Note

Sweet Cucurbitane Glycosides from Fruits of *Siraitia siamensis* (chi-zi luo-han-guo), a Chinese Folk Medicine

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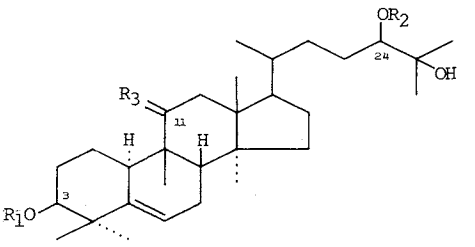
Cucurbitane-type triterpenes are known as bitter principles of cucurbitaceous plants, while some of the glycosides of triterpenes of this type are sweet tasting; bryodulcoside[1] from roots of *Bryonia dioica* JACQ., mogrosides IV (**1**) and V (**2**)[2～4] from fruits of *Siraitia grosvenori* SWINGLE (=*Momordica grosvenori* SWINGLE; Chinese name of the fruit, luo-han-guo) and glycosides from rhizomes of *Hemsleya carnosiflora* C. Y. WU *et* Z. L. CHEN sp. nov.[5] and *H. panacis-scandens* C. Y. WU *et* Z. L. CHEN.[6] In our series of studies on Chinese cucurbitaceous plants, the present paper describes the characterization of sweet glycosides from fruits of *Siraitia siamensis* CRAIB. (Chinese name, chi-zi lou-han-guo) which grows in Xi-shuang-bana, South-Yunnan, China and is closely related to *S. grosvenori*.

Dried and powdered fruits were extracted with petroleum ether to remove the lipophilic materials and then extracted with methanol. The sweet methanolic extract was separated by chromatography on a highly porous polymer, then on silica gel and finally by high-performance liquid chromatography (HPLC) on a reverse-phase column to give six glycosides, A-F, in yields of 0.036, 0.57, 0.047, 0.12, 0.013 and 0.055%, respectively.

Glycosides B and D were identified as **2** and **1**, respectively, both of which have already been isolated from Luo-han-guo by Takemoto *et al.*[2～4]

A new sweet glycoside A (**4**) afforded D-glucose on acid hydrolysis. The ^{1}H- and ^{13}C-NMR spectra of **4** exhibited signals due to anomeric protons and carbons of five glucoside units (see the experimental section).

The ^{1}H- and ^{13}C-NMR signals of mogrol (**3**), which is the common aglycone of **1** and **2**, were characterized by means of ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY and 2D-INADEQUATE procedures as shown in Tables I and II. In a comparison of the ^{13}C-NMR spectra of **1** and **3**, the signal due to the 24-carbinyl carbon was displaced downfield by 9.0 ppm on glycosylation (glycosylation



	R_1	R_2	R_3
1	-Glc^{6}—Glc	-Glc^{2}—Glc	H, OH
2	-Glc^{6}—Glc	-Glc^{2}(6-Glc)—Glc	H, OH
3	-H	-H	H, OH
4	-Glc^{6}—Glc	-Glc^{2}(6-Glc)—Glc	=O
5	-H	-H	=O
6	-Glc	-Glc^{2}(6-Glc)—Glc	H, OH

Glc: β-D-glucopyranosyl

Chart 1.

Table I. ^{1}H-NMR SPECTRAL DATA OF MOGROL (**3**)

(400 MHz, d_6-acetone with TMS)

H		
3	3.40 t	J=2.7 Hz
6	5.47 d	J=6.1 Hz
7α	1.78 dd	J=10.4, 6.1 Hz
8	1.67 d	J=7.3 Hz
10	2.55 br. d	J=12.1 Hz
11	3.87 dd	J=11.4, 5.5 Hz
17	1.59 br. t	J=8.2 Hz
18	0.91 s	
21	0.95 d	J=6.2 Hz
22	1.30 dd	J=9.4, 1.7 Hz
23	1.35 br. d	J=10.3 Hz
24	3.25 dd	J=10.3, 2.0 Hz

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Table II. ^{13}C-NMR SPECTRAL DATA OF THE AGLYCONE MOIETIES OF **1**~**6**

(100 MHz, C_5D_5N with TMS)

C-No.	**3**	**1**	**2**	**6**	**5**	**4**
1	25.8	26.8	26.8	26.8	21.2	22.2
2	30.8	29.4	29.4	29.4[b]	29.7	29.2
3	76.2	87.4	87.4	87.9	75.5	86.6
4	42.2	42.3	42.2	42.3	41.8	41.9
5	144.3	144.3	144.3	144.2	141.3	141.2
6	119.1	118.4	118.4	118.4	118.9	118.4
7	24.5	24.5	24.6	24.5	24.2	24.0
8	43.6	43.5	43.5	43.5	44.0	44.0
9	40.2	40.1	40.1	40.1	49.1	49.0
10	36.9	36.7	36.7	36.7	35.9	35.9[b]
11	77.8	77.9	77.9	77.9	213.8	213.9
12	41.2	41.0	41.0	41.1	48.7	48.8
13	47.4	47.4	47.4	47.4	49.1	49.0
14	49.8	49.7	49.7	49.6	49.6	49.7
15	34.5	34.5	34.5	34.5	34.5	34.5
16	28.4	28.5	28.5	28.5	28.1	28.3
17	51.0	50.9	51.0	51.0	49.8	49.9
18	17.0	17.0	17.1	17.1	16.9[a]	17.0[a]
19	26.7[a]	27.0	27.0	27.0	20.1	20.3
20	36.3	36.7	36.3	36.3	35.9	36.2[b]
21	18.9	19.1	19.1	19.0	18.2[a]	18.3[a]
22	34.2	33.8	33.2	33.2	33.9	33.0
23	29.0	28.5	29.4	29.5[b]	28.6	28.5
24	79.0	88.0	91.9	92.1	78.9	92.0
25	72.7	72.4	72.7	72.8	72.7	72.7
26	25.8[a]	25.8[a]	24.6[a]	24.5[a]	25.9	25.8
27	26.2[a]	26.2[a]	26.2[a]	26.3[a]	26.1	24.6
28	19.3[a]	19.3[a]	19.4[a]	19.4[a]	18.5[a]	18.7[a]
29	27.3	27.6	27.6	27.6	27.9	28.2
30	26.2[a]	26.2[a]	26.2[a]	26.3[a]	27.0[a]	27.0[a]

[a,b] These signals may be interchanged in each column.

shift).[7~9] On the other hand, an unexpectedly large glycosylation shift (+12.9 ppm) of the 24-C signal was observed for **2** which had a bulky sugar moiety (branched chain) at the 24-hydroxyl group. This anomalous shift is useful for allocating the sugar moiety of the related glycosides.

A comparison of aglycone carbon signals for **4** with those of 11-oxomogrol[3] (=bryoducosigenin, **5**[1]) and **2** (Table II) indicated that **4** must be a 3,24-*O*-bisglycoside of **5**. The carbon signals due to the sugar moiety of **4** were almost superimposable on those of **2**. The anomalous glycosylation shift of 24-C (*vide supra*) was observed from **5** to **4**, leading to the structure of 11-oxomogroside V as shown in Chart 1.

A new sweet glycoside C named siamenoside I (**6**) afforded D-glucose after acid hydrolysis. The ^{1}H- and ^{13}C-NMR spectra showed signals due to anomeric protons and carbons of four β-glucoside units (see the experimental section). The EI-MS of an acetate (**7**) of **6** exhibited fragment ions due to Glc-Ac_4 (*m/z* 331) and Glc_3-Ac_{10} (*m/z* 907) but no fragment ion due to Glc_2-Ac_7 (*m/z* 619) suggesting the presence of a branched chain glucotrioside moiety like that in **2**. All of the carbon signals due to the aglycone moiety of **6** appeared at very similar chemical shifts to those of **2**, including the anomalous glycosylation shift of 24-C (Table II). These results indicated that **6** must be 3-*O*-β-glucosyl-24-*O*-glucotriosyl (branched)-mogrol. The structure of the 24-*O*-glucotriosyl moiety of **6** was elucidated by means of the ^{1}H-^{1}H COSY and ^{1}H-^{1}H NOESY spectra (in acetone-d_6) of **7**. In the ^{1}H-NMR spectrum of **7**, most of the carbinyl proton signals of the glycosyl moiety appeared downfield by acetylation shift, while signals which were not displaced downfield [δ 3.71 (1H, dd, J=7.7, 8.3 Hz) and δ 3.68 (1H, dd, J=4.0, 12.1 Hz) and 3.79 (1H, dd, J=2.3, 12.1 Hz)] were respectively characterized as 2-H and 6-H_2 in the same glucoside unit based on the ^{1}H-^{1}H COSY procedure. The presence of NOE between this 2-H signal and an anomeric proton signal at δ 4.51 (1H, d, J=7.9 Hz), as well as between one of the 6-H_2 signals (δ 3.68, *vide supra*) and an anomeric proton signal at δ 4.79 (1H, d, J=8.1 Hz), was substantiated by the ^{1}H-^{1}H NOESY procedure. These results indicated the presence of a 2,6-di-*O*-β-glucosyl-β-glucoside moiety. A signal at δ 4.45 (1H, d, J=7.7 Hz) was assigned as an anomeric proton of the foregoing 2,6-linked glucosyl unit by the ^{1}H-^{1}H COSY procedure. The presence of NOE between this anomeric proton signal and the 24-carbinyl proton signal of the aglycone moiety at δ 3.40 (1H, br. d, J=8.3 Hz) was observed by the ^{1}H-^{1}H NOESY procedure. This revealed the allocation of the 2,6-linked glucoside unit at the 24-hydroxyl group. The presence of NOE between a signal due to the 3-carbinyl proton of the aglycone moiety at δ 3.34 (1H, br. s) and a remaining anomeric proton signal at δ 4.60 (1H, d, J=8.0 Hz) was also observed by the ^{1}H-^{1}H NOESY procedure. Thus, **6** could be formulated as mogrol-3-*O*-β-D-glucopyranosido-24-*O*-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside.

A structural elucidation of compounds E and F has not yet been done due to the shortage of materials.

Experimental

NMR spectra were recorded with a JEOL GX 400 spectrometer at 400 MHz for protons and at 100 MHz for carbon-13 in C_5D_5N unless otherwise stated.

Plant material. The plant was collected at Xi-shuang-bana, South Yunnan, China and identified by Emeritus Professor C. Y. Wu of the Kunming Institute of Botany, Chinese Academy of Science. A voucher specimen has been deposited in the herbarium of this institute.

Conditions for preparative HPLC. Reverse-phase column, TSK-GEL ODS-120T (21 mm i.d. × 30 cm); detection, R.I. and UV (210 nm); flow rate, 6 ml/min.

Acid hydrolysis and subsequent identification of the resulting monosaccharide. A solution of a few milligram of each glycoside in 1 N H_2SO_4/50% EtOH (1 ml) was heated at 100°C in a sealed tube for 4 hr. The reaction mixture was treated with $BaCO_3$ and then with Amberlite MB-3, and concentrated to dryness. The residue was subjected to the identification of monosaccharides including the absolute configuration that was reported by Ohshima *et al.*[10] Each compound (**1**, **2**, **4**, **6**, and glycosides E and F) afforded D-glucose.

Extraction and separation. Dried and powdered fruits (207 g) were defatted by extracting with petroleum ether and then extracted with hot MeOH. This extract (65 g) was chromatographed on highly porous polymer resin (DA-201, made in China) by eluting with H_2O and then with 70% MeOH. The 70% MeOH eluate was dissolved in MeOH to remove the MeOH-insoluble substances to give an MeOH-soluble sweet fraction (10.5 g), which was chromatographed on silica gel by eluting with $CHCl_3$–MeOH (9 : 1 then 5 : 5) to separate into seven fractions (I ~ VII). A part (1.5 g) of fraction V (2.7 g) was further chromatographed on silica gel by eluting with $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous) to give fractions V-1, -2 and -3. Fraction V-2 (544 mg) was subjected to HPLC (mobile phase, 57% MeOH) to give **2** (140 mg) and **6** (54 mg). Fraction V-3 (917 mg) was separated by chromatography on silica gel [$CHCl_3$–MeOH–H_2O solvent (6 : 4 : 1, homogeneous)] and followed by HPLC (54% MeOH mobile phase) to give **1** (372 mg) and glycoside E (15 mg).

A part (503 mg) of fraction VI (910 mg) was separated by chromatography on silica gel [$CHCl_3$–MeOH–H_2O solvent (6 : 4 : 1, homogeneous)] and followed by HPLC (mobile phase, 54% MeOH) to give **4** (42 mg) and **1** (270 mg). A part (503 mg) of fraction VII (1.24 g) was subjected to chromatography on silica gel by eluting with $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous) and followed by HPLC (mobile phase, 56% MeOH) to give glycoside F (60 mg).

1 (mogroside IV): a white powder, $[\alpha]_D^{18}$ −4.4° (c=0.91, MeOH), NMR δ_H anomeric H: 4.81 (1H, d, J=7.6 Hz), 5.07 (1H, d, J=7.3 Hz), 5.16 (1H, d, J=7.6 Hz), 5.33 (1H, d, J=7.3 Hz). **2** (mogroside V): a white powder, $[\alpha]_D^{23}$ −6.3° (c=0.50, H_2O), NMR δ_H anomeric H: 4.80 (1H, d, J=7.8 Hz), 4.86 (1H, d, J=7.5 Hz), 4.89 (1H, d, J=7.8 Hz), 5.15 (1H, d, J=7.8 Hz), 5.47 (1H, d, J=7.5 Hz). Identification of **1** and **2** was conducted by comparing the optical rotation, and ^{1}H- and ^{13}C-NMR spectra with those of respective authentic samples. **4**: A white powder, $[\alpha]_D^{18}$ +24.3° (c=1.07, MeOH). *Anal.* Found: C, 53.41; H, 7.79%. Calcd. for $C_{60}H_{100}O_{29}$ 7/2 H_2O: C, 53.44; H, 8.00%. NMR δ_H anomeric H: 4.81 (1H, d, J=8.6 Hz), 4.86 (1H, d, J=7.6 Hz), 4.90 (1H, d, J=7.3 Hz), 5.13 (1H, d, J=7.9 Hz), 5.49 (1H, d, J=7.3 Hz); δ_C anomeric C: 103.6 (1C), 104.8 (1C), 105.4 (2C), 106.9 (1C). **6**: A white powder, $[\alpha]_D^{18}$ +3.5° (c=0.52, MeOH). *Anal.* Found: C, 54.58; H, 8.10%. Calcd. for $C_{54}H_{92}O_{24}$ 7/2 H_2O: C, 54.58; H. 8.40%. NMR δ_H anomeric H: 4.86 (1H, d, J=7.6 Hz), 4.87 (1H, d, J=8.0 Hz), 4.91 (1H, d, J=7.3 Hz), 5.47 (1H, d, J=7.6 Hz); δ_C anomeric C: 103.7 (1C), 104.8 (1C), 105.4 (1C), 107.3 (1C).

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References

1) P. Tunmann, W. Gerner and G. Stapel, *Arch. Pharm.*, **299**, 597 (1966).
2) T. Takemoto, S. Arihara, T. Nakajima and M. Okuhira, *Yakugaku Zasshi*, **103**, 1151 (1983).
3) T. Takemoto, S. Arihara, T. Nakajima and M. Okuhira, *Yakugaku Zasshi*, **103**, 1155 (1983).
4) T. Takemoto, S. Arihara, T. Nakajima and M. Okuhira, *Yakugaku Zasshi*, **103**, 1167 (1983).
5) R. Kasai, K. Matsumoto, R.-L. Nie, T. Morita, A. Awazu, J. Zhou and O. Tanaka, *Phytochemistry*, **26**, 1371 (1987).
6) R. Kasai, K. Matsumoto, R.-L. Nie, J. Zhou and O. Tanaka, *Chem. Pharm. Bull.*, **36**, 234 (1988).
7) R. Kasai, M. Suzuo, J. Asakawa and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175.
8) R. Kasai, M. Okihara, J. Asakawa, K. Mizutani and O. Tanaka, *Tetrahedron*, **35**, 1427 (1979).
9) K. Mizutani, Z. Kasai and O. Tanaka, *Carbohydr. Res.*, **87**, 19 (1980).
10) R. Ohshima, J. Kumanotani and C. Watanabe, *J. Chromatogr.*, **259**, 159 (1983).