Chemical and Pharmaceutical Bulletin Advance Publication by J-STAGE
DOI:10.1248/cpb.c19-00210

Advance Publication
April 13, 2019

Chem. Phar. Bull.

Note

Two New Cucurbitane Glycosides from the Fruits of *Siraitia grosvenori* Swingle

Donghai Chu,[#,a] Aftab Yaseen,[#,b] Lun Wang,[b] Bin Chen,[b] Mingkui Wang,[b] Weicheng Hu,[*,c] and Fu Li,[*,b]

[a]*School of Biomedical & Chemical Engineering, Liaoning Institute of Science and Technology; Benxi 117004, China;* [b]*Natural Products Research Center, Chengdu Institute of Biology, Chinese Academy of Sciences; Chengdu 610041, China; and* [c]*Jiangsu Collaborative Innovation Center of Regional Modern Agriculture & Environmental protection/Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake, Huaiyin Normal University; Huaian 223300, China.*

[#] These authors contributed equally to this work. To whom correspondance should be addressed. e-mail: hu_weicheng@163.com; andy197971@163.com

Two novel cucurbitane glycosides, named as 11-oxomogroside III A_1 and 7β-methoxy-mogroside V, along with sixteen known ones were isolated from the fruits of *Siraitia grosvenori* Swingle. The structures of the new compounds were characterized by chemical and extensive spectral methods.

Key words cucurbitane glucoside; *Siraitia grosvenori* Swingle; structural elucidation

Introduction

Siraitia grosvenori Swingle, belonging to the famiy Cucurbitaceae, is chiefly distributed in the south of China and the north of Thailand. The fruits of *Siraitia grosvenori* are rich in cucurbitane glycosides,[1-5] which showed broad biological activities including anticarcinogenic, anti-virus, antioxidative and anti-diabetic properties.[6-9] We had previously obtained a series of cucurbitane glycosides with anti-diabetic activities from 20 kg of the fruits of *Siraitia grosvenori*.[10] The present paper is a continuation of our previous work and describes the isolation and structural elucidation of two new cucurbitane glycosides (**1** and **2**) (Fig. 1), together with sixteen known ones (**3**-**18**) from 100 kg of the fruits of this plant.

Results and discussion

Compound **1** was obtained as a white amorphous powder. The molecular formula $C_{48}H_{80}O_{19}$ for compound **1** was determined by HR-ESI-MS at *m/z* 983.5220 $[M+Na]^+$ in positive ion mode. Acid hydrolysis result suggested that the sugar residues in compound **1** were composed of only D-glucose according to the method previously described.[10] The ^{1}H-NMR spectrum (Table 1) displayed three anomeric proton signals at δ 4.86 (1H, d, J = 7.6 Hz, glcIII H-1), 4.91 (1H, d, J = 7.4 Hz, glcI H-1) and 5.49 (1H, d, J = 7.7 Hz, glcII H-1), while the ^{13}C-NMR spectrum (Table 2) showed three anomeric carbon signals at δ 103.7, 104.9 and 105.6, demonstrating the presence of three glucose residues in **1**. The coupling constants of the anomeric protons suggested a β configuration for all the glucose moieties. The ^{1}H-NMR data of **1** suggested the presence of eight methyl hydrogen signals at δ 0.77 (3H, s, H-18), 1.02 (3H, d, J = 6.2 Hz, H-21), 1.08 (3H, s, H-30), 1.13 (3H, s, H-28), 1.28 (3H, s, H-19), 1.36 (3H, s, H-27), 1.43 (3H, s, H-29) and 1.47 (3H, s, H-26), two isolated oxymethine hydrogen signals at δ 3.71 (1H, br s, H-3) and 3.76 (1H, d, J = 8.5 Hz, H-24), and an olefinic methine hydrogen signal at δ 5.68 (1H, br s, H-6), while the ^{13}C-NMR data indicated the existance of an obvious oxymethine carbon signal at δ 92.1 (C-24), two double bond carbon signals at δ 141.5 (C-5) and 119.1 (C-6), and a carbonyl carbon signal at δ 214.2. The ^{1}H-NMR and ^{13}C-NMR spectra of the aglycone of **1** resembled those of 11-oxomogrol besides the glycosylation shifts of the C-24, suggesting the aglycone of **1** was 11-oxomogrol and the sugar residues might be connected to C-24 of the aglycone.[5] A trisaccharide unit

composed of β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl was attached to C-24 of the aglycone based on the following important HMBC correlations between the H-1 (d, J = 7.4 Hz, δ 4.91) of GlcI and C-24 (δ 92.1) of the aglycone, between the H-1 (d, J = 7.7 Hz, δ 5.49) of GlcII and C-2 (δ 82.2) of GlcI, and between H-1 (d, J = 7.6 Hz, δ 4.86) of GlcIII and C-6 (δ 70.2) of GlcI (Fig. 2). Accordingly, **1** was characterized and named as 11-oxomogroside III A_1.

Compound **2** was isolated as a white amorphous powder. The molecular formula $C_{61}H_{104}O_{30}$ for compound **2** was determined by HR-ESI-MS at *m/z* 1339.6501 $[M+Na]^+$ in positive ion mode. The monosaccharides obtained by acid hydrolysis also revealed the presence of only glucose in **2**.[10] The ^{1}H-NMR spectrum (Table 1) displayed five anomeric proton signals at δ 4.82 (1H, d, J = 7.7 Hz, glcIV H-1), 4.86 (1H, d, J = 7.7 Hz, glcIII H-1), 4.94 (1H, d, J = 7.7 Hz, glcI H-1), 5.14 (1H, d, J = 7.7 Hz, glcV H-1) and 5.45 (1H, d, J = 7.7 Hz, glcII H-1), while the ^{13}C-NMR spectrum (Table 2) showed five anomeric carbon signals at δ 103.7, 104.9, 105.5, 105.7 and 107.1, demonstrating the presence of five glucose residues in **2**. The ^{1}H-NMR data of **2** suggested the presence of eight methyl hydrogen signals at δ 0.84 (3H, s, H-18), 1.01 (3H, s, H-30), 1.11 (3H, d, J = 6.3 Hz, H-21), 1.14 (3H, s, H-28), 1.35 (3H, s, H-27), 1.47 (3H, s, H-26), 1.59 (3H, s, H-29) and 1.60 (3H, s, H-19), three isolated oxymethine hydrogen signals at δ 3.45 (1H, d, J = 5.4 Hz, H-7), 3.76 (1H, br s, H-3) and 3.78 (1H, d, J = 9.8 Hz, H-24), and an olefinic methine hydrogen signal at δ 5.93 (1H, d, J = 5.4 Hz, H-6), while the ^{13}C-NMR data indicated the existance of two obvious oxymethine carbon signal at δ 87.4 (C-3) and 92.1 (C-24), two double bond carbon signals at δ 149.9 (C-5) and 118.7 (C-6). The proton signal at δ 3.29 (3H, s, -OCH_3) in ^{1}H-NMR spectrum and the carbon signal at δ 56.2 in ^{13}C-NMR spectrum indicated the presence of a methoxy group in **2**. The ^{13}C-NMR data of **2** were similar to those of mogroside V except those of C-5, C-7 and C-8 downfielding from δ 144.7, 25.0 and 44.0 in mogroside V to 149.9, 77.9 and 47.8 in **2**, indicating the methoxy group was located at C-7.[11] This was further verified by the HMBC correlations between -OCH_3 (s, δ 3.29) and C-7 (δ 77.9), and between H-6 (d, J = 5.4 Hz, δ 5.93) and C-7 (δ 77.9). The other part of **2** was the same as that of mogroside V according the HMBC correlations (Fig. 2). NOE correlations between -OCH_3 (s, δ 3.29) and H-8 (br s, δ

2.14), H-19 (s, δ 1.60) clearly confirmed the -OCH_3 group was in β orientation (Fig. 2). Thus, **2** was elucidated and named as 7β-methoxy-mogroside V.

The sixteen known cucurbitane glycosides were elucidated as mogroside II A_1 (**3**),[12] mogroside II A_2 (**4**),[13] mogroside III (**5**),[14] mogroside III E (**6**), [14] mogroside III A_1 (**7**),[15] siamenoside I (**8**), [15] mogroside IVa (**9**), [15] mogroside IVe (**10**), [15] mogroside V (**11**), [15] isomogroside V (**12**),[4] 11-*O*-mogroside V (**13**),[15] 11-*epi*-mogroside V (**14**),[10] mogroside VI (**15**),[11] 11-*O*-mogroside VI (**16**),[10] mogroside VI A (**17**)[1] and mogroside VI B (**18**) [1] according to their spectroscopic data compared with those reported in the literatures.

Experimental

General Procedures Optical rotations were measured with a Perkin-Elmer 341 polarimeter. The HRESIMS spectra were acquired on a Vion IMS QT of (Waters Corp., Milford, Massachusetts, USA) in positive ion mode. 1D and 2D NMR data were obtained on a Bruker Avance-600 spectrometer in C_5D_5N. Macroporous resin (HPD-100A, 26-60 mesh) was used to enrich total saponins (Cangzhou Bon Adsorber Technology Co. Ltd., Cangzhou, China). Normal phase column chromatography was carried out with silica gel (100-200 mesh, Qingdao Haiyang Chemical Factory, Qingdao, China). Preparative-scale HPLC was implemented on a CXTH system, equipped with a C_{18} column (50 × 250 mm i.d., 10 μm, Daiso SP-100-10-ODS-P) from Daiso Co., Ltd. (Osaka, Japan) at a flowrate of 90 mL/min.

Plant Material The fruits of *Siraitia grosvenori* Swingle were purchased in February 2017 from Lotus Pond Chinese Herbal Medicine Market, Sichuan province, China.

Extraction and Isolation The fruits of *Siraitia grosvenori* Swingle (100 kg) were extracted with distilled water (3 × 300 L, each 4 h) at 80 °C. The extracted water solution was passed through an HPD-100A macroporous resin column eluted with H_2O, 20% EtOH, 70% EtOH and 95% EtOH (100 L for each gradient elution), respectively. The 70% EtOH eluant solution was concentrated under reduced pressure to give a crude saponin (1220 g), which was further separated by silica gel column chromatography with a gradient solvent system of H_2O saturated MeOH/$CHCl_3$ (1:5→1:4→1:3→1:2→1:1), affording nine fractions. Fraction 2 was isolated by preparative HPLC (26% CH_3CN) to afford compounds **3** (0.12 g) and **4** (1.21 g). Fraction 3 was further separated by preparative HPLC (25% CH_3CN) to yield

compounds **1** (620.6 mg), **5** (0.30 g), **6** (1.27 g) and **7** (2.24 g). Part of fraction 5 was isolated by preparative HPLC (24% CH_3CN) to afford compounds **8** (3.38 g), **9** (1.56 g) and **10** (3.61 g). Part of fraction 7 was further purified by preparative HPLC (23% CH_3CN) to give compounds **2** (133.2 mg), **11** (20.2 g), **12** (0.46 g), **13** (4.88 g) and **14** (0.23 g). Fraction 9 was separated by preparative HPLC (22% CH_3CN) to yield compounds **15** (0.15 g), **16** (0.32 g), **17** (1.24 g) and **18** (0.55 g).

Compound 1 A white amorphous powder. $[\alpha]_D^{20}$ +20.3° (*c* 0.20, MeOH). HR-ESI-MS *m/z* 983.5220 (Calcd. for $C_{48}H_{80}O_{19}Na^+$: 983.5186).

^{1}H-NMR (pyridine-d_5) δ: Table 1.

^{13}C-NMR: Table 2.

Compound 2 A white amorphous powder. $[\alpha]_D^{20}$ +7.9° (*c* 0.15, MeOH). HR-ESI-MS *m/z* 1339.6501 (Calcd. for $C_{61}H_{104}O_{30}Na^+$: 1339.6505).

^{1}H-NMR (pyridine-d_5) δ: Table 1.

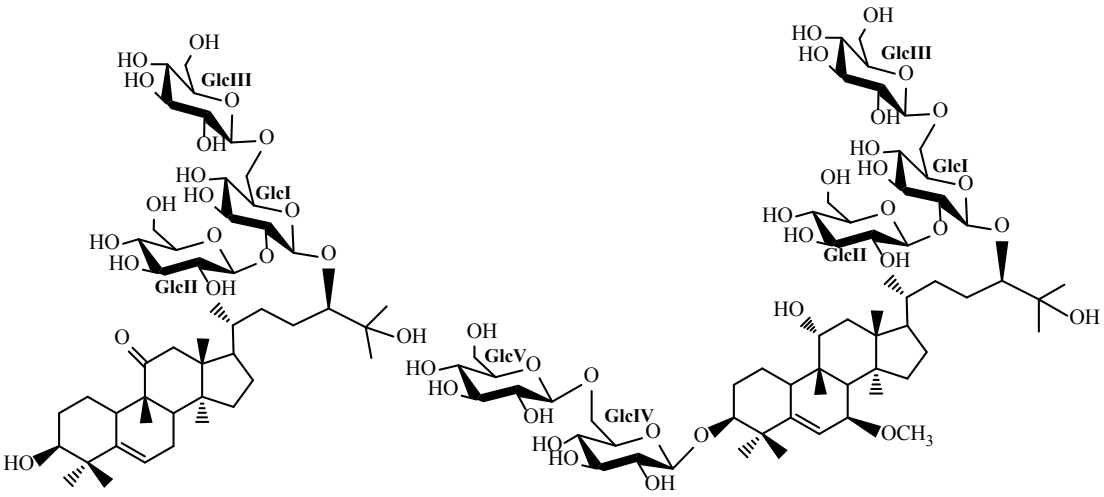
^{13}C-NMR: Table 2.

Identification of sugars for 1 and 2 Compounds **1** and **2** (each 5.0 mg) were mixed and heated with 5% H_2SO_4 (5 mL) under reflux for 8 h. The reaction mixture was extracted with EtOAc. The H_2O layer was neutralized with $Ba(OH)_2$, filtered and subjected to TLC analysis with authentic glucose sample (R_f = 0.35, mobile phase: ethyl acetate:pyridine:ethanol:water = 8:1:1:2). The optical rotation of the acid hydrolysis solution was measured as $[\alpha]_D^{20}$ +48.7° (c 0.05, H_2O). Therefore, the configuration of the glucose in the new compounds should be in *D*-form.

Confilct of interest The authors declare no conflict of interest.

References

1) Niu B., Ke C.-Q., Li B.-H., Li, Y.-Y., Yi Y.-J., Luo Y.-W., Shuai L., Yao S., Lin L.-G., Li J., Ye Y., *J. Nat. Prod.*, **80**, 1428-1435 (2017).
2) Li D.-P., Ikeda T., Matsuoka N., Nohara T., Zhang H.-R., Sakamoto T., Nonaka G.-I., *Chem. Pharm. Bull.*, **54**, 1425-1428 (2006).
3) Li D.-P., Ikeda T., Nohara T., Liu J.-L., Wen Y.-X., Sakamoto T., Nonaka G.-I., *Chem. Pharm. Bull.*, **55**, 1082-1086 (2007).
4) Jia Z.-H., Yang X.-G., *Nat. Prod. Commun.*, **4**, 769-772 (2009).
5) Kasai R., Nie R.-L., Nashi K.-J., Ohtani K., Zhou J., Tao G.-D., Tanaka O., *Agric. Biol. Chem.*, **53**, 3347-3349 (1989).
6) Takasaki M., Konoshima T., Murata Y., Sugiura M., Nishino H., Tokuda H., Matsumoto K., Kasai R., Yamasaki, K., *Cancer Lett.*, **198**, 37-42 (2003).
7) Akihisa T., Hayakawa Y., Tokuda H., Banno N., Shimizu N., Suzuki T., Kimura Y., *J. Nat. Prod.*, **70**, 783−788 (2007).
8) Xiang-Yang Q.-I., Chen W.-J., Zhang L.-Q., Shan X.-F., Song Y.-F., *Sci. Agric. Sin.*, **39**, 382−388 (2006).
9) Suzuki Y.-A., Murata Y., Inui H., Sugiura M., Nakano, Y., *J. Agric. Food Chem.,* **53**, 2941-2946 (2005).
10) Li F., Yang F.-M., Liu X., Wang L., Chen B., Li L.-H., Wang M.-K., *Food Chem.*, **228**, 567-573 (2017).
11) Prakash Chaturvedula V.-S., Prakash I., *J. Carbohydr. Chem.*, 30, 16-26 (2011).
12) Yang X.-W., Zhang J.-Y., Qian Z.-M., *Chin. Tradit. Herb. Drugs*, **39**, 810-814 (2008).
13) Takemoto T., Arihara S., Nakajima T., Okuhira M., *Yakugaku Zasshi*, **103**, 1167-1173 (1983).
14) Matsumoto K., Kasai R., Ohtani K., Tanaka O., *Chem. Pharm. Bull.*, **38**, 2030-2032 (1990).
15) Li C., Lin L.-M., Luo M., Ma C.-F., Wang Z.-M., China J. Chin. Mater. Med., **36**, 721-724 (2011).



1 **2**

Fig. 1 Chemical Structures of **C**ompounds **1** and **2**

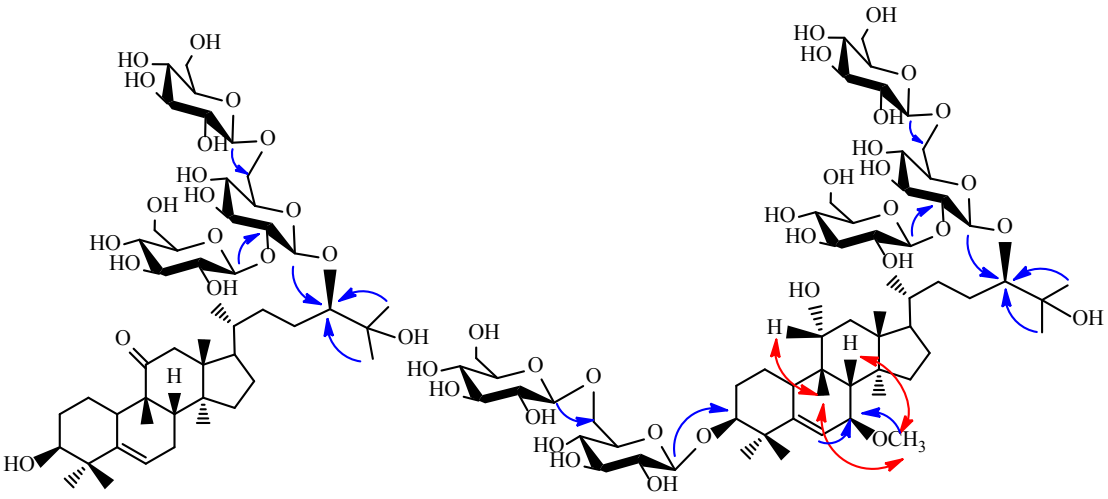


Fig. 2 Key HMBC and NOE correlations of Compounds **1** and **2**

Table 1. ^{1}H-NMR data for compounds **1** and **2**

	1	**2**		**1**	**2**
1	1.62 (o), 2.07 (o)	2.07 (m), 3.04 (m)	GlcI 1	4.91 (d, 7.4)	4.94 (d, 7.7)
2	1.84 (o), 1.92 (o)	1.91 (m), 2.46 (m)	2	4.19 (o)	4.18 (o)
3	3.71 (br s)	3.76 (br s)	3	4.23 (o)	4.24 (o)
4			4	3.94 (o)	3.94 (o)
5			5	4.08 (o)	4.08 (o)
6	5.68 (br s)	5.93 (d, 5.4)	6	3.94(o), 4.90 (o)	3.96 (o), 4.90 (o)
7	1.83 (o), 2.30 (o)	3.45 (d, 5.4)	GlcII 1	5.49 (d, 7.7)	5.45 (d, 7.7)
8	1.84 (o)	2.14 (br s)	2	4.05 (o)	4.04 (o)
9			3	4.22 (o)	4.22 (o)
10	2.55 (o)	2.87 (br d, 11.6)	4	4.23 (o)	4.23 (o)
11		4.21 (o)	5	3.92 (o)	3.91 (o)
12	2.58 (d, 14.2),	2.17 (o)	6	4.34 (o), 4.51(o)	4.34 (o), 4.51(o)
	3.07 (d, 14.2)	2.18 (o)			
13			GlcIII 1	4.86 (d, 7.6)	4.86 (d, 7.7)
14			2	4.06 (o)	4.06 (o)
15	1.19 (m), 1.31 (m)	1.20 (m), 1.25 (m)	3	4.22 (o)	4.22 (o)
16	1.50 (m), 2.25 (m)	1.50 (m), 2.16 (o)	4	4.23 (o)	4.23 (o)
17	1.89 (m)	1.81 (m)	5	3.96 (o)	3.96 (o)
18	0.77 (s)	0.84 (s)	6	4.34 (o), 4.51(o)	4.37 (o), 4.52 (o)
19	1.28 (s)	1.60 (s)	GlcIV 1		4.82 (d, 7.7)
20	1.46 (o)	1.57 (o)	2		3.85 (t, 8.3)
21	1.02 (d, 6.2)	1.11 (d, 6.3)	3		4.14 (o)
22	1.78 (o), 1.93 (o)	1.70 (o), 1.90 (o)	4		4.03 (o)
23	1.60 (m), 1.90 (m)	1.64 (o), 1.90 (o)	5		4.09 (o)
24	3.76 (d, 8.5)	3.78 (d, 9.8)	6		4.31 (o), 4.75 (o)
25			Glc V 1		5.14 (d, 7.7)
26	1.47 (s)	1.47 (s)	2		4.00 (o)
27	1.36 (s)	1.35 (s)	3		4.22 (o)
28	1.13 (s)	1.14 (s)	4		4.25 (o)
29	1.43 (s)	1.59 (s)	5		3.95 (o)
30	1.08 (s)	1.01 (s)	6		4.30 (o), 4.49 (o)
-OCH_3		3.29 (s)			

Table 2. ^{13}C-NMR data for compounds **1** and **2**

	1	**2**		**1**	**2**
1	21.3	26.9	GlcI 1	103.7	103.7
2	29.8	29.5	2	82.2	82.6
3	75.7	87.4	3	78.8	78.7
4	41.9	42.7	4	71.6	71.6
5	141.5	149.9	5	76.5	76.5
6	119.1	118.7	6	70.2	70.2
7	24.3	77.9	GlcII 1	105.6	105.7
8	44.2	47.8	2	76.0	76.0
9	49.2	40.0	3	78.1	78.1
10	36.3	37.9	4	72.5	72.6
11	214.2	78.2	5	78.5	78.5
12	48.9	41.0	6	62.7	62.7
13	49.2	47.2	GlcIII 1	104.9	104.9
14	49.8	48.5	2	75.5	75.5
15	34.7	34.6	3	78.4	78.4
16	28.4	28.6	4	71.6	71.6
17	50.0	51.1	5	78.1	78.1
18	17.1	17.1	6	63.6	63.6
19	20.3	26.3	GlcIV 1		107.1
20	36.0	36.7	2		75.1
21	18.8	19.1	3		78.6
22	33.1	33.3	4		71.7
23	29.3	29.6	5		77.4
24	92.1	92.1	6		70.3
25	72.8	72.9	Glc V 1		105.5
26	24.7	24.7	2		75.3
27	27.1	27.1	3		78.5
28	28.0	28.2	4		71.8
29	26.4	27.3	5		78.4
30	18.5	19.4	6		62.8
-OCH_3		56.2			