2H, d, J=7.3 Hz), 3.66 (1H, dd, J=3.4, 9.4 Hz), 5.08 (1H, dt, J=0.8, 6.8 Hz), 5.16 (1H, dt, J=1.3, 6.8 Hz), 5.17 (1H, s), 5.31 (1H, dt, J=1.2, 7.3 Hz), 5.35—5.38 (2H, m), 6.65 (1H, d, J=2.8 Hz), 6.71 (1H, d, J=2.8 Hz).

Preparation of 4 Compound **9** (17 mg, 23 μmol) in MeOH (4 ml) was treated with $TsOH \cdot H_2O$ (3 mg) at r.t. under N_2. After stirring for 4 h, pyridine (1 drop) was added, and the mixture was concentrated. The residue was purified with silica gel column chromatography [hexane–ethyl acetate (1 : 1)] to obtain compound **3** (6.8 mg, 43% yield), and the starting acetonide was also recovered (8 mg).

Compound **4**: $[\alpha]_D^{25}$ +6.1° (c=0.34, $CHCl_3$). The spectral data of the synthetic compound were identical to those of isolated **4**.

Animal Male Wistar rats (5 weeks of age) were purchased from Sankyo Labo Co., Ltd. and were used. The animals were maintained in a thermostatically controlled room at 24±2 °C during the experimental period.

Effects on Lipid Peroxidation in Rat Liver Homogenates[4] The standard incubation mixture consisted of 10 μl of rat liver homogenates (contained 5 mg of microsomal protein), 500 μl of 0.1 M tris/HCl buffer (containing 14 mM $MgCl_2$, pH 7.5), 20 μl of 0.2 M ADP (containing 12 mM $FeSO_4 \cdot 7H_2O$), 40 μl of 10 mM NADPH (containing 12.5 U/ml of glucose-6-phosphate dehydrogenase, 160 mM glucose-6-phosphate) and 420 μl of water. Ten milliliters of each sample ethanol solution was added to the standard incubation mixtures. After incubation at 37 °C for 10 min, reaction was stopped by adding 2 ml of 15% trichloroacetic acid (containing 0.375% thiobarbituric acid, 0.25 M HCl). The solution was boiled for 15 min, and the amount of lipid peroxidation was determined by measuring the concentration of thiobarbituric acid reactive substance (TBA-RS). Inhibitory activity was determined from the difference in optical density of TBA-RS at 535 nm, with or without rat liver homogenates.

Reductive Activities on DPPH[5] Reaction mixture contained 3.0 ml of 0.5 M 1,1-diphenyl-2-picrylhydrazyl ethanol solution and 0.5 ml of ethanol with or without each sample. After incubation at 37 °C for 20 min, the optical absorption was measured at 517 nm. Reductive activity on DPPH was determined by the decreasing ratio of absorption with or without each sample.

Cells and Cell Culture Murine colon 26-L5 adenocarcinoma cell line, derived from colon 26, was kindly provided by Prof. I. Saiki (Toyama Med. & Pharm. Univ., Inst. of Natural Medicine, Toyama, Japan). The cell line is highly liver metastatic compared with the colon 26 parental cell line.[6] The cell line was maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 2-mercaptoethenol, 100 U/ml penicillin, and 0.1 mg/ml streptomycin.

Cell Proliferation Assay Colon 26-L5 cells (2×10^3) suspended in RPMI-1640 containing 5% FBS were seeded onto wells of 96 multiwell plates with vehicle or various concentrations of test compounds and incubated at 37 °C for 72 h. Then, the culture medium was exchanged to fresh 10% FBS-medium with WST-1 solution (Wako Pure Chemicals Ind. Ltd.) and incubated for an additional 2 h at 37 °C. Absorbance of each well was measured at 450 nm.

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