Asian Journal of Chemistry; Vol. 25, No. 2 (2013), 1083-1085

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13488

Glycerol Derivatives of Fatty Acids from the Fruits of *Lycium chinense*

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(*Received*: 21 January 2012; *Accepted*: 30 August 2012) AJC-12048

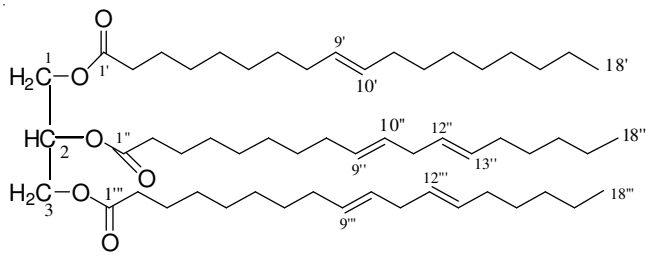
Two compounds 1-oleo-2, 3-dilinoleioglyceride (**1**) and 1,2-dioleo-3-linolenioglyceride (**2**) have been isolated from the ethyl acetate extract of fruits of *Lycium chinense*. Their structures have been elucidated with the help of 600 MHz NMR using 1D spectral method *viz.*, ^{1}H and ^{1}3C, aided by FAB MS and IR spectroscopy. The compounds **1-2** are reported for the first time in the fruits of *L. chinense*.

Key Words: *Lycium chinense*, Solanaceae, Fruits, 1-Oleo-2, 3-dilinoleioglyceride, 1,2-Dioleo-3-linolenioglyceride.

INTRODUCTION

Lycium chinense Miller fruits (Fructus Lycii) known as "Gou-Qi-Zi" in Chinese, has long history of application as a valuable tonic and health food supplement for improving vision and maintaining good health. It is reputed to have the properties of nourishing the blood, enriching the yin, tonifying the kidney and liver, moistening the lungs[1,2]. Fruits of *L. chinense* (Solanaceae), distributed in northeast Asia, specially China, Japan, Korea and Taiwan, have been widely used as a tonic in traditional medicine. Potentially isolated constituents were reported to exhibit hypotensive, hypoglycemic and antipyretic activities[3,4]. Several compounds like cerebrosides and lyciumamide in this plant are known to display various bioactivities[5,6]. Potentially hepatoprotective glycolipid constituents and determination of betain in *L. chinense* fruits have been reported[7,8]. Antimicrobial compounds have also been reported from *L. chinense* roots[9]. Specific α-galactosidase inhibitors, N-methylcalystegines structure/activity relationship of calystegines from *L. chinense* have been reported[10]. The plant is reported to possess antibacterial, anticancer and antioxidant properties[9,11,12]. Antihepatotoxic activity and chemical constituents from *L. chinense* fruits have been reported[5,13].

Several compounds like cyclic peptides, acyclic diterpene glycosides and other compounds from *L. chinense*[14], glycoconjugates from *L. barbarum* have been reported[15]. Variation in fruit sugar composition of *L. barbarum* and *L. chinense* of different regions and varieties were also reported[16]. Evaluation of antioxidant and other activities of compounds from *L. barbarum* and *L. chinense* has been reported[17,18]. Due to significance of fruits of this plant in medicinal use, the work in this area has already been done. The aim of the present investigation is to report some of the findings in the form of natural products first time from the fruits of *L. chinense*. This paper describes the isolation and characterization of two glyceride derivatives of unsaturated fatty acids as 1-oleo-2,3-dilinoleioglyceride (**1**) and 1,2-dioleo-3-linolenioglyceride (**2**) from the fruits of *L. chinense*. Isolated compounds data are compared with previously reported similar compounds[19] and glycolipid[7], glycerogalactolipids[20].



Chemical structures of compounds **1** and **2**

EXPERIMENTAL

All chemicals used were of analytical grade. Hexane, ethyl acetate, methanol, ethanol, sulfuric acid and vanillin were purchased from Daejung Chemicals and Metals Co., Ltd, Korea. Precoated TLC plates (layer thickness 0.25 mm) and silica gel used for column chromatography (70-230 mesh ASTM) and LiChroprep RP-18 (40-63 μm) were from Merck (Darmstadt, Germany). Authentic samples of standards were purchased from Sigma-Aldrich (USA). Optical rotation was measured on an AA-10 model polarimeter (Instruments Ltd, Seoul, South Korea). Both ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance 600 high resolution spectrometer operating at 600 and 150 MHz, respectively. This NMR machine was available at National Instrumentation Center for Environmental Management (NICEM) Seoul National University (SNU), Seoul, South Korea. NMR spectra were obtained in deuterated methanol and chloroform using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in ppm (δ) and coupling constants (*J*) in hertz (Hz). FABMS data were recorded on a JMS-700 (Jeol, Japan) spectrometer instrument which was available at Korea Basic Science Institute (KBSI) Daegu, South Korea. IR spectra were recorded on a Infinity Gold FT-IR (Thermo Mattson, USA) spectrophotometer, which was available at Korea Institute of Science and Technology, Seoul, South Korea.

Fruits of *Lycium chinense* were purchased from local medicinal plants shop market in Seoul, Korea and were identified by the Department of Pharmacognosy. Voucher specimen No. KU/LC/2010 has been deposited in Department of Applied Life Science, Konkuk University.

Extraction of fruits: The fruits of *L. chinense* (3.1 kg) were immersed in methanol (8 L) for three days at room temperature and then the supernatant was concentrated under vacuum to yield 230 g of the extract, which was suspended in water and extracted with hexane, ethyl acetate and *n*-butanol successively to produce 20 g, 10.1 g and 40 g extract, respectively.

Isolation of the compounds from ethyl acetate extract: The entire ethyl acetate extract was subjected to normal phase column chromatography over silica gel (600 g) to yield 24 fractions (each of 500 mL) with the following eluants: fractions 1-2 with hexane, fractions 3-4 with hexane:EtOAc (9:1), fractions 5-6 with hexane:EtOAc (8:2), fractions 7-8 with hexane: EtOAc (7:3), fractions 9-10 with hexane:EtOAc (6:4), fractions 11-12 with hexane:EtOAc (1:1), fractions 13-14 with hexane:EtOAc (4:6), fractions 15-16 with hexane:EtOAc (3:7), fractions 17-18 with hexane:EtOAc (2:8), fractions 19-20 with hexane:EtOAc (1:9) and fractions 21-24 with EtOAc. All fractions were examined by TLC. Fractions 3-4 (0.78 g) were rechromatographed over silica gel with hexane:ethyl acetate: fractions 1-2 with hexane, fractions 3-4 with hexane:EtOAc (9.8:0.2), fractions 5-6 with hexane:EtOAc (9.6:0.4), fractions 7-8 with hexane:EtOAc (9.4:0.6), fractions 9-10 with hexane:EtOAc (9.2:0.8), fractions 11-12 with hexane:EtOAc (9:1). Fractions 11-12 to yield two compounds **1** and **2**.

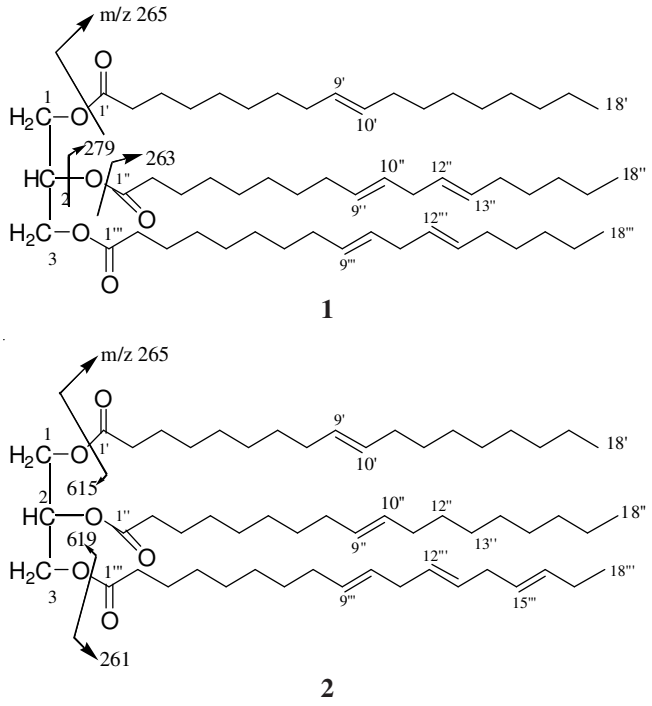
1-Oleo-2,3-dilinoleioglyceride (1): Dark yellow solid; R_f: 0.32 (hexane:EtOAc; 1:1); $[\alpha]_D$ -32.4 (c 0.3, MeOH); IR (KBr, ν_{max}, cm^{-1}): 2923, 2853, 1735, 1728, 1658, 1463, 1379, 1258, 1072; ^{1}H NMR (MeOD): δ 5.36 (1H, m, H-9'), 5.36 (1H, m, H-10'), 5.32 (2H, m, H-9'', H-9'''), 5.30 (2H, m, H-12'', H-12'''), 5.28 ((2H, m, H-10'', H-10'''), 5.23 (2H, m, H-13'', H-13'''), 4.08 (1H, m, H-2), 3.88 (2H, m, H_2-1), 3.62 (2H, m, H_2-3), 2.81 (2H, m, H_2-11''), 2.76 (2H, m, H_2-11'''), 2.34 (2H, t, *J* = 7.2 Hz, H_2-2'), 2.32 (2H, t, *J* = 10.5 Hz, H_2-2''), 2.30 (2H, t, *J* =7.2 Hz, H_2-2'''), 2.07 (4H, m, H_2-8', H_2-11'), 2.05 (4H, m, H_2-8'', H_2-14''), 2.02 (4H, m, H_2-8''', H_2-14'''), 1.59 (16H, br s, 8 × CH_2), 1.36 (4H, br s, 2 × CH_2), 1.36 (14H, br s,7 × CH_2), 1.28 (20H, br s, 10 × CH_2), 0.89 (3H, t, *J* = 6.6 Hz, Me-18'), 0.87 (3H, t, *J* = 6.6 Hz, Me-18''), 0.85 (3H, t, *J* = 5.7 Hz, Me-18'''); ^{13}C NMR (MeOD): δ 175.15 (C-1'), 174.83 (C-1''), 169.49 (C-1'''), 133.74 (C-12''), 132.89 (C-12'''), 132.55 (C-10''), 131.10 (C-10'''), 131.02 (C-9'), 130.01 (C-10'), 129.27 (C-9''), 129.27 (C-9'''), 129.20 (C-13''), 128.41 (C-13'''), 78.76 (C-2), 73.42 (C-1), 69.26 (C-3), 54.39 (CH_2), 40.33 (CH_2), 35.28 (CH_2), 35.11 (CH_2), 33.23 (CH_2), 32.83 (CH_2), 31.77 (CH_2), 30.93 (17 × CH_2), 30.81 (CH_2), 30.63 (CH_2), 30.54 (CH_2), 30.44 (CH_2), 30.38 (CH_2), 30.29 (CH_2), 28.33 (2 × CH_2), 26.72 (CH_2), 26.18 (CH_2), 25.10 (CH_2), 24.17 (CH_2), 23.89 (CH_2), 23.79 (CH_2), 14.80 (Me-18'), 14.60 (Me-18''), 14.55 (Me-18'''); FAB MS (positive mode) m/z 881 $[M+H]^+$ ($C_{57}H_{101}O_6$) (2.5), 265 (11.5), 263 (10.2), 279 (23.6).

1,2-Dioleo-3-linolenioglyceride (2): Light yellow semi-solid; R_f: 0.43 (Hexane:EtOAc; 1:1); $[\alpha]_D$ -22.4 (*c* 0.2, $CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 2924, 2855, 1738, 1725, 1645, 1463, 1380, 1265, 1071, 721; ^{1}H NMR ($CDCl_3$): δ 5.38 (2H, m, H-9', H-9''), 5.36 (2H, m, H-10', H10''), 5.35 (2H, m, H-9''', H-16'''), 5.32 (2H, m, H-10''', H-12'''), 5.30 ((2H, m, H-13''', H-15'''), 2.80 (2H, m, CH_2-11'''), 2.76 (2H, m, CH_2-14'''), 2.29 (6H, br s, CH_2-2', CH_2-2'', CH_2-2'''), 2.08 (2H, m, CH_2-8'), 2.07 (2H, m, H_2-11'), 2.05 (4H, m, H_2-8'', H_2-11''), 2.03 (2H, m, H_2-8'''), 2.01 (2H, m, H_2-17'''), 1.57 (4H, m, 2 × CH_2), 1.35 (2H, m, CH_2), 1.33 (4H, m, 2 × CH_2), 1.29 (20H, br s, 10 × CH_2), 1.25 (24H, br s, 12 × CH_2), 0.97 (3H, t, *J* = 7.8 Hz, Me-18'''), 0.89 (3H, t, *J* = 6.0 Hz, Me-18'), 0.87 (3H, t, *J* = 6.6 Hz, Me-18''); ^{13}C NMR ($CDCl_3$): δ 173.90 (C-1'), 173.88 (C-1''), 171.25 (C-1'''), 131.90 (C-12'''), 130.18 (C-13'''), 129.91 (C-15'''), 128.28 (C-9'''), 128.17 (C-10'''), 128.04 (C-9''), 127.85 (C-10''), 127.85 (C-9'), 127.73 (C-16'''), 127.08 (C-10'), 34.11 (CH_2), 31.92 (CH_2), 31.49 (CH_2), 29.76 (CH_2), 29.69 (6 × CH_2), 29.37 (5 × CH_2), 29.33 (8 × CH_2), 27.23 (CH_2), 27.16 (CH_2), 25.60 (CH_2), 25.50 (CH_2), 24.89 (CH_2), 22.68 (CH_2), 22.56 (CH_2), 20.52 (CH_2), 14.26 (Me-18'), 14.10 (Me-18''), 14.06 (Me-18'''); FAB MS (positive mode) m/z 881 $[M+H]^+$ ($C_{57}H_{101}O_6$) (18.3), 619 (7.8), 615 (13.3), 265 (18.1), 261 (23.2).

RESULTS AND DISCUSSION

The EtOAc extract of *L. chinense* fruits was chromatographed on a SiO_2 gel using hexane-EtOAc and to yield two compounds (**1-2**).

Compound **1**, was obtained as a dark yellow solid from hexane-ethyl acetate (9:1) eluants. Its IR spectrum showed characteristic absorption bands for 2923, 2853 cm^{-1}, ester function (1735, 1728 cm^{-1}) and double bonds (1658, 1463 cm^{-1}). The FAB mass and ^{13}C NMR spectral data led to established molecular formula ion peak at m/z 880 consistent with the molecular formula $C_{57}H_{100}O_6$ of a glycerol esterified with three unsaturated fatty acids. The mass fragmentation pattern of **1** is shown in Fig. 1.



1 **2**

Fig. 1. Mass fragmentation pattern of compounds **1** and **2**

The ^{1}H NMR spectrum of **1** showed a multiplets between δ 4.13-5.38 were assigned to H-9', H-10', H-9", H-10", H-12", H-13", H-9"', H-10"', H-12"' and H-13"'. Four proton multiplets at δ 4.08, 3.88 and 3.62 were assigned for H-2, H_2-1 and H_2-3. Four proton multiplets at δ 2.81 and 2.76 were assigned for H_2-11" and H_2-11"'. Two proton triplets at δ 2.34 (J = 7.2 Hz), 2.32 (J = 10.5 Hz) and 2.30 (J = 7.5 Hz) were assigned for H_2-2', H_2-2" and H_2-2"'. Other multiplets at δ 2.07, 2.05 and 2.02 were assigned for H_2-8', H_2-11', H_2-8", H_2-14", H_2-8"' and H_2-14"'. The methylene protons were resonated between at δ 1.59-1.28. Three protons each at δ 0.89 (J = 6.6 Hz), 0.87 (J = 6.6 Hz) and 0.85 (J = 5.7 Hz) were assigned for Me-18', Me-18" and Me-18"'.

The ^{13}C NMR spectrum of **1** exhibited three deshielded carbon at δ 175.15, 174.83 and 169.49 was assigned to ester carbon C-1', C-1" and C-1"'. The deshielded carbon signals at δ 133.74-128.41 were associated with vinylic carbons H-9', H-10', H-9", H-10", H-12", H-13", H-9"', H-10"', H-12"' and H-13"' of the fatty acids. More details of proton and carbon assignments are showed in experimental section. On the basis of spectral data analysis, the structure of **1** has been established as 1-oleo-2,3-dilinoleioglyceride.

Compound **2**, was obtained as a light yellow semi-solid from hexane-ethyl acetate (9:1) eluants. Its IR spectrum showed characteristic absorption bands for 2924, 2855 cm^{-1}, ester function (1725 cm^{-1}) and double bonds (1645, 1463 cm^{-1}). The FAB mass and ^{13}C NMR spectral data led to established molecular formula ion peak at m/z 880 consistent with the molecular formula $C_{57}H_{100}O_6$ of a glycerol esterified with three unsaturated fatty acids. The mass fragmentation pattern of **2** is shown in Fig. 1.

The ^{1}H NMR spectrum of **2** showed multiplets between δ 5.38-5.30 assigned to H-9', H-9", H-10', H-10", H-9"', H-10"', H-12"', H-13"', H-15"' and H-16"'. Four proton multiplets at δ 2.80 and 2.76 were assigned for CH_2-14"', CH_2-11"'. Six protons broad singlet at δ 2.29 was assigned for CH_2-2', CH_2-2" and CH_2-2"'. Several multiplets at δ 2.08, 2.07, 2.05, 2.03 and 2.01 were assigned for CH_2-8' and H_2-11', H_2-11", H_2-8"', H_2-17"'. Several multiplets between at δ 1.57-1.25 was assigned for methylene protons. Three protons each at δ 0.97 (J = 6.6 Hz), 0.89 (J = 6.6 Hz) and 0.87 (J = 5.7 Hz) were assigned for Me-18' Me-18" and Me-18"'.

The ^{13}C NMR spectrum of 2 exhibited three deshielded carbon at δ 173.90, 173.88 and 171.25 was assigned to ester carbon C-1', C-1" and C-1"'. The deshielded carbon signals at δ 131.90-127.08 were associated with vinylic carbons H-9', H-9", H-10', H-10", H-9"', H-10"', H-12"', H-13"', H-15"' and H-16"' of the fatty acids. More details of proton and carbon assignments are given in experimental. On the basis of spectral data analysis, the structure of **2** has been established as 1,2-dioleo-3-linolenioglyceride.

ACKNOWLEDGEMENTS

This work was carried out with the support of Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ 906938), Rural Development Administration, Republic of Korea.

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