Notes

New Diacylgalactolipids from the Marine Cyanophycean Microalga *Oscillatoria* sp.

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Glycolipids are widely distributed in plants[1] and in microorganisms[2,3,4] as components of the cell wall. In addition they perform many interesting biological activities[5] including antitumor-promoting, antiinflammatory, antialgal, hemolytic, antiviral properties, and inhibitory effects on platelet aggregation[6] and reverse transcriptase of HIV-1.[3]

As part of our search to find new bioactive compounds from marine microalgi, we have investigated the metabolites of the marine blue-green alga *Oscillatoria* sp. (strain #: KMCC CY-6), and have found new glycolipids, diacylgalactolipids I (**1**), II (**2**) and inseparable III (**3**) and IV (**4**).

Diacylgalactolipid I (**1**) showed a hydroxyl (3422 cm^{-1}) and ester functions (1735, 1245 cm^{-1}) in the IR spectrum. Diacylgalactolipid I (**1**) also gave a sodiated molecular ion of *m/z* 775 $(M+Na)^+$ in FABMS. The ^1H- and ^{13}C NMR spectra of **1** showed signals assignable for a monogalactopyranosyl-1,2-diacylglycerol (Tables 1 and 2). Alkaline hydrolysis (3% NaOMe in dry MeOH) of **1** afforded a galactopyranosyl glycerol (**1b**), together with a mixture of fatty acid methyl esters. The fatty acid composition in **1** was determined to be a mixture of methyl 9*z*,12*z*-octadecadienoate and methyl 9*z*-hexadecenoate by GC-MS analysis.[7] The galactopyranosyl glycerol, $[\alpha]_D$ -7[0] (H_2O), was shown to be identical with (2*R*)-1-*O*-*β*-D-galactopyranosyl glycerol (**1b**), which was previously obtained by NaOMe treatment of glyceroglycolipid,[8] isolated from the marine brown alga *Sargassum thunbergii*. Therefore, the absolute configuration at C-2 of **1** has been determined to be *S*. ^{13}C NMR analysis of the galactopyranosyl glycerol moiety for **1**, in comparison with that of **1b**, showed that fatty acid residues were connected at C-1 and C-2 of diacylgalactolipid I (**1**) (Table 2).[8]

In order to determine the locations of the two fatty acid residues in diacylgalactolipid I (**1**), we carried out enzymatic hydrolysis (lipase type XIII, dioxane/H_2O, 1 : 1).[9] The lipase catalyzed hydrolysis of **1** afforded 1-*O*-deacylated monoacylgalactolipid [**1a**, *m/z* 513 $(M+Na)^+$] and 9*z*,12*z*-octadecadienoic acid. The ^1H- and ^{13}C NMR spectra of **1a** revealed that the signals, due to both H_2-1 and C-1, were observed at higher fields than those in **1** (Tables 1 and 2). Furthermore, alkaline treatment (3% NaOMe in dry MeOH) of **1a** afforded (2*R*)-1-*O*-*β*-D-galactopyranosyl glycerol (**1b**) and methyl 9*z*-hexadecenoate.

Based on the above evidence, the chemical structure of diacylgalactolipid I was determined to be (2*S*)-3-*O*-*β*-D-galactopyranosyl-1-*O*-(9*z*,12*z*-octadecadienoyl)-2-*O*-(9*z*-hexadecenoyl)glycerol (**1**).

Diacylgalactolipid II (**2**) gave a sodiated molecular ion of *m/z* 773 $(M+Na)^-$ in FABMS. The ^1H- and ^{13}C NMR spectra of **2** closely resembled of those of **1** and showed signals which were characteristic of a monogalactopyranosyl-1,2-diacylglycerol (Tables 1 and 2). Alkaline treatment of **2**, as carried out for **1**, provided (2*R*)-1-*O*-*β*-D-galactopyranosyl glycerol (**1b**) and a mixture of methyl 9*z*,12*z*,15*z*-octadecatrienoate and methyl 9*z*-hexadecenoate, which was identified by GC-MS analysis.[10] The enzymatic regioselective deacylation of **2** using lipase type XIII furnished **1a** and 9*z*, 12*z*,15*z*-octadecatrienoic acid as a single fatty acid. As a result, the chemical structure of diacylgalactolipid II was determined to be (2*S*)-3-*O*-*β*-D-galactopyranosyl-1-*O*-(9*z*,12*z*, 15*z*-octadecatrienoyl)-2-*O*-(9*z*-hexadecenoyl)glycerol (**2**).

The inseparable diacylgalactolipids III (**3**) and IV (**4**) gave sodiated molecular ions of *m/z* 751 $(M+Na)^-$ and 777 $(M+Na)^+$ in FABMS. The ^1H- and ^{13}C NMR spectra of inseparable **3** and **4** closely resembled those of **1** and **2** except for the signals derived from the fatty acid residues (Tables 1 and 2). Treatment of inseparable **3** and **4** with 3% NaOMe-dry MeOH furnished the (2*R*)-1-*O*-*β*-D-galactopyranosyl glycerol (**1b**) and a mixture of fatty acid methyl esters. The fatty acid methyl esters were analyzed by GC-MS and were found to be a mixture of methyl 9*z*-hexadecenoate, methyl hexadecanoate and methyl 9*z*-octadecenoate.[11]

The lipase-catalyzed hydrolysis of inseparable **3** and **4** afforded 1-*O*-deacylated monogalactolipids [inseparable **3a** and **4a**, *m/z* 515 $(M+Na)^-$ and 513 $(M+Na)^+$] and a mixture of fatty acids, which gave methyl esters on treatment with CH_2N_2. Comparing the ^1H- and ^{13}C NMR data of inseparable **3a** and **4a** with those of inseparable **3** and **4** showed that the regioselective deacylation occurred at the C-1 position of inseparable **3** and **4** (Tables 1 and 2). The methyl esters were determined by GC-MS analysis to be methyl 9*z*-hexadecenoate and methyl 9*z*-octadecenoate.

Alkaline treatment of inseparable **3a** and **4a** gave the (2*R*)-

Table 1. ^{1}H NMR data (δ, mult, J) for diacylgalactolipids I (**1**), II (**2**) and inseparable III (**3**) and IV (**4**), and 1-*O*-deacylated galactolipids **1a** and inseparable **3a** and **4a**[a,b]

C#	1	1a	2	3	3a and 4a
1	4.42 (dd, 12.0, 3.0)	4.20 (dd, 7.8, 2.0)	4.42 (dd, 12.0, 3.0)	4.42 (dd, 12.0, 3.0)	4.21 (dd ,7.5, 2.0)
	4.22 (dd, 12.0, 6.5)	3.75 (m)	4.22 (dd, 2.0, 6.5)	4.22 (dd, 12.0, 6.5)	3.75 (m)
2	5.26 (m)	5.05 (dddd, 5.2, 5.2, 4.8, 4.4)	5.26 (m)	5.26 (m)	5.04 (dddd, 5.4, 5.4, 5.3, 4.6)
3	3.98 (dd, 11.0, 5.5)	3.94 (dd, 10.9, 5.5)	3.98 (dd, 11.0, 5.5)	3.98 (dd, 11.0, 5.5)	3.96 (dd, 10.9, 5.6)
	3.73 (m)	3.75 (m)	3.73 (m)	3.73 (m)	3.75 (m)
1'	4.23 (d, 7.5)	4.23 (d, 7.5)	4.23 (d, 7.5)	4.23 (d, 7.5)	4.22 (d, 7.5)
2'	3.51 (m)	3.50 (m)	3.51 (m)	3.50 (m)	3.50 (m)
3'	3.44 (dd, 9.5, 3.2)	3.47 (dd, 9.5, 3.0)	3.45 (dd, 9.5, 3.2)	3.44 (dd, 9.5, 3.2)	3.45 (dd, 10.0, 3.0)
4'	3.82 (d, 3.2)	3.80 (d, 3.0)	3.82 (d-like, 3.2)	3.82 (d, 3.2)	3.81 (d, 3.0)
5'	3.51 (m)	3.50 (m)	3.51 (m)	3.51 (m)	3.50 (m)
6'	3.73 (m)	3.75 (m)	3.73 (m)	3.73 (m)	3.75 (m)

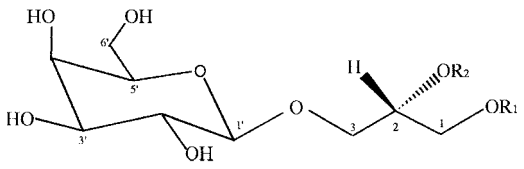
[a]Recorded in CD_3OD at 400 MHz and chemical shifts are relative to CD_3OD (δ=3.3 ppm). [b]Assignments aided by DEPT, COSY, HMQC, and HMBC.

Table 2. ^{13}C NMR data (δ, mult) for diacylgalactolipids I (**1**), II (**2**) and inseparable III (**3**) and IV (**4**), and their derivatives, **1a**, **1b**, and inseparable **3a** and **4a**[a,b]

C#	1	1a	1b	2	3 and 4	3a and 4a
1	64.0(t)	61.7	64.0	64.0	64.0	61.7
2	71.9(d)	74.8	71.9	71.8	71.7	74.7
3	68.7(t)	68.8	72.1	68.7	68.6	68.8
1'	105.4(d)	105.3	105.1	105.4	105.0	105.3
2'	72.4(d)	72.4	72.5	72.4	72.3	72.4
3'	74.9(d)	74.9	74.7	74.9	74.5	74.9
4'	70.2(d)	70.3	70.2	70.2	70.1	70.3
5'	76.8(d)	76.8	76.6	76.8	76.4	76.8
6'	62.5(t)	62.5	62.4	62.5	62.3	62.5

[a]Recorded in CD_3OD at 100 MHz and chemical shifts are relative to CD_3OD (δ=49.0 ppm). [b]Assignments aided by DEPT, COSY, HMQC, and HMBC.

1-*O*-β-D-galactopyranosyl glycerol (**1b**) and a mixture of fatty acid methyl esters, which was characterized by GC-MS to be a mixture of methyl 9*z*-hexadecenoate and methyl hexadecanoate. Therefore, the chemical structures of inseparable diacylgalactolipids III and IV were elucidated as being inseparable (2*S*)-3-*O*-β-D-galactopyranosyl-1-*O*-(9*z*-hexadecenoyl)-2-*O*-(hexadecanoyl)glycerol (**3**) and (2*S*)-3-*O*-β-D-galactopyranosyl-1-*O*-(9*z*-octadecenoyl)-2-*O*-(9*z*-hexadecenoyl)glycerol (**4**).



1 : R_1 = 9*z*,12*z*-octadecadienoyl, R_2 = 9*z*-hexadecenoyl

1a : R_1 = H, R_2 = 9*z*-hexadecenoyl

1b : R_1 = R_2 = H

2 : R_1 = 9*z*,12*z*,15*z*-octadecatrienoyl, R_2 = 9*z*-hexadecenoyl

3 and **4** (inseparable) : R_1 = 9*z*-hexadecenoyl, R_2 = hexadecanoyl

R_1 = 9*z*-octadecenoyl, R_2 = 9*z*-hexadecenoyl

3a and **4a** (inseparable): R_1 = H, R_2 = hexadecanoyl

R_1 = H, R_2 = 9*z*-hexadecenoyl

The biological functions of these diacylgalactolipids (**1**, **2**, and inseparable **3** and **4**) would be an interesting subject for further investigation.

Experimental Section

Culture. Cyanophycean microalga *Oscillatoria* sp. (strain #. KMCC CY-6) was obtained from Korea Marine Microalgae Culture Center. Institute of Fisheries Science, Pukyong National University. The strain was cultured for 28 days at 23 °C in a f/2 medium with aeration (filtered air. 0.3 L/min) under cool-white fluorescent illumination of 5000 lux. The f/2 medium composed of $NaNO_3$ (150 mg). NaH_2PO_4 (8.69 mg). Ferric EDTA (10.0 mg), $MnCl_2$ (0.22 mg). $CoCl_2$ (0.11 mg). $CuSO_4 \cdot 5H_2O$ (0.0196 mg). $ZnSO_4 \cdot 7H_2O$ (0.044 mg), $Na_2SiO_3 \cdot 9H_2O$ (50.0 mg), $Na_2MoO_4 \cdot 2H_2O$ (0.012 mg), vitamin B_{12} (1.0 μg). biotin (10.0 μg). thiamine HCl (0.2 mg) per seawater (1 L). After 4 weeks. the alga was harvested by centrifugation at 10.000 g and by filtration with filterpaper from the 100 liter culture, and lyophilized.

Isolation of diacylgalactolipids I (1), II (2) and inseparable III (3) and IV (4). The lyophilized alga (10.0 g) was extracted with CH_2Cl_2-MeOH (1 : 1) at r.t. and concentrated under reduced pressure to yield an extract (2.0 g). This extract (1.0 g) was subjected to flash silica gel column chromatography developing with EtOAc-MeOH (5 : 1) to furnish glycolipid fractions (160 mg). which was decolorized by activated-carbon column chromatography using MeOH-CH_3COCH_3 (100% $\rightarrow$ 0%) as the eluent. and purified by successive reverse phase column chromatography (ODS-A) (MeOH/H_2O, 20 : 1) and HPLC (YMC, ODS-A, MeOH) to furnish **1** (7.0 mg), **2** (7.1 mg) and inseparable **3** and **4** (10.0 mg).

1: colorless viscous solid; $[\alpha]_D$ -5° (*c* 0.3. $CHCl_3$); HRFABMS *m/z* 775.5175 $[M+Na]^+$ (calcd for $C_{43}H_{76}O_{10}Na$, 775.5163); LRFABMS *m/z* 775 $[M+Na]^+$; IR (neat): 3422, 1735. 1638, 1245, 1154, 1074 cm^{-1}; See Tables 1 and 2 for NMR spectral data of galactopyranosyl glycerol moiety; NMR data for fatty acid moiety (9*z*,12*z*-octadecadienoyl and

9*z*-hexadecenoyl) of **1**. ^{1}H NMR (400 MHz, CD_3OD): δ 5.30-5.44 (6H, m), 2.77 (t-like, J = 6.0 Hz), 2.35 (m), 2.31 (t, J = 7.4 Hz), 2.05 (q-like, J = 6.5 Hz), 1.60 (m), 1.30 (m), 0.90, 0.89 (each 3H, dd, J = 7.0, 6.8 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ_C 175.0 (s), 174.1 (s), 132.4 (d), 131.0 (d), 130.9 (d), 129.1 (d), 129.0 (d), 128.6 (d), 35.3 (t), 35.0 (t), 33.1 (t), 32.7 (t), 30.8 (t), 30.7 (t), 30.5 (t), 30.4 (t), 30.3 (t), 30.2 (t), 30.1 (t), 26.6 (t), 26.0 (t), 23.8 (t), 23.7 (t), 23.6 (t), 14.5 (q).

2: colorless viscous solid; $[\alpha]_D$ -6° (*c* 0.3, $CHCl_3$); HRFABMS *m/z* 773.5181 $[M+Na]^-$ (calcd for $C_{43}H_{74}O_{10}Na$, 773.5180); LRFABMS *m/z* 773 $[M+Na]^-$; IR (neat): 3401, 1738, 1159, 1071 cm^{-1}; See Tables 1 and 2 for NMR spectral data of galactopyranosyl glycerol moiety; NMR data for fatty acid moiety (9*z*,12*z*,15*z*-octadecatrienoyl and 9*z*-hexadecenoyl) of **2**. ^{1}H NMR (400 MHz, CD_3OD) δ 5.32-5.39 (8H, m), 2.80 (t-like, J = 6.0 Hz), 2.35 (m), 2.31 (t, J = 7.5 Hz), 2.07 (m), 1.59 (m), 1.32 (m), 1.28 (s-like), 0.96 (3H, t, J = 7.5 Hz), 0.89 (3H, dd, J = 7.1, 6.7 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ_C 175.0 (s), 174.1 (s), 132.7 (d), 132.4 (d), 131.1 (d), 129.2 (d), 129.2 (d), 128.9 (d), 128.6 (d), 128.2 (d), 35.3 (t), 35.0 (t), 33.1 (t), 30.8 (t), 30.7 (t), 30.6 (t), 30.5 (t), 30.4 (t), 30.3 (t), 30.2 (t), 30.1 (t), 28.2 (t), 26.6 (t), 26.4 (t), 26.0 (t), 23.8 (t), 23.7 (t), 21.5 (t), 14.7 (q), 14.5 (q).

Inseparable 3 and 4: colorless viscous solid; $[\alpha]_D$ -7° (*c* 0.3, $CHCl_3$); HRFABMS *m/z* 777.5495 $[M+Na]^-$ (calcd for $C_{43}H_{78}O_{10}Na$, 777.5493) and *m/z* 751.5334 $[M+Na]^+$ (calcd for $C_{41}H_{76}O_{10}Na$, 751.5336); LRFABMS *m/z* 777 $[M+Na]^+$ and 751 $[M+Na]^-$; IR (neat): 3420, 1736, 1164, 1070 cm^{-1}; See Tables 1 and 2 for NMR spectral data of galactopyranosyl glycerol moiety; NMR data for fatty acid moiety (9*z*-hexadecenoyl, hexadecanoyl and 9*z*-octadecenoyl) of inseparable **3** and **4**. ^{1}H NMR (400 MHz, CD_3OD) δ 5.30-5.39 (6H, m), 2.35 (m), 2.32 (t, J = 7.5 Hz), 2.04 (m), 1.59 (m), 1.31 (m), 0.90 (6H, dd, J = 7.5, 6.5 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ_C 175.0 (s), 174.1 (s), 132.4 (d), 130.9 (d), 130.8 (d), 128.6 (d), 35.0 (t), 33.1 (t), 30.8 (t), 30.7 (t), 30.6 (t), 30.5 (t), 30.4 (t), 30.3 (t), 30.2 (t), 28.2 (t), 26.0 (t), 23.7 (t), 14.5 (q).

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7. A mixture of fatty acid methyl esters was identified by GC-MS (HP-5 capillary column, 50 m, and gradient temp. (3 °C/min) from 150 °C to 190 °C): methyl 9*z*-hexadecenoate, t_R (min)=32.786, MS (*m/z*) 268 (M^+), 236, 207, 194, 166, 152, 141, 110, 97, 83, 69, 55, and methyl 9*z*,12*z*-octadecadienoate, t_R (min)=36.434, MS (*m/z*) 294 (M^+), 263, 164, 150, 136, 123, 109, 95, 81, 67, 55.
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10. A mixture of fatty acid methyl esters was identified by GC-MS as described for compound **1**: methyl 9*z*-hexadecenoate, t_R (min)=32.787, MS (*m/z*) 268 (M^+), 236, 194, 166, 152, 138, 123, 110, 97, 84, 74, 69, 55, and methyl 9*z*,12*z*,15*z*-octadecatrienoate, t_R (min)=37.112, MS (*m/z*) 292 (M^-), 261, 236, 173, 163, 149, 135, 121, 108, 95, 79, 67, 55.
11. A mixture of fatty acid methyl esters was identified by GC-MS as described for compound **1**: methyl 9*z*-hexadecenoate, t_R (min)=32.718, MS (*m/z*) 268 (M^+), 236, 207, 194, 179, 165, 152, 138, 123, 110, 97, 83, 69, 55, and methyl hexadecanoate, t_R (min)=33.261, MS (*m/z*) 270 (M^+), 239, 227, 199, 185, 171, 157, 143, 129, 115, 97, 87, 74, 55, and methyl 9*z*-octadecenoate, t_R (min)=36.531, MS (*m/z*) 296 (M^-), 264, 222, 180, 166, 152, 137, 123, 110, 96, 83, 69, 55.