Minor Cucurbitane-Glycosides from Fruits of *Siraitia grosvenori* (Cucurbitaceae)

Kazuhiro MATSUMOTO,[a] Ryoji KASAI,*[,b] Kazuhiro OHTANI[b] and Osamu TANAKA[b]

Central Research Laboratories, Asahi Breweries, Ltd,[a] 2–13–1 Omorikita, Ota-ku, Tokyo 143, Japan and Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[b] Kasumi, Minami-ku, Hiroshima 734, Japan. *Received December 12, 1989*

From fruits of *Siraitia grosvenori*, a Chinese medicinal plant, a new minor glycoside and four known minor cucurbitane glycosides, siamenoside I (sweet), 11-oxo-mogroside V (sweet), and mogrosides IIE and IIIE (both tasteless) were isolated together with mogrosides IV and V (both sweet) previously isolated from this fruit by Arihara *et al.* Structure of the new tasteless glycoside called mogroside III was elucidated as 3-*O*-β-D-glucopyranosyl-24-*O*-β-gentiobiosyl-mogrol. The relative sweetness of siamenoside I to sucrose was estimated × 563, making this the sweetest compound among the cucurbitane-glycosides so far isolated. The structure–taste relationship of cucurbitane-glycosides is also described.

Keywords *Siraitia grosvenori*; *Momordica grosvenori*; Cucurbitaceae; sweet principle; cucurbitane-glycoside; Chinese medicinal plant; mogroside; siamenoside

A fruit of *Siraitia grosvenori* SWINGLE (*Momordica grosvenori* SWINGLE., 羅漢果, Cucurbitaceae) growing in Kwangshi, China is used as an expectorant as well as a natural sweet food in that country. Two major sweet cucurbitane-glycosides called mogrosides IV (**1**) and V (**2**) have been isolated by Takemoto, Arihara *et al.*[1–3] Very recently, in our serial studies on Chinese cucurbitaceous medicinal plants, two new minor sweet glycosides called siamenoside I (**3**) and 11-oxo-mogroside V (**4**)[4] were isolated together with **1** and **2** from *Siraitia siamensis* CRAIB (翅子羅漢果) collected in South-Yunnan, China. The present paper reports the isolation and identification of minor cucurbitane glycosides from fruits of *Siraitia grosvenori*. The structure–taste relationship of glycosides of this type is also described.

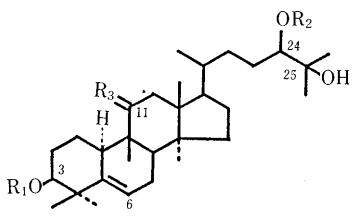
The dried fruits were extracted with methanol. A suspension of methanol-extract in water was defatted with hexane and then chromatographed on a highly porous synthetic polymer, Diaion HP-20. The fractions eluted with 50% and 80% methanol were respectively separated by repeated chromatography to give seven glycosides, A—G in yields of 0.025%, 0.008%, 0.029%, 0.044%, 0.034%, 0.18% and 0.45%, respectively.

Glycosides D, E, F and G were identified as **3**, **1**, **4** and **2**, respectively. Glycosides A and C were identified as mogrosides IIE (**5**) and IIIE (**6**), respectively, which were obtained from **2** by partial hydrolysis with maltase.[3] This is the first example of the occurrence of **5** and **6** in nature.

Comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of a new glycoside B (**7**) called mogroside III with that of mogrol (**8**), the common aglycone of **1**, **2** and **3**, showed the glycosylation shifts[5] for C-2, C-3 and C-24 as in the case of **2**, indicating that **7** is 3- and 24-diglycosyl-mogrol. On acid hydrolysis, **7** yielded D-glucose and the coupling constants of three anomeric proton signals demonstrated anomeric configurations of three glucopyranosyl units to be β. In the electron impact mass spectrum (EI-MS) of acetylated **7**, the fragment ions associated with terminal glycosyl (*m/z* 331) and glycobiosyl units (*m/z* 619) were observed. The sequencing analysis of permethylated **7** revealed the presence of terminal and 6-linked glucopyranosyl residues.[6] These results indicated the presence of β-D-glucosyl and β-gentiobiosyl residues in **7**.

The allocation of β-D-glucosyl and β-gentiobiosyl groups on the aglycone (**8**) was elucidated by means of the nuclear Overhauser effect (NOE) spectrum of acetylated **7**. The assignment of signals due to three sets of β-glucosyl protons of acetylated **7** was established by means of ^{1}H–^{1}H two dimensional correlation spectroscopy (2D COSY) as summarized in Table I. In the NOE spectrum, cross peaks were observed between an anomeric proton at δ 4.98 (1H, d, J=8.0 Hz) and 6-H_2 signals at δ 3.65 (1H, dd, J=7.3, 12.0 Hz) and 3.74 (1H, dd, J=2.2, 12.0 Hz), and were proved by ^{1}H–^{1}H 2D COSY to be located in the same glucosyl unit as that of an anomeric proton which appeared at δ 4.84 (1H, d, J=7.6 Hz). Accordingly, signals at δ 4.84 and 4.98 were assigned as anomeric protons of inner and terminal β-glucosyl units of a β-gentiobiosyl moiety, respectively. It follows that the remaining signal at δ 4.71 (1H, d, J=8.0 Hz) is assigned as an anomeric proton of an unsubstituted β-glucosyl unit. In the NOE spectrum, cross peaks were observed between the signal at δ 4.84 and the signal due to H-24 of the aglycone moiety at δ 3.48 (1H, dd, J=10.5, 5.4 Hz), and also between the signal at δ 4.71 and the signal due to H-3 of the aglycone moiety at δ 3.42 (1H, br s). Based on these results, **7** was formulated as 3-*O*-β-D-glucopyranosyl-24-*O*-β-gentiobiosyl-mogrol.

The relative sweetness to sucrose was determined in an 0.012% aqueous solution for **1** and **2**, 0.01% solution for **3** and 0.05% solution for **4** by a panel of five professional tasters in the manner described previously.[7] This panel also determined taste quality using an aqueous solution of



Glc: β-D-glucopyranosyl

	R_1	R_2	R_3		R_1	R_2	R_3
1	–Glc^{6}Glc	–Glc^{2}Glc	···OH H	**5**	–Glc	–Glc	···OH H
2	–Glc^{6}Glc	–Glc^{2}Glc ^{6}Glc	···OH H	**6**	–Glc	–Glc^{2}Glc	···OH H
3	–Glc	–Glc^{2}Glc ^{6}Glc	···OH H	**7**	–Glc	–Glc^{6}Glc	···OH H
4	–Glc^{6}Glc	–Glc^{2}Glc ^{6}Glc	=O	**8**	–H	–H	···OH H

TABLE I. ^{1}H-NMR Chemical Shifts (δ) for Sugar Moiety of Acetylated **7** in Acetone-d_6[a]

3-O-Sugar		24-O-Sugar			
		Inner		Terminal	
H-1	4.71 (d, J=8.0)	H-1	4.84 (d, J=7.6)	H-1′	4.98 (d, J=8.0)
H-2	4.82 (dd, J=8.0, 9.8)	H-2	4.89 (dd, J=7.6, 9.6)	H-2′	4.84 (dd, J=8.0, 9.6)
H-3	5.18 (dd, J=9.3, 9.8)	H-3	5.19 (dd, J=9.3, 9.6)	H-3′	5.21 (dd, J=9.6, 9.6)
H-4	4.91 (dd, J=9.3, 10.2)	H-4	4.82 (dd, J=9.3, 10.2)	H-4′	4.94 (dd, J=9.6, 10.2)
H-5	3.85 (ddd, J=2.5, 5.5, 10.2)	H-5	3.87 (ddd, J=2.2, 7.3, 10.2)	H-5′	3.86 (ddd, J=2.5, 5.2, 10.2)
H-6	4.04 (dd, J=2.5, 12.0)	H-6	3.65 (dd, J=7.3, 12.0)	H-6′	4.06 (dd, J=2.5, 12.2)
	4.18 (dd, J=5.5, 12.0)		3.74 (dd, J=2.2, 12.0)		4.19 (dd, J=5.2, 12.2)

a) Measured at 400 MHz with TMS as the internal standard; coupling constants are given in Hz (d, doublet).

TABLE II. Taste and Relative Sweetness

Compound	Conc.[a] (%)	Taste	Relative sweetness[b]
1	0.012	Sweet	392
2	0.012	Sweet	425
3	0.010	Sweet	563
4	0.05	Sweet	84
5		Tasteless	
6		Tasteless	
7		Tasteless	

a) Concentration of sample in aqueous solution (w/v%). b) Sucrose = 1.

samples at a concentration which exhibited similar sweetness to that of a 5% aqueous solution of sucrose. The results are summarized in Table II.

We previously isolated a number of cucurbitane glycosides from the rhizomes of *Hemsleya carnosiflora* C. Y. WU *et* Z. L. Chen[8] and *H. panacis-scandens* C. Y. WU *et* Z. L. Chen[6] collected in Yunnan, China. In those studies on the structure–taste relationship of these compounds and their derivatives, it was suggested that the oxygen function at the 11-position of the aglycone moiety is responsible for the occurrence of taste; glycosides of 11α-hydroxy-compounds taste sweet, while glycosides of 11β-hydroxy-compounds are tasteless and 11-keto-glycosides taste bitter.[6,8] In the present study, glycosides of 11α-hydroxy-aglycone, **1**, **2** and **3** were found to taste very sweet, while a remarkable decrease in sweetness was observed for the glycoside (**4**) of 11-keto-aglycone. The quality of taste of **4** was also significantly poorer than **2** and **3**.

It has been observed that the number of glucose units is also responsible for the occurrence of taste.[6,8] Mogrol-glycosides, **5**, **6** and **7** which have less than three glycosyl units, are almost tasteless. The relationship between the allocation of glucosyl units and sweetness is also noteworthy. Siamenoside I (**3**) which has four glucosyl units, is the sweetest compound among glycosides of this type so far isolated and shows a similar good the taste quality to **2** which has five glucosyl units, while the sweetness of mogroside IV (**1**) with the same number of glucosyl units as **3** is even less than **3**.

Experimental

Optical rotations were measured with a Union PM-101 automatic digital polarimeter and a Jasco DIP-360 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 spectrometer in C_5D_5N solution (acetates of **7**: in acetone-d_6) using tetramethylsilane (TMS) as an internal standard. EI-MS and fast atom bombardment mass spectrum (FAB-MS) were recorded on a JEOL SX-102 spectrometer. Gas liquid chromatography mass spectrometry (GC-MS) were recorded on a Hitachi M-80B mass spectrometer. Acetylation procedure for the EI-MS and ^{1}H-NMR: A solution of a few mg of glycoside in Ac_2O (1 ml) and C_5H_5N (2 ml) was allowed to stand at room temperature overnight. After work-up in the usual way, the resulting acetate was subjected to spectrometry. High performance liquid chromatography (HPLC) was carried out with a Tosoh CCPM pump equipped with a Tosoh UV 8010 UV/VIS as a detector on a YMS packed column D-ODS-R (20 mm × 250 cm), flow rate: 5 ml/min. For column chromatography, silica gel: Kieselgel 60 (Merck, 70—230 mesh), silanized silica gel: Cosmosil 75C_{18}-OPN (Nacalai Tesque) and highly porous synthetic resin: Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used. All solvent systems for chromatography were homogeneous. Identification of known compounds was made by comparison of the ^{1}H- and ^{13}C-NMR spectra and optical rotation with those of a respective authentic sample. In the case of **5** and **6**, identification was established by comparison of the EI-MS, ^{1}H- and ^{13}C-NMR spectra of their acetates and $[\alpha]_D$ with the reference data. Acid hydrolysis of glycosides and identification of the resulting monosaccharides including absolute configuration were carried out as reported previously.[6] Permethylation and the sequencing analysis of sugar moiety were also referred to in the previous paper.[6]

Extraction and Separation The dried fruits of *Siraitia grosvenori* (240 g) purchased in Macao were extracted with MeOH. A suspension of the MeOH-extract (34 g) in H_2O was defatted with C_6H_{12}. The H_2O layer was successively chromatographed on Diaion HP-20 with H_2O, 50% MeOH, 80% MeOH, MeOH and $(CH_3)_2CO$.

The 50% MeOH eluate was separated by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (6:4:1) and then successively on silanized silica gel eluted with 56% and 70% MeOH to give **4** and **2**.

The 80% MeOH eluate was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (6:4:1) to give fractions I—IX. Fraction I was subjected to HPLC; mobile phase: 62% MeOH to give **5**. Fraction IV was subjected to chromatography on silica gel with $CHCl_3$–MeOH–H_2O (10:5:1) followed by HPLC; mobile phase: 65% MeOH to give **7**. Fraction V was separated in the same way as fraction IV, yielding **6**. Fraction VII was chromatographed on silanized silica gel with 60% MeOH to afford **3** and **1**. Chromatography of fraction VIII on silanized silica gel with 60% MeOH afforded **1** and **4**. Fraction IX was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (6:4:1) and then on silanized silica gel with 56% MeOH to give **2**.

Mogroside IV (**1**): A white powder, $[\alpha]_D^{22}$ −5.8° (c=1.04, MeOH). IR (Nujol) cm^{-1}: 3400 (OH), 1640, 890 (C=C). ^{1}H-NMR δ: anomeric H 5.34 (1H, d, J=7.7 Hz), 5.17 (1H, d, J=7.9 Hz), 5.09 (1H, d, J=7.7 Hz), 4.82 (1H, d, J=7.9 Hz). ^{13}C-NMR δ: anomeric C 107.0, 106.4, 105.5, 101.8.

Mogroside V (**2**): A white powder, $[\alpha]_D^{22}$ −11.7° (c=1.02, MeOH). IR (Nujol) cm^{-1}: 3400 (OH), 1640, 890 (C=C). ^{1}H-NMR δ: anomeric H 5.41 (1H, d, J=7.7 Hz), 4.88 (1H, d, J=8.6 Hz), 4.86 (1H, d, J=6.1 Hz), 4.84 (1H, d, J=7.4 Hz), 4.79 (1H, d, J=7.7 Hz). ^{13}C-NMR δ: anomeric C 106.8, 105.5, 105.2, 104.7, 103.5.

Siamenoside I (**3**): A white powder, $[\alpha]_D^{22}$ +4.9° (c=1.03, MeOH). IR (Nujol) cm^{-1}: 3400 (OH), 1640, 890 (C=C). ^{1}H-NMR δ: anomeric H 5.38 (1H, d, J=7.7 Hz), 4.99 (1H, d, J=7.7 Hz), 4.86 (1H, d, J=7.7 Hz), 4.84 (1H, d, J=7.5 Hz). ^{13}C-NMR δ: anomeric C 107.3, 105.7, 104.9, 103.6.

11-Oxo-mogroside V (**4**): A white powder, $[\alpha]_D^{25}$ +20.5° (c=0.51, MeOH). IR (Nujol) cm^{-1}: 3400 (OH), 1640, 890 (C=C). ^{1}H-NMR δ: anomeric H 5.46 (1H, d, J=7.7 Hz), 5.12 (1H, d, J=7.7 Hz), 4.88 (1H, d, J=7.9 Hz), 4.84 (1H, d, J=7.7 Hz), 4.77 (1H, d, J=7.5 Hz). ^{13}C-NMR δ: anomeric C 106.9, 105.6, 105.5, 104.9, 103.6.

Mogroside IIE (**5**): A white powder, $[\alpha]_D^{23}$ +35.2° (c=0.88, MeOH). IR (Nujol) cm^{-1}: 3400 (OH), 1640, 890 (C=C). ^{1}H-NMR δ: anomeric H 4.98 (1H, d, J=8.0 Hz), 4.87 (1H, d, J=7.8 Hz). ^{13}C-NMR δ: anomeric

C 107.0, 105.8.

Mogroside IIIE (**6**): A white powder, $[\alpha]_D^{23}$ +4.5° (c=0.88, MeOH). IR (Nujol) cm^{-1}: 3400 (OH), 1640, 890 (C=C). ^{1}H-NMR δ: anomeric H 5.28 (1H, d, J=7.7 Hz), 5.03 (1H, d, J=7.7 Hz), 4.84 (1H, d, J=7.7 Hz). ^{13}C-NMR δ: anomeric C 107.2, 106.2, 101.7.

Mogroside III (**7**): A white powder, $[\alpha]_D^{20}$ +2.5° (c=0.36, MeOH). High resolution FAB-MS m/z: Calcd for $C_{48}H_{82}O_{19}$+Na 985.5349. Found 985.5344 $(M+Na)^+$. IR (Nujol) cm^{-1}: 3400 (OH), 1640, 890 (C=C). ^{1}H-NMR δ: anomeric H 4.86 (1H, d, J=7.9 Hz), 4.84 (1H, d, J=7.7 Hz), 4.80 (1H, d, J=7.5 Hz). ^{13}C-NMR δ: aglycone moiety 26.2 (C1), 29.5 (C2), 87.9 (C3), 42.4 (C4), 144.2 (C5), 118.5 (C6), 24.6 (C7), 43.5 (C8), 40.1 (C9), 36.9 ((C10), 77.8 (C11), 41.1 (C12), 47.4 (C13), 49.7 (C14), 34.6 (C15), 28.2 (C16), 51.1 (C17), 17.1 (C18),[a] 26.7 (C19),[a] 36.2 (C20), 18.8 (C21), 33.1 (C22), 27.5 (C23), 92.8 (C24), 72.7 (C25), 24.2 (C26),[a] 26.3 (C27),[a] 19.3 (C28), 27.7 (C29),[a] 26.3 (C30),[a] glucosyl moiety 107.4 (C1), 75.5 (C2),[b] 78.5 (C3),[c] 71.5 (C4),[d] 78.1 (C5)[c] 63.1 (C6),[e] gentiobiosyl moiety: inner Glc 104.8 (C1), 75.1 (C2), [b] 78.1 (C3),[c] 72.1 (C4),[d] 76.4 (C5), 70.4 (C6), terminal Glc 106.3 (C1), 75.5 (C2),[b] 78.7 (C3),[c] 71.8 (C4),[d] 78.6 (C5),[c] 62.5 (C6).[e] (a—l: are interchangeable).

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