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New Aliphatic Glycoside Constituent from the Fruits of *Lycium chinense* Miller

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One new compound as *n*-henecosanoyl-β-D-arabinofuranosyl-2'-(1→2)-β-D-arabinopyranosyl-(1→2)-2''-β-D-arabinopyranosyl (1→2)-2'''-β-D-arabinopyranoside (**1**), along with known *n*-eicosanoic acid were isolated from the fruits of *Lycium chinense*. The chemical structure of new compound was elucidated by 1D and 2D NMR spectroscopic techniques (COSY, HSQC, HMBC) aided by FAB-MS and IR spectra.

Key Words: *Lycium chinense* M, Solanaceae, Fruits compounds, Aliphatic glycoside.

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 hypertensive, 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 were 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* were 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].

In continuation of our previous work[14-17] on *L. chinense* fruits one more new compound (**1**) was isolated as a natural product. The aim of the present investigation is to report new aliphatic natural product from the fruits of *L. chinense*. This paper deals with the isolation and structural elucidation of one new compound aliphatic glycoside (**1**, Fig. 1) on the basis of spectral methods, *viz*: 1D, 2D NMR, in combination with IR, FAB-MS analysis.

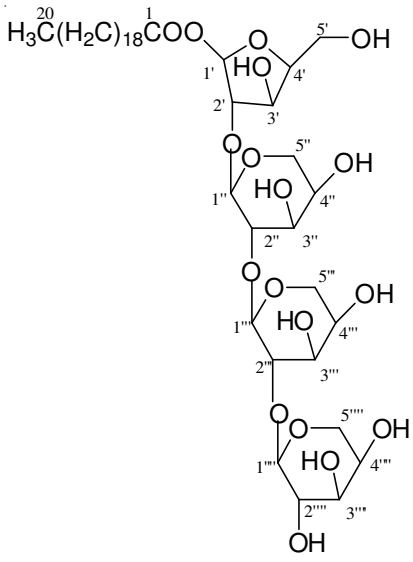

Fig. 1. Structure of compound **1**

EXPERIMENTAL

All chemicals used were of analytical grade. Hexane, ethyl acetate, chloroform, methanol, ethanol, water, sulphuric acid and vanillin were purchased from Daejung Chemicals and Metals Co. Ltd., Shiheung (Gyeonggi-do) Korea. Pre-coated TLC plates (layer thickness 0.25 mm), silica gel for column

chromatography (70-230 mesh ASTM) and LiChroprep RP-18 (40-63 μm) were from Merck, Darmstadt, Germany. An authentic standard of *n*-eicosanoic acid was purchased from Sigma-Aldrich, St. Louis, Missourri, USA. Both ^{1}H 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 Seoul National University, Seoul, South Korea and all NMR spectra were recorded at Seoul National University (Instrument, Bruker, Germany). NMR spectra were obtained in deuterated solvents using tetramethylsilane as an internal standard, with chemical shifts expressed in ppm (δ) and coupling constants (*J*) in Hz. FAB MS data were recorded on a JMS-700 (Jeol, Japan) spectrometer instrument which was available at SNU, Seoul, South Korea. IR spectra were recorded on an Infinity Gold FT-IR (Thermo Mattson, USA) spectrophotometer, which was available at Korea Institute of Science and Technology, Seoul, South Korea.

Fruits of *L. 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, Seoul, South Korea.

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 *in vacuo* to yield 230 g of extract. This material 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 hexane extract: The entire hexane extract (20 g) was subjected to normal phase CC over silica gel (600 g) to yield 28 fractions (each of 500 mL) with the following eluants: fractions 1-2 with hexane, fractions 3-4 with hexane:chloroform (9.5:0.5), fractions 5-6 with hexane:chloroform (9:1), fractions 7-8 with hexane: chloroform (8:2), fractions 9-10 with hexane: chloroform (7:3), fractions 11-12 with hexane:chloroform (6:4), fractions 13-14 with hexane:chloroform (1:1), fractions 15-16 with hexane: chloroform (4:6), fractions 17-18 with hexane:chloroform (3:7), fractions 19-20 with hexane:chloroform (2:8), fractions 21-22 with hexane:chloroform (1:9) and fractions 23-28 with chloroform. All fractions were examined by TLC. Fractions 1-2 were not further separated due to the low amount of the substance. Fractions 3-4 (1.2 g) was further chromatographed over silica gel with chloroform and obtained known compound eicosanoic acid (54 mg). Fractions 7-8 (4.4 g) was re-chromatographed over LiChroprep RP-18 (ODS silica gel; 40-63 μm: 100 g; each fraction 100 mL). The elution was sequentially performed with methanol and water to yield 20 fractions. Fractions 1-4 with water:methanol (8:2), fractions 5-8 with water:methanol (6:4), fractions 9-12 with water:methanol (4:6), fractions 13-16 with water:methanol (2:8), fractions 17-20 with methanol. Fractions 17-20 after rechromatography over Lichroprep RP-18 ODS (80 g, each fraction of 50 mL). The elution was sequentially performed with methanol containing 80, 60, 40, 20, 10 and 0 % of water to yield one new compound **1** (21 mg).

***n*-Henecosanoyl-β-D-arabinofuranosyl-2'-(1→2)-β-D-arabinopyranosyl-(1→2)-2''-β-D-arabinopyranosyl-(1→2)-2'''-β-D-arabinopyranoside (1):** Colourless semi-solid, R_f 0.34 ($CHCl_3$:MeOH; 9.8:0.2); ^{1}H (600 MHz) and ^{13}C NMR (150 MHz, MeOD, δ) (Table-1); IR (KBr, ν_{max}, cm^{-1}): 3425, 3352, 3165, 2929, 2845, 1725, 1462, 1365, 1255, 1073, 774; FAB MS (positive ion mode) *m/z* (rel. int.): 841 $[M+H]^+$ ($C_{40}H_{73}O_{18}$) (22.7), 295 (40.2), 443 (21.3), 575 (10.1), 707 (9.6).

TABLE-1
^{1}H AND ^{13}C NMR SPECTRAL DATA FOR COMPOUND (**1**)[a]

Position	^{1}H NMR	^{13}C NMR
1	-	174.62
2	2.28 t (6.8)	50.99
3	2.03 m	37.78
4	1.57 br s	36.37
5	1.57 br s	33.54
6	1.57 br s	31.28
7	1.28 br s	31.02
8	1.28 br s	28.85
9	1.28 br s	28.85
10	1.28 br s	28.56
11	1.28 br s	28.56
12	1.28 br s	28.56
13	1.28 br s	28.56
14	1.28 br s	28.56
15	1.25 br s	28.56
16	1.25 br s	27.31
17	1.25 br s	25.33
18	1.22 br s	24.38
19	1.22 br s	22.02
20	0.85 t (6.5)	13.34
1'	5.12 d (7.0)	107.43
2'	4.06 m	88.26
3'	3.82 m	75.48
4'	3.70 m	83.18
5'	3.28 d (8.7)	60.23
1''	4.46 d (7.8)	102.13
2''	4.01 m	80.47
3''	3.78 m	74.28
4''	3.68 m	70.03
5''	3.33 br s	60.87
1'''	4.42 d (7.6)	96.19
2'''	3.95 m	76.07
3'''	3.76 m	73.14
4'''	3.71 m	68.76
5'''	3.35 br s	60.68
1''''	4.37 d (7.1)	91.97
2''''	3.86 m	75.84
3''''	3.73 m	71.88
4''''	3.66 m	70.93
5''''	3.63 br s	67.86

[a]Coupling constants in Hz are provided in parenthesis

***n*-Eicosanoic acid:** White crystalline solid; ^{1}H NMR ($CDCl_3$; 600 MHz) δ_H: 2.35 (2H, t, *J* = 7.5 Hz, H_2-2), 1.64 (4 H, m, 2 × CH_2), 1.29 (10 H, br s 5 × CH_2), 1.25 (20 H, br s, 10 × CH_2), 0.89 (3H, t, *J* = 6.6 Hz, Me-20); ^{13}C NMR ($CDCl_3$; 150 MHz): δ_C 179.72 (C-1), 33.96 (CH_2), 31.90 (CH_2), 29.68 (9 × CH_2), 29.58 (CH_2), 29.42 (CH_2), 29.35 (CH_2), 29.23 (CH_2), 29.03 (CH_2), 24.64 (CH_2), 22.68 (CH_2), 14.13 (Me-20); IR (KBr, ν_{max}, cm^{-1}): 3490, 2916, 2848, 1707, 1463, 1297, 725; FAB MS (positive mode): *m/z* (rel. int.): 312 $[M]^+$ ($C_{20}H_{40}O_2$) (12.6), 295 (18.3).

RESULTS AND DISCUSSION

Compound **1** gave positive tests for glycosides. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3425, 3352, 3165 cm^{-1}), ester function (1725 cm^{-1}) and long aliphatic chain (774 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular ion peak of **1** has been determined at *m/z* 841 $[M+H]^+$ consistent to the molecular formula of a C_{20} fatty acid tetraglycoside, $C_{40}H_{73}O_{18}$. The ion fragments generating at *m/z* 295 $[CH_3(CH_2)_{18}CO]^+$, 443 $[CH_3(CH_2)_{18}COOC_5H_8O_4]^+$, 575 $[CH_3(CH_2)_{18}COOC_5H_8O_4$-$C_5H_8O_4]^+$ and 707 $[CH_3(CH_2)_{18}COO\ (C_5H_8O_4)_3]^+$ suggested that tetraarabinosidic chain was linked to the arachidic acid unit. The mass fragmentation pattern of **1** is shown in Fig. 2.

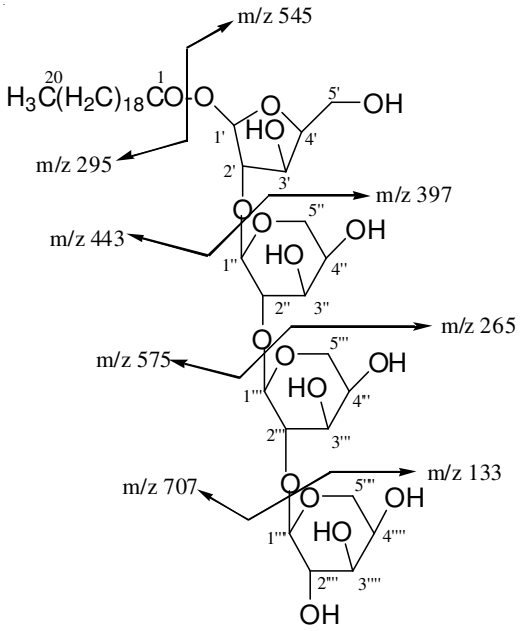

Fig. 2. Fragmentation patterns of compound **1**

The ^1H NMR spectrum of **1** displayed four one-proton doublets at δ 5.12 (J = 7.0 Hz), 4.46 (J = 7.8 Hz), 4.42 (J = 7.6 Hz) and 4.37 (J = 7.1 Hz) assigned to anomeric H-1', H-1", H-1'" and H-1"", respectively. The other sugar protons appeared from δ 4.06 to 3.33. The signals from δ 2.28 to 1.22 were associated with the methylene protons. A three proton triplet at δ 0.85 (J = 6.5 Hz) was accounted to C-20 primary methyl protons. The deshielding nature of H-1' at δ 5.12 suggested that C-1' was linked to the ester function. The ^{13}C NMR spectrum of **1** showed signals for anomeric carbons at δ 107.43 (C-1'), 102.12 (C-1"), 96.19 (C-1'") and 91.97 (C-1"") and other sugar carbons from δ 88.26 to 60.23. The aliphatic chain carbons appeared from δ 50.99 to 22.02 for methylene unit, ester carbon at δ 174.62 (C-1) and methyl carbon at δ 13.34 (C-20). The presence of one of the sugar carbons in the deshielded region at δ 107.43 (C-1'), 88.26 (C-2') and 83.18 (C-4'), indicated furanose form of the sugar unit. The existence of C-2', C-2" and C-2'" carbons in the downfield region at δ 88.26, 80.47 and 76.07, respectively, in the ^{13}C NMR spectrum and at δ 4.06, 4.01 and 3.95 for H-2', H-2" and H-2'", respectively in the ^1H NMR spectrum suggested (1→2) linkages of the sugar units. The position of the carbon signals with corresponding proton signals were determined by HSQC spectrum. The ^1H-^1H COSY spectrum showed correlations of H-1' with H2-2, H-2' and H-3'; H-1" with H-2', H-2", H-3" and H_2-5"; H-1'" with H-2" and H_2-5'"; and H-1"" with H-2'" and H_2-5'". The HMBC spectrum of **1** exhibited that C-1 interacted with H_2-2, H_2-3 and H-1'; C-1" interacted with H-2', H-2", H-3" and H_2-5"; C-1'" interacted with H-2" and H_2-5'"; and C-1"" interacted with H-2'", H-2"" and H_2-5"". On the basis of these evidences, the structure of this aliphatic tetraglycoside has been elucidated as *n*-henecosanoyl-β-D-arabinofuranosyl-2'-(1→2)-β-D-arabinopyranosyl-(1→2)-2"-β-D-arabinopyranosyl-(1→2)-2'"-β-D-arabinopyranoside. This is a new aliphatic tetraglycoside.

Conclusion

L. chinense has been used as traditional Chinese medicine and it has long history of applications. One new compound (*n*-henecosanoyl-β-D-arabinofuranosyl-2'-(1→2)-β-D-arabinopyranosyl-(1→2)-2"-β-D-arabinopyranosyl (1→2)-2'"-β-D-arabinopyranoside (**1**) was reported in this paper. There is need of this *L. chinense* fruits for further investigations.

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