Note

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

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Two novel cucurbitane glycosides, named as 11-oxomogroside III A₁ and 7 β -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*; structural elucidation

Introduction

Siraitia grosvenori SWINGLE, belonging to the family 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 C₄₈H₈₀O₁₉ for compound **1** was determined by high resolution-electrospray ionization (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 ¹H-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 ¹³C-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 ¹H-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, brs, H-3) and 3.76 (1H, d, *J* = 8.5 Hz, H-24), and an olefinic methine hydrogen signal at δ 5.68 (1H, brs, H-6), while the ¹³C-NMR data indicated the existence 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 ¹H-NMR and ¹³C-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 $\rightarrow$ 2)-[β -D-glucopyranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranosyl was attached to C-24 of the aglycone based on the following important heteronuclear multiple bond connectivity (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 A₁.

Compound **2** was isolated as a white amorphous powder. The molecular formula C₆₁H₁₀₄O₃₀ 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 ¹H-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 ¹³C-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 ¹H-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, brs, 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 ¹³C-NMR data indicated the existence 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, -OCH₃) in ¹H-NMR spectrum and the carbon signal at δ 56.2 in ¹³C-NMR spectrum indicated the pres-

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Fig. 1. Chemical Structures of Compounds **1** and **2**Table 1. ¹H-NMR Data for Compounds **1** and **2**

1			2		
1	1.62 (o), 2.07 (o)		2.07 (m), 3.04 (m)	Glc I 1	4.91 (d, 7.4)
2	1.84 (o), 1.92 (o)		1.91 (m), 2.46 (m)	2	4.19 (o)
3	3.71 (brs)		3.76 (brs)	3	4.23 (o)
4				4	3.94 (o)
5				5	4.08 (o)
6	5.68 (brs)		5.93 (d, 5.4)	6	3.94(o), 4.90 (o)
7	1.83 (o), 2.30 (o)		3.45 (d, 5.4)	Glc II 1	5.49 (d, 7.7)
8	1.84 (o)		2.14 (brs)	2	4.05 (o)
9				3	4.22 (o)
10	2.55 (o)		2.87 (brd, 11.6)	4	4.23 (o)
11			4.21 (o)	5	3.92 (o)
12	2.58 (d, 14.2), 3.07 (d, 14.2)		2.17 (o), 2.18 (o)	6	4.34 (o), 4.51(o)
13				Glc III 1	4.86 (d, 7.6)
14				2	4.06 (o)
15	1.19 (m), 1.31 (m)		1.20 (m), 1.25 (m)	3	4.22 (o)
16	1.50 (m), 2.25 (m)		1.50 (m), 2.16 (o)	4	4.23 (o)
17	1.89 (m)		1.81 (m)	5	3.96 (o)
18	0.77 (s)		0.84 (s)	6	4.34 (o), 4.51(o)
19	1.28 (s)		1.60 (s)	Glc IV 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)		

ence of a methoxy group in **2**. The ¹³C-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 –OCH₃ (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 to the HMBC correlations (Fig. 2). Nuclear Overhauser effect (NOE) correlations between –OCH₃ (s, δ 3.29) and H-8 (brs, δ 2.14),

H-19 (s, δ 1.60) clearly confirmed the –OCH₃ group was in β orientation (Fig. 2). Thus, **2** was elucidated and named as 7 β -methoxy-mogroside V.

The sixteen known cucurbitane glycosides were elucidated as mogroside II A₁ (**3**),¹²⁾ mogroside II A₂ (**4**),¹³⁾ mogroside III (**5**),¹⁴⁾ mogroside III E (**6**),¹⁴⁾ mogroside III A₁ (**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

Fig. 2. Key HMBC and NOE Correlations of Compounds **1** and **2**Table 2. ^{13}C -NMR Data for Compounds **1** and **2**

	1	2		1	2
1	21.3	26.9	Glc I 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	Glc II 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	Glc III 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	Glc IV 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			

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 HR-ESI-MS spectra were acquired on a Vion IMS QT of (Waters Corp., Milford, MA, U.S.A.) in positive ion mode. 1D and two-dimensional NMR (2D-NMR) data were obtained on a Bruker Avance-600

spectrometer in $\text{C}_5\text{D}_5\text{N}$. Macroporous resin (HPD-100 A, 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., $10 \mu\text{m}$, 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-100 A 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. $[\alpha]_{\text{D}}^{20} + 20.3^\circ$ (c 0.20, MeOH). HR-ESI-MS m/z 983.5220 (Calcd for $\text{C}_{48}\text{H}_{80}\text{O}_{19}\text{Na}^+$: 983.5186).

^1H -NMR (pyridine- d_5) δ : Table 1.

^{13}C -NMR: Table 2.

Compound 2 A white amorphous powder. $[\alpha]_{\text{D}}^{20} + 7.9^\circ$ (c 0.15, MeOH). HR-ESI-MS m/z 1339.6501 (Calcd for $\text{C}_{61}\text{H}_{104}\text{O}_{30}\text{Na}^+$: 1339.6505).

^1H -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% H₂SO₄ (5 mL) under reflux for 8 h. The reaction mixture was extracted with EtOAc. The H₂O 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 $[\alpha]_D^{20} + 48.7^\circ$ (c 0.05, H₂O). Therefore, the configuration of the glucose in the new compounds should be in D-form.

Conflict of Interest The authors declare no conflict of interest.

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