Studies on Glycolipids. VII.[1] Isolation of Two New Sulfoquinovosyl Diacylglycerols from the Green Alga *Chlorella vulgaris*

Takashi MORIMOTO, Nobutoshi MURAKAMI,[2] Akito NAGATSU, and Jinsaku SAKAKIBARA*

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan.
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Two new sulfoquinovosyl diacylglycerols (SQDGs, 1, 2) one of which contained a branched fatty acid as an acyl substituent were isolated from the green alga *Chlorella vulgaris*, along with three known SQDGs (3—5). Their structures were determined on the basis of physicochemical evidence and enzymatic hydrolysis using lipase (from *Rhizopus arrhizus*).

Keywords sulfoquinovosyl diacylglycerol (SQDG); glyceroglycolipid; green alga; *Chlorella vulgaris*; branched fatty acid

Together with galactosyl diacylglycerols, sulfoquinovosyl diacylglycerols (SQDGs) are present in photosynthetic tissue of plants, and are structural components of thylakoid membrane.[3] Furthermore, they are concerned with the electron transport chain in photosynthesis as well as being involved in the construction of chloroplasts. On the other hand, SQDGs have been found to exhibit several biological activities such as anti-human immunodeficiency virus (HIV)-I activity,[4] resistance to a complement fixation reaction,[5] and antitumor-promoting activity.[6] In spite of their physicochemically and pharmacologically unique properties, most investigations on SQDGs have been conducted with mixtures containing various acyl substituents because of the difficulty of separating a component possessing a single pair of acyl residues. Thus, SQDG with a single acyl pair was synthesized to investigate its anti-HIV-I activity.[7] Analytical studies of SQDG to elucidate the acyl composition have so far been performed only after tedious chemical derivatization.[8] As a part of our investigation on glycolipids in microalgae, we undertook structural elucidation and establishment of a practical separation procedure of SQDGs in the fresh-water green alga *Chlorella vulgaris*. In this paper, we report two new sulfoquinovosyl diacylglycerols (**1**, **2**) from *C. vulgaris* and a practical separation method of SQDGs.

The fresh-water green alga *C. vulgaris* was cultured in modified Detmer (MD) medium for 3 weeks in our laboratory. The alga was harvested by centrifugation and lyophilized. The lyophilized alga (6.8 g) was extracted with $CHCl_3$: MeOH = 1 : 2. The extract (1.2 g) was successively subjected to silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower layer→$CHCl_3$: MeOH : 28% aqueous NH_3 = 13 : 4 : 1) to give a mixture of SQDGs. Separation of the mixture of SQDGs by reversed-phase HPLC using a usual octadecyl silica (ODS) column gave the result shown in Fig. 1A. Tailing on the chromatogram in Fig. 1A was presumably caused by partial dissociation of the highly polar sulfonic group of the sugar moiety in neutral media. Namely, taking into consideration that the pK_a of SQDG was *ca.* 5.7, about 5% of SQDG would be undissociated under a neutral condition. Therefore, we attempted to separate SQDGs in alkaline medium (pH 8.0—9.0), in which the sulfonic group would be completely dissociated. The separation of SQDGs by HPLC was carried out by use of a Capcell pak C_{18} column with MeOH : aqueous Na_2HPO_4 (pH 9.0) as the mobile phase. Figure 1B shows that the mixture of SQDGs is efficiently separated into eight peaks without tailing; five peaks consist of a pure SQDG [**1** (peak 2), **2** (peak 6), **3** (peak 5), **4** (peak 8), **5** (peak 7)] and the other three peaks are mixtures of a few SQDGs.

Compound **1** gave a quasimolecular ion peak at *m/z* 885 $(M+Na)^+$ in the positive FAB-MS. In the negative FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 839 $(M-Na)^-$. High-resolution FAB-MS indicated the molecular formula of **1** to be $C_{45}H_{75}NaO_{12}S$. The IR spectrum of **1** showed the presence of hydroxyl (3420 cm^{-1}), ester (1737 cm^{-1}), and sulfonic (1121, 1026 cm^{-1}) functions. The ^{1}H-NMR spectrum exhibited signals characteristic of glyceroglycolipid: *e.g.*, two terminal methyl signals (0.90 ppm, 6H, m), two methylene protons linked to a carbonyl function (2.35 ppm, 4H, m), and a 12H signal due to a monoglycosylglycerol moiety. Coupling constant analysis of the signals due to the sugar moiety showed that the relative configuration of hydroxy groups was the same as that of α-D-glucopyranoside, but the chemical shifts assignable to the sugar moiety were partly different. Namely, the signals of C_6-methylene protons at 3.34 and 2.91 ppm and C_6-carbon at 54.2 ppm in the sugar proton indicated the attachment of a sulfonic group on the C_6-carbon. Thus, the sugar moiety of **1** was determined to be sulfoquinovose. Taking into consideration that alkaline treatment of **1** afforded 3-α-D-6-sulfoquinovosyl-*sn*-glycerol,[9] compound **1** was concluded to be the sulfoquinovosyl diacylglycerol possessing 2*S*-configuration in the glycerol portion.

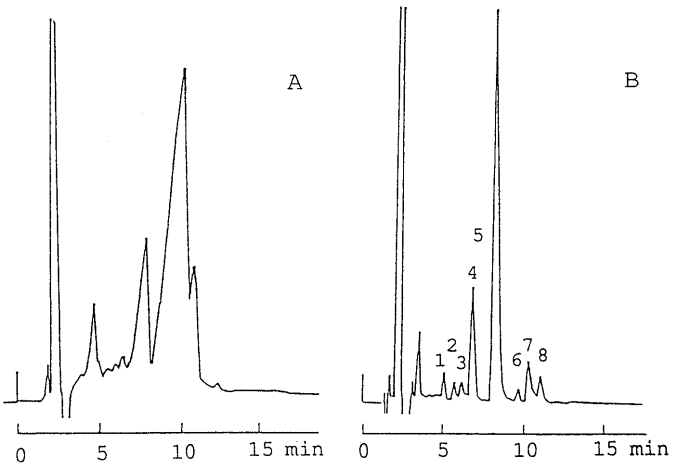


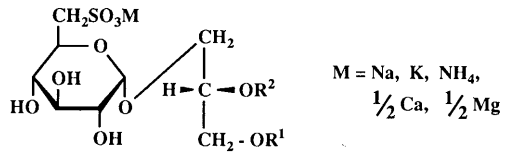
Fig. 1. HPLC Chromatograms of SQDGs

A. Column, Develosil ODS-5; Mobile phase, MeOH : H_2O = 91.5 : 8.5, B. Column, Capcell Pak C_{18}; Mobile phase, MeOH : aqueous Na_2HPO_4 (pH 9.0) = 91.5 : 8.5.

TABLE I. NMR Data for the Sulfoquinovosyl Glycerol Moiety in **1** and **1a**

C	**1** ^{1}H	**1** ^{13}C	**1a** ^{1}H
1	4.76 (d, J=3.8)	100.1	4.76 (d, J=3.8)
2	3.40 (dd, J=3.8, 9.7)	73.5	3.40 (dd, J=3.8, 9.7)
3	3.19 (dd, J=9.0, 9.7)	75.0	3.08 (t-like)
4	3.63 (dd, J=9.0, 9.3)	74.9	3.61 (t-like)
5	4.06 (ddd, J=2.2, 9.2, 9.3)	69.9	4.07 (m)[a]
6	3.34 (dd, J=2.2, 14.3)	54.2	3.35 (dd, J=2.0, 14.3)
	2.91 (dd, J=9.2, 14.3)		2.92 (dd, J=9.1, 14.3)
sn-1	4.69 (dd, J=2.9, 12.1)	64.3	3.74 (dd, J=4.3, 12.2)
	4.18 (dd, J=6.8, 12.1)		3.71 (dd, J=5.4, 12.2)
sn-2	5.36 (m)	71.7	5.09 (m)
sn-3	4.10 (dd, J=5.3, 10.8)	67.1	4.07 (m)[a]
	3.57 (dd, J=6.2, 10.8)		3.53 (dd, J=6.2, 10.7)

The NMR spectra were recorded in CD_3OD (^{1}H, 400 MHz; ^{13}C, 100 MHz).
a) These signals overlapped.



1 : R^1 = linolenoyl, R^2 = linoleoyl
1a : R^1 = H, R^2 = linoleoyl
1b : $R^1 = R^2$ = H
2 : R^1 = linoleoyl, R^2 = isoheptadecanoyl
2a : R^1 = H, R^2 = isoheptadecanoyl
3 : R^1 = linoleoyl, R^2 = palmitoyl
3a : R^1 = H, R^2 = palmitoyl
4 : R^1 = oleoyl, R^2 = palmitoyl
5 : R^1 = palmitoyl, R^2 = palmitoyl

Chart 1

We next examined regioselective deacylation by use of lipase to determine the distribution of the two fatty acid residues. On enzymatic hydrolysis using lipase type XI (from *Rhizopus arrhizus*, Sigma Co., Ltd.) in the presence of Triton X-100 in boric acid–borax buffer (pH 7.7), **1** gave exclusively *sn*-1 lysoglycolipid (**1a**) and linolenic acid.[10] The structure of **1a** was confirmed by the ^{1}H-NMR spectrum, in which the signals due to *sn*-1 methylene protons appeared at higher field than those of **1**. Treatment of **1a** with 5% NaOMe–MeOH liberated methyl linolate. Accordingly, the structure of **1** was determined as (2*S*)-1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-2-*O*-(9*Z*,12*Z*-octadecadienoyl)-3-*O*-α-D-6-sulfoquinovosyl-*sn*-glycerol.

Compound **2** gave a quasimolecular ion peak m/z 877 $(M+Na)^+$ in the positive FAB-MS. The ^{1}H-NMR spectrum of **2** showed three terminal methyl signals. GC-MS analysis of the methyl ester of the fatty acid attached to the *sn*-2 position gave a molecular ion peak at m/z 284 and a fragment ion peak at m/z 241 $(M-C_3H_7)^+$ indicative of the presence of a terminal isopropyl group. Therefore, the fatty acid residue linked to *sn*-2 position was determined to be a branched fatty acid, isoheptadecanoic acid. Consequently, the structure of **2** was characterized as (2*S*)-1-*O*-(9*Z*,12*Z*-octadecadienoyl)-2-*O*-(isoheptadecanoyl)-3-*O*-α-D-6-sulfoquinovosyl-*sn*-glycerol. The chemical structures of the other SQDGs (**3**—**5**) were similarly elucidated by enzymatic hydrolysis followed by treatment with 5% NaOMe–MeOH (Chart 1).

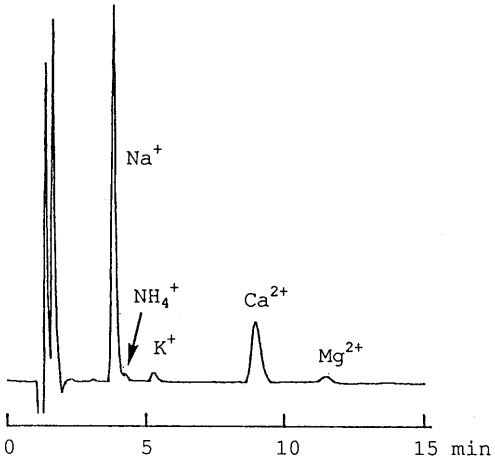


Fig. 2. Positive Ion Chromatogram of SQDGs
Column, Shodex IC YD-521; eluent, 5 mM tartaric acid, 1 mM dipicolinic acid; flow rate, 1.0 ml/min, detection; electric conductivity detector.

Some of the SQDGs were characterized as the sulfonic acid, and others as alkaline metal salts. Thus, we finally examined whether the SQDGs from the cyanobacterium were free sulfonic acids[11] or metal salts.[12] When the FAB-MS of the mixture of **1**—**5** was taken without adding inorganic salts, the quasimolecular ion peak at m/z 817 [R^3=Na, $(M+H)^+$, $R^1=C_{16:0}$, $R^2=C_{16:0}$] was intense. However, the quasimolecular ion peak corresponding to the molecular formula $C_{41}H_{79}O_{12}S$ [R=H, $(M+H)^+$, $R^1=C_{16:0}$, $R^2=C_{16:0}$] was weak. Diazomethane treatment of the SQDG mixture in the presence of a slight excess of AcOH afforded the corresponding methyl esters, while the reaction in the absence of AcOH gave little of the methyl esters. These findings suggest that the SQDG mixture was isolated as metal salts by the above procedure. To confirm this, the SQDG mixture before HPLC separation was subjected to positive ion chromatography to give the chromatogram in Fig. 2, which shows the existence of Na^+, K^+, NH_4^+, Ca^{2+}, and Mg^{2+} as counter cations. Quantification of the cations revealed that the SQDG mixture contained approximately an equimolar amount of the cations. The contents of counter cations in 1 mg (*ca.* 1.2 μm) of the SQDG mixture were as follows: Na^+ 0.79 μM, NH_4^+ 0.02 μM, K^+ 0.04 μM, Ca^{2+} 0.10 μM, Mg^{2+} 0.01 μM. Thus, most of the SQDGs isolated by the above procedure formed salts with several cations. Counter cations were presumed to have been exchanged during the isolation procedure. In particular, ammonium ion would be derived from aqueous NH_3 during column chromatography. The SQDG purified by the present HPLC procedure may include a larger proportion of sodium cation as the counter ion than the SQDG mixture before HPLC separation since aqueous Na_2HPO_4 was used as the mobile phase.

In summary, we have characterized two novel sulfoquinovosyl diacylglycerols (**1**, **2**) from the fresh-water green alga *C. vulgaris* and established a practical separation method of SQDGs containing various pairs of fatty acids. Among the five SQDGs, compound **2** was a novel type of glyceroglycolipid containing a branched fatty acid. To our knowledge, this is the first report of the isolation and characterization of a glyceroglycolipid possessing a

branched fatty acid as the acyl residue.

Experimental

IR spectra were recorded on a JASCO IRA-2 spectrometer. ^{1}H-NMR 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 a JEOL SX-102 spectrometer. Optical rotations were measured on a JASCO DIP-4 digital polarimeter. Gas liquid chromatography (GLC) was carried out on a Shimadzu GC-14A. 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, DB1 (0.25 mm i.d. × 30 m, J & W Scientific); injection temperature, 300 °C; column temperature, 160—300 °C, 3 °C/min; carrier gas, He, 1.0 ml/min. Mass spectrometry: ionizing energy, 70 eV; ion source temperature, 250 °C. HPLC was carried out using a JASCO 880-PU pump equipped with a JASCO 830-RI differential refractometer. Ion chromatography was performed using a JASCO PU-980 pump equipped with a Shodex CD-5 electric conductivity detector. 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% $HClO_4$ or 1% $Ce(SO_4)_2$–10% H_2SO_4 followed by heating. Column chromatography was performed on silica gel BW-200 (Fuji Davison Chemicals Co., Ltd.).

Culture Condition The strain of *C. vulgaris* (NIES-227) used for the present investigation was purchased from the National Institute for Environmental Studies of the Environment Agency. Axenic *C. vulgaris* was cultured in 5-l Erlenmeyer flasks containing MDM medium adjusted to pH 8.0 at 25 °C with cool-white fluorescent illumination of 3000 lux. MDM medium: KNO_3 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, K_2HPO_4 0.25 g, NaCl 0.1 g, $CaCl_2 \cdot 2H_2O$ 0.1 g, Fe solution ($FeSO_4 \cdot 7H_2O$ 1.0 g and concentrated H_2SO_4 2 drops were diluted to 500 ml with water) 1.0 ml, and A_5 solution (A_5 solution: H_3BO_4 286 mg, $CuSO_4 \cdot 5H_2O$ 7.9 mg, and $Na_2MoO_4 \cdot 2H_2O$ 2.1 mg were diluted to 100 ml with water) 1.0 ml were diluted to 1 l with water. The pH of the medium was adjusted to 8.0 with 1 N aqueous NaOH prior to autoclaving. The cultures were aerated with sterilized air passed through 0.2 μm membrane filters (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 36 l culture solution and lyophilized (yield, 6.88 g).

Isolation The lyophilized alga (6.88 g) was homogenized in $CHCl_3$: MeOH = 1 : 2 (300 ml). After being held at room temperature for 12 h, the mixture was separated into supernatant and precipitate by centrifugation (3000 × ***g***). The precipitate was further extracted twice with $CHCl_3$: MeOH = 1 : 2. The combined supernatants were evaporated under reduced pressure to give the extract. The extract (1.2 g) was purified by silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower layer) to give a crude SQDG fraction (459 mg), which was further purified by silica gel column chromatography ($CHCl_3$: MeOH : 28% aq. NH_3 = 13 : 4 : 1) to give a mixture of SQDG (152 mg). Then, the SQDG mixture was subjected to HPLC [Capcell Pak C_{18} column (4.6 mm i.d. × 250 mm), MeOH : aqueous Na_2HPO_4 (pH 9.0) = 91.5 : 8.5, flow rate 1.3 ml/min, detector refractive index (RI)] to furnish **1** (9.1 mg), **2** (10.4 mg), **3** (89.7 mg), **4** (9.6 mg), and **5** (15.5 mg). Compounds **3** and **4** were characterized as sulfonic acids.[4] Although compound **5** has been chemically synthesized,[13] this is the first report of its isolation from a natural source. The physicochemical properties of **5** except for elemental analysis are not available in the literature. **1**: a white amorphous powder. $[\alpha]_D^{26}$ +44.7° (*c* = 0.52, MeOH). IR (film): 3420, 1737, 1238, 1073 cm^{-1}. FAB-MS *m/z*: 885 (M + Na)$^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{45}H_{75}Na_2O_{12}S$: 886.1318. Found: 886.1362 (M + Na)$^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, m), 2.05 (8H, m), 2.32 (4H, m), 2.73 (6H, m), 5.26—5.44 (11H, m, olefinic protons, *sn*-2-H). The proton signals ascribable to the sulfoquinovosyl glycerol moiety are shown in Table I. **2**: a white amorphous powder. $[\alpha]_D^{26}$ +43.2° (*c* = 0.40, MeOH). IR (film): 3420, 1737, 1121, 1028 cm^{-1}. FAB-MS *m/z*: 877 (M + Na)$^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{44}H_{79}Na_2O_{12}S$: 878.1526. Found: 878.1579 (M + Na)$^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (9H, m), 2.05 (4H, m), 2.32 (4H, m), 2.77 (2H, m), 2.91 (1H, *J* = 9.1, 14.5 Hz, 6-H), 3.08 (1H, dd, *J* = 9.0, 9.6 Hz, 3-H), 3.34 (1H, *J* = 2.2, 14.5 Hz, 6-H), 3.40 (1H, dd, *J* = 3.9, 9.6 Hz, 2-H), 3.57 (1H, dd, *J* = 6.5, 10.8 Hz, *sn*-3-H), 3.63 (1H, m, 4-H), 4.06 (1H, m, 5-H), 4.10 (1H, dd, *J* = 5.1, 10.8 Hz, *sn*-3-H), 4.17 (1H, dd, *J* = 6.9, 12.1 Hz, *sn*-1-H), 4.50 (1H, *J* = 2.9, 12.1 Hz, *sn*-1-H), 4.76 (1H, d, *J* = 3.9 Hz, 1-H), 5.27—5.44 (5H, m, olefinic protons, *sn*-2-H). **3**: a white amorphous powder. $[\alpha]_D^{26}$ +43.8° (*c* = 0.50, MeOH). IR (film): 3420, 1736, 1121, 1028 cm^{-1}. FAB-MS *m/z*: 863 (M + Na)$^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{43}H_{77}Na_2O_{12}S$: 864.1255. Found: 864.1288 (M + Na)$^+$. ^{1}H-NMR (CD_3OD) δ: 0.89 (6H, m), 2.35 (4H, m), 2.76 (2H, m), 2.91 (dd, *J* = 9.2, 14.3 Hz, 6-H), 3.10 (dd, *J* = 9.0, 9.7 Hz, 3-H), 3.34 (dd, *J* = 2.2, 14.3 Hz, 6-H), 3.40 (dd, *J* = 3.8, 9.7 Hz, 2-H), 3.57 (dd, *J* = 6.2, 10.8 Hz, *sn*-3-H), 3.63 (dd, *J* = 9.0, 9.3 Hz, 4-H), 4.07 (ddd, *J* = 2.7, 9.3, 9.4 Hz, 5-H), 4.10 (dd, *J* = 5.3, 10.8 Hz, *sn*-3-H), 4.18 (dd, *J* = 6.8, 12.1 Hz, *sn*-1-H), 4.69 (dd, *J* = 2.9, 12.1 Hz, *sn*-1-H), 4.76 (d, *J* = 3.8 Hz, 1-H), 5.39—5.28 (5H, m, olefinic protons, *sn*-2-H). **4**: a white amorphous powder. $[\alpha]_D^{26}$ +41.9° (*c* = 0.06, MeOH). IR (film): 3383, 1737, 1121, 1028 cm^{-1}. FAB-MS *m/z*: 865 (M + Na)$^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{43}H_{79}Na_2O_{12}S$: 866.1414. Found: 866.1470. ^{1}H-NMR (CD_3OD) δ: 0.89 (6H, m), 2.33 (4H, m), 2.91 (dd, *J* = 9.1, 14.3 Hz, 6-H), 3.08 (dd, *J* = 9.0, 9.7 Hz, 3-H), 3.34 (dd, *J* = 2.2, 14.3 Hz, 6-H), 3.40 (dd, *J* = 3.9, 9.7 Hz, 2-H), 3.57 (dd, *J* = 6.3, 11.0 Hz, *sn*-3-H), 3.63 (dd, *J* = 9.0, 9.7 Hz, 4-H), 4.07 (ddd, *J* = 2.1, 9.1, 9.7 Hz, 5-H), 4.10 (dd, *J* = 5.3, 11.0 Hz, *sn*-3-H), 4.17 (dd, *J* = 6.9, 12.1 Hz, *sn*-1-H), 4.50 (dd, *J* = 3.0, 12.1 Hz, *sn*-1-H), 4.75 (d, *J* = 3.9 Hz, 1-H), 5.37—5.28 (3H, m, olefinic protons, *sn*-2-H). **5**: a white amorphous powder. $[\alpha]_D^{26}$ +48.0° (*c* = 0.15, $CHCl_3$: MeOH = 5 : 1). IR (film): 3420, 1736, 1121, 1028 cm^{-1}. FAB-MS *m/z*: 839 (M + Na)$^+$. High-resolution FAB-MS: Calcd for $C_{41}H_{77}Na_2O_{12}S$: 840.1032. Found: 840.1064. ^{1}H-NMR ($CDCl_3$: CD_3OD = 2 : 1) δ: 0.89 (6H, t, *J* = 6.8 Hz), 2.33 (4H, m), 3.08 (dd, *J* = 8.0, 14.3 Hz, 6-H), 3.22 (dd, *J* = 9.0, 9.7 Hz, 3-H), 3.34 (m, 6-H), 3.40 (dd, *J* = 3.7, 9.7 Hz, 2-H), 3.60 (dd, *J* = 6.4, 10.8 Hz, *sn*-3-H), 3.65 (t-like, *J* = 9.0 Hz, 4-H), 4.04 (m, 5-H), 4.04 (m, *sn*-3-H), 4.19 (dd, *J* = 7.1, 12.1 Hz, *sn*-1-H), 4.48 (dd, *J* = 3.0, 12.1 Hz, *sn*-1-H), 4.81 (d, *J* = 3.7 Hz, 1-H), 5.32 (m, *sn*-2-H).

Enzymatic Hydrolysis of the SQDGs (1—5) by the Use of Lipase (from *Rhizopus arrhizus*) A solution of **1** (2.0 mg), lipase type XI (720 units), and Triton X-100 (1.0 mg) in boric acid–borax buffer (0.25 ml, pH 7.7) was stirred at 38°C for 5 h. The reaction was quenched with 5% aqueous acetic acid (0.2 ml), then EtOH was added to the reaction mixture. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel with $CHCl_3$: MeOH : H_2O (65 : 35 : 10, lower layer) to yield **1a** (1.2 mg) and linolenic acid (0.6 mg). Linolenic acid was converted into a methyl ester by CH_2N_2 treatment and identified by GLC comparison with the authentic sample. In a similar manner, **2a** (1.3 mg) and linoleic acid (0.6 mg) from **2** (2.0 mg), **3a** (1.3 mg) and linoleic acid (0.6 mg) from **3** (2.0 mg), **3a** (1.3 mg) and oleic acid (0.6 mg) from **4**, **3a** (1.3 mg) and palmitic acid (0.6 mg) from **5** (2.0 mg) were obtained. **1a**: a white amorphous powder. $[\alpha]_D^{26}$ +51.5° (*c* = 0.15, MeOH). IR (film): 3384, 1736, 1121, 1029 cm^{-1}. FAB-MS *m/z*: 625 (M + Na)$^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{27}H_{47}Na_2O_{11}S$: 625.7086. Found: 625.7135 (M + Na)$^+$. ^{1}H-NMR (CD_3OD) δ: 0.89 (3H, t, *J* = 7.0 Hz), 2.05 (4H, m), 2.36 (2H, m), 2.70 (4H, m), 5.29—5.31 (4H, m). The proton signals ascribable to the sulfoquinovosyl glycerol moiety are shown in Table I. **2a**: a white amorphous powder. $[\alpha]_D^{26}$ +50.8° (*c* = 0.025, MeOH). IR (film): 3346, 1734, 1121, 1029 cm^{-1}. FAB-MS *m/z*: 615 (M + Na)$^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{26}H_{49}Na_2O_{11}S$: 615.7134. Found: 615.7189 (M + Na)$^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, m), 2.36 (2H, t, *J* = 7.4 Hz), 2.91 (dd, *J* = 9.1, 14.5 Hz, 6-H), 3.08 (t-like, 3-H), 3.34 (dd, *J* = 2.0, 14.5 Hz, 6-H), 3.40 (dd, *J* = 3.8, 9.7 Hz, 2-H), 3.53 (dd, *J* = 6.2, 10.6 Hz, *sn*-3-H), 3.61 (t-like, 4-H), 3.71 (dd, *J* = 5.5, 12.2 Hz, *sn*-1-H), 3.74 (dd, *J* = 4.3, 12.2 Hz, *sn*-1-H), 4.08 (m, *sn*-3-H, 5-H), 4.76 (d, *J* = 3.7 Hz, 1-H), 5.09 (m, *sn*-2-H). **3a**: a white amorphous powder. $[\alpha]_D^{26}$ +47.1° (*c* = 0.035, MeOH). IR (film): 3346, 1734, 1121, 1029 cm^{-1}. FAB-MS *m/z*: 601 (M + Na)$^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{25}H_{47}Na_2O_{11}S$: 601.6863. Found: 601.6898 (M + Na)$^+$. ^{1}H-NMR (CD_3OD) δ: 0.90 (3H, t, m), 2.36 (4H, m), 2.79 (2H, m), 2.91 (dd, *J* = 9.1, 14.5 Hz, 6-H), 3.08 (t-like, 3-H), 3.34 (dd, *J* = 2.1, 14.5 Hz, 6-H), 3.39 (dd, *J* = 3.7, 9.8 Hz, 2-H), 3.53 (dd, *J* = 6.0, 10.4 Hz, *sn*-3-H), 3.62 (t-like, 4-H), 3.71 (dd, *J* = 5.5, 12.1 Hz, *sn*-1-H), 3.76 (dd, *J* = 4.3, 12.1 Hz, *sn*-1-H), 4.06 (2H, m, 5-H, *sn*-3-H), 4.76 (d, *J* = 3.7 Hz, 1-H), 5.09 (m, *sn*-2-H).

Alkaline Treatment of *sn*-1 Lysosulfolipids (1a—3a) A solution of **1a** (1.0 mg) was treated with 5% NaOMe–MeOH (0.5 ml) at room temperature for 10 min. The reaction mixture was neutralized with an ion-exchange resin (Dowex 50W × 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 methyl linolate. The methyl ester was identified by GLC comparison with authentic samples. Removal of the solvent from the MeOH layer gave 3-α-D-6-sulfoquinovosyl-*sn*-glycerol (**1b**, 0.5 mg).

Similarly, alkaline treatment of **2a** (1.0 mg) and **3a** (1.0 mg) afforded methyl isoheptadecanoate (0.6 mg) and methyl palmitate (0.6 mg), respectively, along with **1b** (0.5 mg).

Diazomethane Treatment of the Mixture of SQDGs Giving the Corresponding Methyl Ester Mixture A solution of the SQDG mixture (5.0 mg) in 5% AcOH–MeOH (0.5 ml) was treated with excess ethereal diazomethane. The reaction mixture was concentrated under reduced pressure to give a residue, which was purified by column chromatography ($CHCl_3$: MeOH = 4 ; 1) to give the methyl ester mixture (5.1 mg). SQDG methyl ester: IR (film): 3384, 1740, 1359, 1169 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, m), 2.07 (4H, m), 2.78 (2H, m), 2.33 (4H, m), 3.27 (t-like, 4-H), 3.28 (dd, J=4.6, 9.4 Hz, 6-H), 3.48 (dd, J=3.7, 10.8 Hz, 2-H), 3.56 (dd, J=6.1, 10.4 Hz, *sn*-3-H), 3.72 (2H, m, 6-H, 3-H), 3.91 (3H, s, $-OCH_3$), 4.00 (dd, J=4.9, 10.4 Hz, *sn*-3-H), 4.13 (2H, m, 5-H, *sn*-1-H), 4.41 (dd, J=3.9, 11.8 Hz, *sn*-1-H), 4.85 (d, J=3.7 Hz), 5.35 (5H, m, olefinic protons, *sn*-2-H).

Fatty Acid Distribution of the SQDG from *Chlorella vulgaris* Lipase-catalyzed deacylation of the SQDG similarly gave the *sn*-1 lysoSQDG and the fatty acid mixture, which was converted into the methyl esters by CH_2N_2 treatment. On alkaline treatment, the *sn*-1 lysoSQDG was hydrolyzed to give the mixture of fatty acid methyl esters. GLC analysis of both mixtures of methyl esters determined the fatty acid distribution of SQDG to be as follows, *sn*-1: palmitic acid (28.4%), hexadecadienoic acid (1.6%), oleinic acid (2.6%), linoleic acid (51.2%), linolenic acid (16.2%), *sn*-2: palmitic acid (62.7%), hexadecadienoic acid (6.5%), isoheptadecanoic acid (3.1%) oleinic acid (7.0%), linoleic acid (15.2%), linolenic acid (5.5%).

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