## 2-O-(α-D-Glucopyranosyl)glycerol, A New Glycerol Glycoside from the Marine Blue-green Alga Oscillatoria sp.

Jin Souk Choi,<sup>†,‡</sup> Dong Ick Lee,<sup>†,‡</sup> Won Kap Lee,<sup>‡</sup> Dong-Soo Kim,<sup>‡</sup> Hong Dae Choi,<sup>§</sup> and Byeng Wha Son<sup>†,\*</sup>

<sup>†</sup>Department of Chemistry, Pukyong National University, Pusan 608-737, Korea <sup>‡</sup>Department of Food Science and Technology, Kyungsung University, Pusan 608-736, Korea <sup>§</sup>Department of Chemistry, Dongeui University, Pusan 614-714, Korea Received August 10, 1999

Microalgae grow autotrophically using light energy and  $CO_2$ , and are the primary producers to the marine food web.

More recently, microalgae have been targeted as a source of bioactive compounds and pharmaceuticals, specialty chemicals, health foods, aquaculture feeds, and for waste treatment and agriculture.<sup>1,2</sup>

In a study on the bioactive metabolites from the marine microalgae, we have undertaken a detailed study of the extract of an assemblage of blue-green alga, *Oscillatoria* sp. (strain #, KMCC CY-13).<sup>3</sup> Microalgae, *Oscillatoria* sp. was cultured under saline conditions,<sup>4</sup> and cells were harvested by continuous centrifugation, and then lyophilized. The cell extract (methanol/dichloromethane, 1 : 1) (260 mg) was fractioned using a flash silica gel column (CH<sub>2</sub>Cl<sub>2</sub> in CH<sub>3</sub>OH, and then CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH-H<sub>2</sub>O, 65 : 35 : 10, lower phase) to yield a glycopyranosyl glycerol fraction. The CH<sub>2</sub>Cl<sub>2</sub>-insoluble portion of this fraction was further purified by HPLC (YMC ODS-A, CH<sub>3</sub>OH/H<sub>2</sub>O, 10 : 1) to give glycopyranosyl glycerol (1) (25 mg).

2-O-( $\alpha$ -D-Glucopyranosyl)glycerol (1)<sup>5</sup> was isolated as a colorless viscous syrup which analyzed for  $C_9H_{19}O_8$  by HRFABMS and <sup>13</sup>C NMR methods. The IR absorption spectrum of 1 showed bands characteristic of a hydroxy (3392 cm<sup>-1</sup>) and a glycosidic linkage (1097, 1024 cm<sup>-1</sup>) functionalities. The <sup>1</sup>H NMR spectrum of **1** showed signals assignable to anomeric proton [ $\delta$  4.97 (1H, d, J = 3.8 Hz)] and eleven protons geminal to oxygen functions (Table 1). The  ${}^{13}C$ NMR spectrum of 1 showed signals ascribable to hexose and monosubstituted glycerol (Table 1). Comparisons in detail of <sup>13</sup>C NMR data for known methyl  $\alpha$ -D-glucopyranose,<sup>6,7</sup> and 2-*O*-galactopyranosyl glycerol<sup>8</sup> with that for **1** showed that **1** was a 2-O-glucopyranosyl glycerol. This conclusion was further supported by the spectral data of hexaacetate of 1. Acetylation of 1 with acetic anhydride in pyridine provided a hexaacetate (2).<sup>9</sup> The IR spectrum of 2 exhibited characteristic absorptions of an ester group at 1728 and 1264 cm<sup>-1</sup>,



but did not show the presence of a free hydroxyl group. The positive FABMS of **2** showed a quasi-molecular ion peak at m/z 507 [M(C<sub>21</sub>H<sub>30</sub>O<sub>14</sub>) + H]<sup>+</sup> and several other peaks (m/z 391, 331, 176, 116). Detailed analyses of the <sup>1</sup>H- and <sup>13</sup>C NMR spectra of **2** revealed several diagnostic signals for hexaacetate of  $\alpha$ -D-glucopyranosyl glycerol, which was further supported by the MS fragment ions, m/z 391, 331, 176 and 116, as illustrated in Figure 1.

The sugar moiety was determined to be glucose by coupling constants in 1 ( $J_{\text{H-2',H-3'}} = 9.7 \text{ Hz}$ ,  $J_{\text{H-3',H-4'}} = 9.1 \text{ Hz}$ ,  $J_{\text{H-4',H-5'}} = 9.5 \text{ Hz}$ ) and in 2 ( $J_{\text{H-2',H-3'}} = 10.4 \text{ Hz}$ ,  $J_{\text{H-3',H-4'}} = 9.5 \text{ Hz}$ ,  $J_{\text{H-4',H-5'}} = 10.3 \text{ Hz}$ ), which showed the diaxial interactions, respectively. Acid hydrolysis of 1 with 9% HCl in dry CH<sub>3</sub>OH afforded methyl D-glucoside and glycerol, which were identified by a GC of their trimethylsilyl derivatives.<sup>10</sup> The physicochemical features outlined above suggested that 1 was a glucopyranosyl glycerol.

The anomeric configuration at the glycosidic linkage was able to define to be  $\alpha$  configuration from the coupling constants of anomeric protons ( $J_{\text{H-1',H-2'}} = 3.8 \text{ Hz in 1}$ ;  $J_{\text{H-1',H-2'}} = 3.7 \text{ Hz in 2}$ ) and the chemical shifts of anomeric carbons ( $\delta_{\text{C}}$  100.2 in 1;  $\delta_{\text{C}}$  95.5 in 2). While, the location of sugar moiety was confirmed to be C-2 of glycerol moiety by comparisons of the <sup>13</sup>C NMR data of floridoside, <sup>8</sup> lilioside C,<sup>11</sup> lilioside D,<sup>12</sup> and 1-*O*-( $\beta$ -D-galactopyranosyl)glycerol<sup>13,14</sup> to those of 1 and 2. On the basis of all of the foregoing evidence, 1 was elucidated as 2-*O*-( $\alpha$ -D-glucopyranosyl)glycerol.

This is the first example of a naturally occurring glycerol glucoside.



**Figure 1**. Diagnostic FABMS fragment ions of 2-O-( $\alpha$ -D-gluco-pyranosyl)glycerol hexaacetate (2).

**Table 1**. <sup>1</sup>H- and <sup>13</sup>C NMR Data for 2-O-( $\alpha$ -D-glucopyranosyl) glycerol (1)<sup>*a,b*</sup>

C#	$\delta_{\mathrm{H}}$ (mult, $J$ (Hz))	$\delta_{ m C}$
1	3.76 (2H, m)	62.8 <sup>c</sup> (CH <sub>2</sub> )
2	3.68 (1H, m)	81.7 (CH)
3	3.67 (2H, m)	62.5 <sup>c</sup> (CH <sub>2</sub> )
1'	4.97 (1H, d, <i>J</i> = 3.8)	100.2 (CH)
2'	3.37 (1H, dd, <i>J</i> = 9.7, 3.8)	73.9 (CH)
3'	3.62 (1H, dd, J = 9.5, 9.1)	75.3 (CH)
4'	3.25 (1H, dd, J = 9.5, 9.1)	72.0 (CH)
5'	3.71 (1H, m)	74.1 (CH)
6'	3.66 (2H, m)	63.1 (CH <sub>2</sub> )

<sup>*a*</sup>Recorded in CD<sub>3</sub>OD at 300 MHz (<sup>1</sup>H) and 22.5 MHz (<sup>13</sup>C). Chemical shifts are relative to internal TMS ( $\delta$ =0 ppm) and CD<sub>3</sub>OD ( $\delta$ =49.15 ppm). <sup>*b*</sup>Assignments aided by DEPT, <sup>1</sup>H- <sup>13</sup>C Hetero COSY, and COLOC. <sup>c</sup>Interchangable in each column.

Glycolipids such as glycosyl diglycerides have been shown to be widely distributed in plants<sup>15</sup> and in microorganisms.<sup>16</sup> Some glycerol glycosides, floridoside and lilioside B, have so far been isolated from the marine red alga<sup>8</sup> and liliaceae plant,<sup>17</sup> respectively.

The metabolites of the marine red algi have galactose, mannose and sulfonoquinovose,<sup>13,18</sup> but the metabolites of higher plant have glucose<sup>19</sup> as their sugar moieties. The locations of sugar moiety were C-2 of glycerol in the marine red algi,<sup>8</sup> but C-1 or C-2 of glycerol in the terrestrial higher plant.<sup>19</sup> The anomeric configuration of glycopyranonsyl glycerol was distinctive between the marine algi<sup>8</sup> and terrestrial higher plant.<sup>19</sup> The marine metabolites showed  $\alpha$  - configuration, but the terrestrial metabolites showed  $\beta$  - configuration.

It is noteworthy that glycopyranosyl glycerol was detected only from blue-green algi among the family of the marine microalgi, Chlorophyceae (green algae), Bacillariophyceae (diatom) and Cyanophyceae (blue-green algae) from the TLC and NMR examinations.

Therefore, this is important in a chemotaxonomic viewpoint of the marine microalgae.

Acknowledgment. The authors thank Professor Sung Bum Hur, The Korean Marine Microalgal Culture Center, Institute of Fisheries Science, Pukyong National University, for providing microalgal samples and for allowing the use of the microalgal bank's facilities. NMR and Mass spectral data were kindly provided by the Korea Basic Science Institute, Taejeon and Pusan. This research was financially supported by grants from the Ministry of Maritime Affairs and Fisheries (1999) and from Pukyong National University, made in the 1998 program year.

## References

- 1. Metting Jr, F. B. J. Indust. Microbiol. 1996, 17, 477.
- Matsunaga, T.; Takeyama, H. J. Chem. Soc. Jpn. 1995, No. 9, 669.
- Microalgae, Oscillatoria sp. (strain No. KMCC CY-13) was obtained from the Korean Marine Microalgal Culture Center, Institute of Fisheries Science, Pukyong National University.
- 4. The strain was cultured for 31 days at 23 °C in a f/2

medium with aeration(filtered air, 0.3 L/min) under coolwhite fluorescent light (5000 Lux). The f/2 medium composed of NaNO<sub>3</sub> (150 mg), NaH<sub>2</sub>PO<sub>4</sub> (8.69 mg), Ferric EDTA (10.0 mg), MnCl<sub>2</sub> (0.22 mg), CoCl<sub>2</sub> (0.11 mg), CuSO<sub>4</sub> · 5H<sub>2</sub>O (0.0196 mg), ZnSO<sub>4</sub> · 7H<sub>2</sub>O (0.044 mg), Na<sub>2</sub>SiO<sub>3</sub> · 9H<sub>2</sub>O (50.0 mg), Na<sub>2</sub>MoO<sub>4</sub> · 2H<sub>2</sub>O (0.012 mg), Vitamin B<sub>12</sub> (1.0  $\mu$ g), Biotin (10.0  $\mu$ g), thiamine HCl (0.2 mg) per seawater (1 L).

- 5. 2-O-( $\alpha$ -D-Glucopyranosyl)glycerol (1) was isolated as a colorless viscous syrup which showed:  $[\alpha]_D$ -37 °C (*c* 0.6, H<sub>2</sub>O): HRFABMS (M+H)<sup>+</sup> m/z obsd. 255.1077, C<sub>9</sub>H<sub>19</sub>O<sub>8</sub>, dev -1.2 ppm; LRFABMS: m/z 277 (M+Na)<sup>+</sup>, 255 (M+H)<sup>+</sup>; IR (KBr) 3392, 1645, 1384, 1148, 1097, 1024, 534 cm<sup>-1</sup>. See Table 1 for NMR spectral data.
- Breitmaier, E.; Voelter, W. Carbon-13 NMR Spectroscopy; VCH Publishers: New York, U.S.A., 1990; p 387.
- Agrawal, P. K.; Bansal, M. C. In *Carbon-13 NMR of fla*vonoids; Agrawal, P. K., Ed.; Elsevier: Tokyo, Japan, 1989; Vol. 39, p 287.
- Roh, Y. S.; Son, B. W.; Im, K. S.; Choi, H. D. Korean J. Pharmacogn. 1994, 25, 117, and references cited therein.
- Acetylation of 2-*O*-(α-D-glucopyranosyl)glycerol (1) (10 mg) in the usual manner (acetic anhydride / pyridine) furnished the hexaacetate (2) (14 mg) as a colorless solid. The following data were recorded for 2: [α]<sub>D</sub> +119° (*c* 0.2, CHCl<sub>3</sub>); IR (neat) 1728, 1264 cm<sup>-1</sup>; LRFABMS: *m/z* 507 (M+H)<sup>+</sup>, 391, 331, 176, 116 (see Figure 1); <sup>1</sup>H NMR (300 MHz, cdcl<sub>3</sub>): δ 5.31 (1H, d, *J* = 3.7 Hz, H-1'), 4.83 (1H, dd, *J* = 10.4, 3.7 Hz, H-2'), 5.47 (1H, dd, *J* = 10.4, 9.5 Hz, H-3'), 5.05 (1H, dd, *J* = 10.3, 9.5 Hz, H-4'), 4.30-4.17 (5H, m), 4.10-4.03 (3H, m), 2.09, 2.09, 2.07, 2.05, 2.03, 2.02 (each 3H s, -COC<u>H</u><sub>3</sub>); <sup>13</sup>C NMR (22.5 MHz, cdcl<sub>3</sub>): δ c 95.5(d), 74.2(d), 70.7(d), 69.9(d), 68.5(d), 67.8(d), 63.6(t), 63.3(t), 61.9(t), 170.6, 170.4, 170.3, 170.1, 170.0, 169.6 (each s, -OCOCH<sub>3</sub>), 20.6 × 6 (each q, -OCO<u>C</u>H<sub>3</sub>)
- 10. A solution of **1** (5 mg) in 9% dry HCl-MeOH (1.5 mL) was refluxed under N<sub>2</sub> atmosphere for 1.5 hr. The reaction mixture was neutralized with Ag<sub>2</sub>CO<sub>3</sub> and filtered. Evaporation of solvent at reduced pressure from the filtrate gave a residue, which was dissolved in pyridine (0.5 mL) and treated with N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) (0.5 mL) at r.t. for 1hr. The TMSi derivatives thus obtained was shown to be a mixture of TMSi-glycerol and methyl TMSi-glucoside by GC analysis (2% SE-30 on Chromosorb WAW DMCS, 80-100 mesh, 3 mm × 2 m): TMSi-glycerol (column temp 120 °C, N<sub>2</sub> flow rate 25 mL/min. t<sub>R</sub>(min) = 5.25) and methyl TMSi-glucoside (column temp. 140°, N<sub>2</sub> flow rate 36 mL/min, t<sub>R</sub>(min) = 14.25, 16.03).
- Kaneda, M.; Mizutani, K.; Tanaka, K. *Phytochemistry* 1982, 21, 891.
- 12. Kaneda, M.; Kobayashi, K.; Nishida, K.; Katsuta, S. *Phytochemistry* **1984**, *23*, 795.
- Son, B. W.; Cho, Y. J.; Kim, N. K.; Choi, H. D. Bull. Korean Chem. Soc. 1992, 13, 584.
- 14. Son, B. W. Bull. Korean Chem. Soc. 1988, 9, 264.
- Sassaki, G. L.; Machado, M. J.; Tischer, C. A.; Gorin, P. A. J.; Iacomini, M. J. Nat. Prod. 1999, 62, 844.
- Loya, S.; Reshef, V.; Mizrachi, E.; Silberstein, C.; Rachamim, Y.; Carmeli, S.; Hizi, A. *J. Nat. Prod.* **1998**, *61*, 891.
- 17. Kaneda, M.; Mizutani, K.; Takahashi, Y.; Kurono, G.; Nishikawa, Y. *Tetrahedron Lett.* **1974**, *No. 45*, 3937.
- 18. Son, B. W. Phytochemistry 1990, 29, 307.
- Shimomura, H.; Sashida, Y.; Mimaki, Y.; Kudo, Y.; Maeda, K. *Chem. Pharm. Bull.* **1988**, *36*, 4841, and references cited therein.