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Glucose-lowering activity of novel tetrasaccharide glyceroglycolipids from the fruits of *Cucurbita moschata*

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ABSTRACT

Two new tetrasaccharide glyceroglycolipids were obtained from pumpkin. The structures of the two compounds were determined using chemical methods and spectroscopic analysis. Both compounds demonstrated significant glucose-lowering effects in streptozotocin- and high-fat-diet-induced diabetic mice.

The fruit of the pumpkin, *Cucurbita moschata*, is considered to be a health food in China.[1] Preliminary investigations indicated that a pumpkin-rich diet could reduce blood glucose,[2] and protein-bound polysaccharides from pumpkin reduced blood glucose levels and improved glucose tolerance.[3] However, lower molecular weight compounds from pumpkin have rarely been screened for glucose-lowering activity, possibly because no small molecular weight compounds of interest have been isolated. To clarify the bioactive constituents of pumpkin, we systematically isolated its chemical constituents and identified two new tetrasaccharide glyceroglycolipids which belong to glycolipids possessing possible antithrombotic,[4] antiviral,[5,6] anticancer,[7–10] or antiinflammatory[11,12] activities. In the present study we investigated the glucose-lowering activity of these new tetrasaccharide glyceroglycolipids from pumpkin.

Two new tetrasaccharide glyceroglycolipids, QGMG-3 and QGMG-2, were obtained from pumpkin by extraction and separation. Freeze-dried and powdered pumpkin (5 kg) was extracted with 95% ethanol (10 l × 3) at room temperature. After solvent removal, the crude extract (350 g) was suspended in H_2O (3 l), then extracted with EtOAc and *n*-BuOH (5 × 500 ml each) to produce two extracts. The BuOH-soluble fraction (100 g) was subjected to MCI CHP20P gel (75–150 μm, Mitsubishi Chemical Industries Ltd, Tokyo, Japan) column chromatography 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 high speed countercurrent chromatography using a two-phase solvent system composed of CH_2Cl_2/*n*-hexane/EtOH/H_2O (6:2:2:4, v/v), by eluting the lower organic phase at 1.5 ml/min and 800 rpm to give a mixture of QGMG-3 and QGMG-2 (200 mg). This was further applied to preparative RP-18 HPLC (CH_3CN/H_2O 5.5:4.5, v/v) to afford compounds QGMG-3 (40 mg) and QGMG-2 (50 mg). Their structures were determined by chemical and spectral methods (IR, ^1H and ^{13}C NMR, DEPT, TOCOSY, HSQC, HMBC, and MS spectral data are included in Supplementary data.).

QGMG-3 was obtained as an optically active, white amorphous powder ($[\alpha]_D^{20} = -18.1$). The HRESIMS *m/z* 1023.4628 ($[M+Na]^+$; calcd 1023.4624) revealed a molecular formula of $C_{45}H_{76}O_{24}$, inferring the existence of eight degrees of unsaturation. The IR absorption bands at 3420 and 1722 cm^{-1} were attributed to the hydroxyls and ester carbonyl, respectively. The interpretation of NMR spectra of QGMG-3 indicated that it was a glyceroglycolipid. Analysis of the acid hydrolysis product by TLC showed that the sugar moiety of QGMG-3 contained only galactose. Alkaline hydrolysis of QGMG-3 yielded linolenic methyl ester, identified by GC–MS analysis. The ^{13}C NMR spectrum showed the presence of four anomeric carbons (δ_C 106.0, 105.8, 105.8, and 105.8), one carbonyl (δ_C 173.8), three olefinic (δ_C 132.3, 130.7, 128.9 (2 × C), 128.3, and 127.7), and one terminal methyl (δ_C 14.6). In the ^1H NMR spectrum, four anomeric proton signals (δ_H 4.85 (1H, d, J = 7.7 Hz); 4.75 (1H, d, J = 7.6 Hz); 4.75 (1H, d, J = 7.5 Hz); 4.75 (1H, d, J = 7.6 Hz)) indicated that the configurations of all three galactopyranosyls were β-type. Six olefinic protons and one methyl were also observed in the ^1H NMR spectrum. In addition, the characteristic proton signals observed at δ_H 2.92 (4H, m) and 2.32 (2H, t, J = 7.5 Hz) were easily assigned as the methylene proton signals inserted between double bonds and the methylene proton signals next to carbonyl[13], respectively. The downfield chemical shift of C-6″(δ_C 69.7),

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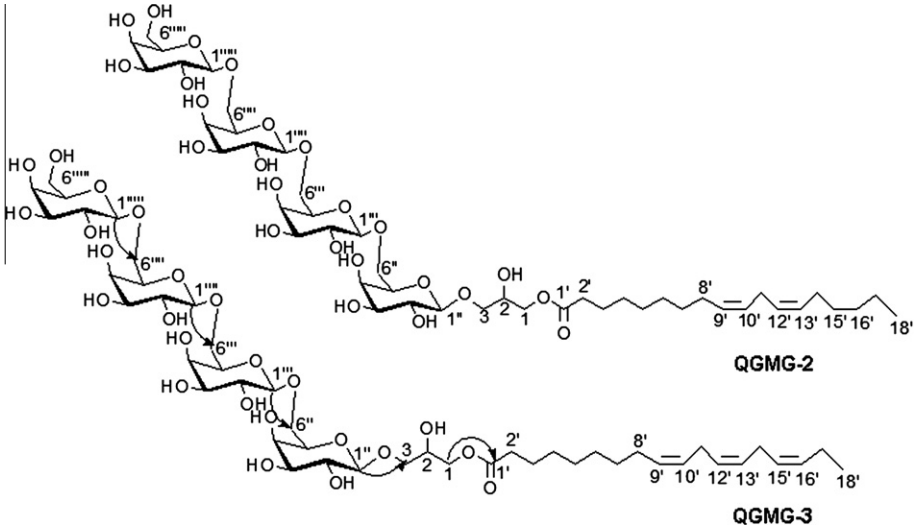

Figure 1. Chemical structures of compounds **1** and **2** with key HMBC correlations (H→C).

Table 1
Serum glucose levels in streptozotocin- and high-fat-diet-induced diabetic mice treated with glyceroglycolipids and metformin (mmol/L, mean ± SD).

Mice (n = 10)	Day 0	7 days	14 days	21 days	28 days
Control	4.90 ± 0. 32**	4.73 ± 0.51**	5.02 ± 0.65**	5.15 ± 0.72**	4.87 ± 0.54**
Diabetic group	19.21 ± 2.35	22.65 ± 6.16	22. 17 ± 5.63	23.89 ± 6.98	23.77 ± 6.12
Metformin group	19.12 ± 2.56	16.12 ± 3.25**	17.09 ± 3.19**	16.97 ± 3.03**	15.27 ± 2.79**
QGMG-2 group	18.99 ± 2.37	21.47 ± 5.05	19.09 ± 4.03*	18.17 ± 3.58**	15.73 ± 2.66**
QGMG-3 group	19.17 ± 3.45	20.89 ± 5.50*	18.27 ± 3.33**	16.36 ± 3.01**	14.61 ± 2.31**

**P <0.01, *P <0.05 versus diabetic group.

C-6‴(δ_C 69.6), and C-6⁗(δ_C 69.6) compared with the carbon signal of C-6⁗(δ_C 62.5), indicated that the three sugars likely formed a linear linkage between C-1⁗ and C-6‴, C-1⁗ and C-6‴, and between C-1‴ and C-6″ by the formation of ether bonds[14], as confirmed by HMBC spectroscopy. The connectivity of sugars, glycerol, and acyl parts was investigated by HMBC analysis; the carbonyl at δ_C 173.8 (C-1′) showed cross-peaks with the H-C (δ_H 4.63 (dd, 10.3, 5.9); 4.61 (dd, 10.3, 5.2)); the anomeric proton signal at δ 4.75 (H-1⁗) correlated with the carbon signal at δ 69.6 (C-6‴); the anomeric proton signal at δ 4.75 (H-1⁗) correlated with the carbon signal at δ 69.6 (C-6‴); the anomeric proton signal at δ 4.75 (H-1‴) correlated with the carbon signal at δ 69.7 (C-6″); and the anomeric proton signal at δ_H 4.85 (H-C (1″)) correlated with C-3 (δ_C 72.8). The structure of QGMG-3 was therefore identified as 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-(1→6)-*O*-β-D-galactopyranosyl] glycerol (Fig. 1).

QGMG-2 had the molecular formula $C_{45}H_{78}O_{24}$, based on HR-ESI-MS analysis (*m/z* 1025.4777 ([M+Na]$^+$; calcd 1025.4780), inferring the existence of seven degrees of unsaturation, one less than that of QGMG-3. The compound was also obtained as a white amorphous powder. The NMR spectra of its sugar and glycerol moieties were almost identical to those of QGMG-3; 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 QGMG-2. Alkaline hydrolysis of QGMG-2 yielded a fatty acid methyl ester, which was identified as linoleic methyl ester by GC-MS analysis. The structure of QGMG-2 was thus characterized as 1-O-(9*Z*,12*Z*-octadecadienoyl)-3-O-[β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl] glycerol (Fig. 1).

To investigate the effects of QGMG-2 and QGMG-3 on blood glucose levels, serum glucose levels in streptozotocin- and high-fat diet-induced diabetic mice treated with the glyceroglycolipids were compared with those after metformin treatment.[15] Blood glucose levels were monitored at 7-day intervals in diabetic mice after intraperitoneal (ip) injection of QGMG-3 and QGMG-2 at doses of 50 mg/kg body weight daily for 4 weeks. After the treatment of 4-weeks, the values of blood glucose of QGMG-3 and QGMG-2 were 14.61 ± 2.31 and 5.73 ± 2.66 mmol/L, respectively, while that of metformin was 15.27 ± 2.79 mmol/L. It is clear that the two tetrasaccharide glyceroglycolipids showed stronger blood glucose-lowering activity similar to that of metformin (250 mg/kg body weight) (Table 1). In comparison, QGMG-3, with a three double-bond side chain, exhibited higher activity than QGMG-2, with a two double-bond side chain. Experimental result also showed that body weight was unaffected by both QGMG-3 and QGMG-2, which showed the two compounds were possibly no potential cytotoxicity.

In conclusion, this study demonstrated that two tetrasaccharide glyceroglycolipids from pumpkin possess glucose-lowering activities. These glyceroglycolipids could represent suitable candidates for the treatment of type II diabetes, though further investigations into the mechanisms responsible for the glucose-lowering effects of these glyceroglycolipids are required.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.030.

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15. *Animals and experimental procedures*: Eight-week-old male C57/BL6J mice were purchased from the Laboratory Animal Center of the Academy of Zhejiang Medical Sciences (Hangzhou, China). The animals were maintained under standard conditions (12 h light–dark cycle, 24 °C) with free access to water and standard laboratory chow. All mice were cared for in accordance with the standards of the Guide for the Care and Use of Laboratory Animals. Diabetes was induced according to the procedure reported by Lian et al.[16] In brief, the animals were fed high-fat diets for 4 weeks before ip injection of 120 mg/kg streptozotocin (STZ, Sigma, USA). An equal volume of vehicle was injected into control mice ($n = 10$). Serum glucose levels were determined using a glucose measuring kit (Biosino Bio-technology and Science, China), using the glucose oxidase method. Mice with fasting glucose levels >11 mmol/l were considered to be diabetic. Diabetic mice with consecutive 10-day hyperglycemia ($\geqslant$11 mmol/l) were used for the experiment. The diabetic mice were randomly divided into four groups: QGMG-3-treated, QGMG-2-treated, and metformin-treated groups ($n = 10$ each), and diabetic group ($n = 10$). The mice in the QGMG-3- and QGMG-2-treated groups were injected ip with QGMG-3 and QGMG-2, respectively, at doses of 50 mg/kg body weight daily for 4 weeks. The metformin-treated group was injected i.p. with metformin at a dose of 250 mg/kg body weight daily for 4 weeks. The diabetic group was injected ip with vehicle solvent (sterilized 0.9% NaCl). An age- and weight-matched normal group ($n = 10$) was used as the control group. Body weight and food consumption in each group were monitored daily. Blood glucose levels were checked every 7 days. Blood samples were obtained from the tail vein, and serum glucose levels were determined, as described above. The results are presented as means ± SEM. The statistical methods used to analyze the data in this study were unpaired Student's *t*-test (two-tailed) using MS-Excel software program. Comparisons with P values <0.05 were considered to be statistically significant.
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