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

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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.[1,2]

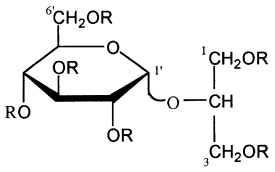
In a study on the bioactive metabolites from the marine microalgae, we have undertaken a detailed study of the extract of an assemblage of blue-green alga, *Oscillatoria* sp. (strain #, KMCC CY-13).[3] Microalgae, *Oscillatoria* sp. was cultured under saline conditions,[4] and cells were harvested by continuous centrifugation, and then lyophilized. The cell extract (methanol/dichloromethane, 1 : 1) (260 mg) was fractioned using a flash silica gel column (CH_2Cl_2 in CH_3OH, and then CH_2Cl_2-CH_3OH-H_2O, 65 : 35 : 10, lower phase) to yield a glycopyranosyl glycerol fraction. The CH_2Cl_2-insoluble portion of this fraction was further purified by HPLC (YMC ODS-A, CH_3OH/H_2O, 10 : 1) to give glycopyranosyl glycerol (**1**) (25 mg).

2-*O*-(α-D-Glucopyranosyl)glycerol (**1**)[5] was isolated as a colorless viscous syrup which analyzed for $C_9H_{18}O_8$ by HRFABMS and ^{13}C NMR methods. The IR absorption spectrum of **1** showed bands characteristic of a hydroxy (3392 cm^{-1}) and a glycosidic linkage (1097, 1024 cm^{-1}) functionalities. The ^1H NMR spectrum of **1** 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 ^{13}C NMR spectrum of **1** showed signals ascribable to hexose and monosubstituted glycerol (Table 1). Comparisons in detail of ^{13}C NMR data for known methyl α-D-glucopyranose,[6,7] and 2-*O*-galactopyranosyl glycerol[8] 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**).[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($C_{21}H_{30}O_{14}$) − H]$^+$ and several other peaks (*m/z* 391, 331, 176, 116). Detailed analyses of the ^1H- and ^{13}C 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 in **1** ($J_{H\text{-}2',H\text{-}3'}$ = 9.7 Hz, $J_{H\text{-}3',H\text{-}4'}$=9.1 Hz, $J_{H\text{-}4',H\text{-}5'}$ = 9.5 Hz) and in **2** ($J_{H\text{-}2',H\text{-}3'}$ = 10.4 Hz, $J_{H\text{-}3',H\text{-}4'}$ = 9.5 Hz, $J_{H\text{-}4',H\text{-}5'}$ = 10.3 Hz), which showed the diaxial interactions, respectively. Acid hydrolysis of **1** with 9% HCl in dry CH_3OH afforded methyl D-glucoside and glycerol, which were identified by a GC of their trimethylsilyl derivatives.[10] 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 α configuration from the coupling constants of anomeric protons ($J_{H\text{-}1',H\text{-}2'}$ = 3.8 Hz in **1**; $J_{H\text{-}1',H\text{-}2'}$ = 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 ^{13}C NMR data of floridoside,[8] lilioside C,[11] lilioside D,[12] and 1-*O*-(β-D-galactopyranosyl)glycerol[13,14] to those of **1** and **2**. On the basis of all of the foregoing evidence, **1** was elucidated as 2-*O*-(α-D-glucopyranosyl)glycerol.

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



1 : R=H
2 : R=Ac

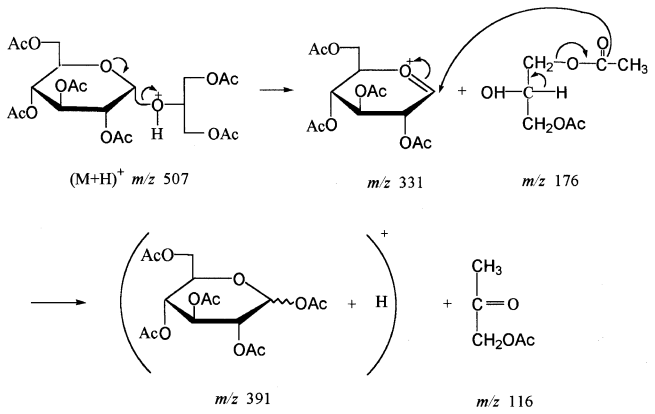


Figure 1. Diagnostic FABMS fragment ions of 2-*O*-(α-D-glucopyranosyl)glycerol hexaacetate (**2**).

Table 1. ^{1}H- and ^{13}C NMR Data for 2-*O*-(α-D-glucopyranosyl) glycerol (**1**)[a,b]

C#	δ_H (mult, *J* (Hz))	δ_C
1	3.76 (2H, m)	62.8[c] (CH_2)
2	3.68 (1H, m)	81.7 (CH)
3	3.67 (2H, m)	62.5[c] (CH_2)
1'	4.97 (1H, d, *J* = 3.8)	100.2 (CH)
2'	3.37 (1H, dd, *J* = 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_2)

[a]Recorded in CD_3OD at 300 MHz (^{1}H) and 22.5 MHz (^{13}C). Chemical shifts are relative to internal TMS (δ = 0 ppm) and CD_3OD (δ = 49.15 ppm). [b]Assignments aided by DEPT, ^{1}H- ^{13}C Hetero COSY, and COLOC. [c]Interchangable in each column.

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

The metabolites of the marine red algi have galactose, mannose and sulfonoquinovose,[13,18] but the metabolites of higher plant have glucose[19] as their sugar moieties. The locations of sugar moiety were C-2 of glycerol in the marine red algi,[8] but C-1 or C-2 of glycerol in the terrestrial higher plant.[19] The anomeric configuration of glycopyranonsyl glycerol was distinctive between the marine algi[8] 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 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.

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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 f/2 medium with aeration(filtered air, 0.3 L/min) under cool-white fluorescent light (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).
5. 2-*O*-(α-D-Glucopyranosyl)glycerol (**1**) was isolated as a colorless viscous syrup which showed: $[\alpha]_D$ -37 °C (*c* 0.6, H_2O); HRFABMS (M+H)$^+$ *m z* obsd. 255.1077, $C_9H_{19}O_8$, dev -1.2 ppm; LRFABMS: *m z* 277 (M+Na)$^+$, 255 (M+H)$^+$; IR (KBr) 3392, 1645, 1384, 1148, 1097, 1024, 534 cm^{-1}. See Table 1 for NMR spectral data.
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9. 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**: $[\alpha]_D$ +119° (*c* 0.2, $CHCl_3$); IR (neat) 1728, 1264 cm^{-1}; LRFABMS: *m z* 507 (M+H)$^+$, 391, 331, 176, 116 (see Figure 1); ^{1}H NMR (300 MHz, $CDCl_3$): 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, -$COCH_3$); ^{13}C NMR (22.5 MHz, $CDCl_3$): 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, -O<u>C</u>OCH$_3$), 20.6 × 6 (each q, -OCO<u>C</u>H$_3$)
10. A solution of **1** (5 mg) in 9% dry HCl-MeOH (1.5 mL) was refluxed under N_2 atmosphere for 1.5 hr. The reaction mixture was neutralized with Ag_2CO_3 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_2 flow rate 25 mL/min, t_R(min) = 5.25) and methyl TMSi-glucoside (column temp. 140°, N_2 flow rate 36 mL/min, t_R(min) = 14.25, 16.03).
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