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Additional cucurbitane glycosides from *Siraitia grosvenorii*

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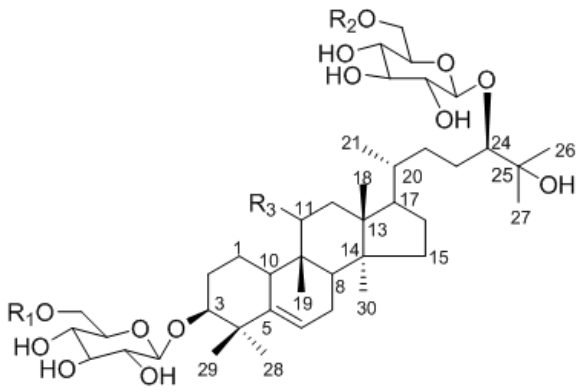
***Abstract*—Continuation of the phytochemical studies of the extract of Luo Han Go (*Siraitia grosvenorii*) furnished two additional known cucurbitane glycosides namely mogroside III A2, and 11-deoxymogroside III. The structures of the two isolated compounds 1-2 were characterized and their complete ^{1}H and ^{13}C NMR spectral assignments were made based on COSY, HMQC, HMBC and NOESY spectroscopic data, hydrolysis studies and in comparison with literature data.**

***Keywords*—*Siraitia grosvenorii*, Cucurbitaceae, Luo Han Go, Cucurbitane glycosides, NMR, MS, Chemical studies**

I. INTRODUCTION

The fruit of *Siraitia grosvenorii* (Swingle) Lu & Zhang (*Momordica grosvenorii*; Cucurbitaceae) is used as an expectorant and as a natural sweetener which grows widely in Guangxi, People's Republic of China [1-3]. The fruit of *S. grosvenorii*, also known as Luo Han Guo, has been used for centuries in traditional Chinese medicine for the treatment of pulmonary demulcent and emollient for the treatment of dry cough, sore throat, and constipation [4]. Luo Han Guo is well known now throughout the world due to its intense sweet taste and has been used as a non-caloric natural sweetener in some countries. Early chemical investigation revealed that the sweet principles of Luo Han Guo were triterpenoid glycosides also known as mogrosides [1-4]. In continuation of our study on the isolation of natural sweeteners from the commercial extracts of various sweet taste plants, we have recently reported isolation and characterization of several diterpene glycosides from *S. rebaudiana* [5-12], cucurbitane glycosides namely mogroside IVa, mogrosides V & VI, isomogroside V, 11-oxomogroside V and siamenoside I apart from the two phenolic glycosides namely kaempferol-3-*O*-α-L-rhamnoside and kaempferol-3,7-*O*-α-L-dirhamnoside from the aqueous alcoholic extract of Luo Han Go extract [13-14] based on their extensive NMR and Mass spectroscopic studies. In this paper we are describing the isolation and structure elucidation of the two additional cucurbitane glycosides, mogrosides III A2 (**1**), and 11-deoxymogroside III (**2**) that were characterized on the basis of COSY, HSQC, HMBC and NOESY spectral data as well as chemical studies (Figure 1).



Mogroside III A2 (**1**): R_1 = β-D-glucosyl; R_2 = H; R_3 = ⋯⋯OH

11-Deoxymogroside III (**2**): R_1 = H; R_2 = β-D-glucosyl; R_3 = H

Figure 1: Structures of Mogroside III A2 (**1**) and 11-Deoxymogroside III (**2**)

II. MATERIALS AND METHODS

General Methods

NMR spectra were acquired on a Varian Unity Plus 600 MHz instrument using standard pulse sequences at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. MS and MS/MS data were generated with a Waters Premier Quadrupole Time-of-Flight (Q-Tof) mass spectrometer equipped with an electrospray ionization source operated in the positive-ion mode and ThermoFisher Discovery OrbiTrap in the positive Positive Mode Electrospray. Samples were diluted with water: acetonitrile (1:1) containing 0.1% formic acid and introduced via infusion using the onboard syringe pump at ~10ul/min. Low pressure chromatography was performed on a Biotage Flash system using a C-18 cartridge (40+ M, 35-70 μm). TLC was performed on Baker Si-C_{18}F plates with mobile phase H_2O-MeOH (35:65). Identification of the spots on the TLC plate was carried out by spraying 10% H_2SO_4 in EtOH and heating the plate at about 80^o C. Analytical HPLC for sugar analysis was carried out with a Waters 600E multisolvent delivery system using a Phenomenex Luna C_{18} non-chiral (150 x 4.6 mm, 5 μm) column.

Materials

The Luo Han Guo commercial extract was purchased from Chengdu Biopurify Phytochemicals, China. A voucher specimen is deposited at The Coca Cola Company, No. VSPC-3166-99.

Isolation and Purification

The Luo Han Guo extract (50 g) was purified on a C-18 column using a Biotage Flash chromatography system (Solvent system: gradient from 20-80% MeOH-water, 60 mL/min, Detection: UV 210 nm) by collecting 60 fractions. Fractions 39-43 (1.2 g) were combined and subjected to repeated Flash Chromatography purification with gradient from 60-40% MeOH-water, 30 mL/min afforded mogroside III A2 (**1**, 80 mg). Fractions 44-48 (2.1 g) were combined and further subjected by Flash Chromatography purification two times (Solvent system: 70% MeOH-water, 20 mL/min, Detection: UV 210 nm) yielded the 11-deoxymogroside III (**2**, 46 mg).

General procedure for acid hydrolysis and determination of sugar configuration.

Each glycoside **1**-**2** (1 mg) was hydrolyzed with 0.5 M HCl (0.5 mL) for 1.5 h. After cooling, the mixture was passed through an Amberlite IRA400 column and the eluate was lyophilized. The residue was dissolved in pyridine (0.25 mL) and heated with L-cysteine methyl ester HCl (2.5 mg) at 60ºC for 1.5 h, and then *O*-tolyl isothiocyanate (12.5 uL) was added to the mixture and heated at 60ºC for an additional 1.5 h. The reaction mixture was analyzed by HPLC: column Phenomenex Luna C_{18}, 150 x 4.6 mm (5 u); 25% acetonitrile-0.2% TFA water, 1 mL/min; UV detection at 250 nm. The sugar was identified as D-glucose in each experiment (*t*R, 12.25 to 12.36 min) [authentic samples, D-glucose (*t*R, 12.34) and L-glucose (*t*R, 11.12 min)] [15].

III. RESULTS AND DISCUSSION

Purification of the commercial extract of Luo Han Guo (*S. grosvenorii*) resulted in the isolation of two additional cucurbitane glycosides **1**-**2**, which were identified as mogrosides III A2, and 11-deoxymogroside III. The structures of the two compounds were characterized from the detailed spectroscopic studies (COSY, HMQC, HMBC and NOESY) as well chemical studies, and in comparison of their spectral data with the reported values in the literature [16].

Compound **1**, was obtained as a white powder. The molecular formula was inferred as $C_{48}H_{82}O_{19}$ from MS studies. The ESI-MS spectrum of this compound was found at m/z 961 to correspond to [M-H] quasi-molecular ion, suggesting that the molecular weight of compound **1** is 962. The ^{1}H NMR spectrum of **1** showed the presence of seven methyl singlets at δ 0.89, 0.90, 1.15, 1.34, 1.47, 1.50, and 1.57, a methyl doublet at 1.06, eight methylene and seven methine groups between δ 1.15-3.86 (including three secondary hydroxyl groups), and a tertiary hydroxyl resonating at δ_C 72.8; characteristic to the aglycone moiety of the triterpenoid mogrol isolated earlier from *S.grosvenorii*. The ^{1}H NMR spectrum of **1** also showed the presence three anomeric protons as doublets at δ 4.96, 5.11 and 5.35 suggesting the presence of three hexose moieties which was supported by the fragment ions observed in the negative mode of ESI-MS/MS spectrum at m/z 799, 637 and 475. Acid hydrolysis of **1** afforded a hexose unit that was identified as glucose and its configuration was confirmed as D-glucose by preparing the corresponding thiocarbamoyl-thiazolidine carboxylate derivative with L-cysteine methyl ester and *O*-tolyl isothiocyanate, and in comparison of its retention time with the standard sugars as described in the literature [15]. The ^{1}H and ^{13}C NMR values for all the protons and carbons were assigned on the basis of COSY, HMQC and HMBC correlations and were given in Tables 1-2.

Table 1: ^{1}H NMR chemical shifts values for the compounds **1-2** in C_5D_5N[a-c]

Proton	**1**	**2**
1	1.48 m, 1.96 m	1.46 m, 1.84 m
2	1.93 m, 2.42 m	1.93 m, 2.43 m
3	3.45 br s	3.64 br s
6	5.49 d (6.2)	5.47 d (6.2)
7	1.81 m, 2.35 m	1.83 m, 2.23 m
8	1.65 br s	1.63 br s
10	2.48 d (12.4)	2.26 d (12.1)
11	3.86 m	1.34 m, 1.62 m
12	1.82 m, 1.86 m	1.43 m, 1.58 m
15	1.15 m, 1.20 m	1.12 m, 1.20 m
16	1.33 m, 1.98 m	1.42 m, 2.06 m
17	1.63 d (9.1)	1.58 d (9.1)
18	0.89 s	0.86 s
19	1.50 s	0.89 s
20	1.44 m	1.48 m
21	1.06 d (6.2)	0.97 d (6.0)
22	1.51 m, 1.58 m	1.72 m, 1.86 m
23	1.85 m	1.80 m, 1.88 m
24	3.40 m	3.72 br d (9.8)
26	1.47 s	1.45 s
27	1.34 s	1.37 s
28	1.15 s	1.12 s
29	1.57 s	1.54 s
30	0.90 s	0.88 s
Glucose-1		
1	5.11 d (7.2)	5.04 d (7.5)
2	4.04 m	3.91 m
3	4.21 m	4.22 m
4	3.94 m	4.10 m
5	4.08 m	3.90 m
6	3.98 m, 4.92 m	4.38 m, 4.56 m
Glucose-2		
1	4.96 d (7.4)	4.98 d (7.6)
2	4.04 m	4.02 m
3	4.18 m	4.17 m
4	4.32 m	3.98 m
5	3.90 m	4.12 m
6	4.14 m, 4.36 m	3.95 m, 4.88 m
Glucose-3		
1	5.35 d (7.6)	4.86 d (7.4)
2	4.03 m	4.01 m
3	4.26 m	4.24 m
4	4.34 m	4.30 m
5	3.93 m	3.85 m
6	4.16 m, 4.38 m	4.22 m, 4.46 m

[a] Assignments were made on the basis of COSY, HMQC and HMBC; [b] Coupling constants are in Hz; [c] Chemical shift values are in δ (ppm).

Table 2: ^{13}C NMR chemical shift values (δ, ppm) for the compounds **1-2** in C_5D_5N[a]

Carbon	**1**	**2**
1	27.2	27.3
2	29.9	29.4
3	88.4	88.2
4	42.7	41.8
5	144.7	143.4
6	118.9	118.7
7	25.0	24.9
8	43.9	44.0
9	40.5	34.6
10	37.1	38.2
11	77.0	32.6
12	41.4	30.5
13	47.8	46.9
14	50.1	49.2
15	34.9	34.9
16	28.9	28.0
17	51.3	51.3
18	17.5	17.6
19	26.7	28.2
20	37.2	36.4
21	19.7	19.0
22	34.2	33.3
23	29.9	30.0
24	92.4	92.6
25	72.8	72.8
26	26.3	24.2
27	21.9	26.9
28	28.0	28.4
29	27.2	26.1
30	19.5	18.1
Glucose-1		
1	107.8	107.5
2	75.7	75.7
3	78.2	78.6
4	71.4	71.7
5	76.3	78.3
6	70.0	63.1
Glucose-2		
1	106.8	106.5
2	75.3	75.3
3	78.2	78.7
4	71.7	71.9
5	78.2	76.7
6	62.8	70.6
Glucose-3		
1	102.3	104.8
2	75.3	75.5
3	78.8	78.0
4	71.7	71.5
5	78.4	78.5
6	62.9	62.7

[a] Assignments were made on the basis of COSY, HMQC and HMBC

The placement of the three sugar units in **1** was confirmed as a 1→6 β-D-glucobiosyl substituent at C-3 with an additional β-D-glucosyl unit at C-24 position on the basis of the key HMBC and NOESY correlations as shown in Figure 2. A close comparison of the NMR spectral data of **1** with the reported literature values for mogroside III A2 confirmed its structure [16].

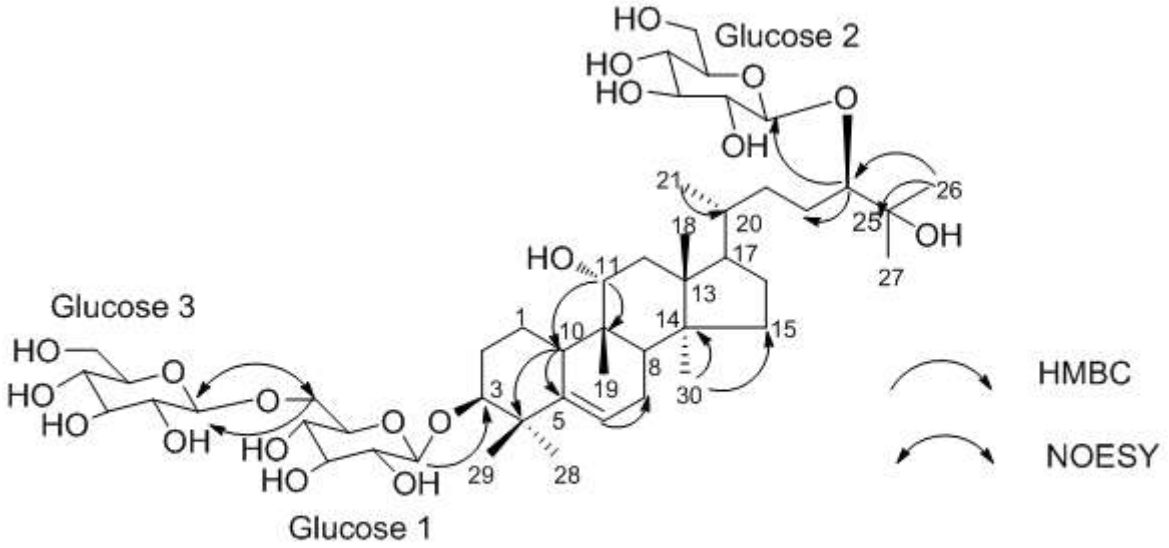


Figure 2: Key HMBC and NOESY correlation of Mogroside III A2 (**1**)

Compound **2** was also obtained as a white amorphous powder and its molecular formula was inferred as $C_{48}H_{82}O_{18}$ from the ESI-MS/MS spectrum. A peak at m/z 945 corresponding to [M-H] in the ESI-MS spectrum, suggested that the molecular weight of compound **2** might be 946. The ^{1}H NMR spectrum of **2** showed the presence of seven methyl singlets, one methyl doublet, eight methylenes and seven methines, identical to **1**. The ^{1}H NMR spectrum of **2** showed the presence three anomeric protons suggesting the presence of three hexose moieties and acid hydrolysis confirmed the sugar and its stereochemistry as D-glucose. A close comparison of the ^{1}H and ^{13}C NMR chemical shift values of **1** and **2** together with their ESI-MS data which has 16 amu difference suggested the absence of the hydroxyl group at C-11 position which was supported by the presence of two secondary hydroxyl groups in its aglycone part from the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2). The three β-D-glucosyl moieties were present as 1→6 linked β-D-glucobiosyl substituent at C-24 with an additional β-D-glucosyl moiety at C-3 were identified on the basis of the key HMBC and NOESY correlations as shown in Figure 3. The ^{1}H and ^{13}C NMR spectral data (Tables 1-2) for compound **2** were assigned based on the COSY, HSQC and HMBC spectra are consistent with 11-deoxymogroside III confirmed the structure completely [16].

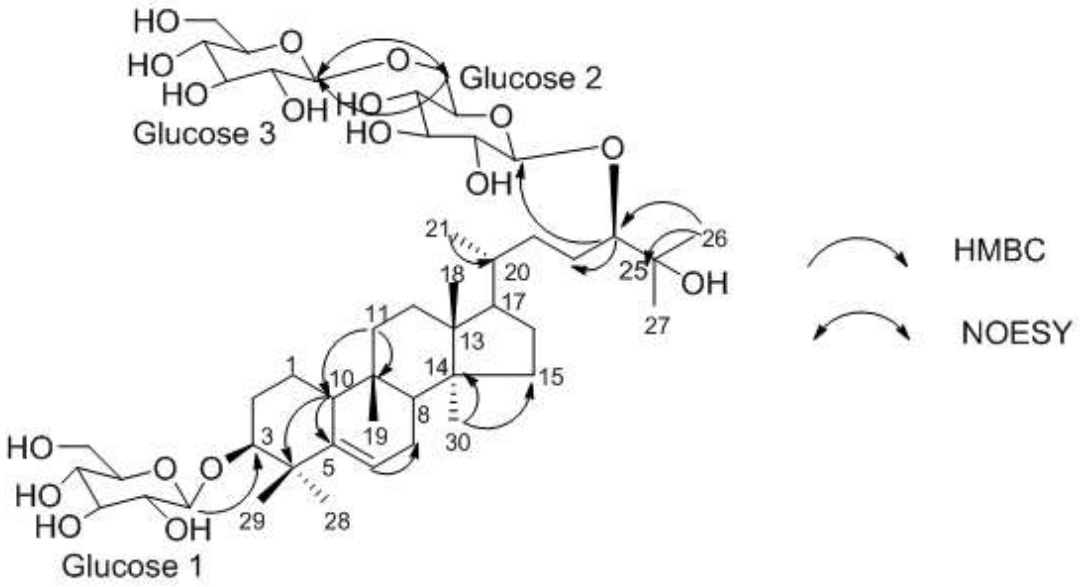


Figure 3: Key HMBC and NOESY correlation of 11-Deoxymogroside III (**2**)

IV. CONCLUSIONS

Though the two isolated compounds are reported in the literature, the detailed NMR characterization has not been studied on the basis of 1D and 2D NMR as well as chemical studies. This is a compilation of the ^{1}H and ^{13}C NMR spectral data for the two compounds mogrosides III A2, and 11-deoxymogroside III in CD_5N_5 based on the extensive 2D NMR spectroscopic data and chemical studies.

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