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Glycosidic ester and tetra- and hexaglycosides from the fruits of *Lycium chinense* Miller

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Abstract

The fruits of *Lycium chinense* (Solanaceae) Miller possess emmenagogue, diuretic, antipyretic and hepatoprotective properties and are used to stimulate the immune system, to increase hormonal growth, to improve blood circulation and eye sight and to cure morning sickness. Phytochemical investigation of an aqueous alcoholic (80%) extract of the fruits gave two new glycosidic aliphatic esters, characterized as 1- linoleoyl-6-linolenoyl β -D- glucopyranoside (1) and arachidyl-O- β -D-arabinopyranosyl-(1 \rightarrow 2)-2'-O- β -D-arabinopyranosyl-(1 \rightarrow 2)-2''-O- β -D-arabinopyranosyl -(1 \rightarrow 2)-2'''-O- β -D-arabinopyranoside (2) and one each tetra- and hexaglycosides identified as α -D-galactopyranosyl-(2 \rightarrow 1')-O- α -D-xylopyranosyl-(2' \rightarrow 1'')-O- α -D-xylopyranosyl-(2'' \rightarrow 1''')-O- α -D-xylopyranoside (3) and β -D-galacturanopyranosyl-(6 \rightarrow 1)-O- β -D-glucopyranosyl-(6 \rightarrow 1)-O- β -D-glucopyranosyl-(6 \rightarrow 1)- β -D-glucopyranosyl-(6 \rightarrow 1)- β -D-glucopyranosyl-(6 \rightarrow 1)- β -D-glucopyranoside (4). The structures of these phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.

Keywords: *Lycium chinense*, fruits, glycosidic aliphatic esters, polyglycosides, structure elucidation

1. Introduction

Lycium chinense Miller (Solanaceae), known as Goji berry or Gou-qu-zi, is a cold-hard, perennial shrub. Its origin is believed to be in the region of south-eastern Europe and south-western Asia, but now it is cultivated throughout the world. Today China is the largest producer of this fruit. The dried form of this fruit is emmenagogue, diuretic, antipyretic and hepatoprotective and marketed in Ayurvedic and herbal health shops as the Tibetan or Himalayan Goji berry. The red-orange ellipsoid fruits stimulate the immune system due to high vitamin C contents, increase human growth hormone and sperm production and improve blood circulation and eye sight. The berries are taken by pregnant females to prevent morning sickness and to alleviate hepatitis and insomnia, to improve memory, to provide longevity and as a tonic in traditional oriental medicine. The fruits exhibited hypotensive, antipyretic and hypoglycemic activities [1,2]. They have properties like nourishing the blood, enriching the yin, tonifying the kidney and the liver, and moistening the lungs [2,3]. They are useful for reducing the risk of certain diseases such as arteriosclerosis, essential arterial hypertension, diabetes and night blindness [4-9]. The dried ripe fruits are an ingredient of herbal drugs and functional foods [10]. Goji berry contained sterols, polysaccharides, zeaxanthin and antioxidants like as lutein, β -carotene, vitamin C and lycopene. Potentially hepatoprotective glycolipid constituents and determination of betain in *L. chinense* fruits have been reported [11, 12]. Di- and tetraterpene glycosides and polyglycosides of fatty acids are published in recent reports from *L. chinense* fruits [13-16]. This paper describes isolation and characterization of glycosidic ester and tetra- and hexaglycosides from the berries of *L. chinense* available locally.

2. Materials and Method

2.1. General procedure

Melting points were determined on a Perfit apparatus without correction. The IR spectra were measured in KBr pellet on a Bio-Red FT-IR spectrometer. Ultraviolet (UV) spectra were obtained in methanol with a Lambda Bio 20 spectrometer. The ^1H (400 MHz), ^{13}C (100 MHz), COSY and HMBC NMR spectra were recorded on Bruker spectropin spectrometer. DMSO- D_6 (Sigma-Aldrich, Bangalore, India) was used as a solvent and TMS as an internal standard. ESI MS analyses were performed on a Waters Q-TOF Premier (Micromass MS Technologies, Manchester, UK) Mass Spectrometer. Column chromatography separations were carried out on silica gel (Merck, 60-120 mesh, Mumbai, India).

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Precoated silica gel plates (Merck, Silica gel 60 F₂₅₄) were used for analytical thin layer chromatography and visualized by exposure to iodine vapours and UV radiations.

2.2. Plant material

The fruits of *L. chinense* were procured from a local market of Delhi and identified by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen of drug was deposited in the herbarium of Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi.

2.3. Extraction and isolation

The fruits of *L. chinense* (500 g) were coarsely powdered and extracted exhaustively with aqueous alcohol (80%) using a Soxhlet apparatus for 18 h. The extract was concentrated under reduced pressure to get dark brown mass (112.5 g). The residue (100 g) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) to obtain a slurry. The slurry was dried in air and chromatographed over silica gel column loaded in chloroform. The column was eluted with chloroform-methanol mixtures to obtain compounds 1-- 4.

2.4. 1-Linoleoyl-6-linolenoyl-β-D-glucopyranoside (1)

Elution of the column with chloroform-methanol (19:1) gave a yellow semisolid mass of **1**, IR λ_{max} (KBr): 3408, 3355, 2928, 2849, 1737, 1721, 1645, 1468, 1331, 1260, 1174, 1023 cm⁻¹; ¹H NMR (DMSO-d₆): δ 5.25 (1H, d, J=7.7 Hz, H-1), 4.37 (1H, m, H-5), 3.94 (1H, m, H-2), 3.67 (1H, m, H-3), 3.60 (1H, m, H-4), 4.25 (1H, d, J=6.7 Hz, H₂-6a), 4.23 (1H, d, J=6.5 Hz, H₂-6b), 5.40-5.31 (10 H, m, H-9', H-10', H-12', H-13', H-9'', H-10'', H-12'', H-13'', H-15''', H-16'''), 2.80 (2H, m, H₂-11'), 2.78 (2H, m, H₂-11''), 2.75 (2H, m, H₂-15'''), 2.41 (2H, t, J=7.3 Hz, H₂-2'), 2.33 (2H, t, J=7.1 Hz, H₂-2''), 2.29-1.34 (6H, m, 6 x CH₂), 1.29 (4H, brs, 2 x CH₂), 1.25 (12H, brs, 6 x CH₂), 1.19 (6H, brs, 3 x CH₂), 0.97 (3H, t, J= 6.9 Hz, Me-18'), 0.89 (3H, t, J=6.2 Hz, Me-18''); ¹³C NMR (DMSO-d₆): δ 103.57 (C-1), 72.39 (C-2), 71.48 (C-3), 68.23 (C-4), 73.11 (C-5), 62.36 (C-6), 173.37 (C-1'), 173.89 (C-1''), 131.90-127.08 (C-9', C-10', C-12', C-13', C-9'', C-10'', C-12'', C-13'', C-15'', C-16''), 34.25 - 20.54 (22 x CH₂), 14.23 (Me-18'), 14.07 (Me-18''). +ve FAB MS *m/z* (ret. int.): 703 [M+ H]⁺ (C₄₂H₇₁O₈) (3.7), 441(7.7), 439 (6.3), 422 (8.2), 280 (12.8), 263 (20.6), 261 (34.5).

2.5. Arachidyl tetra arabinoside (2)

Elution of the column with chloroform-methanol (9:1) afforded colourless crystals of **2**, recrystallized from chloroform-methanol (1:1), 31 mg, m.p. 183-185°C; IR λ_{max} (KBr): 3427, 3349, 3168, 2927, 2842, 1723, 1460, 1363, 1257, 1070, 771 cm⁻¹; ¹H NMR (DMSO-d₆): δ 2.31 (2H, t, J=7.1 Hz, H₂-2), 2.02 (2H, m, H₂-3), 1.55 (6H, brs, 3 x CH₂), 1.29 (16H, brs, 8 x CH₂), 1.26 (6H, brs, 3 x CH₂), 1.23 (4H, brs, 2 x CH₂), 0.86 (3H, t, J=6.6 Hz, Me-20), 5.15 (1H, d, J=7.2 Hz, H-1'), 4.48 (1H, d, J=7.5 Hz, H-1''), 4.45 (1H, d, J=7.4 Hz, H-1'''), 4.39 (1H, d, J=7.3 Hz, H-1'''), 4.08 (1H, m, H-2'), 4.02 (1H, m, H-2''), 3.99 (1H, m, H-2'''), 3.86 (1H, m, H-2'''), 3.83- 3.66 (8H, m, H-3' to H-4'''), 3.63 (2H, d, J=8.3 Hz, H₂-5'), 3.36 (2H, d, J=7.9 Hz, H₂-5''), 3.32 (2H, d, J=7.8 Hz, H₂-5'''), 3.27 (2H, d, J=8.6 Hz, H₂-5'''); ¹³C NMR (DMSO-d₆): δ 174.60 (C-1), 50.97 (C-2), 37.70 (C-3), 36.35 (C-4), 33.52 (C-5), 31.26 (C-6), 31.26 (C-6), 31.05 (C-7), 28.83 (C-8, C-9), 28.54 (C-10 to C-15), 27.35 (C-16), 25.31 (C-17), 24.40 (C-18),

22.06 (C-19), 14.02 (C-20), 107.41 (C-1'), 83.18 (C-2'), 75.46 (C-3'), 70.93 (C-4'), 60.23 (C-5'), 103.15 (C-1''), 80.49 (C-2''), 74.28 (C-3''), 70.05 (C-4''), 60.86 (C-5''), 96.21 (C-1'''), 76.09 (C-2'''), 73.14 (C-3'''), 68.78 (C-4'''), 60.67 (C-5'''), 91.99 (C-1'''), 75.85 (C-2'''), 71.91 (C-3'''), 67.85 (C-4'''), 60.25 (C-5'''). FAB MS *m/z* (ret. int.): 841 [M+ H]⁺ (C₄₀H₇₃O₁₈) (21.8), 707 (9.8), 575 (10.3), 443 (20.9), 295 (41.1).

2.6. α-D-Galactosyl Trixyloside (3)

Elution of the column with chloroform-methanol (4:1) yielded colourless crystals of **3**, recrystallized from chloroform-methanol (1:1), 41 mg, m.p. 246-247°C; IR λ_{max} (KBr): 3427, 3515, 3463, 3361, 3286, 2932, 2851, 1639, 1412, 1056, 814 cm⁻¹; ¹H NMR (DMSO-d₆): δ 5.87 (1H, d, J=6.5 Hz, H-1), 5.22 (1H, d, J=4.2 Hz, H-1'), 5.18 (1H, d, J=6.1 Hz, H-1''), 4.19 (1H, d, J=6.6 Hz, H-1'''), 4.17 (1H, m, H-5), 4.03 (1H, dd, J=6.5, 9.2 Hz, H-2), 3.96 (1H, dd, J=4.2, 9.6 Hz, H-2'), 3.78 (1H, dd, J= 6.1, 9.2 Hz, H-2''), 3.42 (1H, dd, J=6.6, 6.1 Hz, H-2'''), 3.86 (1H, m, H-3), 3.76- 3.71 (5H, m, H-4, 3', H-3'', H-3''', H-4'), 3.62 (1H, m, H-4''), 3.55 (1H, m, H-4'''), 3.55 (1H, m, H-4'''), 3.47 (2H, brs, H₂-5'), 3.37 (2H, brs, H₂-5''), 3.31 (2H, brs, H₂-5'''), 3.10 (2H, brs, H₂-6); ¹³C NMR (DMSO-d₆): δ 105.88 (C-1), 48.03 (C-2), 73.75 (C-3), 65.88 (C-4), 77.88 (C-5), 60.57 (C-6), 103.16 (C-1'), 83.07 (C-2'), 72.75 (C-3'), 72.15 (C-4'), 64.56 (C-5'), 99.35 (C-1''), 77.48 (C-2''), 71.76 (C-3''), 69.35 (C-4''), 64.15 (C-5''), 99.24 (C-1'''), 76.65 (C-2'''), 71.16 (C-3'''), 65.05 (C-4'''), 62.61 (C-5'''). +ve FAB MS *m/z* (ret. int.): 577 [M+ H]⁺ (C₂₁H₃₇O₁₈) (10.7), 443 (13.5), 311 (13.8), 179 (19.1), 163 (100), 149 (21.6).

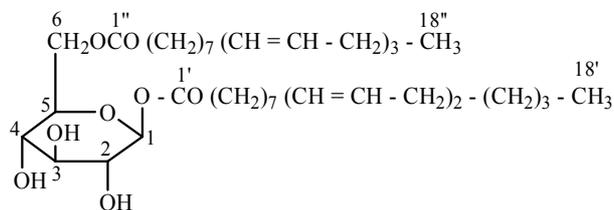
2.7. β-D-Glucuronosyl polyglucoside (4)

Elution of the column with chloroform-methanol (7:3) furnished colourless crystals of **4**, recrystallized from methanol, 53 mg, m.p. 255-257°C (decomp); IR λ_{max} (KBr): 3512, 3427, 3342, 3217, 2935, 2845, 1731, 1635, 1412, 1356, 1073, 815 cm⁻¹; ¹H NMR (DMSO-d₆): δ 5.21 (1H, d, J=7.3 Hz, H₂-1a), 4.96 (1H, d, J=7.2 Hz, H₂-1b), 4.91 (3H, brs, w_{1/2}=7.5 Hz, H-1c, H-1d, H-1e), 4.59 (1H, d, J= 7.3 Hz, H-1f), 4.10-4.02 (6H, m, H-5a to H-5f), 3.90 to 3.81 (12H, m, H-2a to H-2f, H-3a to H-3f), 3.75-3.71 (6H, m, H-4a to H-4f), 3.54 (2H, d, J=12.0 Hz, H₂-6b), 3.38- 3.33 (6H, brs, H₂-6c, H₂-6d, H₂-6e), 3.11 (2H, d, J=12.0 Hz, H₂-6f); ¹³C NMR (DMSO-d₆): δ 103.15 (C-1a), 74.76 (C-2a), 73.49 (C-3a), 71.53 (C-4a), 77.81 (C-5a), 167.06 (C-6a), 99.46 (C-1b), 74.55 (C-2b), 73.57 (C-3b), 71.63 (C-4b), 77.81 (C-5b), 64.77 (C-6b), 97.94 (C-1c), 74.73 (C-2c), 73.56 (C-3c), 69.77 (C-4c), 77.83 (C-5c), 64.24 (C-6c), 94.07 (C-1d), 74.76 (C-2d), 73.52 (C-3d), 69.51 (C-4d), 76.15 (C-5d), 64.07 (C-6d), 94.03 (C-1e), 74.72 (C-2e), 73.06 (C-3e), 71.64 (C-4e), 76.13 (C-5e), 63.88 (C-6e), 93.89 (C-1f), 74.52 (C-2f), 73.01 (C-3f), 72.97 (C-4f), 76.11 (C-5f), 61.12 (C-6f); +ve FAB MS *m/z* (ret. int.): 1005 [M+ H]⁺ (C₃₆H₆₁O₃₂) (3.5), 193 (5.1), 177 (100), 179 (6.2), 163 (32.4).

3. Results and Discussion

Compound **1**, [M+H]⁺ at *m/z* 703 (C₄₂H₇₁O₈), responded positively for glycoside tests and showed IR absorption bands for hydroxyl groups (3408, 3355 cm⁻¹), ester functions (1737, 1721 cm⁻¹) and unsaturation (1645 cm⁻¹). A prominent ion peak arising at *m/z* 280 CH₃ (CH₂)₃(CH=CH=CH)₂-(CH₂)₇COOH]⁺ and a mass ion fragment at *m/z* 422 [M - 280]⁺ indicated the attachment of linoleate ester chain at the anomeric C-1 carbon. The ion peaks produced at *m/z* 263 [O-C₁' fission, CH₃ (C₁₆ H₂₈CO)]⁺, 439 [M-263]⁺, 261 [O-C₁'

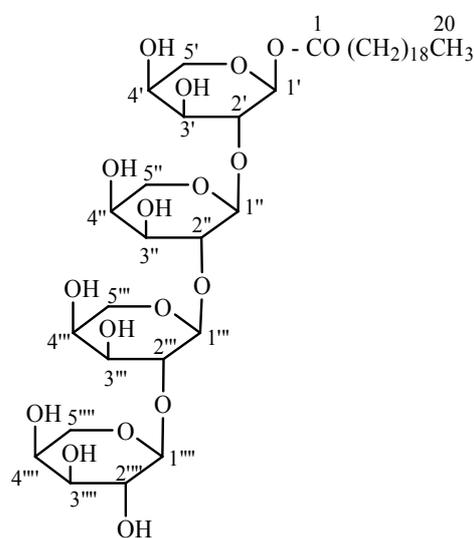
fission, CH_3 ($\text{C}_{16} \text{H}_{26}\text{CO}$)⁺ and $441[\text{M}-261]^+$ suggested the linkage of linoleate and linolenate ester groups to the sugar unit. The ^1H NMR spectrum of **1** exhibited a one-proton doublet at δ 5.25 ($J=7.7$ Hz) assigned to anomeric H-1 proton, four one-proton multiplets between δ 4.37-3.60 ascribed to sugar carbinol H-2, H-3, H-4 and H-5 protons and two one-proton doublets at δ 4.25 ($J=6.5$ Hz) and 4.23 ($J=6.5$ Hz) attributed to oxygenated methylene H₂-6 protons and their presence in the deshielded region supported the attachment of linolenate ester function at C-6. The vinylic proton signals appeared as multiplets between δ 5.40- 5.31. Two two-proton triplets at δ 2.41 ($J=7.3$ Hz) and 2.33 ($J=7.1$ Hz), signals between δ 2.29-1.34 as multiplets and three broad singlets at δ 1.29 (4H), 1.25 (12H) and 1.19 (6H) were associated with the methylene protons. Two three-proton triplets at δ 0.97 ($J=6.9$ Hz) and 0.89 ($J=6.2$ Hz) were accounted terminal primary methyl H₃-18' and H₃-18'' protons, respectively. The ^{13}C NMR spectrum of **1** exhibited signals for anomeric carbon at δ 103.57 (C-1), other sugar carbons between δ 73.11-62.36, ester carbons at 173.37 (C-1') and 173.89 (C-1''), vinylic carbons from δ 131.90 to 127.08, methylene carbons in the range of δ 34.25- 20.54 and methyl carbons at δ 14.23 (C-18') and 14.07 (C-18''). The $^1\text{H}-^1\text{H}$ COSY spectrum of **1** showed co-relations of H-1 with H-2, H-5 and H₂-2'; H₂-6 with H-5 and H₂-2''; H₂-11' with H-9', H-10', H-12' and H-13'; and H₂-11'' with H-9'', H-10'', H-12' and H-13'. The HMBC spectrum of **1** displayed interactions of H-5 and H-2 with C-1; H-1 and H₂-2' with C-1'; H₂-6 and H₂-2'' with C-1''; H-9', H₂-11' and H-12' with C-10'; and H-9'', H₂-11'' and H-12'' with C-10''. On the bases of these evidences the structure of **1** has been elucidated as 1-linoleoyl-6-linolenoyl β -D-glucopyranoside. This is a new glucosidic diester.



(1) 1-Linoleoyl-6-linolenoyl β -D-glucopyranoside

Compound **2** gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3427, 3349, 3168 cm^{-1}), ester function (1723 cm^{-1}) and long aliphatic chain (771 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular ion of **2** has been determined at m/z 841 $[\text{M}+\text{H}]^+$ consistent to molecular formula of a C₂₀ fatty acid tetraglycoside, C₄₀H₇₃O₁₈. The ion fragments generating at m/z 295 $[\text{CH}_3(\text{CH}_2)_{18}\text{CO}]^+$, 443 $[\text{CH}_3(\text{CH}_2)_{18}\text{COO}-\text{C}_5\text{H}_8\text{O}_4]^+$, 575 $[\text{CH}_3(\text{CH}_2)_{18}\text{COO}-\text{C}_5\text{H}_8\text{O}_4-\text{C}_5\text{H}_8\text{O}_4]^+$ and 707 $[\text{CH}_3(\text{CH}_2)_{18}-\text{COO}(\text{C}_5\text{H}_8\text{O}_4)_3]^+$ suggested that a tetra-arabinoside chain was linked to the arachidic acid unit. The ^1H NMR spectrum of **2** displayed four one-proton doublets at δ 5.15 ($J=7.2$ Hz), 4.48 ($J=7.5$ Hz), 4.45 ($J=7.4$ Hz) and 4.39 ($J=7.3$ Hz) assigned to anomeric H-1', H-1'', H-1''' and H-1'''' protons, respectively. The other sugar protons appeared from δ 4.06 to 3.27. The signals from δ 2.31 to 1.23 were associated with the methylene protons. A three-proton triplet at δ 0.86 ($J=6.6$ Hz) was accounted to C-20 primary methyl protons. The deshielding nature of the anomeric H-1' at δ 5.15 suggested that C-1' was linked to the ester function. The ^{13}C NMR spectrum of **2** showed signals for anomeric carbons at δ 107.41 (C-1'), 103.15 (C-1''), 96.21 (C-1''') and 91.99 (C-1''') and

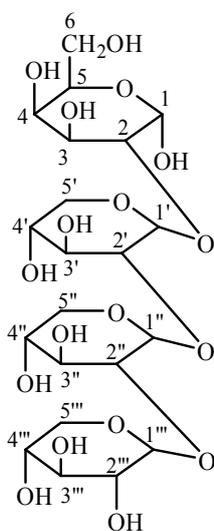
other sugar carbons from δ 83.18 to 60.23. The aliphatic chain carbons appeared from δ 50.57 to 22.06 for methylene unit, ester carbon at δ 174.60 (C-1) and methyl carbon at δ 14.02 (C-20). The existence of C-2', C-2'' and C-2''' carbons in the downfield region at δ 83.18, 80.49 and 76.09, respectively, in the ^{13}C NMR spectrum and at δ 4.08, 4.07, 3.99 for H-2', H-2'' and H-2''', respectively, in the ^1H NMR spectrum suggested (1 \rightarrow 2) linkages of the sugar units. The positions of the carbon signals with corresponding proton signals were determined by HSQC spectrum. The $^1\text{H}-^1\text{H}$ COSY spectrum of **2** showed correlations of H-1' with H₂-2, H-2' and H-3'; H-1'' with H-2'', H-2''', H-3'' and H₂-5''; H-1''' with H-2'' and H₂-5'''; and H-1'''' with H-2''', H-2'''' and H₂-5'''. The HMBC spectrum of **2** exhibited that H₂-2, H₂-3 and H-1' interacted with C-1; H-2', H-2'', H-3'' and H₂-5'' interacted with C-1'; H-2'', H-2''' and H₂-5''' interacted with C-1''; and H-2''', H-2'''' and H₂-5'''' interacted with C-1'''. On the bases of these evidences, the structure of this tetraglycosidic ester has been elucidated as arachidyl-O- β -D-arabinopyranosyl-(1 \rightarrow 2)-2'-O- β -D-arabinopyranosyl-(1 \rightarrow 2)-2''-O- β -D-arabinopyranosyl-(1 \rightarrow 2)-2'''-O- β -D-arabino pyranoside. This is a new acyl tetra-arabinoside.



(2) Arachidyl tetra-arabinoside

Compound **3** responded to glycosidal tests positively and showed IR absorption bands for hydroxyl groups at 3515, 3463, 3361 and 3286 cm^{-1} . On the basis of FAB mass and ^{13}C NMR spectra, the molecular weight of **3** was determined at m/z 577 $[\text{M}+\text{H}]^+$ consistent to the molecular formula of a tetraglycoside, C₂₁H₃₇O₁₈. The ion fragments arising at m/z 163 $[\text{C}_6\text{H}_{11}\text{O}_5]^+$, 179 $[\text{C}_6\text{H}_{11}\text{O}_6]^+$, 311 $[\text{C}_6\text{H}_{11}\text{O}_6 - [\text{C}_5\text{H}_8\text{O}_4]^+]$, 443 $[\text{C}_6\text{H}_{11}\text{O}_6 - \text{C}_5\text{H}_8\text{O}_4 - \text{C}_5\text{H}_8\text{O}_4]^+$ and 149 $[\text{C}_5\text{H}_9\text{O}_5]^+$ suggested three units of pentose sugar linked to a hexose sugar. The ^1H NMR spectrum of **3** showed four one-proton doublets at δ 5.87 ($J=6.5$ Hz), 5.22 ($J=4.2$ Hz), 5.18 ($J=6.1$ Hz) and 4.19 ($J=6.6$ Hz) assigned to α -oriented H-1, H-1', H-1'' and H-1''' anomeric protons, respectively. Four two-proton broad singlets at δ 3.47, 3.37, 3.31 and 3.10 were ascribed to oxygenated methylene H₂-5', H₂-5'' and H₂-5''' and hydroxymethylene H₂-6 protons, respectively. The other sugar protons resonated from δ 4.17 to 3.55. The presence of signals only from δ 5.87 to 3.10 suggested glycosidic nature of the molecule. The ^{13}C NMR spectrum of **3** displayed signals for anomeric carbons at δ 105.88 (C-1), 103.16 (C-1'), 99.35 (C-1'') and 99.24 (C-1'''), oxygenated methylene carbons at δ 60.57 (C-6), 64.56 (C-5'), 64.15 (C-5'') and 62.61 (C-5''') and other sugar carbons from δ 84.03 to 65.05. The $^1\text{H}-^1\text{H}$ COSY

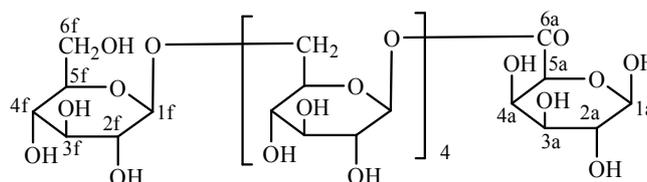
spectrum of **3** showed correlations of H-1 with H-2 and H-5; H-2 with H-3, H-4 and H-1'; H-2' with H-1', H-3' and H-1''; H-2'' with H-1'', H-3'' and H-1'''. The HMBC spectrum of **3** exhibited that H-1, H-3, H-4 and H-1' interacted with C-2; H-2', H-2'' and H-3'' interacted with C-1''; H-2'', H-2''', H-2-5''' and H-3''' interacted with C-1'''. The HSQC spectrum of **3** showed important correlations of H-1 at δ 5.87 with C-1 at 105.88, H-2 at δ 4.03 with C-2 at δ 84.03, H-1' at δ 5.22 with C-1' at δ 103.16; H-2-5' at 3.47 with C-5' at δ 64.56, H-1'' at δ 5.18 with C-1'' at δ 99.35 and H-1''' at δ 4.19 with C-1''' at δ 99.24. The presence of C-2 at δ 83.03, C-2' at δ 83.07 and C-2'' at δ 77.48 in the deshielded region suggested (2 \rightarrow 1) linkages of the sugar units. Acid hydrolysis of **3** yielded α -D-galactose and α -D-xylose. On the basis of spectral data analysis and chemical reactions, the structure of **3** has been established as α -D-galactopyranosyl-(2 \rightarrow 1')-O- α -D-xylopyranosyl-(2' \rightarrow 1'')-O- α -D-xylopyranosyl-(2'' \rightarrow 1''')- α -D-xylopyranoside. This is new tetracyclic glycoside.



(3) α -D-Galactosyl trixyloside

Compound **4** gave positive tests for glycosides and had IR absorption bands for hydroxyl groups (3512, 3427, 3342, 3217 cm^{-1}) and ester function (1731 cm^{-1}). Its molecular ion peak was established at m/z 1005 $[\text{M}+\text{H}]^+$ on the basis FAB mass and ^{13}C NMR spectra corresponded to a molecular formula of a hexaglycoside $\text{C}_{36}\text{H}_{61}\text{O}_{32}$. The ion peaks produced at m/z 193 $[\text{C}_6\text{H}_9\text{O}_7]^+$, 177 $[\text{C}_6\text{H}_9\text{O}_6]^+$, 179 $[\text{C}_6\text{H}_{11}\text{O}_6]^+$ and 163 $[\text{C}_6\text{H}_{11}\text{O}_5]^+$ indicated the existence of glucuronide unit at one terminal and glucosyl moiety at another end of the sugar chain. The ^1H NMR spectrum of **4** exhibited three one-proton doublets at δ 5.21 ($J=7.3$ Hz), 4.96 ($J=7.2$ Hz) and 4.59 ($J=7.3$ Hz) and a three-proton broad singlet at δ 4.91 with half-width of 7.5 Hz assigned to anomeric H-1a, H-1b, H-1f and to H-1c, H-1d and H-1 protons, respectively. The oxygenated methine protons of the sugar units appeared between δ 4.10-3.71. Two two-proton doublets at δ 3.54 ($J=12.0$ Hz) and 3.11 ($J=12.0$ Hz) and three two-proton singlets from δ 3.38 to 3.33 were ascribed to oxygenated methylene H₂-6b, H₂-6f and to H₂-6c, H₂-6d and H₂-6e protons, respectively. The ^{13}C NMR spectrum of **4** displayed signals for ester carbon at δ 167.06 (C-6a), anomeric carbons from δ 103.15 to 93.89, oxygenated methine carbons in the range of δ 77.81- 69.51 and oxygenated methylene carbons between δ 64.77 – 61.12. The presence of C-6b at δ 64.77, C-6c at δ 64.24, C-6d at δ 64.07 and C-6e at δ 63.88 in the deshielded region of ^{13}C NMR spectrum of **4** indicated (6 \rightarrow 1) linkages of the sugar units. Its ^1H - ^1H COSY

spectrum showed co-relations of H-1a with H-2a and H-5a; H₂-6b with H-5b and H-1c; H₂-6c with H-5c and H-1d; and H-1f with H-2f, H-5f and H₂-6e. The HMBC spectrum of **4** exhibited interactions of H-2a and H-5a with C-1a; H-5a and H-1b with C-6a; H₂-6b, H-5c and H-3c with C-1c; H₂-6d with C-1e; and H₂-6e, H-2f and H-5f with C-1f. Acid hydrolysis of **4** yielded D-galacturonic acid and D-glucose, co-TLC comparable. On the basis of above discussion the structure of **4** has been established as β -D-galacturonopyranosyl-(6 \rightarrow 1)-O- β -D-glucopyranosyl-(6 \rightarrow 1)-O- β -D-glucopyranosyl-(6 \rightarrow 1)- β -D-glucopyranosyl-(6 \rightarrow 1)- β -D-glucopyranosyl-(6 \rightarrow 1)- β -D-glucopyranoside. This is a new hexaglycoside.



(4) D-Galacturonoylpentaglycoside

4. Conclusion

The present work reported that the phytochemical investigation of an aqueous methanolic extract of the fruits of *Lycium chinensis* led to isolation of two acyl glycosides and one each tetra- and hexaglycosides for the first time. This study has enhanced the phytochemical nature of the plant.

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6. References

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