Microalgae grow autotrophically using light energy and CO₂, 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.¹,²

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).³ Microalgae, Oscillatoria sp. was cultured under saline conditions, and cells were harvested by continuous centrifugation, and then lyophilized. The cell extract (methanol/dichloromethane, 1:1) was fractioned (methanol/dichloromethane, 1:1) (260 mg) was fractioned sequentially using a flash silica gel column (CH₂Cl₂ in CH₃OH, and then CH₂Cl₂-CH₃OH-H₂O, 65:35:10, lower phase) to yield a colorless viscous syrup which analyzed for C₉H₁₉O₈ by HRFABMS and ¹³C NMR methods. The IR absorption spectrum of the CH₂Cl₂-insoluble portion of this fraction was further purified by HPLC (YMC ODS-A, CH₃OH/H₂O, 10:1) to give glycopyranosyl glycerol fraction. The CH₂Cl₂-insoluble portion of this fraction was further purified by HPLC (YMC ODS-A, CH₃OH/H₂O, 10:1) to give glycopyranosyl glycerol (I) (25 mg).

2-O-(α-D-Glucopyranosyl)glycerol (I)³ was isolated as a colorless viscous syrup which analyzed for C₉H₁₉O₈ by HRFABMS and ¹³C NMR methods. The IR absorption spectrum of I showed bands characteristic of a hydroxy (3392 cm⁻¹) and a glycosidic linkage (1097, 1024 cm⁻¹) functionalities. The ¹H NMR spectrum of I showed signals assignable to anomeric proton [δ 4.97 (1H, d, J = 3.8 Hz)] and eleven protons geminal to oxygen functions (Table 1). The ¹³C NMR spectrum of I showed signals attributable to hexose and monosubstituted glycerol (Table 1). In comparison of ¹³C NMR data for known methyl α-D-glucopyranose,⁶,⁷ and 2-O-galactopyranosyl glycerol⁸ with that for I showed that I was a 2-O-glycopyranosyl glycerol. This conclusion was further supported by the spectral data of hexaacetate of I. Acetylation of I with acetic anhydride in pyridine provided a hexaacetate (2).⁹ The IR spectrum of 2 exhibited characteristic absorptions of an ester group at 1728 and 1264 cm⁻¹, 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(C₂₁H₃₀O₁₄) + H]⁺ and several other peaks (m/z 391, 331, 176, 116). Detailed analyses of the ¹H- and ¹³C NMR spectra of 2 revealed several diagnostic signals for hexaacetate of α-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 of anomeric protons (J₁H-J₃H-2 = 9.7 Hz, J₄H-J₅H-3 = 9.5 Hz) in 1 (J₁H-J₃H-2 = 9.1 Hz, J₄H-J₅H-3 = 9.5 Hz) and in 2 (J₁H-J₃H-2 = 10.4 Hz, J₄H-J₅H-3 = 9.5 Hz, J₆H-J₇H-5 = 10.3 Hz), which showed the diaxial interactions, respectively. Acid hydrolysis of I with 9% HCl in dry CH₂OH afforded methyl D-glucoside and glycerol, which were identified by a GC of their trimethylsilyl derivatives.¹⁰ The physicochemical features outlined above suggested that I was a glycopyranosyl glycerol.

The anomeric configuration at the glycosidic linkage was able to define to be α configuration from the coupling constants of anomeric protons (J₁H-J₃H-2 = 3.8 Hz in 1; J₁H-J₃H-2 = 3.7 Hz in 2) and the chemical shifts of anomeric carbons (δ 100.2 in 1; δ 95.5 in 2). While, the location of sugar moiety was confirmed to be C-2 of glycerol moiety by comparisons of the ¹³C NMR data of floridoside,⁶ lilioside C,¹¹ lilioside D,¹² and 1-O-(β-D-galactopyranosyl)glycerol¹³,¹⁴ to those of 1 and 2. On the basis of all of the foregoing evidence, I was elucidated as 2-O-(α-D-glucopyranosyl)glycerol.

This is the first example of a naturally occurring glycerol glucoside.
Table 1. 1H- and 13C NMR Data for 2-O-(α-D-glucopyranosyl)glycerol (1)*, b

<table>
<thead>
<tr>
<th>C#</th>
<th>δH (mult, J (Hz))</th>
<th>δC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.76 (2H, m)</td>
<td>62.8 (CH2)</td>
</tr>
<tr>
<td>2</td>
<td>3.68 (1H, m)</td>
<td>81.7 (CH)</td>
</tr>
<tr>
<td>3</td>
<td>3.67 (2H, m)</td>
<td>62.5 (CH2)</td>
</tr>
<tr>
<td>1'</td>
<td>4.97 (1H, d, J = 3.8)</td>
<td>100.2 (CH)</td>
</tr>
<tr>
<td>2'</td>
<td>3.37 (1H, dd, J = 9.7, 3.8)</td>
<td>73.9 (CH)</td>
</tr>
<tr>
<td>3'</td>
<td>3.62 (1H, dd, J = 9.5, 9.1)</td>
<td>75.3 (CH)</td>
</tr>
<tr>
<td>4'</td>
<td>3.25 (1H, dd, J = 9.5, 9.1)</td>
<td>72.0 (CH)</td>
</tr>
<tr>
<td>5'</td>
<td>3.71 (1H, m)</td>
<td>74.1 (CH)</td>
</tr>
<tr>
<td>6'</td>
<td>3.66 (2H, m)</td>
<td>63.1 (CH)</td>
</tr>
</tbody>
</table>

*Recorded in CD3OD at 300 MHz (1H) and 75.5 MHz (13C). Chemical shifts are relative to internal TMS (δ = 0 ppm) and CD3OD (δ = 49.15 ppm). a Assignments aided by DEPT. b 13C Hetero COSY, and COLOC. Interchangeable in each column.

Glycolipids such as glycosyl diglycerides have been shown to be widely distributed in plants and in microorganisms. Some glycerol glycosides, floridoside and lilioside B, have been isolated from the marine red alga8 and liliaceous plant,17 respectively.

The metabolites of the marine red algae have galactose, mannose and sulfonoquinovose,13,18 but the metabolites of higher plant have glucose19 as their sugar moieties. The location of sugar moiety are C-2 of glycerol in the marine red alga2 but C-1 or C-2 of glycerol in the terrestrial higher plant.19 The anomeric configuration of glycopyranosyl glycerol was distinctive between the marine algae and terrestrial higher plant.19 The marine metabolites showed α-configuration, but the terrestrial metabolites showed β-configuration.

It is noteworthy that glycopyranosyl glycerol was detected only from blue-green algae among the family of the marine microalg, 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.

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References

3. 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 1/2 medium with aeration (filtered air, 0.3 L/min) under cool-white fluorescent light (5000 Lux). The 1/2 medium composed of NaNO3 (150 mg), NaH2PO4 (8.69 mg), Ferric EDTA (10.0 mg), MnCl2 (0.22 mg), CoCl2 (0.11 mg), CuSO4 · 5H2O (0.0196 mg), ZnSO4 · 7H2O (0.044 mg), Na2SiO3 · 9H2O (50.0 mg), Na2MoO3 · 2H2O (0.012 mg). Vitamin B12 (1.0 µg), Biotin (10.0 µg), thiamine HCl (0.2 mg) per seawater (1 L).
5. 2-O-(α-D-Glucopyranosyl)glycerol (1) was isolated as a colorless viscous syrup which showed: [α]D −37 °C (c 0.6, H2O); HRFABMS (M+H)+ m/z 391, 331, 176, 116 (see Figure 1); 1H NMR (300 MHz, CDCl3): δH (mult, J (Hz)) = 5.51 (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, each 3H s, -COCH3); 13C NMR (22.5 MHz, CDCl3): δC (mult) = 106.8 (CH2), 101.4 (CH), 54.1 (CH2), 53.5, 53.3 (each s, -OCOC2H5).

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10. A solution of 1 (5 mg) in 9% dry HCl-MeOH (1.5 mL) was refluxed under N2 atmosphere for 1.5 hr. The reaction mixture was neutralized with Ag:CO3 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 NO-bis(trimethylsilyl)trifluoroacacetamide (BSTFA) (0.5 mL) at rt. for 1 hr. 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, N2 flow rate 25 mL/min, tR(min) = 5.25) and methyl TMSi-glucoside (column temp 140 °C, N2 flow rate 36 mL/min, tR(min) = 14.25, 16.03).