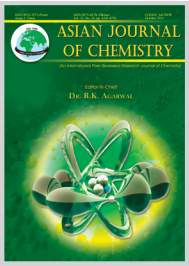
Asian Journal of Chemistry; Vol. 24, No. 7 (2012), 3206-3208

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Glucuronopyranosyl Polyglucopyranosyl Constituent from the Fruits of *Lycium chinense*

ILL-MIN CHUNG[1], SEUNG HYUN KIM[1], YOUNG SUP AHN[2] and ATEEQUE AHMAD[1,*]

[1]Department of Applied Life Science, Konkuk University, Seoul 143-701, South Korea
[2]Department of Herbal Crop Research, NIHHIS, RDA, Eumseong 369-873, South Korea

*Corresponding author: Fax: +82 2 4467856; Tel: +82 2 4503730; E-mail: ateeque97@gmail.com

(*Received*: 29 August 2011; *Accepted*: 22 February 2012) AJC-11102

One compound β-D-glucuronopyranosyl-β-D-(6→1)-glucopyranosyl-β-D-(6→1)-β-D-glucopyranosyl-(6→1)-β-D-glucopyranosyl-(6→1)-β-D-glucopyranosyl-(6→1)-β-D-glucopyranoside (**1**) has been isolated for the first time from the ethyl acetate extract of fruits of *Lycium chinense*. Their structure has been elucidated with the help of 600 MHz NMR using 1D and 2D spectral methods *viz*: ^{1}H and ^{13}C, aided by FAB MS and IR spectroscopy.

Key Words: *Lycium chinense*, Solanaceae, Fruits, Constitutents.

INTRODUCTION

The dried ripe fruits of *Lycium chinense* Miller (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[1,2]. Several compounds, steroids and alkaloids in this plant are known to various bioactivities[3-6]. Potentially hepatoprotective glycolipid constituents and determination of betain in *L. chinense* fruits have been reported[7,8]. Antimicrobial compounds and methyl sterols are also reported from *L. chinense* roots[9,10]. Specific α-galactosidase inhibitors, *N*-methylcalystegines structure/activity relationship of calystegines from *L. chinense* have been reported[11]. The *L. chinense* plant is well known in North East Asia and nowadays has been widely used as a popular functional food with a large variety of beneficial effects, such as antibacterial, antipyretic, cancer; haemostatic, hepatic, kidney, ophthalmic, tonic *etc*. Other useful references of *L. chinense* regarding compounds and activity also reported[12-17].

This paper deals with the isolation and structure elucidation of one compound β-D-Glucuronopyranosyl-β-D-(6→1)-glucopyranosyl-β-D-(6→1)-β-D-glucopyranosyl-(6→1)-β-D-glucopyranosyl-(6→1)-β-D-glucopyranosyl-(6→1)-β-D-glucopyranoside (**1**) on the basis of ^{1}H and ^{13}C NMR, spectroscopic studies, including FAB MS and IR for the first time from the fruits of *L. chinense*. This may be the first report of the isolated compound (**1**) along with other known compounds β-sitosterol and β-sitosterol-β-D-glucoside from the fruits of *L. chinense*. In continuation of our previous work[18] on *L. chinense* fruits, one more compound (**1**) was reported for the first time in the form of natural products.

EXPERIMENTAL

All chemicals used were of analytical grade. Hexane, ethyl acetate, methanol, ethanol, water, sulphuric acid and vanillin were purchased from Daejung Chemicals and Metals Co. Ltd, 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). Previously isolated authentic standard of β-sitosterol, β-sitosterol-β-D-glucoside are available. 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 at Seoul National University (SNU), Seoul, South Korea. NMR spectra were obtained in deuterated methanol using tetramethylsilane (TMS) 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 Seoul National University, 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.

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 20.0 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: ethyl acetate (9:1), fractions 5-6 with hexane: ethyl acetate (8:2), fractions 7-8 with hexane: ethyl acetate (7:3), fractions 9-10 with hexane: ethyl acetate (6:4), fractions 11-12 with hexane: ethyl acetate (1:1), fractions 13-14 with hexane: ethyl acetate (4:6), fractions 15-16 with hexane: ethyl acetate (3:7), fractions 17-18 with hexane: ethyl acetate (2:8), fractions 19-20 with hexane: ethyl acetate (1:9) and fractions 21-24 with ethyl acetate. All fractions were examined by TLC. Fractions 1-4 were not further separated due to the low amount of the substance. Fractions 5-6 (0.9 g) were crystallized after the purification by column chromatography, yielding β-sitosterol (37 mg) whose identity was confirmed through the comparison of TLC and spectroscopic data with those of an authentic sample. Fractions 17-20 (4.4 g) was re-chromatographed over Li Chroprep RP-18 (ODS silica gel; 40-63 μm: 200 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), 17-18 with water:methanol (1:9), 19-20 with methanol. Fractions 13-16 with water: methanol (2:8) yielded β-sitosterol-β-D-glucoside and confirm with authentic sample. Fractions 17-18 after rechromatography over Lichroprep RP18 ODS (15 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 compound **1** in 10 %.

β-D-Glucuronopyranosyl-β-D-(6→1)-glucopyranosyl-β-D-(6→1)-β-D-glucopyranosyl-(6→1)-β-D- glucopyranosyl-(6→1)-β-D- glucopyranosyl-(6→1)-β-D-glucopyranoside (1): IR (KBr, ν_{max} cm^{-1}): 3510, 3425, 3339, 3215, 2936, 2848, 1734, 1690, 1630, 1410, 1345, 1075, 817; FAB MS (positive ion mode) m/z (rel. int.): 1004 $[M]^+$ ($C_{36}H_{60}O_{32}$) (3.6), 177 (100), 163 (32.1); ^1H (600 MHz) and ^{13}C (150 MHz) NMR (Table-1).

RESULTS AND DISCUSSION

Compound **1**, a hexaglycoside was obtained from water:methanol (1:9) eluants. It responded to glycosidal test positively and IR absorptions band for hydroxyl groups 3510, 3425, 3329, 3281 and 3215 cm^{-1}. On the basis of FAB mass and ^{13}C NMR spectra, the molecular weight of **1** has been determined at m/z 1004 consistent to the molecular formula of hexaglycoside, $C_{36}H_{60}O_{32}$. The ion fragments arising at m/z 163 $[C_6H_{11}O_5]^+$, 179 $[C_6H_{11}O_6]^+$, 177 $[C_6H_9O_6]^+$.

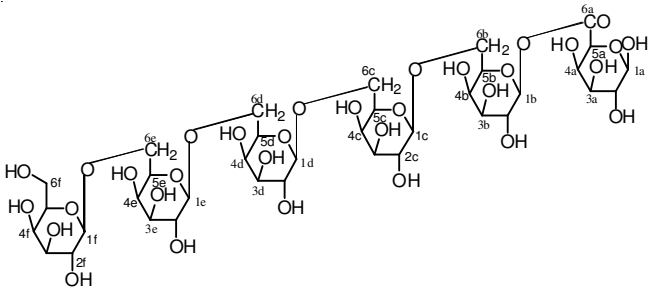


Fig. 1. Chemical structure of compound **1**

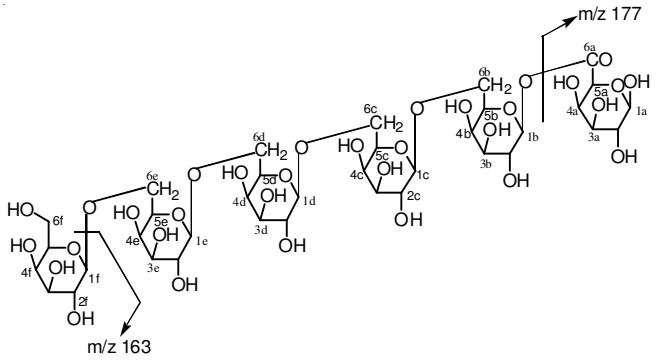


Fig. 2. Fragmentation pattern of compound **1**

The ^1H NMR spectrum of **1** showed four two-protons doublets each at δ 3.54 (J = 12.0 Hz), 3.56 (J = 12.0 Hz) and 3.24 (J = 12.0 Hz), 3.22 (J = 12.0) were assigned to H-6b and H-6f protons, respectively. Three one-proton broad signals at δ 4.94, two protons at δ 4.58 and 5.20 were ascribed to anomeric protons for H-1a to H-1e. One doublet at δ 4.58 (J = 7.0) was assigned for anomeric proton for H-1f. The other protons multiplets were resonated from δ 3.75 to 4.05 for H-2a to H-5f. More details of proton values are given Table-1.

TABLE-1
^1H (600 MHz) AND ^{13}C (150 MHz) NMR DATA FOR COMPOUND 1 MEOD (J IN Hz IN PARENTHESIS)

Position	^1H NMR	^{13}C NMR
1a	4.94 br s	99.44
2a	3.90 m	74.74
3a	3.84 m	73.51
4a	3.75 m	71.56
5a	4.10 m	77.83
6a	-	162.09
1b	5.20 br s	103.16
2b	3.90 m	74.54
3b	3.84 m	73.58
4b	3.75 m	71.66
5b	4.05 m	77.83
6b	3.54 d (12), 3.56 d (12)	64.79
1c	4.94 br s	97.96
2c	3.87 m	74.71
3c	3.86 m	73.54
4c	3.72 m	69.75
5c	4.02 m	77.81
6c	3.38 br s	64.21
1d	4.94 br s	94.09
2d	3.87 m	74.74
3d	3.86 m	73.54
4d	3.71 m	69.52
5d	4.02 m	76.13
6d	3.38 brs	64.05
1e	4.94 br s	94.01
2e	3.94 m	74.74
3e	3.81 m	73.05
4e	3.73 m	71.66
5e	4.05 m	76.15
6e	3.33 br s	63.86
1f	4.58 d (7.0)	93.85
2f	3.94 m	74.54
3f	3.81 m	73.03
4f	3.73 m	72.95
5f	4.02 m	76.13
6f	3.24 d (12.0), 3.22 d (12.0)	62.62

Coupling constants in hertz are provided in parenthesis

The ^{13}C NMR spectrum of **1** displayed signals for anomeric carbons at δ 99.44 (C-1a), 103.16 (C-1b), 97.96 (C-1c), 94.09 (C-1d), 94.01 (C-1e) and 93.85 (C-1f), oxygenated methylene carbons at δ 64.79 (C-6b), 64.21 (C-6c), 64.05 (C-6d), 63.86 (C-6e) and 62.62 (C-6f), other sugar carbons were resonating from 69.52-76.15. The carbonyl carbon displayed at δ 162.09 for C-6a. The ^{1}H-^{1}H COSY spectrum of **1** showed correlations of H-1a with H-2a and H-5a; H-2a with H-3a, H-4a and H-1a; H-2b with H-1b, H-3b and H-2c; and H-2c with H-1c, H-3c and H-1d. The HMBC spectrum of **1** exhibited that C-2a interacted with H-1a, H-3a, H-4a and H-1b; C-1c interacted with H-2b, H-2c, H-3c and C-1d interacted with H-2c, H-2d, H2-5d and H-3d. The HSQC spectrum of **1** showed important correlations of H-1a at δ 4.94 with C-1 at δ 99.44, H-2a at δ 3.90 with C-2 at δ 74.74, H-1b at δ 5.20 with C-1b at δ 103.16, H-1c (4.94) with C-1c (97.96), H-1d (4.94) with C-1d (94.09), H-1f (4.58) with C-1f (93.85). On the basis of spectral data analysis, the structure of **1** has been established as β-D-Glucuronopyranosyl-β-D-(6→1)-glucopyranosyl-β-D-(6→1)-β-D-glucopyranosyl-(6→1)-β-D-glucopyranosyl-(6→1)-β-D- glucopyranosyl-(6→1)-β-D-glucopyranoside.

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.

REFERENCES

1. S. Funayama, K. Yoshida, C. Konno and H. Hikino, *Tetrahedron Lett*., **21**, 1355 (1980).
2. J. Yamahara, M, Kim, T. Sawada and H. Fujimua, *Shoyakugaku Zasshi* **18**, 33 (1964).
3. A. Sannai, T. Fujimori and K. Kato, *Agric. Biol. Chem.*, **47**, 2397 (1983).
4. S.Y. Kim, Y. Choi, H. Huh, J. Kim, Y.C. Kim and H.S. Lee, *J. Nat. Prods.*, **60**, 274 (1997).
5. M. Noguchi, K. Mochida, M. Shingu, K. Kozuka and K. Fujitani, *Chem. Pharm. Bull.*, **32**, 3584 (1984).
6. L.L. Yanfg, K.Y. Yen, Y. Kiso and H. Kikino, *J. Ethnopharmacol.*, **19**, 103 (1987).
7. K. Jung, Y.W. Chin, Y.C. Kim and J. Kim, *Arch. Pharm. Res.*, **28**, 1381 (2005).
8. Y.G. Shin, K.H. Cho, J.M. Kim, M.K. Park and J.H. Park, *J. Chromatogr. A*, **857**, 331 (1999).
9. D.G. Lee, H.J. Jung and E.R. Woo, *Arch. Pharm. Res.*, **28**, 1031 (2005).
10. T. Itoh, T. Tamura and T. Matsumoto, *Phytochemistry,* **17**, 971 (1978).
11. N. Asano, A. Kato, M. Miyauchi, T. Tomimori, K. Matsu, R.J. Nash and R.J. Molyneux, *Eur. J. Biochem*., **248**, 296 (1997).
12. Y.W. Chin, S.W. Lim, S.H. Kim, D.Y. Sin, Y.G. Sur, Y. Kim, Y.C. Kim and J. Kim, *Bioorgan. Biomed. Chem. Lett.*, **13**, 79 (2003).
13. A. Sannai, T. Fujimori and K. Kato, *Phytochemisty,* **21**, 2986 (1982).
14. K.H. Miean and S. Mohamed, *J. Agric. Food Chem.,* **49**, 3106 (2001).
15. S.J. Han, H.H. Lee, I.S. Lee, Y.S. Moon and E.R. Woo, *Arch. Pharm. Res.*, **25**, 433 (2002).
16. P. Weller and D.E. Breithaupt, *J. Agric. Food Chem.*, **51**, 7044 (2003).
17. J.Z. Dong, D.Y. Lu and Y. Wang, *Plant Foods Hum. Nut.*, **64**, 199 (2009).
18. I.M. Chung, N. Praveen and A. Ahmad, *Asian. J., Chem*., **24**, 885 (2012).