# Notes

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

**Byeng Wha Son,[†,*] Jung Chul Kim,[†] Sang Mi Lee,[†] Yong Jin Cho,[†] Jin Seok Choi,[†,‡] Hong Dae Choi,[$] and Jin Cherl Song[#]**

[†]*Department of Chemistry, Pukyong National University, Pusan 608-737, Korea*
[‡]*Department of Food Science and Technology, Kyungsung University, Pusan 608-736, Korea*
[$]*Department of Chemistry, Dongeui University, Pusan 614-714, Korea*
[#]*Department of Polymer Science and Engineering, Pusan National University, Pusan 609-735, Korea*
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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 ($\delta$, 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) |

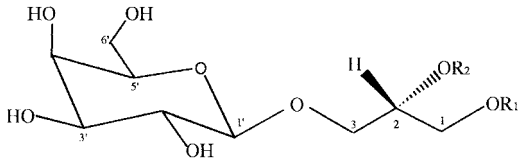
[a]Recorded in $CD_3OD$ at 400 MHz and chemical shifts are relative to $CD_3OD$ ($\delta$=3.3 ppm). [b]Assignments aided by DEPT, COSY, HMQC, and HMBC.

**Table 2**. $^{13}$C NMR data ($\delta$, 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 $CD_3OD$ ($\delta$=49.0 ppm). [b]Assignments aided by DEPT, COSY, HMQC, and HMBC.

1-*O*-$\beta$-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*-$\beta$-D-galactopyranosyl-1-*O*-(9z-hexadecenoyl)-2-*O*-(hexadecanoyl)glycerol (**3**) and (2*S*)-3-*O*-$\beta$-D-galactopyranosyl-1-*O*-(9z-octadecenoyl)-2-*O*-(9z-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 $\mu$g), biotin (10.0 $\mu$g), 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 (9z,12z-octadecadienoyl and

9*z*-hexadecenoyl) of **1**, $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ 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, $CD_3OD$): $\delta_c$ 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, $CD_3OD$) $\delta$ 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, $CD_3OD$) $\delta_c$ 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, $CD_3OD$) $\delta$ 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, $CD_3OD$) $\delta_c$ 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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## References

1. Sassaki, G. L.; Machado, M. J.; Tischer, C. A.; Gorin, P. A. J.; Iacomini, M. *J. Nat. Prod.* **1999**, *62*, 844.
2. Wicke, C.; Huners, M.; Wray, V.; Nimtz, M.; Bilitewski, U.; Lang, S. *J. Nat. Prod*. **2000**, *63*, 621.
3. Loya, S.; Reshef, V.; Mizrachi, E.; Silberstein, C.; Rachamim, Y.; Carmeli, S.; Hizi, A. *J. Nat. Prod*. **1998**, *61*, 891.
4. Murakami, N.; Morimoto, T.; Imamura, H.; Ueda, T.; Nagai, S.; Sakakibara, J. *Chem. Pharm. Bull*. **1991**, *39*, 2277.
5. Reshef, V.; Mizrachi, E.; Maretzki, T.; Silberstein, C.; Loya, S.; Hizi, A.; Carmeli, S. *J. Nat. Prod*. **1997**, *60*, 1251 and references cited therein.
6. Rho, M. -C.; Matsunaga, K.; Yasuda, K.; Ohizumi, Y. *J. Nat. Prod.* **1996**, *59*, 308.
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.
8. Son, B. W.; Cho, Y. J.; Kim, N. K.; Choi, H. D. *Bull. Korean Chem. Soc.* **1992**, *13*, 584.
9. Kobayashi, M.; Hayashi, K.; Kawazoe, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1992**, *40*, 1404.
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.