Arch Pharm Res Vol 28, No 12, 1381-1385, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Potentially Hepatoprotective Glycolipid Constituents of *Lycium chinense* Fruits

Kiwon Jung, Young-Won Chin, Young Choong Kim, and Jinwoong Kim*

College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul 151-742, Korea

(Received August 2, 2005)

Further investigation of *Lycium chinense* fruits gave a mixture of (6'-*O*-palmitoyl)- and (6'-*O*-stearoyl)-β-sitosterol-3-*O*-β-D-glucopyranoside (**1**) and two glycolipids, 1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-2-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol (**2**) and 1-*O*-(9*Z*,12*Z*-octadecadienoyl)-2-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol (**3**). These compounds were newly isolated as constituents of *L. chinense*.

Key words: *Lycium chinense* fruits, (6'-*O*-Palmitoyl)- and (6'-*O*-stearoyl)-β-sitosterol-3-*O*-β-D-glucopyranoside, 1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-2-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol, 1-*O*-(9*Z*,12*Z*-octadecadienoyl)-2-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol

INTRODUCTION

Fruits of *Lycium chinense* Miller (Solanaceae), distributed in northeast Asia, have been used as a tonic in traditional oriental medicine (Kim *et al.*, 1997). These fruits are also known to possess a hypotensive, hypoglycemic, hepatoprotective, and antipyretic activities, and to prevent stress-induced ulceration in experimental animals. A number of neutral volatile compounds, steroids, cerebrosides, and alkaloids were known as constituents of the fruits of this plant (Chin *et al.*, 2003). Because the EtOAc fraction of these fruits was found to show a hepatoprotective activity, this fraction was chosen for further investigation of bioactive materials. As a result, a mixture of (6'-*O*-palmitoyl)- and (6'-*O*-stearoyl)-β-sitosterol-3-*O*-β-D-glucopyranoside (**1**), and two glycolipids (**2** and **3**) were isolated from the fruits of *L. chinense*.

MATERIALS AND METHODS

Plant material

Air-dried fruits of *L. chinense* were purchased from Chungyang Agricultural Cooperatives Federation in Korea and identified by one of the authors. A voucher specimen (SNUPH-0027) was deposited at the College of Pharmacy, Seoul National University.

Correspondence to: Jinwoong Kim, College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul 151-742, Korea
Tel: 82-2-880-7853, Fax: 82-2-887-8509;
E-mail: jwkim@snu.ac.kr

General experimental procedures

NMR spectra were measured on a Varian VXR 300 spectrometer in $CDCl_3$. FAB-MS were obtained on a JMS AX 505WA spectrometer. GC-FID analysis was carried out on an HP 5890II (Hewlett Packard) with an HP 3395 integrator and DB-1, capillary column (J & W Scientific, 30 m×0.25 mm×0.17 μm film thickness). Helium was used as a carrier gas at the flow rate of 2 mL/min. The oven temperature was set at 180°C for 5 min, from 180°C to 230°C at a rate of 2°C /min, then held at 230°C for 1 min, and finally up to 280°C at a rate of 10 °C/min. The injector temperature and the detector temperature were set at 250°C and was 280°C, respectively.

Extraction and isolation

Dried fruits (120 kg) were extracted with EtOH and evaporated *in vacuo*. The extract was suspended in water and partitioned with *n*-hexane and EtOAc. The EtOAc extract (600 g) was subjected to SiO_2 column chromatography with $CHCl_3$-EtOAC gradient system (1:0 to 0:1, 5 L each) to give 18 fractions. Fraction 13 (16.7 g) was filtered and obtained a white precipitate (1.2 g). The precipitate was chromatographed on a Sephadex LH-20 column (CH_2Cl_2-MeOH = 4:1) and then was subjected to HPLC

(*n*-hexane-isopropyl alcohol = 7:1, 2 mL/min, Conosil Silica 7.8×250 mm) to afford compound **1** (70.2 mg). Fraction 16 (26.5 g) was subjected to SiO_2 column chromatography ($CHCl_3$-MeOH=10:1-0:1, 1000 mL each) and then provided 8 sub-fractions (F1601-F1608). F1602 (3.1 g) was applied on vacuum SiO_2 column chromatography giving 10 sub-fractions (F160201-F160210). F160203 (700 mg) was subjected to low pressure column chromatography (MeCN-H_2O 4:1-0:1, 10 mL/min, Lobar RP-8, Art. 11804, 40-60 um, Merck) to give 7 sub-fractions. Sub-fraction 5 (120 mg) was analyzed by HPLC (MeCN-H_2O = 4:1, 2 mL/min, VyDACTM C8, 10×240 mm) to give compounds **2** (32.0 mg) and **3** (17.5 mg).

A mixture of (6'-*O*-palmitoyl)-β-sitosterol-3-*O*-β-D-glucoside and (6'-*O*-stearoyl)-β-sitosterol-3-*O*-β-D-glucopyranoside (1)

An amorphous powder, EI-MS (70 eV, rel. int.) : *m/z* 414 [M-palmitoyl-glucosyl]$^+$ (8), 396 [414-H_2O]$^+$ (13), 255 [$C_{16}H_{31}O_2$]$^+$ (10), 98 (100); IR ν_{max} ($CHCl_3$); 3410 (OH), 1739 (C=O), 1170 (C-O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.65 (3H, s, H-18), 0.80, 0.81 (6H, d, *J* = 7.0 Hz, H-26, H-27), 0.82 (3H, t, *J* = 7.5 Hz, H-29), 0.86 (3H, t, J = 6.4 Hz, H-16"), 0.89 (3H, d, *J* = 6.6 Hz, H-21), 0.97 (3H, s, H-19), 1.25 (brs, (-CH_2-)$_n$), 2.30 (2H, t, *J* = 7.7 Hz, H-2"), 3.34 (1H, m, H-2'), 3.36 (1H, m, H-4'), 3.44 (1H, m, H-3), 3.54 (2H, m, H-3', H-5'), 4.32 (1H, m, H-6'a), 4.35 (1H, d, *J* = 7.6 Hz, H-1'), 4.37 (1H, m, H-6'b), 5.34 (1H, brs, H-6); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 11.8 (C-18), 11.9 (C-29), 14.1 (C-16"), 18.8 (C-26), 19.0 (C-21), 19.4 (C-19), 19.8 (C-27), 21.0 (C-11), 22.7 (C-15"), 23.0 (C-28), 24.3 (C-15), 25.0 (C-3"), 26.2 (C-25), 29.5-29.9 ((-CH_2-)$_n$), 29.7 (C-2), 31.9 (C-7, 8), 34.0 (C-22), 34.3 (C-2"), 36.2 (C-10), 36.7 (C-20), 37.3 (C-1), 39.0 (C-4), 39.8 (C-12), 42.3 (C-13), 45.8 (C-24), 50.1 (C-9), 56.2 (C-17), 56.8 (C-14), 63.7 (C-6'), 70.5 (C-4'), 73.3 (C-2'), 73.7 (C-5'), 76.3 (C-3'), 79.8 (C-3), 101.3 (C-1'), 122.0 (C-6), 140.4 (C-5), 174.1 (C-1").

Acetylation of compound 1

Compound **1** (5 mg) was mixed with dry pyridine-Ac_2O (1:1) and left 1 h at 60°C. Reaction mixture was dried by N_2 flow to yield compound **1a** (5.7 mg).

Compound **1a**: a white amorphous powder, IR ν_{max} ($CHCl_3$); 2923, 2850, 1747, 1227 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.65 (3H, s, H-18), 0.80, 0.81 (6H, d, *J* = 7.0 Hz, H-26, H-27), 0.82 (3H, t, *J* = 7.5 Hz, H-29), 0.86 (3H, t, *J* = 6.4 Hz, H-16"), 0.89 (3H, d, *J* = 6.6 Hz, H-21), 0.97 (3H, s, H-19), 1.25 (brs, (-CH_2-)$_n$), 1.98 (3H, s, -$COCH_3$), 2.00 (3H, s, -$COCH_3$), 2.02 (3H, -$COCH_3$), 2.30 (2H, t, *J* = 7.7 Hz, H-2"), 3.45 (1H, m, H-3), 3.66 (1H, ddd, *J* = 9.7, 5.4, 2.7 Hz, H-5'), 4.10 (1H, dd, *J* = 12.2, 2.7 Hz, H-6a'), 4.20 (1H, dd, *J* = 12.2, 5.4 Hz, H-6b'), 4.56 (1H, d, *J* = 7.8 Hz, H-1'), 4.93 (1H, dd, *J* = 9.7, 7.8 Hz, H-2'), 5.05 (1H, t, *J* = 9.7 Hz, H-4'), 5.18 (1H, t, *J* = 9.7 Hz, H-3'), 5.33 (1H, m, H-6).

Acid hydrolysis of compound 1

Compound **1** (20 mg) was hydrolyzed in 2N HCl-MeOH (4 mL) at 70°C for 4 h. The reaction mixtures were extracted with *n*-hexane (2 mL×3). The *n*-hexane fractions were evaporated *in vacuo* and analyzed by GC-MS.

Alkaline hydrolysis of compound 1

A solution of **1** (15 mg) in 3% KOH-MeOH (4 mL) was left to stand for 15 min at room temperature, and then neutralized with 1N HCl-MeOH. The solution was passed through Sephadex LH-20 with MeOH to remove the salts. The white crystals, m.p. 292-295°C, deposited from the eluate, were identical to β-sitosterol-3-*O*-β-D-glucopyranoside.

1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-2-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol (2)

A white amorphous powder, $C_{45}H_{74}O_{10}$, FAB-MS (positive) : *m/z* 813 [M+K]$^+$, 797 [M+Na]$^+$, 595 [M-$C_6H_{11}O_6$]$^+$; IR ν_{max} ($CHCl_3$) : 3401 (OH), 1741 (C=O), 1166 (C-O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.95 (6H, t, *J* = 7.6 Hz, H-18", 18'"), 1.28 (16H, m, H-4"~7", 4'"~7'"), 1.58 (4H, m, H-3", 3'"), 2.05 (8H, m, H-8", 8'", 17", 17'"), 2.29 (2H, t, *J* = 7.3 Hz, H-2"), 2.30 (2H, t, *J* = 7.3 Hz, H-2'"), 2.78 (8H, t, *J* = 5.8 Hz, H-11", 11'", 14", 14'"), 3.52 (1H, dd, *J* = 6.1, 4.2 Hz, H-5'), 3.56 (1H, dd, *J* = 9.5, 3.3 Hz, H-3'), 3.63 (1H, dd, *J* = 9.5, 7.3 Hz, H-2'), 3.71 (1H, dd, *J* = 11.2, 6.3 Hz, H-3b), 3.83 (1H, dd, *J* = 11.9, 4.2 Hz, H-6'b), 3.89 (1H, dd, *J* = 11.2, 5.6 Hz, H-3a), 3.93 (1H, dd, *J* = 11.9, 6.1 Hz, H-6'b), 3.99 (1H, brs, H-4'), 4.19 (1H, dd, *J* = 11.9, 6.3 Hz, H-1b), 4.25 (1H, d, *J* = 7.3 Hz, H-1'), 4.37 (1H, dd, *J* = 11.9, 3.4 Hz, H-1a), 5.28 (1H, m, H-2), 5.34 (12H, m, H-9", 9'", 10", 10'", 12", 12'", 13", 13'", 15", 15'", 16", 16'"); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 14.3 (C-18", 18'"), 20.6, 24.8, 24.9, 25.5, 25.6, 29.1, 29.2, 29.6 (C-4"-7", 4'"-7'"), 25.5 (C-3", 3'"), 25.6 (C-11"-14", 11'"-14'"), 27.2 (C-8", 8'", 17", 17'"), 34.1, 34.3 (C-2", 2'"), 62.5 (C-6'), 62.8 (C-1), 68.3 (C-3), 69.4 (C-4'), 70.2 (C-2'), 71.6 (C-2), 73.5 (C-3'), 74.6 (C-5'), 104.0 (C-1'), 127.1, 127.8, 128.2, 128.3, 130.2, 132.0 (C-9", 9'", 10", 10'", 12", 12'", 13", 13'", 15", 15'", 16", 16'"), 173.5, 173.8 (C-1", 1'").

1-*O*-(9*Z*,12*Z*-octadecadienoyl)-2-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol (3)

A white amorphous powder, $C_{45}H_{76}O_{10}$, FAB-MS (positive): *m/z* 815 [M+K]$^+$, 799 [M+Na]$^+$, 597 [M-$C_6H_{11}O_6$]$^+$; IR ν_{max} ($CHCl_3$) : 3400 (OH), 1740 (C=O), 1167 (C-O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 7.0 Hz, H-18'"), 0.95 (3H, t, *J* = 7.6 Hz, H-18"), 1.28 (16H, m, H-4"~7", 4'"~7'"), 1.58 (4H, m, H-3", 3'"), 2.05 (8H, m, H-8", 17", 8'",

17'''), 2.29 (2H, t, *J* = 7.3 Hz, H-2''), 2.30 (2H, t, *J* = 7.3 Hz, H-2'''), 2.77 (6H, t, *J* = 5.8 Hz, H-11'', 11''', 14'''), 3.52 (1H, dd, *J* = 6.1, 4.2 Hz, H-5'), 3.56 (1H, dd, *J* = 9.5, 3.3 Hz, H-3') 3.63 (1H, dd, *J* = 9.5, 7.3 Hz, H-2'), 3.71 (1H, dd, *J* = 11.2, 6.3 Hz, H-3b), 3.83 (1H, dd, *J* = 11.9, 4.2 Hz, H-6'b), 3.89 (1H, dd, *J* = 11.2, 5.6 Hz, H-3a), 3.94 (1H, dd, *J* = 11.9, 6.1 Hz, H-6'a), 3.99 (1H, brs, H-4'), 4.19 (1H, dd, *J* = 11.9, 6.3 Hz, H-1b), 4.25 (1H, d, *J* = 7.3 Hz, H-1'), 4.37 (1H, dd, *J* = 11.9, 3.4 Hz, H-1a), 5.28 (1H, m, H-2), 5.34 (10H, m, H-9'', 9''', 10'', 10''',12'', 12''', 13'', 13''', 15'', 16''); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 14.3 (C-18'', 18'''), 25.5 (C-3'', 3'''), 25.6 (C-11'', 11''', 14''), 27.2 (C-8'', 8''', 17'', 17'''), 20.5, 24.8, 24.9, 29.1, 29.2, 29.3, 29.6, 31.6 (C-4''-7'', 4'''-7'''), 34.1, 34.3 (C-2'', 2'''), 62.7 (C-1, 6'), 68.4 (C-3), 69.4 (C-4'), 70.2 (C-2'), 71.6 (C-2), 73.4 (C-3'), 74.5 (C-5'), 104.0 (C-1'), 127.1, 127.7, 128.1, 128.2, 128.3, 130.0, 130.2, 132.0 (C-9'', 9''', 10'', 10''', 12'', 12''', 13'', 13''', 15'', 16''), 173.5, 173.7 (C-1'', 1''').

Acid hydrolysis of compounds 2 and 3

Compound **2** (5 mg) was dissolved in 2N HCl-MeOH (1 mL) and reacted at 70°C for 4 h. The hydrolyzate was extracted with *n*-hexane (1 mL × 3) and the *n*-hexane fraction was evaporated *in vacuo* and analyzed by GC-MS. Acid hydrolysis of compound **3** (3 mg) was performed according to the method described for **2**.

Alkaline hydrolysis of compounds 2 and 3

Compound **2** (8 mg) in 10% NaOMe-MeOH (2 mL) was stirred at 40°C for 2 h. The reaction mixture was neutralized with 2N HCl-MeOH and extracted with *n*-hexane. After removal of *n*-hexane fraction, MeOH extract was evaporated *in vacuo* and was dissolved in H_2O (3 mL). The solution was subjected to C_{18} Sep-Pak cartridge to afford compound **2a**. Compound **3** (9 mg) was hydrolyzed in alkaline condition to the method as described for **2** to yield **3a**.

Compound **2a** : $C_9H_{18}O_8$, $[\alpha]_D^{20}$ -9.5° (*c* 0.1, H_2O); ^{13}C-NMR (100 MHz, CD_3OD) δ: 63.2 (C-6'), 64.7 (C-1), 71.1 (C-4'), 72.7 (C-3), 72.8 (C-2), 73.2 (C-2'), 75.5 (C-3'), 77.4 (C-5'), 105.7 (C-1').

Compound **3a** : $C_9H_{18}O_8$, $[\alpha]_D^{20}$ -9.5° (*c* 0.05, H_2O); ^{13}C-NMR (100 MHz, CD_3OD) δ: 63.2 (C-6'), 64.7 (C-1), 71.1 (C-4'), 72.7 (C-3), 72.8 (C-2), 73.2 (C-2'), 75.5 (C-3'), 77.4 (C-5'), 105.7 (C-1').

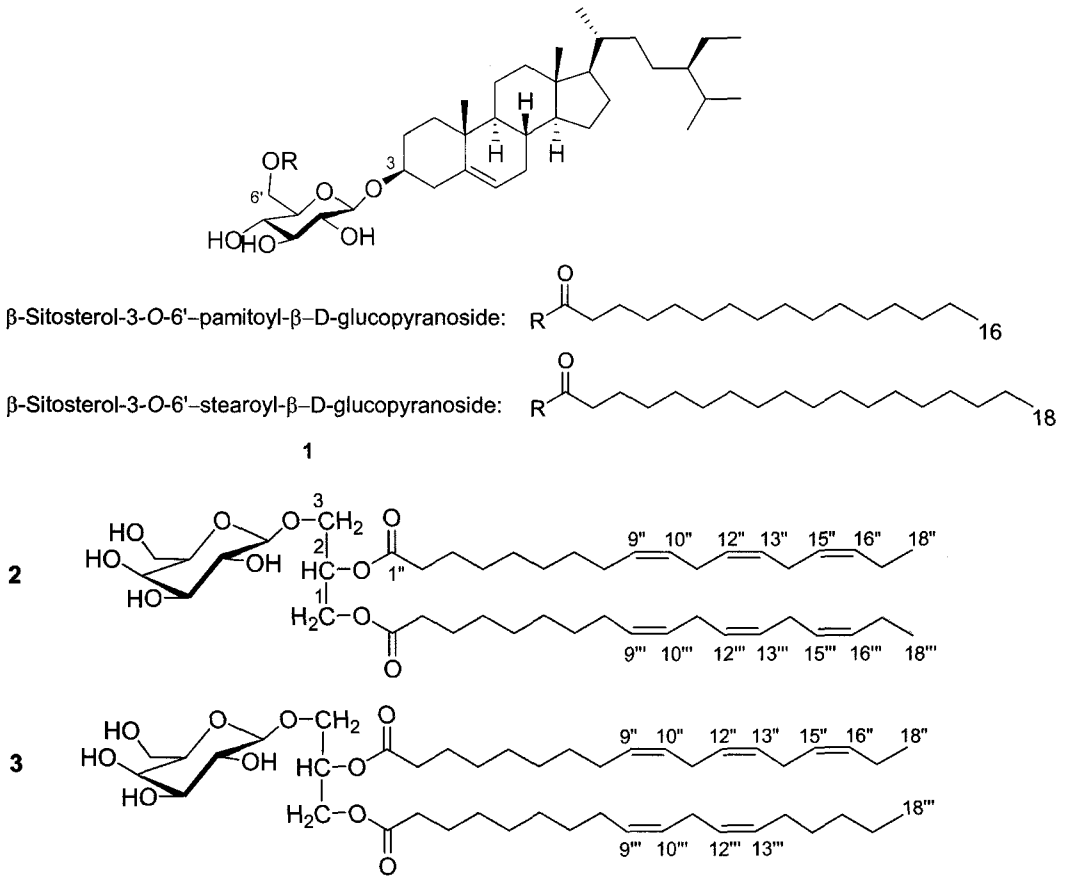

Fig. 1. The structures of compounds 1-3

Enzymic hyrolysis of compounds 2 and 3

A solution of **2** (0.4 mg) and Lipase type XIII (Sigma, 0.2 mg) in 200 μL dioxane-H_2O (1:1) was incubated at 37°C for 3 h. The reaction was quenched by adding 5% HOAc (25 μL), then EtOH was added to the reaction mixture. After removal of solvent *in vacuo*, the resulting residue was dissolved in THF and then esterfied by ethereal CH_2N_2. The reaction mixture was extracted with *n*-hexane. The *n*-hexane layer was concentrated *in vacuo* and analyzed by GC-MS (Jung *et al.*, 1996). Enzymic hydrolysis of **3** was performed according to the method described for **2**.

RESULTS AND DISCUSSION

The EtOAc extract of *L. chinense* fruits was chromatographed on a SiO_2 gel using $CHCl_3$-EtOAc (1:00:1) and then were further subjected to Sephadex LH-20, low pressure column chromatography and reversed-phase HPLC to furnish compounds **1-3**.

Compound **1** in the IR spectrum showed the ester at 1739 and 1170 cm^{-1}, hydroxy at 3410 cm^{-1}. The ^{1}H- and ^{13}C-NMR of **1** were similar to those of β-sitosterol-3-*O*-β-D-glucopyranoside, that was supported by the alkaline hydrolysis of **1**, except that there were the signals at δ 0.86 (terminal methyl, H-16), δ 1.25 (virtually coupled methylenes), and δ 2.30 (methylene proton connected to carbonyl group, H-2) in the ^{1}H-NMR spectrum, and the signal at δ 174.1 (a carbonyl due to an acyl group, C-1) in the ^{13}C-NMR spectrum, which was assigned to a fatty acid moiety (Nakano *et al.*, 1981; Iribarren and Pomilio, 1983). Therefore, **1** was assumed to be β-sitosterol-3-*O*-β-D-glucoside containing a fatty acid moiety. The acetylation of **1** showed three acetyl signals in the ^{1}H-NMR spectrum, and this fact suggested that the fatty acid was located at a hydroxy group of the glucose (Kadota *et al.*, 1989). The position of the fatty acid was determined to be at C-6' based on the downfield shifts of C-6' (α-effect, +1.6 ppm) and upfield shift of C-5' (β-effect, -2.3 ppm) (Chaurasia and Wichti, 1987). Furthermore, acid hydrolysis of **1** afforded the mixture of palmitic acid and stearic acid in the ratio of 4 to 1, which were identified by GC-MS analysis (methyl palmitate at *m/z* 270 and methyl stearate at *m/z* 298, respectively). On the basis of these data, structure of **1** was determined as the mixture of (6'-*O*-palmitoyl)-β-sitosterol-3-*O*-β-D-glucoside and (6'-*O*-stearoyl)-β-sitosterol-3-*O*-β-D-glucoside in the ratio of 4 to 1.

The molecular formula of compound **2**, $C_{45}H_{74}O_{10}$, was obtained by observing quasimolecular ions at *m/z* 813 $[M+K]^+$ and 797 $[M+Na]^+$ in the FAB-MS. The ^{1}H-NMR spectrum of **2** exhibited the signals at δ 3.71 (1H, dd, *J* = 11.2, 6.3 Hz, H-3b), 3.89 (1H, dd, *J* = 11.2, 5.6 Hz, H-3a), 4.19 (1H, dd, *J* = 11.9, 6.3 Hz, H-1b), 4.37 (1H, dd, *J* = 11.9, 3.4 Hz, H-1a), and 5.28 (1H, m, H-2) due to a glycerol moiety. Also, there were two methyl protons at δ 0.95 (6H, t, *J* = 7.6 Hz, H-18 and 18), virtually coupled methylene protons at δ 1.28, two methylene protons at δ 2.29 (2H, t, *J* = 7.3 Hz, H-2) and 2.30 (2H, t, *J* = 7.3 Hz, H-2) adjacent to carbonyl group, and olefinic protons at δ 5.34 (12H, m). These peaks were assigned to aliphatic long chains with double bonds. In addition to these signals, an anomeric signal of the sugar unit was observed at δ 4.25 (1H, d, *J* = 7.1 Hz) and the sugar unit proved to be β-D-galactopyranoside by comparing six oxygenated carbon signals beside a glycerol moiety with the literature (Jung *et al.*,1996). The ^{13}C-NMR spectrum of **2** showed the signals by two terminal methyl signals at δ 14.3, six olefinic signals belonging to 12 carbon signals at δ 127.1-132.0, and two carbonyl carbon signals at δ 173.8 and 173.5, resulting from the presence of two unsaturated fatty acids. The geometry of the double bonds in the fatty acid moieties was determined to be *cis* based on the chemical shift (δ 27.2) of the carbons next to double bonds in the ^{13}C-NMR data (Jung *et al.*, 1996). Thus, **2** was assumed to be a diacyl glycerol with a β-D-galactopyranoside. Acid hydrolysis of **2** with 2N HCl-MeOH gave a methyl 9*Z*,12*Z*,15*Z*-octadecatrienoate (*m/z* 292), which was identified by GC-MS analysis. Also, treatment of **2** with 10% sodium methoxide in methanol yielded **2a**, that was identical in specific rotation and ^{13}C-NMR data to (2*R*)-1-*O*-β-D-galactopyranosyl glycerol confirming the stereochemistry of both sugar and glycerol parts in **2** (Jung *et al.*, 1996; Murakami *et al.*, 1993; Oshima *et al.*, 1994). Therefore, the structure of **2** was determined to be (2*S*) 1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-2-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol.

The spectral data of **3** were similar to those of **2**, except for the signals at δ 0.95 (t, *J* = 7.6 Hz, H-18") and 0.87 (t, *J* = 7.0 Hz, H-18"') in the fatty acid residue, which suggested that two kinds of the fatty acid residues were present in **3** (Jung *et al.*, 1996). Acid hydrolysis of **3** with 2N HCl-MeOH furnished a methyl 9*Z*,12*Z*,15*Z*-octadecatrienoate (*m/z* 292) and a methyl 9*Z*,12*Z*-octadecadienoate (*m/z* 294), which were identified by GC-MS analysis. Also, treatment of **3** with 10% sodium methoxide in methanol afforded **3a**, (2*R*)-1-*O*-β-D-galactopyranosyl glycerol, corroborated by comparison with an optical rotation and ^{13}C-NMR data (Jung *et al.*, 1996; Murakami *et al.*, 1993; Oshima *et al.*, 1994). The sequence of fatty acid residues in **3** was determined by *regio*-selective enzyme hydrolysis using Lipase type XIII that selectively hydrolyzed fatty acid attached to C-1 of glycerol moiety, and 9*Z*,12*Z*-octadecadienoate was obtained as a major compound (Jung *et al.*,1996; Kitagawa *et al.*, 1989). On the basis of these data, structure of **3** was determined as (2*S*) 1-*O*-(9*Z*,12*Z*-octadecadienoyl)-2-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol.

ACKNOWLEDGEMENT

This work was supported by a research grant from (HMP-96-D-1-1005) of the 96-97 Good Health R&D Project, the Ministry of Health & Welfare, Korea.

REFERENCES

Chaurasia, N. and Wichti, M., Sterols and steryl glycosides from *Urtica dioica*. *J. Nat. Prod.*, 50, 881-885 (1987).

Chin, Y.-W., Lim, S. W., Kim, S.-H., Sin, D.-Y., Sur, Y.-G., Kim, Y. B., Kim, Y. C., and Kim, J., Hepatoprotective pyrrole derivatives of *Lycium chinense* fruits. *Bioorg. Med. Chem. Lett.*, 13, 79-81 (2003).

Iribarren, A. M. and Pomilio, A. B., Components of *Bauhinia candicans*. *J. Nat. Prod.*, 46, 752-752 (1983).

Jung, J. H., Lee, H., and Kang, S. S., Diacylglycerylgalactosides from *Arisaema amurese*. *Phytochemistry*, 42, 447-452 (1996).

Kadota, S., Lami, N., Tezuka, Y., and Kikuchi, T., Constituents of the roots of *Boerhaavia diffusa* L. I. Examination of sterols and structures of new rotenoids, boeravinones A and B. *Chem. Pharm. Bull.*, 37, 3214-3220 (1989).

Kim, S. Y., Choi, Y. H., Huh, H., Kim, J., Kim, Y. C., and Lee, H. S., New antihepatotoxic cerebroside from *Lycium chinenese* fruits. *J. Nat. Prod.*, 60, 274-276 (1997).

Kitagawa, I., Hayashi, K., and Kobayashi, M., Heterosigmaglycolipids I and II, two new galactolipids containing octadecatetraenoyl and eicosapentaenoyl residues, from a raphidophyte dinoflagellate *Heterosigma* sp. *Chem. Pharm. Bull.*, 37, 849-851 (1989).

Murakami, N., Shirahashi, H., Nagatsu, A., and Sakakibara, J., Studies on Glycolipids. VI. new acyl-distributed glycerolglycolipids from the nitrogen-fixing cyanobacterium *Anabaena flos-aquae*. *Chem. Pharm. Bull.*, 41, 1177-1179 (1993).

Nakano, K., Murakami, K., Nohara, T., Tomimatsu, T., and Kawasaki, T., The constituents of *Paris verticillata* M.v. Bieb. *Chem. Pharm. Bull.*, 29, 1445-1451 (1981).

Oshima, Y., Yamada, S.-H., Matsunaga, K., Moriya, T., and Ohizumi, Y., A monogalactosyl diacylglycerol from a cultured marine dinoflagellate, *Scrippsiella trochoides*. *J. Nat. Prod.*, 57, 534-536 (1994).