Studies on Glycolipids. III.[1] Glyceroglycolipids from an Axenically Cultured Cyanobacterium, *Phormidium tenue*

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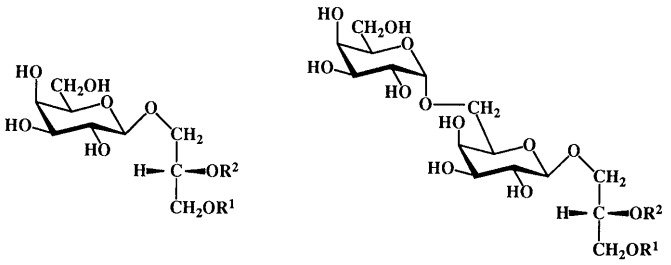
Seven new monogalactosyl diacylglycerols (1—7) and six new digalactosyl diacylglycerols (11—16) were isolated from an axenically cultured cyanobacterium, *P. tenue*. Their structures were elucidated on the basis of physicochemical evidence and the results of enzymatic hydrolysis using a lipase (from *Rhizopus arrhizus*). Comparison of antialgal activity for *P. tenue* between monogalactosyl diacylglycerols (1—8) and digalactosyl diacylglycerols (11—19) revealed that the former showed more potent activity than the latter.

Keywords axenic culture; cyanobacterium; *Phormidium tenue*; glycolipid; monogalactosyl diacylglycerol (MGDG); digalactosyl diacylglycerol (DGDG); antialgal activity; lipase; enzymatic hydrolysis

In a previous paper, we reported that fatty acids induced lysis of an axenically cultured cyanobacterium, *P. tenue*, and unsaturated fatty acids such as linoleic and linolenic acids showed higher antialgal activity (lysis inducing activity) than saturated ones.[2] Furthermore, we demonstrated that monogalactosyl diacylglycerols induced the lysis of this cyanobacterium, although their activities were less potent than those of fatty acids.[1] On the other hand, the cyanobacteria contain digalactosyl diacylglycerols as well as monogalactosyl diacylglycerols as major membrane lipids.[3] In this paper, we would like to present the full details of our studies on the structural elucidation of monogalactosyl diacylglycerols (MGDGs, **1**—**8**) and digalactosyl diacylglycerols (DGDGs, **11**—**19**), and their antialgal activity.

P. tenue was collected from the moat around the Nagoya Castle and an axenic culture was prepared by repeated capillary pipetting.[4] Mass culture was conducted in CT medium for 3 weeks in a laboratory. The alga was harvested by centrifugation and lyophilized. The lyophilized cells were extracted by the Bligh–Dyer method to obtain total lipid components.[5] The resulting extract was successively subjected to silica gel column chromatography to give MGDG and DGDG fractions in 1.3 and 0.9% yields, respectively. The MGDG fraction was further separated by reversed-phase high-performance liquid chromatography (HPLC) to furnish compounds **1**—**8** and a mixture of **9a** and **9b**.

Compound **1**, $[\alpha]_D^{24}$ −2.7° ($CHCl_3$, $c=0.6$), gave a quasimolecular ion peak at m/z 747 $(M+Na)^+$ in its fast atom bombardment mass spectrum (FAB-MS). The infrared (IR) spectrum of **1** showed the presence of hydroxyl and ester groups, while the proton nuclear magnetic resonance (^{1}H-NMR) spectrum exhibited two terminal methyl signals (δ 0.88, 0.96, 3H each, both t), a broad methylene signal at 1.26 ppm, and the signal (δ 2.41, 4H, m) due to two methylene protons linked to a carbonyl function. In addition, coupling constant analysis in the



1: R^1=linolenoyl; R^2=myristoyl
2: R^1=linolenoyl; R^2=palmitelaidoyl
3: R^1=linoleoyl; R^2=myristoyl
4: R^1=palmitoleoyl; R^2=myristoyl
5: R^1=oleoyl; R^2=myristoyl
6: R^1=linoleoyl; R^2=palmitoyl
7: R^1=palmitoyl; R^2=myristoyl
8: R^1=linolenoyl; R^2=linolenoyl
9a: R^1=linolenoyl; R^2=palmitoyl
9b: R^1=linoleoyl; R^2=palmitelaidoyl
(9a:9b=76:24)
10: R^1=R^2=H

11: R^1=linolenoyl; R^2=myristoyl
12: R^1=linoleoyl; R^2=myristoyl
13: R^1=palmitoleoyl; R^2=myristoyl
14: R^1=oleoyl; R^2=myristoyl
15: R^1=palmitoyl; R^2=myristoyl
16: R^1=linolenoyl; R^2=linoleoyl
17: R^1=linolenoyl; R^2=linolenoyl
18: R^1=linoleoyl; R^2=linoleoyl
19: R^1=linoleoyl; R^2=palmitoyl
20a: R^1=linolenoyl; R^2=palmitoyl
20b: R^1=linoleoyl; R^2=palmitelaidoyl
(20a:20b=72:28)

Chart 1

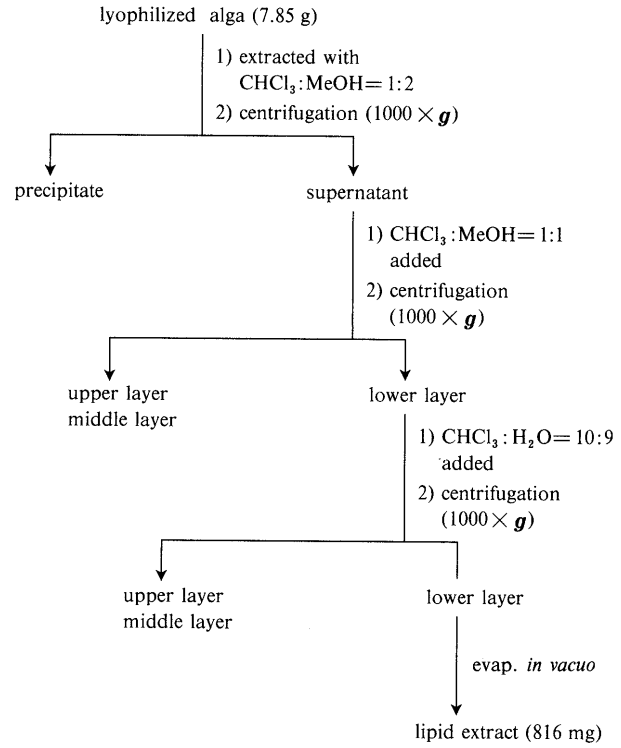
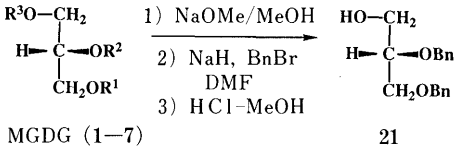


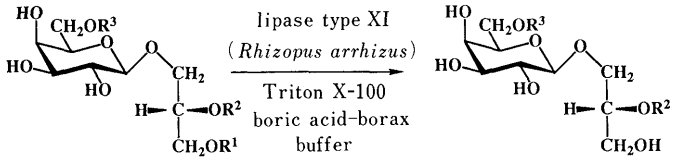
Chart 2



MGDG (1—7)
DGDG (11—19)

21

Chart 3



1 : R^3=H
11 : R^3=α-D-galactopyranosyl

1a : R^3=H
11a : R^3=α-D-galactopyranosyl

Chart 4

homonuclear decoupling spectra defined a sugar component as a galactose; this was consistent with the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I). Treatment of **1** with NaOMe–MeOH gave monogalactosyl-*sn*-glycerol (**10**),[6] methyl myristate and methyl linolenate. Detailed comparison of the ^{1}H-NMR spectrum of **1** with that of **10** disclosed that the proton signals due to 2-H and 1-H_2 in **1** were observed at a lower field than those in **10**.[7] Moreover, the ^{13}C-NMR spectra showed that the carbon signal due to C-1 of **10** appeared at a higher field than that of **1** and the carbon signals ascribable to C-2 and C-3 of both were observed at similar chemical shifts. On the basis of the above findings, it was concluded that the fatty acid residues were attached to C-1 and C-2 in the glycerol portion. The absolute configuration at C-2 in the glycerol portion of **1** was determined by Meguro's method.[8] Namely, the positive Cotton effect at 204 nm in the circular dichroism (CD) spectrum of 1,2-*O*-dibenzyl-*sn*-glycerol (**21**), which was prepared from **1** as illustrated in Chart 3, established 2*S*-configuration.

We next attempted regioselective deacylation of **1** under various conditions to determine the locations of the two fatty acid residues. On enzymatic hydrolysis using Lipase type XI from *Rhizopus arrhizus* in the presence of Triton X-100 in boric acid-borax buffer (pH 7.7), the galactolipid (**1**) liberated only linolenic acid to yield 2-monoacyl-3-β-D-galactopyranosyl-*sn*-glycerol (**1a**) quantitatively.[1b,9] The ^{1}H-NMR spectrum of **1a** showed the signals due to *sn*-1 methylene protons at higher field than those of **1**. The structure of **1a** was also confirmed by its ^{13}C-NMR spectrum; δ 68.8 (*sn*-1-C), 74.7 (*sn*-2-C), 61.7 (*sn*-3-C). Treatment of **1a** with NaOMe–MeOH afforded methyl myristate as a fatty acid component. Accordingly, the structure of the galactolipid (**1**) was determined as (2*S*)-1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-2-*O*-tetradecanoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol.

Compound **2**, $[\alpha]_D^{24}$ −3.4° (c=0.4, $CHCl_3$), gave a quasimolecular ion peak at m/z 773 $(M+Na)^+$ in its FAB-MS. The ^{1}H-NMR spectrum of **2** closely resembled that of **1** except for the signals ascribable to the fatty acid residues. The lipase-catalyzed hydrolysis of **2** afforded linolenic acid and a 1-*O*-deacylated galactolipid, which gave a methyl ester on treatment with NaOMe–MeOH.

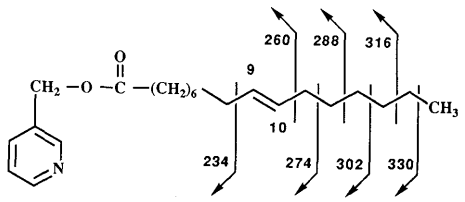


Fig. 1. Fragmentation Pattern of Picolinyl Ester of Palmitelaidic Acid

This methyl ester was found to have a molecular ion m/z 268 ($C_{17}H_{32}O_2$) by GC-MS analysis, but it was not methyl palmitoleate. So we applied the picolinyl method to clarify the location of its double bond.[10] Enzymatic hydrolysis and subsequent alkaline treatment (KOH–MeOH) of **2** gave an unidentified fatty acid, which was led to the corresponding acid chloride by thionyl chloride treatment. The acid chloride was converted to the picolinyl ester by using 3-pyridylcarbinol in CH_3CN. The fragmentation pattern in the GC-MS of the picolinyl ester revealed that the double bond was present between C-9 and C-10 (Fig. 1). The fatty acid, therefore, was determined to be palmitelaidic acid (9*E*-hexadecenoic acid) and the chemical structure of **2** was established as (2*S*)-1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-2-*O*-(9*E*-hexadecenoyl)-3-β-D-galactopyranosyl-*sn*-glycerol. With respect to other monogalactosyl diacylglycerols, the structures were elucidated in the same manner (Chart 1).

Separation of the DGDG fraction was performed by reversed-phase HPLC to furnish nine digalactosyl diacylglycerols (**11**—**19**) and a mixture of **20a** and **20b**. Compound **11**, $[\alpha]_D^{24}$ +43.9° (c=0.8, MeOH), showed a substantial molecular ion peak at m/z 909 $(M+Na)^+$ in its FAB-MS. The IR spectrum of **10** was fairly similar to those of monogalactosyl diacylglycerols. Compound **11** showed close similarity to the galactolipid (**1**) in ^{1}H-NMR signals of the glycerol portion. Detailed analysis of the remaining signals in the ^{1}H- and ^{13}C-NMR spectra suggested that the galactolipid (**11**) possessed another galactose residue attached to the inner galactose unit. The α-configuration of the glycosidic bond between the two galactose residues was determined on the basis of the anomeric carbon signal at 100.7 ppm. The observation of the downfield shift of the C′-6 carbon confirmed a 1—6 linkage of the two galactose units. Deacylation of **11** with the lipase gave linolenic acid and a 1-*O*-deacylated galactolipid (**11a**), which was treated with NaOMe to afford methyl myristate. The stereochemistry of C-2 was defined to be *S* by the previous described procedure. On the basis of the above findings, the structure of **11** was clarified to be (2*S*)-1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-2-*O*-tetradecanoyl-3-*O*-[α-D-galactopyranosyl(1—6)-β-D-galactopyranosyl]-*sn*-glycerol. The chemical structures of other digalactosyl diacylglycerols (**12**—**19**) were similarly elucidated, as illustrated in Chart 1.

We finally examined the antialgal activities of the monogalactosyl diacylglycerols (**1**—**8**) and the digalactosyl diacylglycerols (**11**—**19**) for *P. tenue*. The antialgal activity was determined by measuring chlorophyll a as described by Parsons and Strickland.[11] Relative growth rate of the alga was expressed as a percentage of the control, and IC_{50} of the test compounds was determined from dose-response curves. The concentrations (ppm) of the test compounds

TABLE I. ^{13}C-NMR Data[a] for **1**, **1a**, **10**, **11** and **11a**

Carbon	1	1a	10	11	11a
sn-1	64.0	68.8	64.1	64.0	68.9
sn-2	71.8	74.7	72.2	71.8	74.6
sn-3	68.7	61.7	72.1	68.8	61.8
1′	105.4	105.3	105.3	105.3	105.2
2′	72.4	72.5	72.6	72.6	72.5
3′	74.9	74.9	74.9	74.7[b]	74.7[b]
4′	70.2	70.3	70.4	70.1	70.2
5′	76.8	76.8	76.8	74.6[b]	74.6[b]
6′	62.5	62.5	62.6	67.9	67.9
1″				100.7	100.6
2″				70.3	70.2
3″				71.5	71.5
4″				71.2	71.1
5″				72.5	72.5
6″				62.9	62.8

a) The spectra were measured in CD_3OD at 100 MHz. *b*) Assignments may be interchangeable.

TABLE II. Concentration of Each Galactolipid Giving 50% Inhibition of Growth of *P. tenue* (ppm)

MGDG	ppm	DGDG	ppm
1	26	**11**	>100
2	32	**12**	>100
3	45	**13**	>100
4	>100	**14**	>100
5	>100	**15**	>100
6	27	**16**	>100
7	>100	**17**	>100
8	18	**18**	>100
		19	>100

causing 50% inhibition of growth of the alga after 3 d are summarized in Table II. The activities of the galactolipids containing unsaturated fatty acid residues were more potent than those of the galactolipids having saturated fatty acid residues in the case of the monogalactosyl diacylglycerols. In contrast, the digalactosyl diacylglycerols (**11**—**19**) exhibited no lysis-inducing activity regardless of the fatty acid residues.

In conclusion, we characterized seven new MGDGs (**1**—**7**) and six new DGDGs (**11**—**16**), and isolated three DGDGs (**17**—**19**)[12] as well as an identified galactolipid (**8**).[13] An examination of the antialgal activities of the isolated glyceroglycolipids for *P. tenue* revealed that the monogalactosyl diacylglycerols, which are major membrane lipids, induced the lysis of this cyanobacterium. However, the digalactosyl diacylglycerols showed no antialgal activity. Previously, we reported that unsaturated fatty acids such as linoleic and linolenic acids exhibited more potent antialgal activity than saturated fatty acids.[2] This result suggests that the lysis-inducing activities of the MGDGs are due to the fatty acids liberated by esterase in the alga. It is noteworthy that the galactolipids containing myristic acid are rare, and this is the first report of isolation of galactolipids containing palmitelaidic acid, in spite of the wide distribution of glyceroglycolipids in the plant kingdom.[14]

Experimental

IR spectra were recorded on a JASCO IRA-2 spectrometer. ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL GSX-400 (400 MHz) spectrometer using tetramethylsilane as an internal standard. FAB-MS were determined with JEOL DX-300 and JEOL DX-303 spectrometers. Optical rotations were measured on a JASCO DIP-4 digital polarimeter. Gas-liquid chromatography (GLC) was carried out on a Shimadzu GC-8A. The conditions for identification of methyl esters of fatty acids were as follows: column, ULBON HR-SS-10 (0.25 mm i.d. × 50 m, Shinwa Kako Co., Ltd.); column temperature, 150—220 °C, 3 °C/min; injection temperature, 250 °C; carrier gas, N_2, 2.2 kg/cm^2. For GC-MS analysis, a JEOL D-300 mass spectrometer interfaced to a Hewlett Packard 5710A gas chromatograph with a JMA 2000 data processing system was employed. The conditions for GC-MS measurement were as follows. Gas chromatography: column, Silicone OV-101 (0.25 mm i.d. × 50 m); injection temperature, 300 °C; column temperature, 250—280 °C, 3 °C/min; carrier gas, He, 1.0 ml/min. Mass spectrometry: ionizing energy, 70 eV; ion source temperature, 230 °C. HPLC was performed using a JASCO 880-PU pump equipped with a Shodex RI, SE-11 differential refractometer. Medium pressure chromatography (MPLC) was carried out on a C.I.G. column system (Kusano Scientific Co., Ltd.; pump KPW-20, UV detector KU-331) with a prepacked column (20 mm i.d. × 100 mm, octadecyl silica, 20 μm). Thin layer chromatography (TLC) was performed on Merck precoated Kieselgel 60F_{254}, and spots were detected by illumination with an ultraviolet lamp, or by spraying 5% vanillin–70% $HClO_4$, 1% $Ce(SO_4)_2$–10% H_2SO_4 followed by heating. Column chromatography was performed on silica gel BW-200 or BW-300 (Fuji Davison Chemicals Co., Ltd.).

Culture Conditions *P. tenue* was isolated from a water sample collected from the moat around Nagoya Castle in 1981. Axenic cultures were prepared by the repeated capillary pipette washing method and have been maintained in CT medium adjusted to pH 8.0 at 25 °C with cool-white fluorescent illumination of 1000 lux. The alga was cultured in 5-l Erlenmeyer flasks containing CT medium, *viz.* in g/l $Ca(NO_3)_2 \cdot 4H_2O$ 0.15; KNO_3 0.1; β-Na_2 glycerophosphate 0.05; $MgSO_4 \cdot 7H_2O$ 0.04; minor element solution 0.1 ml/l; trace elements solution 0.1 ml/l. The minor elements solution was composed of, in g/l $FeCl_3 \cdot 6H_2O$ 0.196; $MnCl_2 \cdot 4H_2O$ 0.036; $ZnSO_4 \cdot 7H_2O$ 0.022; $CoCl_2 \cdot 6H_2O$ 0.004; $Na_2MnO_4 \cdot 2H_2O$ 0.0025; $Na_2EDTA \cdot 2H_2O$ 1.0. The trace element solution consisted of, in g/l, vitamin B_{12} 0.1; biotin 0.1; thiamine·HCl 10.0. The pH of the medium was adjusted to 8.0 with sodium hydroxide prior to autoclaving. Cultures were illuminated continuously at an incident intensity of 1000 lux with cool-white fluorescent lamps and vigorously aerated with sterilized air passed through a 0.2 μm membrane filter (Millipore, Mirex FG-50) at the rate of 0.5 l/min. After three weeks, the alga was harvested by centrifugation at 20000 × *g* from the combined 40-l culture and lyophilized. Yields of lyophilized cells were typically in the range of 0.2—0.22 g/l of culture.

Isolation The lyophilized alga (7.85 g) was homogenized in $CHCl_3$:MeOH = 1:2 (300 ml), then the whole mixture was stirred for 20 min. The mixture was separated into supernatant and precipitate by centrifugation (1000 × *g*) and the resulting precipitate was extracted with $CHCl_3$:MeOH = 1:2 (300 ml) twice by the same procedure. $CHCl_3$:MeOH = 1:1 (600 ml) was added to the supernatant and the mixture was centrifuged at 1000 × *g* for 15 min. The upper and middle layers were discarded, then MeOH:H_2O = 10:9 (300 ml) was added to the lower layer. The mixture was centrifuged at 1000 × *g* for 15 min and the upper and middle layers were discarded. The resulting lower layer was concentrated under reduced pressure to yield a lipid extract (816 mg). The extract was subjected to silica gel column chromatography using $CHCl_3$:MeOH:H_2O = 10:3:1 (lower phase) as the eluent to afford a crude MGDG fraction. The column was then eluted with $CHCl_3$:MeOH:H_2O = 7:3:1 (lower phase) to yield a crude DGDG fraction. The crude MGDG fraction was applied to a column of Sephadex LH-20 using $CHCl_3$ as the eluent to furnish the pure MGDG fraction (101 mg). The MGDG fraction was purified by HPLC (Develosil ODS A-5, MeOH:acetone:H_2O = 60:40:5, 10 mm i.d. × 250 mm, Nomura Chemical Co., Ltd.) to furnish **1** (31.2 mg), **2** (7.2 mg), **3** (37.2 mg), **4** (6.4 mg), **5** (4.9 mg), **8** (6.0 mg), a mixture of **6** and **7** (4.8 mg), and a mixture of **9a** and **9b** (8.6 mg). The mixture of **6** and **7** was further separated by HPLC (Develosil ODS K-5, 7.2 mm i.d. × 250 mm, MeOH:acetone:H_2O = 60:40:5) to give **6** (2.0 mg) and **7** (2.8 mg). The crude DGDG fraction was subjected to MPLC (MeOH:H_2O = 50:1) to afford pure DGDG fraction (70 mg). The fraction was purified by HPLC (Develosil ODS-5, 10 mm i.d. × 250 mm, MeOH:acetone:H_2O = 60:40:5) to furnish **11** (13.4 mg), **12** (27.2 mg), **13** (2.8 mg), **16** (4.8 mg), **17** (2.5 mg), **18** (2.9 mg), **19** (2.5 mg), a mixture of **14** and **15** (9.2 mg), and a mixture of **20a** and

20b (2.4 mg). The mixture of **14** and **15** were further separated by HPLC (Develosil ODS A-5, MeOH : CH_3CN : H_2O = 940 : 59 : 1, 10 mm i.d. × 250 mm) to yield **14** (5.1 mg) and **15** (4.0 mg). **1**: A colorless oil. $[\alpha]_D^{24}$ −2.7° (c = 0.7, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500, 1730. FAB-MS m/z: 747 $(M+Na)^+$. ^{1}H-NMR (d_5-pyridine) δ: 0.88 (3H, t, J = 6.8 Hz), 0.96 (3H, t, J = 7.5 Hz), 2.12 (4H, m), 2.41 (4H, m), 2.95 (4H, m), 4.08 (1H, dd, J = 5.5, 5.7 Hz, 5′-H), 4.09 (1H, dd, J = 5.0, 10.8 Hz, *sn*-3-H), 4.16 (1H, dd, J = 3.4, 9.4 Hz, 3′-H), 4.40 (1H, dd, J = 5.3, 10.8 Hz, *sn*-3-H), 4.42 (2H, m, 6′-H_2), 4.46 (1H, m, 2′-H), 4.54 (1H, dd, J = 6.2, 10.8 Hz, *sn*-1-H), 4.57 (1H, d, J = 3.4 Hz, 4′-H), 4.72 (1H, dd, J = 3.1, 10.8 Hz, *sn*-1-H), 4.84 (1H, d, J = 7.7 Hz), 5.42—5.46 (6H, m), 5.71 (1H, m, *sn*-2-H). Assignments for this compound were made with the aid of the homonuclear decoupling spectra. ^{13}C-NMR: Table I. **2**: A colorless oil. $[\alpha]_D^{24}$ −3.4° (c = 0.8, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500, 1730. FAB-MS m/z: 773 $(M+Na)^+$. ^{1}H-NMR (d_5-pyridine) δ: 0.88 (3H, t, J = 6.8 Hz), 0.96 (3H, t, J = 7.5 Hz), 2.09 (4H, m), 2.36 (2H, t, J = 7.4 Hz), 2.49 (4H, br s), 2.94 (4H, m), 4.09 (1H, dd, J = 5.7, 5.7 Hz, 5′-H), 4.10 (1H, dd, J = 5.4, 10.9 Hz, *sn*-3-H), 4.16 (1H, dd, J = 3.3, 9.3 Hz, 3′-H), 4.40 (1H, dd, J = 4.8, 10.9 Hz, *sn*-3-H), 4.42 (2H, m, 6′-H_2), 4.46 (1H, m, 2′-H), 4.54 (1H, dd, J = 3.3, 11.9 Hz, *sn*-1-H), 4.57 (1H, d, J = 3.3 Hz, 4′-H), 4.70 (1H, dd, J = 3.3, 11.9 Hz, *sn*-1-H), 4.84 (1H, d, J = 7.7 Hz), 5.42—5.56 (8H, m), 5.70 (1H, m, *sn*-2-H). Assignments for this compound were made with the aid of the homonuclear decoupling spectra. **3**: A colorless oil. $[\alpha]_D^{24}$ −2.3° (c = 0.8, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500, 1735. FAB-MS m/z: 749 $(M+Na)^+$. ^{1}H-NMR (d_5-pyridine) δ: 0.87 (3H, t, J = 6.8 Hz), 0.88 (3H, t, J = 6.8 Hz), 2.12 (4H, m), 2.40 (4H, m), 2.93 (2H, dd, J = 5.6, 5.8 Hz), 4.05—4.13 (2H, m, 5′-H, *sn*-3-H), 4.17 (1H, dd, J = 3.4, 9.5 Hz, 3′-H), 4.40 (1H, dd, J = 5.4, 11.0 Hz, *sn*-3-H), 4.43—4.50 (3H, m, 2′-H, 6′-H_2), 4.52—4.60 (2H, m, 4′-H, *sn*-1-H), 4.72 (1H, dd, J = 3.4, 12.0 Hz, *sn*-1-H), 4.85 (1H, d, J = 7.8 Hz, 1′-H), 5.52 (4H, m), 5.70 (1H, m, *sn*-2-H). Assignments for this compound were made with the aid of the homonuclear decoupling spectra. **4**: A colorless oil. $[\alpha]_D^{24}$ −2.8° (c = 0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500, 1730. FAB-MS m/z: 723 $(M+Na)^+$. ^{1}H-NMR (d_5-pyridine) δ: 0.88 (6H, m), 2.10 (4H, m), 2.39 (4H, m), 2.93 (2H, dd, J = 5.6, 5.8 Hz), 4.06—4.12 (2H, m, 5′-H, *sn*-3-H), 4.17 (1H, dd, J = 3.5, 9.4 Hz, 3′-H), 4.40 (1H, dd, J = 5.4, 10.9 Hz, *sn*-3-H), 4.43—4.59 (2H, m, 4′-H, *sn*-1-H), 4.72 (1H, dd, J = 3.3, 11.9 Hz, *sn*-1-H), 4.85 (1H, d, J = 7.7 Hz, 1′-H), 5.52 (2H, m), 5.70 (1H, m, *sn*-2-H). **5**: A colorless oil. $[\alpha]_D^{24}$ −2.5° (c = 0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3520, 1730. FAB-MS m/z: 751 $(M+Na)^+$. ^{1}H-NMR (d_5-pyridine) δ: 0.89 (6H, m), 2.12 (4H, m), 4.05—4.12 (2H, m, 5′-H, *sn*-3-H), 4.17 (1H, dd, J = 3.3, 9.5 Hz, 3′-H), 4.40 (1H, dd, J = 5.4, 10.8 Hz, *sn*-3-H), 4.42—4.50 (3H, m, 2′-H, 6′-H_2), 4.52—4.60 (2H, m, 4′-H, *sn*-1-H), 4.73 (1H, dd, J = 3.1, 11.9 Hz, *sn*-1-H), 4.85 (1H, d, J = 7.7 Hz, 1′-H), 5.51 (2H, m), 5.72 (1H, m, *sn*-2-H). **6**: A colorless oil. $[\alpha]_D^{24}$ −3.4° (c = 0.6, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3530, 1735. FAB-MS m/z: 777 $(M+Na)^+$. ^{1}H-NMR (d_5-pyridine) δ: 0.88 (6H, m), 2.12 (4H, m), 2.40 (4H, m), 2.93 (2H, t-like), 4.06—4.13 (2H, m, 5′-H, *sn*-3-H), 4.17 (1H, dd, J = 3.1, 9.5 Hz, 3′-H), 4.40 (1H, dd, J = 5.3, 10.8 Hz, *sn*-3-H), 4.43—4.51 (3H, m, 2′-H, 6′-H_2), 4.53—4.59 (2H, m, 4′-H, *sn*-1-H), 4.73 (1H, dd, J = 3.1, 11.7 Hz, *sn*-1-H), 4.85 (1H, d, J = 7.5 Hz, 1′-H), 5.51 (4H, m), 5.70 (1H, m, *sn*-2-H). **7**: A colorless oil. $[\alpha]_D^{24}$ −2.8° (c = 0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3530, 1735. FAB-MS m/z: 725 $(M+Na)^+$. ^{1}H-NMR (d_5-pyridine) δ: 0.89 (6H, m), 2.39 (4H, m), 4.07—4.13 (2H, m, 5′-H, *sn*-3-H), 4.17 (1H, dd, J = 3.3, 9.5 Hz, 3′-H), 4.40 (1H, dd, J = 5.5, 11.2 Hz, *sn*-3-H), 4.43—4.52 (3H, m, 2′-H, 6′-H_2), 4.54—4.60 (2H, m, 4′-H, *sn*-1-H), 4.73 (1H, dd, J = 3.1, 11.9 Hz, *sn*-1-H), 4.85 (1H, d, J = 7.9 Hz, 1′-H), 5.70 (1H, m, *sn*-2-H). **11**: A colorless oil. $[\alpha]_D^{24}$ +43.9° (c = 0.8, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1735. FAB-MS m/z: 909 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (3H, t, J = 7.1 Hz), 0.97 (3H, t, J = 7.5 Hz), 2.09 (4H, m), 2.32 (4H, m), 2.81 (4H, t, J = 5.7 Hz), 3.49 (1H, dd, J = 4.1, 9.7 Hz, 3′-H), 3.51 (1H, dd, J = 6.8, 9.7 Hz, 2′-H), 3.63—3.80 (8H, m), 3.83—3.92 (4H, m), 3.94 (1H, dd, J = 5.5, 11.0 Hz, *sn*-3-H), 4.22 (1H, dd, J = 6.8, 12.1 Hz, *sn*-1-H), 4.24 (1H, d, J = 6.8 Hz, 1′-H), 4.43 (1H, dd, J = 2.9, 12.1 Hz, *sn*-1-H), 5.25 (1H, m, *sn*-2-H), 5.30 (6H, m), ^{13}C-NMR: Table I. **12**: A colorless oil. $[\alpha]_D^{24}$ +44.6° (c = 0.9, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1740. FAB-MS m/z: 911 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (3H, t, J = 7.0 Hz), 0.91 (3H, t, J = 7.0 Hz), 2.06 (4H, m), 2.32 (4H, m), 2.77 (2H, t, J = 6.2 Hz), 3.48 (1H, dd, J = 3.4, 9.8 Hz, 3′-H), 3.51 (1H, dd, J = 6.6, 9.8 Hz, 2′-H), 3.63—3.80 (8H, m), 3.83—3.93 (4H, m), 3.93 (1H, dd, J = 5.4, 10.8 Hz, *sn*-3-H), 4.22 (1H, dd, J = 6.6, 12.0 Hz, *sn*-1-H), 4.24 (1H, d, J = 6.6 Hz, 1′-H), 4.43 (1H, dd, J = 2.9, 12.1 Hz, *sn*-1-H), 5.24 (1H, m, *sn*-2-H), 5.33 (4H, m). **13**: A colorless oil. $[\alpha]_D^{24}$ +42.1° (c = 0.6, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1750. FAB-MS m/z: 885 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, t, J = 7.0 Hz), 2.06 (4H, m), 2.32 (4H, m), 3.47 (1H, dd, J = 3.3, 9.7 Hz, 3′-H), 3.51 (1H, dd, J = 7.0, 9.7 Hz, 2′-H), 3.63—3.80 (8H, m), 3.83—3.92 (4H, m), 3.93 (1H, dd, J = 5.3, 10.6 Hz, *sn*-3-H), 4.22 (1H, dd, J = 6.8, 12.0 Hz, *sn*-1-H), 4.24 (1H, d, J = 7.0 Hz, 1′-H), 4.43 (1H, dd, J = 2.9, 12.0 Hz, *sn*-1-H), 5.25 (1H, m, *sn*-2-H), 5.35 (2H, m). **14**: A colorless oil. $[\alpha]_D^{24}$ +49.0° (c = 0.6, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1725. FAB-MS m/z: 913 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.89 (6H, t, J = 6.2 Hz), 2.03 (4H, m), 2.32 (4H, m), 3.47 (1H, dd, J = 3.3, 9.7 Hz, 3′-H), 3.51 (1H, dd, J = 7.0, 9.7 Hz, 2′-H), 3.63—3.80 (8H, m), 3.83—3.92 (4H, m), 3.93 (1H, dd, J = 5.3, 10.6 Hz, *sn*-3-H), 4.22 (1H, dd, J = 6.8, 12.1 Hz, *sn*-1-H), 4.24 (1H, d, J = 7.0 Hz, 1′-H), 4.43 (1H, dd, J = 2.9, 12.1 Hz, *sn*-1-H), 5.25 (1H, m, *sn*-2-H), 5.34 (2H, m). **15**: A colorless oil. $[\alpha]_D^{24}$ +44.2° (c = 0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3410, 1725. FAB-MS m/z: 887 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, t, J = 6.6 Hz), 2.32 (4H, m), 3.47 (1H, dd, J = 3.3, 9.7 Hz, 3′-H), 3.51 (1H, dd, J = 7.0, 9.7 Hz, 2-H), 3.63—3.80 (8H, m), 3.83—3.92 (4H, m), 3.93 (1H, dd, J = 5.5, 11.0 Hz, *sn*-3-H), 4.22 (1H, dd, J = 6.8, 12.1 Hz, *sn*-1-H), 4.24 (1H, d, J = 7.0 Hz, 1′-H), 4.43 (1H, dd, J = 2.9, 12.1 Hz, *sn*-1-H), 5.25 (1H, m, *sn*-2-H). **16**: A colorless oil. $[\alpha]_D^{24}$ +46.1° (c = 0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3410, 1740. FAB-MS m/z: 961 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.91 (3H, t, J = 7.0 Hz), 0.96 (3H, t, J = 7.5 Hz), 2.08 (8H, m), 2.32 (4H, m), 2.80 (6H, m), 3.47 (1H, dd, J = 3.3, 9.7 Hz, 3′-H), 3.51 (1H, dd, J = 7.0, 9.7 Hz, 2′-H), 3.63—3.80 (8H, m), 3.83—3.92 (4H, m), 3.93 (1H, dd, J = 5.5, 11.0 Hz, *sn*-3-H), 4.22 (1H, dd, J = 6.8, 12.0 Hz, *sn*-1-H), 4.23 (1H, d, J = 7.0 Hz, 1-H), 4.43 (1H, dd, J = 2.9, 12.0 Hz, *sn*-1-H), 5.25 (1H, m, *sn*-2-H), 5.35 (10H, m). **17**: A colorless oil. $[\alpha]_D^{24}$ +33.7° (c = 0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3390, 1740. FAB-MS m/z: 959 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.91 (3H, t, J = 7.3 Hz), 0.98 (3H, t, J = 7.5 Hz), 2.07 (8H, m), 2.32 (4H, m), 2.81 (8H, m), 3.47 (1H, dd, J = 3.0, 9.7 Hz, 3′-H), 3.51 (1H, dd, J = 7.0, 9.7 Hz, 2′-H), 3.63—3.80 (8H, m), 3.83—3.92 (4H, m), 3.93 (1H, dd, J = 5.5, 11.0 Hz, *sn*-3-H), 4.22 (1H, dd, J = 7.0, 12.1 Hz, *sn*-1-H), 4.42 (1H, d, J = 7.0 Hz, 1′-H), 4.43 (1H, dd, J = 2.9, 12.1 Hz, *sn*-1-H), 5.26 (1H, m, *sn*-2-H), 5.36 (12H, m). **18**: A colorless oil. $[\alpha]_D^{24}$ +51.2° (c = 0.6, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1730. FAB-MS m/z: 963 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.91 (6H, t, J = 6.8 Hz), 2.06 (8H, m), 2.34 (4H, m), 2.77 (4H, m), 3.47 (1H, dd, J = 3.3, 9.7 Hz, 3-H), 3.51 (1H, dd, J = 7.0, 9.7 Hz, 2′-H), 3.63—3.80 (8H, m), 3.83—3.92 (4H, m), 3.93 (1H, dd, J = 5.3, 10.6 Hz, *sn*-3-H), 4.22 (1H, dd, J = 6.7, 12.1 Hz, *sn*-1-H), 4.24 (1H, d, J = 7.0 Hz, 1-H), 4.43 (1H, dd, J = 2.9, 12.1 Hz, *sn*-1-H), 5.25 (1H, m, *sn*-2-H), 5.34 (8H, m). **19**: A colorless oil. $[\alpha]_D^{24}$ +47.6° (c = 0.7, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3420, 1730. FAB-MS m/z: 939 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, t, J = 6.8 Hz), 2.06 (4H, m), 2.32 (4H, m), 2.78 (2H, m), 3.47 (1H, dd, J = 3.3, 9.7 Hz, 3′-H), 3.51 (1H, dd, J = 7.1, 9.7 Hz, 2′-H), 3.63—3.80 (8H, m), 3.83—3.92 (4H, m), 3.93 (1H, dd, J = 5.5, 11.0 Hz, *sn*-3-H), 4.22 (1H, dd, J = 6.9, 11.9 Hz, *sn*-1-H), 4.24 (1H, d, J = 7.1 Hz, 1′-H), 4.43 (1H, dd, J = 2.9, 11.1 Hz, *sn*-1-H), 5.24 (1H, m, *sn*-2-H), 5.33 (4H, m).

Alkaline Treatment of 1 A solution of **1** (5.0 mg) in dry MeOH (1.0 ml) was treated with 5% NaOMe–MeOH (0.2 ml) at room temperature for 10 min. The reaction mixture was neutralized by using ion-exchange resin (Dowex 50 W × 8) and the resin was removed by filtration. The filtrate was extracted with hexane and the hexane layer was concentrated under reduced pressure to yield a mixture of methyl myristate and methyl linolenate (3.5 mg). The mixture of methyl esters was identified by GLC comparison with authentic samples. Removal of the solvent from the MeOH layer under reduced pressure gave a residue, which was purified by SiO_2 column chromatography ($CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to furnish **10** (1.7 mg). **10**: A colorless oil. $[\alpha]_D^{27}$ −8.0° (c = 0.8, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3500. FAB-MS m/z: 277 $(M+Na)^+$. ^{1}H-NMR (d_5-pyridine) δ: 4.08 (1H, dd, J = 5.3, 6.6 Hz, 5′-H), 4.13 (1H, d, J = 5.5 Hz, *sn*-1-H), 4.14 (1H, d, J = 4.9 Hz, *sn*-1-H), 4.17 (1H, dd, J = 3.3, 9.3 Hz, 3′-H), 4.27 (1H, dd, J = 3.8, 9.7 Hz, *sn*-3-H), 4.45 (3H, m, 6′-H_2, *sn*-2-H, *sn*-3-H), 4.52 (1H, dd, J = 7.7, 9.3 Hz, 2′-H), 4.56 (1H, d, J = 3.3 Hz, 4′-H), 4.91 (1H, d, J = 7.7 Hz, 1′-H). ^{13}C-NMR: Table I.

1,2-*O*-Dibenzyl-*sn*-glycerol (21) A solution of **1** (2.0 mg) in dry MeOH (1.0 ml) was treated with 5% NaOMe–MeOH (0.1 ml) at room temperature for 10 min. The reaction mixture was neutralized by using ion-exchange resin (Dowex 50 W × 8) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to give a product. A solution of it in dry DMF (0.5 ml) was treated with NaH (1.0 mg) at room temperature for 30 min. BnBr (6.4 μl) was added to the reaction solution, then the mixture was stirred at room temperature for a further 7.5 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl, and the mixture was poured into water. The whole was extracted with EtOAc, then the EtOAc extract was washed with saturated aqueous NaCl and dried over $MgSO_4$. Removal of the solvent under reduced

pressure gave a benzyl ether. A solution of it in 10% dry HCl–MeOH (1.0 ml) was heated under reflux for 3 h. The reaction mixture was poured into water and the whole was extracted with EtOAc. The EtOAc extract was washed successively with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a residue, which was purified by silica gel column chromatography (hexane : acetone = 5 : 1) to furnish 1,2-*O*-dibenzyl-*sn*-glycerol (**21**, 2.6 mg). **21**: A colorless oil. $[\alpha]_D^{25}$ −16.0° (c = 0.3, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3410. ^{1}H-NMR ($CDCl_3$) δ: 2.05 (1H, t-like, *sn*-3-OH), 3.58—3.78 (5H, m), 4.53—4.56 (2H, ABq, J = 12.1 Hz, Ph–CH_2), 4.62—4.72 (2H, ABq, J = 11.7 Hz, Ph–CH_2), 7.32 (10H, m, Ph–CH_2 × 2). UV λ_{max}^{MeOH} nm (ε): 212.5 (8800). CD (c = 0.01, MeOH) $[\theta]^{23}$ (nm): $+1.3 \times 10^4$ (204) (positive maximum). MS m/z (%): 272 (M^+, 46). High resolution MS m/z: Calcd for $C_{17}H_{20}O_3$: 272.347, Found: 272.346.

Enzymatic Hydrolysis of 1 and 11 by the Use of Lipase (from *Rhizopus arrhizus*) A solution of **1** (5 mg) and Lipase type XI (700 unit) in the presence of Triton X-100 (2.5 mg) in boric acid–borax buffer (0.63 ml, pH 7.7) was stirred at 38 °C for 1 h. The reaction was quenched with acetic acid (0.1 ml), then EtOH was added to the reaction mixture. The solvent was removed under reduced pressure and the resulting residue was chromatographed on silica gel using $CHCl_3$–MeOH (7 : 1) as the eluent to yield **1a** (3.2 mg) and linolenic acid (1.8 mg). In the case of lipase-catalyzed hydrolysis of **11** (5.0 mg), **11a** (3.6 mg) was isolated by silica gel column chromatography with $CHCl_3$: MeOH : H_2O (7 : 3 : 1, lower phase) as the eluent. Enzymatic hydrolysis of the other MGDGs and DGDGs was carried out in the same manner. **1a**: A colorless oil. $[\alpha]_D^{26}$ −7.1° (c = 0.7, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3425, 1730. FAB-MS m/z: 487 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (3H, t, J = 6.8 Hz), 2.35 (2H, t, J = 7.5 Hz), 3.45 (1H, dd, J = 3.3, 9.7 Hz, 3′-H), 3.50 (1H, ddd-like, 5-H), 3.51 (1H, dd, J = 7.3, 9.7 Hz, 2′-H), 3.66—3.79 (5H, m, 6′-H_2, *sn*-1-H_2, *sn*-3-H), 3.82 (1H, dd, J = 0.9, 3.3 Hz, 4-H), 3.96 (1H, dd, J = 5.7, 11.0 Hz, *sn*-3-H), 5.04 (1H, m, *sn*-2-H). ^{13}C-NMR: Table I. **11a**: A colorless oil. $[\alpha]_D^{26}$ +17.0° (c = 0.4, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1740. FAB-MS m/z: 649 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (3H, t, J = 7.0 Hz), 2.35 (2H, m), 3.49 (2H, m, 2-H, 3-H), 3.63—3.80 (10H, m), 3.84—3.95 (5H, m), 4.24 (1H, d, J = 7.3 Hz, 1-H). ^{13}C-NMR: Table I.

Identification of Palmitelaidic Acid by the Picolinyl Method Enzymatic hydrolysis of **2** by the lipase was carried out as above to yield 1-*O*-deacylmonogalactosylglycerol. A solution of it in dry MeOH (1.0 ml) was treated with 10% KOH–MeOH (1.0 ml) at room temperature for 10 min. Work-up of the reaction mixture as described above furnished palmitelaidic acid (0.6 mg). This fatty acid was treated with $SOCl_2$ (1.0 ml) at room temperature for 5 min. Excess $SOCl_2$ was removed, and the corresponding acid chloride in dry CH_3CN (0.1 ml) was treated with a 10% solution of 3-pyridylcarbinol in CH_3CN (0.1 ml) at room temperature for 10 min. Removal of the solvent by a stream of nitrogen gave a picolinyl ester, which was subjected to GC-MS analysis.

Assay of the Antialgal Activity Algal cultures for bioassay were grown to the late logarithmic growth phase for 10 d. A MeOH solution of each test compound (200 μl) sterilized through a membrane filter (Bio-Rad Inc., Micro Prep-disk, 0.2 μm pore size) was added to the CT-medium of the alga (20 ml). After incubation for 3 d, the cultures was filtered and chlorophyll a was extracted from the collected alga with 95% acetone. The concentration of chlorophyll a was determined photometrically as described by Strickland.[11] The IC_{50} of each galactolipid was determined from dose–response curves obtained with six different concentrations of test compounds (1, 5, 10, 25, 50, 100 ppm). The bioassay was carried out in duplicate.

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