Two new glyceroglycolipids from the fruits of *Cucurbita moschata*

Zhi-Guo Jiang[a,b] and Qi-Zhen Du[a]*

[a]*College of Food Science and Biotechnology Engineering, Zhejiang Gongshang University, Hangzhou, 310035, P.R. China*

[b]*College of Food Science, Hainan University, Haikou, 570228, P.R. China*

A phytochemical study of *Cucurbita moschata* resulted in the characterisation of two new glyceroglycolipids, 1-*O*-(9*Z*, 12*Z*,15*Z*-octadecatrienoyl)-3-*O*-[β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl] glycerol **(1)** and 1-*O*-(9*Z*,12*Z*-octadecadienoyl)-3-*O*-[β-D-galactopyranosyl-(1→6)- *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl] glycerol **(2)**. Their structures were elucidated on the basis of spectroscopic analysis especially 2D-NMR (TOCSY, HMQC, HMBC, and NOESY) combined with chemical methods.

Keywords: *Cucurbita moschata*, glyceroglycolipid, linolenic acid, linoleic acid

Glyceroglycolipids are widely distributed in edible plants such as cereals, legumes, vegetables and fruits. Glyceroglycolipids consist of hydrophilic carbohydrate and hydrophobic fatty acid moieties which are bound to glycerol. They are major components of biomembranes, where they play important roles, such as enhancing membrane stability.[1] Some glyceroglycolipids have been reported to exhibit antithrombotic,[2] antiviral,[3,4] cancer chemoprevention,[5-7] antitumor,[8] anti-inflammatory[9,10] and immunosuppressive[11] activities. Pumpkin (*Cucurbita moschata*), which belongs to the family of Cucurbitaceae, is widely grown and consumed in China. In Chinese traditional medicine, it is believed that pumpkin has the function of invigorating the spleen and benefiting the lungs.[12] Pumpkin has received considerable attention in recent years because of the nutritional and health protective value of the proteins and oils from the seeds[13] as well as the polysaccharides from the fruits. The seeds themselves are eaten and show good results in curing several prostate diseases.[14] Ground pumpkin seed flour has been used as a protein supplement in a variety of local foods.[15] Preliminary investigations showed that a pumpkin-rich diet can reduce blood glucose and the polysaccharides from pumpkin have hypoglycemic activity.[16] However, very little information is available concerning the physicochemical and physiological properties of pumpkin glyceroglycolipids. In the present investigation, we reported the isolation and structure elucidation of two new glyceroglycolipids, 1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-3-*O*-[β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl] glycerol **(1)** and 1-*O*-(9*Z*,12*Z*-octadecadienoyl)-3-*O*-[β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl] glycerol **(2)**. Their structures were established on the basis of chemical and spectroscopic methods.

Compound **1** was obtained as an optically active, white amorphous powder ($[\alpha]^{20}_D$ = –15.0°). The HR–ESI–MS (*m/z* 861.4101 ([M + Na]$^+$; calc 861.4096) revealed a molecular formula of $C_{39}H_{66}O_{19}$, implying the existence of seven degrees of unsaturation. IR absorption at 3404 and 1735 cm^{-1} was attributed to the hydroxyl and ester carbonyl groups respectively. Examination of the NMR spectra of **1** indicated that it was a glyceroglycolipid. The analysis of acid hydrolysis product by TLC showed that the sugar moiety of **1** contained only galactose. The alkaline methanolysis of **1** yielded linolenic methyl ester identified by GC-MS analysis (see experimental part). The ^{13}C NMR spectrum showed the presence of three anomeric carbons (δ(C) 106.5, 106.6 and 106.7), one carbonyl (δ(C) 174.5), three olefinic (δ(C) 133.0, 131.5, 129.5(2×C), 129.0 and 128.5), and one terminal methyl (δ(C) 15.3). In the ^{1}H NMR spectrum, three anomeric proton signals (δ(H) 4.96 (1H, d, J = 7.8 Hz); 4.86 (2H, d, J = 7.7 Hz)) indicated that the configuration of all three galactopyranosyls

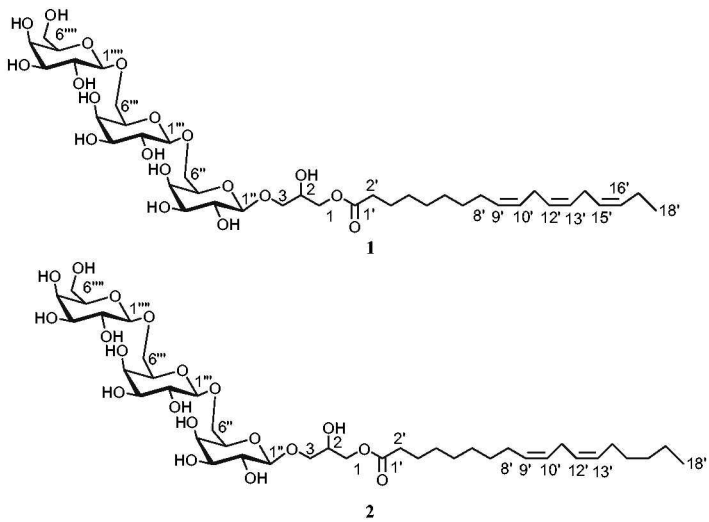

Fig. 1 Structures of glyceroglycolipids from *Cucurbita moschata*.

* Correspondent. E-mail: jiangzhiguo6666@163.com

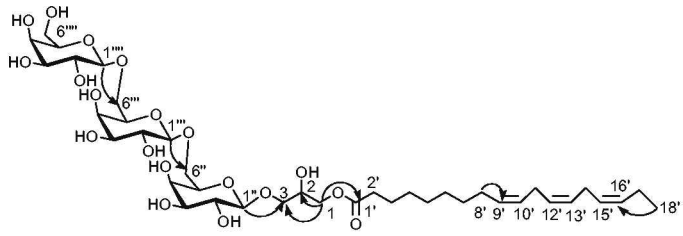

Fig. 2 Selected HMBC correlations (H→C) of **1**.

were β-configuration. Six olefinic protons and one methyl were also observed in the ^{1}H NMR spectrum. In addition the characteristic proton signals observed at δ(H) 3.00 (4H, t, J = 5.7 Hz) and 2.40 (2H, t, J = 7.5 Hz) were readily assigned to methylene proton signals between the double bonds and those of methylene proton signals next to the carbonyl group,[17] respectively. The downfield chemical shift of C-6"(δ(C) 70.5) and C-6'''(δ(C) 70.2) compared to the carbon signal of C-6'''' (δ(C) 63.2) indicated that the three sugars formed a linear linkage between C-1''''and C-6''', and between C-1''' and C-6" by the formation of ether bonds.[18] This was confirmed by the HMBC spectrum (Fig. 2). The HMBC correlation of H-C(1'''')/C(6''') indicated that the C(1'''') and C(6''') were linked by O-atom. The linkage of C(1''') and C(6") by O-atom was established by the HMBC correlations of H-C(1''')/C(6"). The connectivity of sugars, glycerol, and acyl parts was established by an HMBC experiment, in which the carbonyl at δ(C) 174.5 (C-1') showed cross-peaks with the H-C(1) (δ(H) 4.77 (dd, 10.4, 6.0); 4.71 (dd, 10.4, 5.8)); the anomeric proton signal at δ(H) 4.86 (H-C(1")) correlated with C(3) (δ(C) 73.5). The structure of **1** was thus established as 1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-3-*O*-[β-D-galactopyranos yl-(1→6)-*O*-β-D-

Table 1 ^{1}H and ^{13}C NMR spectroscopic data[a] of compounds **1** and **2** (pyridine-d5)

Position	1		2	
	δ (H)	δ (C)	δ (H)	δ (C)
H–C(1)	4.77 (1H, dd, 10.4, 6.0) 4.71 (1H, dd, 10.4, 5.8)	67.2	4.77 (1H, dd, 10.4, 5.9) 4.70 (1H, dd, 10.4, 5.8)	67.1
H–C(2)	4.57 (1H, m)	70.5	4.54 (1H, m)	70.3
H–C(3)	4.36(1H, m) 4.15 (1H, m)	73.5	4.36 (1H, m) 4.14 (1H, m)	73.3
C(1')	–	174.5	–	174.5
H–C(2')	2.40 (2H, t, 7.5)	35.1	2.41 (2H, t, 7.5)	35.0
H–C(3')	1.67 (2H, m)	26.1	1.69(2H, m)	25.9
H–C(4')	1.31 (2H, m)	30.2	1.35 (2H, m)	30.0[a]
H–C(5')	1.37 (2H, m)	30.3	1.35 (2H, m)	30.1[a]
H–C(6')	1.39(2H, m)	30.7[b]	1.39 (2H, m)	30.3
H–C(7')	1.30 (2H, m)	30.8[b]	1.39(2H, m)	30.6
H–C(8')	2.13 (2H, m)	28.4	2.13 (2H, m)	27.5
H–C(9')	5.57 (1H, m)	131.5	5.55 (1H, m)	131.0
H–C(10')	5.57 (1H, m)	128.5	5.55 (1H, m)	128.9
H–C(11')	3.00 (2H, t, 5.7)	26.8[a]	3.00 (2H, t, 5.7)	26.7
H–C(12')	5.57 (1H, m)	129.5	5.57 (1H, m)	128.9
H–C(13')	5.57 (1H, m)	129.5	5.55 (1H, m)	131.0
H–C(14')	3.00 (2H, t, 5.7)	26.9[a]	2.13 (2H, m)	27.5
H–C(15')	5.53 (1H, m)	129.0	1.45 (2H, m)	30.7
H–C(16')	5.53 (1H, m)	133.0	1.43 (2H, m)	32.4
H–C(17')	2.17 (2H, m)	21.7	1.32 (2H, m)	23.5
H–C(18')	1.02 (3H, t, 7.5)	15.3	0.96 (3H, t, 7.0)	14.9
H–C(1")	4.86 (1H, d, 7.7)	106.6[c]	4.83 (1H, d, 7.7)	106.3
H–C(2")	4.52 (1H, m)	73.3	4.52 (1H, m)	73.1[c]
H–C(3")	4.15 (1H, m)	75.8	4.14 (1H, m)	75.6[b]
H–C(4")	4.52 (1H, m)	70.5	4.50 (1H, m)	70.3
H–C(5")	4.12 (1H, m)	77.8	4.10 (1H, m)	77.6
H–C(6")	4.47 (1H, m)	70.5	4.47 (1H, m)	70.3
H–C(1''')	4.86 (1H, d, 7.7)	106.7[c]	4.83 (1H, d, 7.7)	106.4
H–C(2''')	4.54 (1H, m)	73.4	4.52 (1H, m)	73.2[c]
H–C(3''')	4.15 (1H, m)	75.8	4.14 (1H, m)	75.6[b]
H–C(4''')	4.53 (1H, m)	73.3	4.52 (1H, m)	73.0[c]
H–C(5''')	4.15 (1H, m)	75.9	4.14 (1H, m)	75.7[b]
H–C(6''')	4.52 (2H, m)	70.2	4.52 (2H, m)	70.0
H–C(1'''')	4.96 (1H, d, 7.8)	106.5	4.94 (1H, d, 7.7)	106.3
H–C(2'''')	4.63 (1H, m)	71.1	4.62 (1H, m)	70.4
H–C(3'''')	4.53 (1H, m)	73.6	4.52 (1H, m)	73.2
H–C(4'''')	4.63 (1H, m)	70.0	4.62 (1H, m)	69.8
H–C(5'''')	4.24 (1H, dd, 9.5, 3.1)	76.1	4.22 (1H, dd, 9.5, 3.1)	75.9
H–C(6'''')	4.46 (2H, m)	63.2	4.48 (2H, m)	63.0

[a]Assignments with the same superscript (a, b, c) and in the same column may be interchangeable.

galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl] glycerol. The complete assignment of their spectroscopic data were made by the use of two-dimensional (2D) NMR techniques, including TOCSY, HMQC, HMBC and NOESY spectra.

Compound **2** had the molecular formula $C_{39}H_{68}O_{19}$ based on HR–ESI–MS analysis (*m/z* 863.4255 ([M + Na]$^+$; Calcd 863.4252), indicating the existence of six degrees of unsaturation, one less degree of unsaturation than that of **1**. The compound was also obtained as white amorphous powder. The NMR data of the sugar and glycerol moiety were almost identical with those of **1**. The only difference was the presence of one less double bond in the acyl part judging from the existence of two less olefinic protons in **2** (Table). Alkaline methanolysis of **2** yielded the fatty acid methyl ester, which was identified as linoleic methyl ester by GC-MS analysis (see experimental part). The structure of **2** was thus 1-*O*-(9*Z*,12*Z*-octadecadienoyl)- 3-*O*-[β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl] glycerol. The complete assignment of their spectroscopic data followed from the interpretation of 2D-NMR techniques, including the TOCSY, HMQC, HMBC, and NOESY spectra.

Experimental

High-speed counter-current chromatography (HSCCC) was performed on a GS-1000 instrument (Institue of Food Biological Engineering, Zhejiang Gongshang University, Hangzhou, People's Republic of China). IR spectra were recorded on a Thermo Nicolet 6700 spectrometer (Thermo, America) with KBr disks. Optical rotations were measured on a PerkinElmer 341 polarimeter at room temperature. NMR spectra (^{1}H, ^{13}C, and 2D NMR) were recorded on a Bruker AM-500 spectrometer with TMS as an internal standard. ESI-MS was recorded on a Finnigan LCQ^{DECA} Mass spectrometer. GC-MS was performed on a Agilent 5975/6890 gas chromatograph-mass spectrometer in the electron impact (EI) mode (ionising potential, 70 eV) with an HP-INNOWax capillary column (30 m × 0.25 mm i.d.,0.25 μm) (Agilent, America). Helium was used as carrier gas, and the column temperature was ramped from 220 to 240 °C at 1 °C/min. TLC analysis was carried out on silica gel GF254 aluminum plates (Merk, Germany). All solvents used were of analytical grade (Hangzhou Gaojing Fine Chemical Plant, Hangzhou, P. R. China)

Plant material

The plant material used for this study was collected from Hangzhou City, Zhejiang Province, P. R. China, in May 2007, and identified by Prof. Xiao-Qin Hu of Hangzhou Botanical Garden where a voucher specimen (No.20070623H) was deposited in the Herbarium.

Extraction and isolation

The freeze-dried and powdered pumpkin (3 kg) was extracted with 95% ethanol (3 × 10 L) at room temperature. After removal of the solvent, the crude extract (350 g) was suspended in H_2O (3 L), then extracted with EtOAc and n-BuOH (5 × 500 mL) to afford two extracts. The BuOH-soluble fraction (100 g) was subjected to MCI CHP20P gel CC by eluting with MeOH/H_2O (2:8-8:2, v/v) to give two major fractions, Fr.1 (3.2 g) and Fr.2 (7.5 g). Fr. 2 was purified by HSCCC with a two-phase solvent system composed of CH_2Cl_2/n-hexane/EtOH/H_2O (6:2:2:4) by eluting the lower aqueous phase at 1.5 mL/min and 800 rpm to give a mixture of **1** and **2** (200 mg), which was further applied to preparative RP-18 HPLC (CH_3CN/H_2O 5.5 : 4.5, v/v) to afford **1** (40 mg) and **2** (50 mg).

Acid hydrolysis of **1** *and* **2**

Purified glyceroglycolipid was hydrolysed with 0.1 M H_2SO_4 (3 mL) in a sealed tube for 24 h in a boiling water bath. The reaction mixture was neutralised with $BaCO_3$ and filtered. The filtrate was concentrated, and then analysed by TLC on silica gel with a solvent system EtOAc–pyridine–EtOH–H_2O (8 : 1 : 1 : 2; v/v), comparing with monosaccharide standards and stained by aniline/diphenylamine/phosphoric acid at 100 °C, which showed the sugar moiety of **1**/**2** contained only galactose.

Alkaline hydrolysis and GC-MS analysis of **1** *and* **2**

Compound **1** (5.0 mg) in dry MeOH (3 mL) was treated with 5% NaOMe–MeOH (0.5 mL) at room temperature for 10 min. The reaction mixture was neutralised with 0.1 M HCl and diluted with water (10 mL). The aqueous solution was extracted with *n*-hexane for three times, and the organic phase was combined and dried with anhydrous Na_2SO_4. The *n*-hexane layer was concentrated to yield the fatty acid methyl ester, which was identified as linolenic acid methyl ester based on the analysis of GC-MS by comparison with authentic sample (t_R, 9.078 min). The same procedure was used for **2**, and the fatty acid methyl ester which was obtained, was identified as linoleic acid methyl ester (t_R, 7.834 min).

1-O-(9Z,12Z,15Z-octadecatrienoyl)-3-O-[β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl] glycerol **(1)**: White amorphous powder. $[\alpha]^{20}_D$ = –15.0° (*c* = 0.1, MeOH). IR (KBr): ν_{max} 3404, 3007, 2923, 1735, 1645, 1460, 1139, 1069. ^{1}H and ^{13}C NMR see Table 1 ESI-MS (pos.): 861 [M + Na]$^+$. HR-ESI-MS: 861.4101 ([M + Na]$^+$, $C_{39}H_{66}NaO_{19}^+$; calcd 861.4096).

1-O-(9Z,12Z-octadecadienoyl)-3-O-[β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl] glycerol **(2)**: White amorphous powder. $[\alpha]^{20}_D$ = – 11.4° (*c* = 0.1, MeOH). IR (KBr): ν_{max} 3420, 3010, 2923, 1729, 1633, 1453, 1130, 1069. ^{1}H and ^{13}C NMR see Table 1 ESI-MS (pos.): 863 [M + Na]$^+$. HR-ESI-MS: 863.4255 ([M + Na]$^+$, $C_{39}H_{68}NaO_{19}^+$; calcd 863.4252).

The financial support by a Grant (GZ051-4 (148)) from the Sino-German Centre for Research Promotion is gratefully acknowledged.

Received 17 December 2008; accepted 5 January 2009
Paper 08/0344 doi: 10.3184/030823409X416938
Published online: 6 April 2009

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