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A Monoacyldigalactosyl Glycerol from the Green Alga *Enteromorpha prolifera*

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Abstract – A monoacyldigalactosyl glycerol was isolated from the CH_2Cl_2 soluble fraction of the MeOH extract from the green alga *Enteromorpha prolifera*. The structure was established as 1-*O*-palmitoyl-3-*O*-[α-D-galactopyranosyl(1→6)-β-D-galactopyranosyl]-*sn*-glycerol (**1**) by chemical and spectroscopic methods.
Keywords – *Enteromorpha prolifera*, Ulvaceae, Green alga, Monoacyldigalactosyl glycerol

Introduction

In our previous studies, the extracts of various Korean edible seaweeds exhibited inhibitory activity on rat lens aldose reductase (Shin, 2004). Among these seaweeds, *Enteromorpha prolifera* J. Agardh (Ulvaceae) exhibited relatively strong activity. The green alga, *E. prolifera* widely spreads all over the world. In Korea, this green alga is distributed in the intertidal zone of the seashores and has been used as the seasoned sea greens (Oh *et al.*, 1990). Few reports have appeared in the literature on the chemistry and biological activity of this alga. Biological experiments indicated that MeOH-acetone (1:1) extract of *E. prolifera* possessed anticarcinogenic activity (Higashi-Okai *et al.*, 1999). *E. prolifera* has been investigated for their chemical constituents, and four components, 2,6,10-trimethyl-7-(3-methylbutyl)-dodecane and related hydrocarbons (Rowland *et al.*, 1985), free amino acids, two lectins (Ambrosio *et al.*, 2003), and heavy metals (Muse *et al.*, 1999) have been reported. We report here the isolation and structural determination of a monoacyl digalactosyl glycerol (**1**) by means of chromatographic and spectroscopic techniques.

Materials and Methods

General – The optical rotations were determined on a JASCO P-1020 polarimeter. The EI mass spectra were obtained on a Hewlett-Packard 5989B spectrometer. The FAB mass spectrum was obtained in a 3-nitrobenzyl alcohol matrix in positive ion mode on a JEOL-700 spectrometer. The NMR spectra were measured on a Varian Gemmi 2000 instrument (300 MHz) or a Bruker AM-500 (500 MHz), and the chemical shifts were referenced to TMS. GC-MS analysis was performed as previously described (Kang *et al.*, 1999) using a Hewlett Packard 5989B mass spectrometer equipped with a 5890 Series II$^+$ gas chromatograph. TLC was performed on silica gel $60F_{254}$ (Merck).

Algal material – The sample of *E. prolifera* was provided by Seokwon Life Science Research Institute in August, 2003 which was collected at Mokpo area, Chunnam Province, Korea. The botanical identification was made by Prof. Jong-Ahm Shin, Yosu National University. A voucher specimen (No. WSG 2003-3) was deposited in the Seokwon Life Science Research Institute, World Sea Green Co. Ltd.

Extraction and isolation – The chopped pieces of *E. prolifera* (1.8 kg) were extracted five times, with MeOH under reflux, to give an extract (180 g). The MeOH extract was suspended in water and successively partitioned with hexane, dichloromethane, ethyl acetate, and butanol, to yield 46.7, 9.9, 3.7, 12.7, and 103.4 g of fractions, respectively. The dichloromethane fraction (9 g) was separated by a silica gel column chromatography with $CHCl_3$-MeOH-H_2O (7:1:0.5→7:3:1), as the eluent, to give 15 fractions (C01–C15). Fraction C11 (1.2 g) underwent a silica gel column chromatography, with increasing amounts of EtOAc saturated with water-MeOH (1→10%) as the eluent, to yield 14 fractions (C11-01–C11-14). Fraction

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C11-07 (100 mg) was purified by a RP_{18} column chromatography, with MeOH-H_2O (8:2) as the eluent, to yield 6 purified subfractions (C11-07-A – F). Fraction C11-07-F was further purified by crystallization from MeOH to yield pure **1** (8 mg). Other fractions were obtained in small amounts (*ca* 1 – 2 mg).

Compound 1 – Amorphous white powder. $[\alpha]_D^{22}$ +56.3° (*c* 0.4, MeOH), ^{1}H- and ^{13}C-NMR : see Table 1, (+)-FABMS *m/z* 677 $[M + Na]^+$, 515 $[M + Na - 162]^+$, 421 $[M + Na - 256]^+$, (+)-HR-FABMS *m/z* 677.3723 (calcd for $C_{31}H_{58}O_{14}$ + Na, 677.3724).

Alkaline hydrolysis of 1 – A solution of **1** (5 mg) in MeOH (1 ml) was treated with 3% NaOMe-MeOH (2 ml), and the mixture was stirred at 40°C for 2 hr. The reaction mixture was neutralized with 2N-HCl/MeOH solution and partitioned with *n*-hexane. The hexane solubles were evaporated at reduced pressure to give methyl palmitate as a colorless oil, which showed a single peak on GC-MS, EIMS *m/z* 270. The MeOH layer was evaporated under reduced pressure followed by a RP_{18} column chromatography with MeOH as an eluent to yield digalactosyl glycerol **2**, $[\alpha]_D^{27}$ +83.5° (*c* 0.2, H_2O).

Results and Discussion

Repeated column chromatography of the CH_2Cl_2 soluble fraction of the MeOH extract from *E. prolifera* gave compound **1**. Compound **1** gave in high resolution FABMS a quasimolecular ion peak at *m/z* 677.3723 $[M + Na]^+$, which indicated the molecular formula as $C_{31}H_{58}O_{14}$. In its ^{1}H-NMR spectrum, there was a triplet methyl signal at δ 0.89 (3H, t, J = 6.9 Hz), a mass of oxymethylene and oxymethine hydrogen signals between δ 3.4 and 4.2, and long-chain $(CH_2)_n$ signals at δ 1.28. No signals for olefinic protons were observed. These features are characteristic for glycolipids bearing saturated fatty acids. Signals at δ 4.24 (d, J = 7.2 Hz) and 4.86 (d, J = 3.9 Hz) indicated the presence of two β- and α-glycosidic linkages, respectively. Fragmention peaks in the FAB-MS showed at *m/z* 515 $[M + Na - 162]^+$ and *m/z* 421 $[M + Na - 256]^+$, corresponding to the loss of a hexose unit and a fatty acid moiety from the quasimolecularion at *m/z* 677 $[M + Na]^+$, respectively. Analysis of the ^{1}H- and ^{13}C-NMR, ^{1}H-^{1}H COSY, and HMQC spectra of **1** allowed the assignment of all the ^{13}C-NMR signals and ^{1}H-NMR signals for sugars and the glycerol moieties as shown in Table 1. These NMR spectroscopic findings were consistent with **1** bearing a 1-*O*-acyl-3-*O*-[α-D-galactopyranosyl (1→6)-β-D-galactopyranosyl]-*sn*-glycerol moiety (Jung *et al.*, 1996). Furthermore the ^{1}H-NMR spectrum of **1** was characteristic of the 2*S* type, as indicated in Table 1 the chemical shifts of H-1 methylene protons are very close (δ 4.12 and 4.16) and the coupling constant value between H-2 and H-3a is 4.5 Hz (Rho *et al.*, 1996). Acid hydrolysis of **1** by using TLC plate method yielded galactose. In the HMBC spectrum, long-range connectivities were observed between the ester carbonyl carbon (δ 175.6) and the H-1 protons (δ 4.12 and 4.16), indicating that the compound is acylated at C-1. To confirm the structure, **1** was treated with NaOMe-MeOH according to a reported method (Hiraga *et al.*, 2002), yielding a fatty acid methyl ester and digalactosyl glycerol (**2**). The fatty

Table 1. NMR data of 1-*O*-palmitoyl-3-*O*-[α-D-galactopyranosyl(1→6)-β-D-galactopyranosyl]-*sn*-glycerol in CD_3OD.

Carbon No.	^{1}H	^{13}C (DEPT)
1	4.12 (dd, 5.7, 11.1)	66.6 (CH_2)
	4.16 (dd, 4.8, 11.1)	
2	3.98 (m)	69.7 (CH)
3	3.66 (dd, 4.5, 10.5)	72.1 (CH_2)
	3.87 (overlap)	
1'	4.24 (d, 7.2)	105.3 (CH)
2'	3.56 (dd, 7.2, 9.6)	72.6 (CH)
3'	3.48 (dd, 3.3, 9.6)	74.7 (CH)
4'	3.84	70.1 (CH)
5'	3.74	74.6 (CH)
6'	3.66 (dd, 4.5, 10.5)	67.8 (CH_2)
	3.87 (dd, 6.3, 10.5)	
1"	4.86 (d, 3.9)	100.5 (CH)
2"	3.72	71.5 (CH)
3"	3.75	70.2 (CH)
4"	3.86	71.0 (CH)
5"	3.84	72.5 (CH)
6"	3.66 (dd, 6.3, 10.2)	62.7 (CH_2)
	3.70 (dd, 4.5, 10.2)	
1'''	-	175.5 (C)
2'''	2.35 (t, 7.5)	34.9 (CH_2)
3'''	1.61 (m)	26.0 (CH_2)
$(CH_2)_n$	1.28 (br s)	23.7, 30.2, 30.4, 30.5, 30.6, 30.8, 33.1 (all CH_2)
CH_3	0.89 (t, 6.9)	14.4 (CH_3)

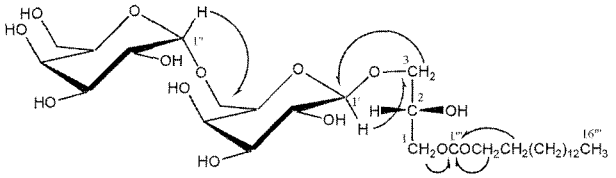

Fig. 1. Structure of 1-*O*-palmitoyl-3-*O*-[α-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl]-*sn*-glycerol (**1**) and key correlations in HMBC.

acid methyl ester was determined by GC-MS analysis and found to be methyl palmitate. Moreover, glyceryl digalactoside (**2**), $[\alpha]_D^{27}$ +83.5° (*c* 0.2, H_2O), was shown to be identical with (2*R*)-3-*O*-(α-D-galactopyranosyl (1→6)-β-D-galactopyranosyl)-*sn*-glycerol on the basis of comparison with the optical rotation and NMR data of previously reported data for this compound (Jung *et al.*, 1996; Hiraga *et al.*, 2002). Consequently, the chemical structure of **1** was determined as 1-*O*-palmitoyl-3-*O*-[α-D-galactopyranosyl(1→6)-β-D-galactopyranosyl]-*sn*-glycerol. This compound has been described in the green alga, *Ulva pertusa* (Fusetani and Hashimoto, 1975), but is now described for the first time in *E. prolifera*. The presence of other types of acylgalactosyl glycerols in *E. prolifera* has been observed by using the ^{1}H-NMR experiment, but these were not characterized due to the shortage of pure materials.

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