Cucurbitane glycosides from the fruit of *Siraitia grosvenori* and their effects on glucose uptake in human HepG2 cells *in vitro*

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Abstract

The mogrosides in the fruit of *Siraitia grosvenori* can serve as a sugar substitute for diabetics due to their sweetness, low calorie and positive effects on blood glucose level control. The present study was to purify the mogrosides from the fruit of *S. grosvenori* and evaluate their enhancement of glucose uptake rate in HepG2 cells *in vitro*. As a result, eighteen mogrosides were isolated, including six new ones and a known but new naturally occurring compound. The chemical structures of the new compounds were identified by 1D, 2D-NMR and HR-ESI-MS techniques, together with chemical methods. Compared to the positive control (metformin), all the obtained mogrosides showed equivalent or more potent effects on the glucose uptake in HepG2 cells *in vitro*. These results suggested the mogrosides in the fruit of *S. grosvenori* were worthy of further research to confirm their potential benefits for obese and diabetic patients.

1. Introduction

*Siraitia grosvenori* (Swingle) C. Jeffery (Cucurbitaceae) is a unique plant mainly distributed in Guangxi province, China. The fruit of *S. grosvenori*, also known as Luo Han Guo, has been used for centuries in traditional Chinese medicine to treat dry coughs, sore throats, diarhhea, and constipation (Jia & Yang, 2009). Nowadays, Luo Han Guo is well known throughout the world due to its ability to produce abundant of mogrosides (Jin & Lee, 2012). However, only several high content compounds, such as mogroside V, IV, III and siamenoside I, had been evaluated for their anti-diabetic activity. Hence, the present study focused on the chemical composition of *S. grosvenori*, including not only major mogrosides but also minor high content compounds, such as mogroside V and some minor elements from the fruit of *S. grosvenori* can suppress the rise in blood glucose level after a single oral administration of maltose in rats by inhibiting rat intestinal maltase. Afterwards, Zhou, Zheng, Ebersole, and Huang (2009) demonstrated the *S. grosvenori* extract and pure mogroside V exhibited a significant activity in stimulating insulin secretion in pancreatic beta cells. A recent study by Chen et al. (2011) revealed cucurbitane triterpenoids obtained through acid hydrolysis of *S. grosvenori* mogrosides might be potential AMPK activators.

Modern pharmaceutical studies showed that mogrosides from the fruit of *S. grosvenori* possessed various biological activities, including anti-inflammatory (Di, Huang, & Ho, 2011), anticarcinogenic (Takasaki et al., 2003), anti-virus (Akihisa et al., 2007; Ukiya et al., 2002), antioxidative (Qi, Chen, Zhang, & Xie, 2008) properties. It was interesting that, as natural sweeteners, the *S. grosvenori* mogrosides not only were safe for diabetics but also exhibited anti-diabetic activity in vitro and in vivo. Suzuki, Murata, Inui, Sugiura, and Nakano (2005) firstly advocated that mogroside V and some minor elements from the fruit of *S. grosvenori* can suppress the rise in blood glucose level after a single oral administration of maltose in rats by inhibiting rat intestinal maltase. Afterwards, Zhou, Zheng, Ebersole, and Huang (2009) demonstrated the *S. grosvenori* extract and pure mogroside V exhibited a significant activity in stimulating insulin secretion in pancreatic beta cells. A recent study by Chen et al. (2011) revealed cucurbitane triterpenoids obtained through acid hydrolysis of *S. grosvenori* mogrosides might be potential AMPK activators.

Up to now, nearly thirty cucurbitane glycosides have been reported from *S. grosvenori* (Jin & Lee, 2012). However, only several high content compounds, such as mogroside V, IV, III and siamenoside I, had been evaluated for their anti-diabetic activity. Hence, the present study focused on the chemical composition of *S. grosvenori*, including not only major mogrosides but also minor...
ones. In addition, all the isolated mogrosides were preliminarily evaluated for their anti-diabetic activity. As a result, six new cucurbitane glycosides, a new naturally occurring compound and eleven known ones were obtained. Herein, the isolation and structure elucidation of these compounds and their ability to increase glucose uptake in human HepG2 cells in vitro are reported.

2. Materials and methods

2.1. General procedures

Optical rotations were determined in methanol on a Perkin-Elmer 341 polarimeter (Perkin-Elmer Corporation, Wellesley, MA, USA). Low resolution MS were recorded on a Finnigan LCQDECA ion-trap mass spectrometer (Finnigan Co. Ltd., San Jose, CA, USA). Macroporous resin (25% CH2CN in H2O) was used to enrich total saponins with a flow rate of 90 mL/min. Macroporous resin was analyzed on an Agilent 1100 series liquid chromatography (Shanghai, China). The preparative HPLC column used was a Shim-pack ODS-AQ (Shimadzu, Kyoto, Japan) with a UV3000 detector at 210 nm. The flow rate was 90 mL/min. HPLC purifications were performed on a Shimadzu LC-20AT series apparatus with an SPD-M20A UV–vis spectrophotometric detector, equipped with a mobile phase of 23% CH3CN in 50 mM H3PO4 at a flow rate of 0.8 mL/min. HPLC purifications were performed on a CXTH system, equipped with a UV3000 detector at 210 nm (Beijing Changhai Chemical Group Co. Ltd., Beijing, People’s Republic of China). The preparative HPLC column used was a 50 x 250 mm i.d., 10 μm, YMC-pack ODS-AM (YMC Co. Ltd., Kyoto, Japan). The flow rate was 90 mL/min. Macroporous resin (HPD-100A, 26–60 mesh) was used to enrich total saponins (Cangzhou Bon Adsorber Technology Co. Ltd., Cangzhou, People’s Republic of China). MCI-gel was used for column chromatography (Cangzhou Bon Adsorber Technology Co. Ltd., Cangzhou, People's Republic of China). The flow rate was 90 mL/min. Macroporous resin (25% CH2CN in H2O) was extracted with CHCl3. The H2O layer was neutralized with Ba(OH)2, filtered and subjected to TLC analysis with authentic standards. The optical rotation of the acid hydrolysis solution was measured in methanol on a Perkin-Elmer 341 polarimeter (Perkin-Elmer Corporation, Wellesley, MA, USA). Low resolution MS were recorded on a Finnigan LCQDECA ion-trap mass spectrometer (Finnigan Co. Ltd., San Jose, CA, USA). High resolution MS were measured on a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). NMR spectra, including 1H NMR, 13C NMR, H–H COSY, HMQC and HMBC, NOESY and TOCSY experiments, were recorded on an Advance III spectrometer (Bruker, Germany) operating at 400 MHz for 1H and 100 MHz with TMS as an internal standard. HPLC analysis of the sugar derivatives was performed on a Shimadzu LC-20AT series apparatus with an SPD-M20A UV–vis spectrophotometric detector, equipped with a mobile phase of 23% CH3CN in 50 mM H3PO4 at a flow rate of 0.8 mL/min. HPLC purifications were performed on a CXTH system, equipped with a UV3000 detector at 210 nm (Beijing Changhai Chemical Group Co. Ltd., Beijing, People’s Republic of China). The preparative HPLC column used was a 50 x 250 mm i.d., 10 μm, YMC-pack ODS-AM (YMC Co. Ltd., Kyoto, Japan). The flow rate was 90 mL/min. Macroporous resin (HPD-100A, 26–60 mesh) was used to enrich total saponins (Cangzhou Bon Adsorber Technology Co. Ltd., Cangzhou, People’s Republic of China). The preparative HPLC column used was a 50 x 250 mm i.d., 10 μm, YMC-pack ODS-AM (YMC Co. Ltd., Kyoto, Japan). A voucher specimen (LHG-100) was deposited at the Laboratory of Natural Product Research Center, Chengdu Institute of Biology, Chinese Academy of Sciences.

2.2. Plant material

The fruit of S. grosvenori was purchased in March 2012 from Lotus Pond Chinese Herbal Medicine Market, Sichuan province, People’s Republic of China. The plant material was identified by Professor Weikai Bao, Chengdu Institute of Biology, Chinese Academy of Sciences. A voucher specimen (LHG–100) was deposited at the Laboratory of Natural Product Research Center, Chengdu Institute of Biology, Chinese Academy of Sciences.

2.3. Extraction and isolation

The fruit of S. grosvenori (20.0 kg) was powdered and extracted with distilled water (3 x 60 L, each 4 h) at 80 °C. The extracted water solution was filtered and then poured into a HPD-100A macroporous resin column (HPD-100A macroporous resin, 10 kg; column, 30 x 90 cm) eluted with H2O, 20% EtOH, 70% EtOH and 95% EtOH (50 L for each gradient elution), respectively. The 70% EtOH eluant solution was concentrated under reduced pressure to give a crude saponin (280 g), which was further separated by silica gel column chromatography (silica gel, 2.5 kg; column, 8 x 120 cm) with a gradient solvent system of MeOH/CHCl3/H2O (1:5:0.1, 1:4:0.15, 1:3:0.2, 1:2:0.3, and 1:1:0.4), affording eight fractions (A–H) based on TLC analyses. Fraction C was decolorized with a MCI gel column (65% MeOH in H2O) and separated by preparative HPLC (25% CH2CN in H2O) to give compound 1 (Rf = 38.1 min, 220.3 mg). Fraction D was decolorized by MCI (65% MeOH in H2O) and further purified by preparative HPLC (25% CH2CN in H2O) to afford compounds 2 (Rf = 11.5 min, 480.2 mg), 3 (Rf = 10.6 min, 548.1 mg), and 18 (Rf = 25.7 min, 832.7 mg). Compounds 3 (Rf = 17.2 min, 1.11 g), 4 (Rf = 19.2 min, 730.0 mg), 5 (Rf = 23.1 min, 894.6 mg), 6 (Rf = 28.1 min, 47.4 mg), 7 (Rf = 25.4 min, 63.8 mg) and 13 (Rf = 14.9 min, 113.0 mg) were obtained from fraction E by repeated preparative HPLC using 23% CH2CN in H2O as eluant. Fraction F was separated by preparative HPLC (23% CH2CN in H2O) to give compounds 8 (Rf = 23.1 min, 39.2 g), 9 (Rf = 28.3 min, 751.3 mg), 14 (Rf = 17.1 min, 6.61 g) and 16 (Rf = 49.6 min, 218.8 mg). The purification of fraction G by preparative HPLC (23% CH2CN in H2O) yielded compound 10 (Rf = 13.2 min, 43.3 mg). Fraction H was decolorized by MCI gel (60% MeOH in H2O) and further purified by preparative HPLC (50% MeOH in H2O) to afford compounds 11 (Rf = 17.2 min, 52.0 mg), 12 (Rf = 20.9 min, 60.8 mg), and 15 (Rf = 13.4 min, 47.7 mg).

2.4. Determination the absolute configuration of the sugar residues

The absolute configurations of the sugar moieties were determined using the method previously described (Xu et al., 2010). Compounds 6, 7, 10, 12, 13 and 15 (each 3 mg) were mixed and heated with 5% H2SO4 (3 mL) under reflux for 8 h. The reaction mixture was extracted with CHCl3. The H2O layer was neutralized with Ba(OH)2, filtered and subjected to TLC analysis with authentic glucose sample. The optical rotation of the acid hydrolysis solution was measured as [α]D20 −50.2° (c 0.05, H2O). Therefore, the configuration of the glucose in the new compounds should be in D-form.

2.5. Cell culture

HepG2 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (11.1 mM glucose) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100 mg/ml), l-glutamine (0.03% (w/v)), and NaHCO3 (2.2% (w/v)). Cells were kept in a humified incubator with 5% CO2 at 37 °C. The culture solution was replaced every other day and passaged once for 2–3 d.

2.6. MTT assay

The cytotoxicity of all the isolated mogrosides against HepG2 cells was measured using the MTT assay (Zhang et al., 2016). The compounds showed no or very little cytotoxicity at concentrations used in the functional assay.

2.7. Glucose uptake assay

The glucose uptake assay was done according to the methods previously reported (Li et al., 2007; Lv et al., 2014). The cells were planted into 96-well plates with six wells left as blank wells. After reaching 80–90% confluence, the medium was replaced by RPMI-1640 (11.1 mM glucose) containing 0.2% bovine serum albumin (BSA). The medium was then added 1 μmol/L metformin or individual compounds at different concentrations, and dimethyl sulfoxide (DMSO) was used as the blank control. The glucose concentration in the medium was determined after 24 h treatment. Glucose uptake rate = [(glucose concentrations in blank wells − glucose concentrations of cell plated wells)/glucose concentration in the medium] x 100%.
concentrations in blank wells] × 100. MTT assay was performed to determine the inhibition ratio of cell proliferation and to adjust the glucose uptake values. The inhibition ratio was calculated as [(OD value of control – OD value of the sample)/OD value of control] × 100.

3. Results and discussion

The hot water extract of the fruit of S. grosvenori was sequentially separated by various column chromatography and further purified by preparative HPLC to yield eighteen pure compounds (Fig. 1), including six new ones. The known compounds were measured for 1H and 13C NMR and ESI-MS spectroscopic data, while the new ones were further measured for 2D NMR and HR-ESI-MS data.

3.1. Spectroscopic data of compounds 6, 7, 10, 12, 13 and 15

3.1.1. Isomogroside IVA (6)

3-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl mogrol 24-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl. White amorphous powder; [α]D20 +3.8° (c 0.21, MeOH); ESI-MS m/z 1147 [M+Na]+; HR-ESI-MS m/z 1123.5966 [M–H]– (calcd. for C54H91O24, 1123.5968); 1H NMR (400 MHz, C5D5N) and 13C NMR (100 MHz, C5D5N) data, see Tables 1 and 2.

3.1.2. Isomogroside IVa (7)

3-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl mogrol 24-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside. White amorphous powder; [α]D20 +3.3° (c 0.48, MeOH); ESI-MS m/z 1147 [M+Na]+; HR-ESI-MS m/z 1123.5890 [M–H]– (calcd. for C54H91O24, 1123.5892); 1H NMR (400 MHz, C5D5N) and 13C NMR (100 MHz, C5D5N) data, see Tables 1 and 2.

3.1.3. 11-epi-Mogroside V (10)

3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl 11-epimuogrol 24-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside. White amorphous powder; [α]D20 +4.2° (c 0.33, MeOH); ESIMS m/z 1285 [M–H]–; HR-ESI-MS m/z 1285.6412 [M–H]– (calcd. for C60H101O29, 1285.6434); 1H NMR (400 MHz, C5D5N) and 13C NMR (100 MHz, C5D5N) data, see Tables 1 and 2.
3.1.4. Mogroside Vla (12)

3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl mogro 24-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl. White amorphous powder; $\delta_C^{13}$C NMR (400 MHz, C5D5N) and $\delta_C^{1}H$ NMR (100 MHz, C5D5N) data, see Tables 1 and 2.

Table 1

| Position | 6 | 7 | 10 | 12 | 13 | 15 |
|----------|---|---|----|----|----|----|
|          | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) |
| 3-O-sugars | Glc (inner) | | | | | | | | | |
| 1 | 107 | 4.82 (d, 7.8) | 107.0 | 4.82 (d, 7.7) | 107.1 | 4.82 (d, 7.7) | 107.0 | 4.80 (d, 7.8) | 107.3 | 4.85 (d, 7.7) | 104.7 | 4.82 (d, 7.0) |
| 2 | 74.9 | 3.95 | 74.9 | 3.96 | 75.5 | 3.89 | 75.4 | 3.91 | 75.5 | 4.09 | 82.0 | 4.20 |
| 3 | 76.9 | 4.04 | 76.9 | 4.04 | 78.5 | 4.14 | 78.4 | 4.18 | 78.7 | 4.17 | 78.6 | 4.11 |
| 4 | 81.3 | 4.26 | 81.4 | 4.27 | 72.3 | 4.02 | 71.7 | 3.98 | 71.5 | 4.25 | 71.6 | 3.97 |
| 5 | 77.9 | 3.92 | 76.3 | 3.92 | 78.0 | 4.09 | 78.5 | 4.05 | 78.1 | 3.99 | 77.1 | 4.09 |
| 6a/b6b | 62.6 | 4.38, 4.51 | 62.7 | 4.28, 4.43 | 70.2 | 4.31, 4.78 | 70.2 | 4.32, 4.78 | 63.0 | 4.35, 4.54 | 70.2 | 4.28, 4.76 |
| Glc (4) | | | | | | | | | | | |
| 1 | 105 | 5.19 (d, 7.8) | 105.0 | 5.18 (d, 7.7) | 105.4 | 5.18 (d, 7.8) | 105.6 | 5.05 (d, 7.8) | 105.5 | 5.12 (d, 7.8) |
| 2 | 76.3 | 4.06 | 75.2 | 4.09 | 78.1 | 4.22 | 78.4 | 4.17 | 77.9 | 4.23 |
| 3 | 78.5 | 4.18 | 78.6 | 4.23 | 78.5 | 4.24 | 78.5 | 4.24 | 78.4 | 4.24 |
| 4 | 72.2 | 4.10 | 72.1 | 4.24 | 71.8 | 4.24 | 71.8 | 4.24 | 71.8 | 4.24 |
| 5 | 78.2 | 3.95 | 78.2 | 3.99 | 78.5 | 3.95 | 78.4 | 4.04 | 78.3 | 3.97 |
| 6a/b6b | 62.4 | 4.29, 4.49 | 62.4 | 4.34, 4.51 | 62.9 | 4.33, 4.54 |
| Glc (6') | | | | | | | | | | | |
| 1 | 105.4 | 5.15 (d, 7.8) | 105.6 | 5.05 (d, 7.8) | 105.5 | 5.12 (d, 7.8) |
| 2 | 75.3 | 4.05 | 75.4 | 3.91 | 75.5 | 4.03 |
| 3 | 78.1 | 4.20 | 78.4 | 4.23 | 77.9 | 4.23 |
| 4 | 71.8 | 4.24 | 71.7 | 4.23 | 71.5 | 4.24 |
| 5 | 78.5 | 3.96 | 77.7 | 4.04 | 77.6 | 3.96 |
| 6a/b6b | 62.8 | 4.37, 4.51 |

24-O-sugars

| Glc (inner) | | | | | | | | | | | |
| 1 | 106.3 | 5.36 (d, 7.7) | 106.4 | 5.49 (d, 7.6) | 106.5 | 5.47 (d, 7.6) | 106.0 | 5.49 (d, 7.6) | 105.5 | 5.34 (d, 7.7) |
| 2 | 75.0 | 4.09 | 76.5 | 4.09 | 76.6 | 4.09 | 76.0 | 4.08 | 76.5 | 4.09 |
| 3 | 78.5 | 4.19 | 78.4 | 4.18 | 78.3 | 4.21 | 78.3 | 4.20 | 78.1 | 4.20 |
| 4 | 72.5 | 4.14 | 72.6 | 4.19 | 72.6 | 4.22 | 72.5 | 4.20 | 72.2 | 4.21 |
| 5 | 78.3 | 3.92 | 78.5 | 3.95 | 78.5 | 3.98 | 78.5 | 3.97 | 78.6 | 3.92 |
| 6a/b6b | 63.4 | 4.33, 4.50 | 62.7 | 4.32, 4.49 | 62.7 | 4.36, 4.54 | 62.6 | 4.37, 4.51 | 62.7 | 4.36, 4.49 |

Glc (6) | | | | | | | | | | | |
| 1 | 104.9 | 4.83 (d, 7.8) | 104.9 | 4.85 (d, 7.7) | 104.9 | 4.79 (d, 7.8) | 104.9 | 4.86 (d, 7.6) | 104.9 | 4.86 (d, 7.6) |
| 2 | 75.5 | 4.07 | 75.3 | 4.05 | 75.3 | 4.04 | 75.6 | 4.04 | 75.3 | 4.05 |
| 3 | 78.1 | 4.24 | 78.4 | 4.24 | 78.4 | 4.20 | 78.4 | 4.24 | 78.3 | 4.21 |
| 4 | 71.6 | 4.25 | 71.7 | 4.24 | 71.8 | 4.22 | 71.8 | 4.24 | 71.8 | 4.24 |
| 5 | 78.7 | 3.91 | 78.5 | 3.89 | 78.5 | 3.93 | 78.2 | 3.93 | 78.5 | 3.96 |
| 6a/b6b | 62.5 | 4.35, 4.51 | 63.7 | 4.34, 4.49 | 63.6 | 4.32, 4.55 | 63.5 | 4.32, 4.49 | 63.5 | 4.31, 4.53 |

3.1.5. 11-Oxo-siamesonoside I (13)

3-O-β-D-glucopyranosyl 11-oxo-mogro 24-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl. White amorphous powder; $\delta_C^{13}$C NMR (400 MHz, C5D5N) and $\delta_C^{1}H$ NMR (100 MHz, C5D5N) data, see Tables 1 and 2.

3.1.6. 11-Oxo-mogroside VI (15)

3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl 11-oxo-mogro 24-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl. White amorphous powder; $\delta_C^{13}$C NMR (400 MHz, C5D5N) and $\delta_C^{1}H$ NMR (100 MHz, C5D5N) data, see Tables 1 and 2.
3.2. Structure elucidation

Compound 6, amorphous white powder, was assigned the molecular formula of C_{54}H_{92}O_{24} by HR-ESI-MS at m/z [M-H]^- 1123.5896 (calc. for C_{54}H_{92}O_{24} 1123.5906). The ^1H NMR spectrum (Table 1) showed four anomeric proton signals at δ_H 4.82 (1H, d, J = 7.8 Hz), 5.06 (1H, d, J = 7.7 Hz), 5.19 (1H, d, J = 7.8 Hz) and 5.36 (1H, d, J = 7.7 Hz). The ^13C NMR spectrum of compound 6 also displayed 4 anomeric carbon signals at δ_C 102.0, 105.0, 106.3 and 107.0. In ESI-MS² spectrum, the pseudo-molecular ion at m/z 1123 [M-H]^- of compound 6 produced fragment ions at m/z 961, 799, 637 and 475, suggesting the presence of four hexose moieties. Acid hydrolysis experiment indicated the sugar residues are α-glucoses (Section 2.4). The coupling constants (Table 1) of the anomeric protons suggested a β-configuration for all the glucose moieties (Li, Sun, Chen, Ding, & Wang, 2011). The ^1H NMR data (Table 2) of the aglycone of 6 were similar to those of morgol except for the glycosylation shifts of the C-3 and C-24 (Kasai et al., 1989). The ^1H and ^13C NMR signals of the glucose residues and the aglycone of compound 6 were assigned using 2D-NMR including COSY, HSQC HMBC and TOCSY experiments. The HMBC correlations between H-1 (δ_H 4.82) of the Glc-1 and C-3 (δ_C 88.1) of the aglycone, between H-1 (δ_H 4.82) of the Glc-3 and C-24 (δ_C 88.2) of the aglycone indicated Glc-1 and Glc-3 were attached to C-3 and C-24 of the aglycone, respectively (Fig. 2). Glc-2 was connected to C-4 of Glc-1 due to the HMBC correlation between H-1 (δ_H 5.19) of the Glc-2 and C-4.
Compound 7 was obtained as an amorphous white power. The HR-ESI-MS showed a pseudo-molecular ion at $m/z$ [M–H]− 1123.5890 (calcd. for C54H89O24). The easily distinguished anomeric proton signals at δH 4.82 (1H, d, J = 7.7 Hz), 4.83 (1H, d, J = 7.8 Hz), 4.89 (1H, d, J = 7.8 Hz), and 5.18 (1H, d, J = 7.7 Hz) and anomeric carbon signals at δC 104.9, 105.0, 106.4, 107.0 (Table 1) both showed the presence of four sugar moieties, which were determined as β-D-glucopyranosyl by detailed analysis of the COSY, HSQC, HMBC, and TOCSY experiments, indicating the disaccharide at C-24 was formed by a 1, 6-glycosidic bond. According to the disaccharide anomeric configuration of the C-11 hydroxyl group (Kasai, Matsumoto, Nie, Zhou, & Tanaka, 1988). The structure of compound 8 was elucidated as 3-O-β-D-glucopyranosyl-11-oxo-mogrol 24-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl and named as isomogroside IVa.

Compound 9 exhibited a [M−H]− ion at m/z 1285.6412 (calcd. for C66H109O34). The 1H and 13C NMR data of the sugar moieties of compound 9 (Table 1) were in accordance with those of the known mogroside V (8), which was the main component in the fruit of S. grossenovi. This suggested that compounds 9 and 10 shared the same sugar chains. Comparison of the 13C NMR data of the aglycone of 10 (Table 2) with those of mogrol and scandenoside R7 suggested that the aglycone of 10 was an epimer of mogrol differing only in the configuration of the C-11 hydroxyl group (Kasai, Matsumoto, Nie, Zhou, & Tanaka, 1988). The structure of compound 10 was further verified by extensive 2D-NMR experiments. Accordingly, compound 10 was elucidated as 3-O-β-D-glucopyranosyl-11-epi-24-O-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl and named as 11-epi-mogroside V.

Compound 12 showed a [M−H]− ion at m/z 1447.6945 (calcd. for C66H112O34), corresponding to the molecular formula C66H112O34. The 1H and 13C NMR data of compound 12 (Tables 1 and 2) were similar to those of compound 8 except for signals attributed to one more glucose residue. The appearance of one more methylene signal at δC 70.4 in 13C NMR spectrum of 12 indicated that this glucose moiety was linked to C-6 of a glucose residue. Compound 12 was elucidated as 3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-11-epi-24-O-β-D-glucopyranosyl mogrol 24-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl and named as 11-epi-mogroside V by detailed analysis of the COSY, HSQC, HMBC, TOCSY and NOESY spectra, and named as mogroside VIA.

Compound 13, amorphous white power, was assigned the molecular formula of C66H109O34 by HR-ESI-MS at m/z [M−H]− 1121.5179 (calcd. for C66H109O34 1121.5174). The 1H and 13C NMR data (Table 1) of the sugar moieties of compound 13 were identical to those of compound 3, while the NMR data due to the aglycone (Table 2) were the same as those of 11-oxo-mogrol. Thus, compound 13 was identified as 3-O-β-D-glucopyranosyl 11-oxo-mogrol 24-O-β-D-glucopyranosyl-11-epi-24-O-β-D-glucopyranosyl and named as 11-oxo-scandenoside I.

Compound 15 was obtained as an amorphous white powder. The HR-ESI-MS showed a pseudo-molecular ion at m/z [M−H]− 1445.6826 (calcd. for C66H112O34). Comparison of the NMR data of 15 with those of 11 suggested that they shared similar chemical properties.

### Table 3

| No. | Concentration (M) | Inhibition ratio (%) | Glucose consumption rate (%) |
|-----|------------------|----------------------|-----------------------------|
| 1   | 1 × 10⁻⁵         | 3.88 ± 2.23          | 37.4 ± 5.25                 |
| 2   | 1 × 10⁻⁶         | 0                    | 39.3 ± 3.34                 |
| 3   | 1 × 10⁻⁷         | 0                    | 31.4 ± 4.78                 |
| 4   | 1 × 10⁻³         | 33.3 ± 2.40          | 3.70 ± 2.79                 |
| 5   | 1 × 10⁻⁴         | 32.3 ± 2.54          | 2.97 ± 0.11                 |
| 6   | 1 × 10⁻⁵         | 6.69 ± 3.56          | 40.4 ± 5.23                 |
| 7   | 1 × 10⁻⁶         | 6.57 ± 2.63          | 40.0 ± 1.41                 |
| 8   | 1 × 10⁻⁷         | 5.10 ± 6.75          | 34.7 ± 1.13                 |
| 9   | 1 × 10⁻⁸         | 0                    | 33.7 ± 4.88                 |
| 10  | 1 × 10⁻⁹         | 5.06 ± 3.54          | 38.9 ± 4.83                 |
| 11  | 1 × 10⁻⁵         | 4.10 ± 3.95          | 41.4 ± 5.73                 |
| 12  | 1 × 10⁻⁶         | 3.90 ± 3.29          | 37.2 ± 5.88                 |
| 13  | 1 × 10⁻⁷         | 6.04 ± 0.38          | 31.7 ± 3.22                 |
| 14  | 1 × 10⁻⁸         | 0                    | 32.8 ± 2.35                 |
| 15  | 1 × 10⁻⁹         | 3.60 ± 2.79          | 36.6 ± 2.22                 |
| 16  | 1 × 10⁻⁴         | 5.71 ± 2.63          | 34.7 ± 2.35                 |
| 17  | 1 × 10⁻⁵         | 6.70 ± 4.48          | 33.2 ± 3.78                 |
| 18  | 1 × 10⁻⁶         | 10.48 ± 3.77         | 33.5 ± 2.68                 |
| 19  | 1 × 10⁻⁷         | 0                    | 33.2 ± 2.81                 |

**P < 0.05, **P < 0.01, compared to DMSO blank control.
structures except for the aglycone of 11-oxo-mogrol in 15 instead of mogrol in 11. This was verified by extensive 2D-NMR analyses. Consequently, compound 15 was determined as 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl 11-oxo-mogrol 24-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-β-D-glucopyranoside, and named as 11-oxo-mogroside VI.

By analyses of the NMR spectroscopic data and comparison with those reported in the literatures, the remaining known compounds were identified as mogroside III (1) (Matsumoto et al., 1990), mogroside III (2) (Matsumoto et al., 1990), sienosamono I (3) (Li, Lin, Luo, & Wang, 2011), mogroside IVa (4) (Li et al., 2011), mogroside IVe (5) (Li et al., 2011), isomogroside V (9) (Jia & Yang, 2009), mogroside VI (11) (Prakash Chaturvedula & Prakash, 2011), 11-oxo-mogroside V (14) (Prakash Chaturvedula & Prakash, 2011), 11-deoxymogroside V (16) (Prakash & Prakash Chaturvedula, 2014), mogroside IIa (17) (Takemoto, Arihara, Nakajima, & Okuhira, 1983) and mogroside IIa (18) (Li et al., 2011).

3.3. Effect of compounds 1–18 on glucose uptake in HepG2 cells

To explore the anti-hyperglycaemic potential of the isolated mogrosides, the increasing glucose uptake effect of compounds 1–18 was investigated in HepG2 cells in vitro (Table 3). The MTT assay was used to assess the cell proliferation. Compared to the hyperglycaemic drug metformin (positive control), compounds 1–18 showed comparative or stronger activity in promoting the glucose uptake at a concentration of 1 × 10^{-6} mol/L. However, all of the compounds did not present a dose-dependent manner and showed the strongest activity at 1 × 10^{-6} mol/L, compared to those at 1 × 10^{-5} mol/L and 1 × 10^{-7} mol/L, respectively.

4. Conclusions

In conclusion, the phytochemical investigation of the fruit of S. grosvenori led to the isolation and characterization of 18 cucurbitane glycosides, including 6 new ones and 1 known but naturally occurring compound. The chemical structures of the new compounds were elucidated on the basis of extensive 1D and 2D-NMR data analysis. All the isolates exhibited considerable bioactivities in promoting glucose uptake in human HepG2 cells in vitro. These results suggested that the fruit of S. grosvenori was abundant in various sweet mogrosides and worthy of further research to confirm their potential benefits for obese and diabetic patients. However, due to the greater tendency of the mogrosides to undergo rapid degradation in vivo (Murata et al., 2010), the structures and anti-hyperglycaemic activity of the metabolites of these mogrosides in vivo should be studied in future. Moreover, the underlying mechanism of the mogrosides to increase glucose uptake in HepG2 cells remains unclear and needs further investigation.

Conflict of interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.02.018.

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