Two New Cucurbitane-Type Glycosides Obtained from Roots of *Siraitia grosvenori* SWINGLE

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Novel Cucurbitane-type glycosides, 5β,19β-epoxy-29-nor-3,11-dioxo-cucurbit-24-ene-27-oic acid 27-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (1) and 19,29-nor-3,11-dioxo-cucurbit-4,24-diene-27-oic acid 27-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (2) were isolated from the roots of *Siraitia grosvenori* SWINGLE.

Key words *Siraitia grosvenori*; cucurbitacine-type glycoside; root

Siraitia grosvenori SWINGLE belongs to the cucurbitaceae family. This plant is a special product found in the southern part of China, Guangxi province, and is greatly expected to be used as a sweetener in the near future. We have studied the seasonal variation of the chemical constituents of the fruits of this plant.[1—3] As part of a series of studies on this plant, we present the chemical constituents of its roots. The roots of this plant are used as raw material in cultivated areas. It is used as a folk medicine for the treatment of rheumatoid arthritis. Pharmacological tests have revealed that its extract exhibits antiinflammatory, analgesic, and anticarcinogenic activities.[4] In this paper, we report two novel norcucurbitane triterpene glycosides.

The roots of *Siraitia grosvenori* were extracted with MeOH to obtain an extract, which was then passed through Diaion HP-20 with water, 50% MeOH, and MeOH, successively. The MeOH eluate was chromatographed on silica gel with a mixture of $CHCl_3$, MeOH, and water and on octadecyl silica (ODS) with 60% MeOH to give compounds **1** and **2**.

Compound **1**, which was obtained as an amorphous powder showing $[\alpha]_D$ +10.4° (MeOH), was found to have molecular formula $C_{41}H_{62}O_{15}Na$ at *m/z* 817.4073 $[M+Na]^+$ in the positive HR-FAB-MS. The ^{1}H-NMR spectrum showed signals attributable to two tertiary methyl groups at δ 0.65, 1.22 (each 3H, s); a vinyl methyl at δ 1.92 (3H, s); two secondary methyl groups at δ 0.82 (3H, d, J=6.3 Hz) , 1.34 (3H, d, J=6.8 Hz); an oxygenated methylene group at δ 3.63, 4.71 (each 1H, d, J=8.6 Hz); an olefinic proton at δ 7.06 (1H, dd, J=5.8, 6.8 Hz), together with two sugar anomeric protons at δ 5.01 (1H, d, J=7.5 Hz), 6.44 (1H, d, J=8.0 Hz). Compound **1** was acid-hydrolyzed to give a sapogenol (**1a**) and D-glucose. The ^{13}C-NMR spectrum of **1** displayed signals due to a β-gentiobiosyl residue, β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl moiety at δ 96.2, 74.2, 78.5, 70.8, 77.9, 69.5, 105.3. 75.1, 78.4, 71.4, 78.5, 62.5 (glc I C-1′—6′, glc II C-1″—6″, respectively) and the following signals arising from the sapogenol moiety: five methyl groups at δ 8.4, 12.5, 16.6, 18.2, 19.9; five methine carbons at δ 35.8, 44.8, 45.5, 49.5 50.3; nine methylene carbons at δ 19.9, 25.6, 25.8, 28.1, 33.8, 34.8, 35.9, 39.9, 50.6; three quaternary carbons at δ 48.7, 49.1, 59.9; an oxygen-bearing quaternary carbon at δ 90.1; an oxygen-bearing methylene carbon at δ 74.6; an α,β-unsaturated carboxylate system at δ 144.6, 127.5, 167.1; and two carbonyl carbons at δ 209.8. The heteronuclear multiple bond connectivity (HMBC) from five methyl groups to the respective neighboring carbons established that the skeleton of the sapogenol to be 29-nor-cucurbit-24-ene-27-oic acid. Moreover, the HMBC examination revealed the occurrence of an epoxy function group between C-19 and C-5 and two carbonyl groups at C-3 and C-11. Next, the sequential ^{1}H–^{1}H correlation spectroscopy (COSY) correlations were observed from H-10 at δ 2.79 (br t, J=9.2 Hz) through H_2-1 at δ 1.13, 1.52 (each 1H, m) to H_2-2 at δ 1.52, 2.12; from H-4 at δ 2.61 (q, J=6.8 Hz) to H_3-28 at δ 1.34 (3H, d, J=6.8 Hz); from H_2-6 at δ 1.91, 2.10 (each 1H) through H_2-7 at δ 1.18, 1.95 (each 1H) to H-8 at δ 2.04 (1H, br t, J=9.7 Hz); and from H_2-15 at δ 1.29 through H_2-16 at δ 1.13, H-17 at δ 1.56, H-20 at δ 1.30, H_2-22 at δ 2.20, 2.32, and H_2-23 at δ 2.59, 2.79 to H-24 at δ 7.06 (1H, dd, J=5.8, 6.8 Hz). The nuclear Overhauser effect spectroscopy (NOESY) correlations between H_2-19 at δ 3.63, 4.71 (each 1H, d, J=8.6 Hz) and H-8, and between H-8 and H_3-18 disclosed that CH_2-19 and H-8 are oriented on β. The structure of the sapogenol (**1a**) was similarly verified by the ^{1}H- and ^{13}C-NMR to be 5β,19β-epoxy-29-nor-3,11-dioxo-cucurbit-24-ene-27-oic acid methyl ester, whose desmethyl compound was identical with siraitic acid B isolated from the same source and determined by X-ray analysis by Wang *et al.*[4] The connection of the sugar moiety to the sapogenol was also apparent due to the fact that the signal due to H-1′ at δ 6.44 correlated to that of C-27 at δ 167.1. Therefore, the structure of **1** was characterized as 5β,19β-epoxy-29-nor-3,11-dioxo-cucurbit-24-ene-27-oic acid 27-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside as shown in Fig. 1.

Compound **2**, which was obtained as an amorphous powder showing $[\alpha]_D$ +23.5° (MeOH), was found to have the molecular formula $C_{40}H_{60}O_{14}Na$ at *m/z* 787.3889 $[M+Na]^+$ in the positive HR-FAB-MS. The ^{1}H-NMR spectrum showed two tertiary methyl groups at δ 0.72, 1.08 (each 3H, s); two vinyl methyl groups at δ 1.89 (6H, s); a secondary methyl group at δ 0.79 (3H, d, J=6.7 Hz); and an olefinic proton at δ 7.04 (1H, m), together with two sugar anomeric protons at δ 4.97 (1H, d, J=7.2 Hz), 6.38 (1H, d, J=7.9 Hz). The ^{13}C-NMR spectrum displayed signals due to the β-gentiobiosyl

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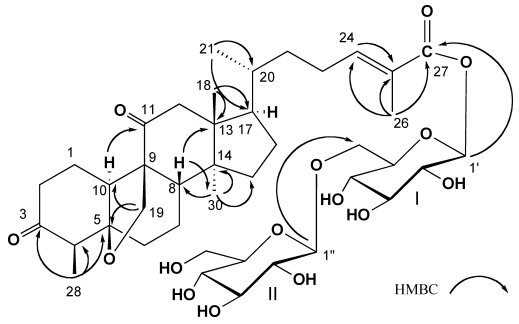

Fig. 1. Structure of Compound **1** with Key HMBC

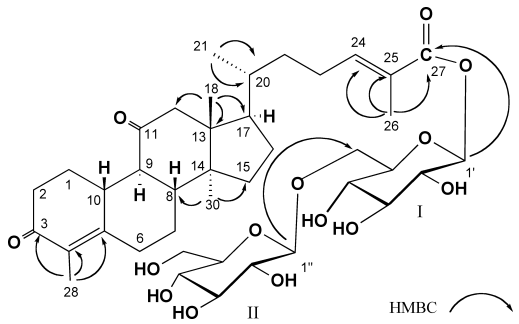

Fig. 2. Structure of Compound **2** with Key HMBC

moiety at δ 96.2, 74.1, 78.5, 70.8, 77.9, 69.5, 105.3, 75.1, 78.4, 71.5, 78.5, 62.6 (glc I C-1′—6′, glc II C-1″—6″, respectively), which were identical to those displayed in the ^{13}C-NMR spectrum of **1**. The ^{13}C-NMR spectrum also displayed signals due to five methyl groups at δ 11.2, 12.4, 16.1, 17.0, 18.1; five methine carbons at δ 36.0, 37.7, 44.8, 49.6, 54.6; nine methylene carbons at δ 25.8, 27.5, 28.5×2, 30.9, 32.0, 34.8, 37.3, 50.6; two quaternary carbons at δ 48.4, 50.2; an α,β-unsaturated carboxylate system at δ 144.5, 127.5, 167.1; two carbonyl carbons at δ 198.5, 210.8; and a tetra-substituted double bond at δ 129.9, 157.8. The HMBC of five methyl groups revealed the structure of the surrounding carbon atoms. In addition, the fundamental framework of compound **2** was found to be the cucurbitane-type triterpene, 19,29-nor-3,11-dioxo-cucurbit-4,24-diene-27-oic acid as shown in Fig. 2, whose sapogenol was also obtained from the same source and named as siraitic acid C.[5] Moreover, it was suggested that the sugar moiety combined with the C-27 carboxyl group by the HMBC. Therefore, the structure of **2** was characterized as 19,29-nor-3,11-dioxo-cucurbit-4,24-diene-27-oic acid 27-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside as shown in Fig. 2.

These major glycosides **1** and **2** obtained from the roots were 3,11-dioxo-29- or 3,11-dioxo-19,29-nor-cucurbit-24-ene-27-oic acid type triterpene derivatives having the β-gentiobiosyl moiety at C-27-OH, which differ from the sweet normal 3β,11α,24*R*,25-tetrahydroxy-cucurbitane glycosides carrying the glucosidic linkages at C-3-OH and C-24-OH such as mogrosides IV and V included in the mature fruits.[6] Actually, we could not taste their sweetness.

Experimental

General Optical rotations were performed with a JASCO DIP-1000 KYU digital polarimeter (JASCO, Tokyo). MS were recorded on a JEOL JMS-700. ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL-α-500 spectrometer at 500 and 125 MHz, respectively, chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Column chromatographies were carried out on MCI gel CHP-20P (75—150 mm, Mitsubishi Chem. Ind.), silica gel 60 (230—400 mesh, Merck), and Chromatorex ODS (30—50 μm, Fuji Silysia Chem., Ltd.), and TLC was performed on silica gel plates (Kieselgel 60 F_{254}, Merck) and RP C_{18} silica gel plates (Merck).The spots on TLC were visualized by UV light (254/366 nm) and sprayed with 10% H_2SO_4, followed by heating.

Plant Materials Roots of *Siraitia grosvenori* Swingle were collected from Longjiang country, Guilin city of Guangxi province, China, in December 2006 and identified by Prof. Wei Huanan. A voucher specimen (RSG1202) of the plant is deposited at the Herbarium of Guangxi Institute of Botany, China.

Extraction and Isolation Dried roots of *S. grosvenori* (3.0 kg) were exteracted with methanol (6 l×3) at room temperature for 10 d. The extract was evaporated under reduced pressure to afford methanol extract (120.0 g). The extract was chromatographed on Diaion HP-20, in successive elution with (H_2O→20%→ 60%→100% MeOH). The 100% MeOH eluate (2.4 g) was subjected to silica gel column and eluted with ($CHCl_3$–MeOH–H_2O= 20 : 2 : 0.2→8 : 2 : 0.2→7 : 3 : 0.5), gradiently, to provide 9 fractions. Fraction 6 (100 mg) was further chromatographed on Chromatorex ODS (60% MeOH) followed by silica gel column eluted with ($CHCl_3$–MeOH–H_2O= 8 : 2 : 0.2) to give compound **1** (164.6 mg) and compound **2** (41.2 mg).

Compound **1**: An amorphous powder, $[\alpha]_D^{25}$ +10.4° (c=0.20, MeOH), positive FAB-MS *m*/*z*: 817 $[M+Na]^+$, positive HR-FAB-MS *m*/*z*: 817.4073 $[M+Na]^+$ (Calcd for $C_{41}H_{62}O_{15}Na$: 817.3986), ^{1}H-NMR (in pyridine-d_5) δ: 0.65 (3H, s, H_3-18), 0.82 (3H, d, J=6.3 Hz, H_3-21), 1.13 (1H, m, H-1), 1.22 (3H, s, H_3-30), 1.34 (3H, d, J=6.8 Hz, H_3-28), 1.52 (1H, m, H′-1), 1.92 (3H, s, H_3-26), 2.04 (1H, br t, J=9.7 Hz, H-8), 2.61 (1H, q, J=6.8 Hz, H-4), 2.79 (1H, br t, J=9.2 Hz, H-10), 3.63 (1H, d, J=8.6 Hz, H-19), 3.88 (1H, m, glc II H-3), 4.02 (1H, dd, J=7.5, 6.8 Hz, glc II H-2), 4.10 (1H, m, glc I H-5), 4.12 (1H, dd, J=7.5, 6.3 Hz, glc I H-2), 4.18 (2H, dd, J=7.9, 6.3 Hz, glc I H-3, glc II H-5), 4.19 (1H, dd, J=7.9, 6.3 Hz, glc II H-4), 4.28 (1H, d, J=9.1 Hz, glc II H-6), 4.31 (1H, d, J=9.8 Hz, glc I H-6), 4.35 (1H, dd, J=5.1, 6.3 Hz, glc I H-4), 4.42 (1H, d, J=8.6 Hz, glc II H′-6), 4.71 (1H, d, J=8.6 Hz, H′-19), 4.76 (1H, d, J=11.5 Hz, glc I H′-6), 5.01 (1H, d, J=7.5 Hz, glc II H-1), 6.44 (1H, d, J=8.0 Hz, glc I H-1), 7.06 (1H, dd, J=6.8, 5.8 Hz, H-24). ^{13}C-NMR (in pyridine-d_5) δ: aglycone moiety 19.9 (C-1), 25.6 (C-2), 209.8 (C-3), 50.3 (C-4), 90.1 (C-5), 25.8 (C-6), 28.1 (C-7), 45.5 (C-8) , 59.9 (C-9), 44.8 (C-10), 209.8 (C-11), 33.8 (C-12), 48.7 (C-13), 49.1 (C-14), 35.9 (C-15), 34.8 (C-16), 49.5 (C-17), 16.6 (C-18), 74.6 (C-19), 35.8 (C-20), 18.2 (C-21), 39.9 (C-22), 50.6 (C-23), 144.6 (C-24), 127.5 (C-25), 12.5 (C-26), 167.1 (C-27), 8.4 (C-28), 19.9 (C-30); glc I moiety 96.2 (C-1), 74.2 (C-2), 78.5 (C-3), 70.8 (C-4), 77.9 (C-5), 69.5 (C-6); glc II moiety 105.3 (C-1), 75.1 (C-2), 78.4 (C-3), 71.4 (C-4), 78.5 (C-5), 62.5 (C-6).

Compound **2**: An amorphous powder, $[\alpha]_D^{25}$ +23.5° (c=0.25, CH_3OH), positive FAB-MS *m*/*z*: 765 $[M+H]^+$, positive HR-FAB-MS *m*/*z*: 787.3889 $[M+Na]^+$ (Calcd for $C_{40}H_{60}O_{14}$: 787.3881), ^{1}H-NMR (in pyridine-d_5) δ: 0.72 (3H, s, H_3-18), 0.79 (3H, d, J=6.7 Hz, H_3-21), 1.08 (3H, s, H_3-30), 1.89 (3H, s, H_3-26), 1.89 (3H, s, H_3-28), 4.97 (1H, d, J=7.2 Hz, glc I H-1), 6.38 (1H, d, J=7.9 Hz, glc II H-1), 7.04 (1H, m, H-24). ^{13}C-NMR (in pyridine-d_5) δ: aglycone moiety 25.8 (C-1) , 27.5 (C-2), 198.5 (C-3), 129.9 (C-4), 157.8 (C-5), 28.5 (C-6), 28.5 (C-7), 54.6 (C-8), 37.7 (C-9), 44.8 (C-10), 210.8 (C-11), 34.8 (C-12), 48.4 (C-13), 50.2 (C-14), 32.0 (C-15), 30.9 (C-16), 49.6 (C-17), 16.1 (C-18), 36.0 (C-20), 18.1 (C-21), 37.3 (C-22), 52.3 (C-23), 144.5 (C-24), 127.5 (C-25), 11.2 (C-26), 167.1 (C-27), 12.4 (C-28), 17.0 (C-30); glc I moiety 96.2 (C-1), 74.1 (C-2), 78.5 (C-3), 70.8 (C-4), 77.9 (C-5), 69.5 (C-6); glc II moiety 105.3 (C-1), 75.1 (C-2), 78.4 (C-3), 71.5 (C-4), 78.5 (C-5), 62.6 (C-6).

Acid Hydrolysis of 1 and 2 A solution of **1** (20.0 mg) in 5% H_2SO_4–MeOH (5 ml) was refluxed for 2 h. The reaction mixture was diluted with H_2O and passed through Amberlite IRA-400. The aqueous eluate was subjected to HPLC analysis under the following conditions: HPLC column, COSMOSIL Sugar-D, 4.6 mm i.d.×250 mm (Nacalai Tesque, Co., Ltd., Tokyo, Japan); detector, JASCO OR-2090; pump, JASCO PU-2080; mobile solvent: 80% CH_3CN; flow rate, 0.8 ml/min; column oven, Co-2060 plus; column temperature, 35 °C. Identification of D-glucose in the aqueous layer was carried out by comparison of retention time t_R with those of an authentic sample: D-glucose, t_R 13.5 min. On the other hand, the MeOH eluate from Amberlite IRA-400 column gave a sapogenol (**1a**, 3.6 mg). Simultaneously, compound **2** was also hydrolyzed to check D-glucose as the same way.

Sapogenol **1a**: An amorphous powder, $[\alpha]_D^{25}$ +56.5° (c=0.20, MeOH), ^{1}H-NMR (in pyridine-d_5) δ: 0.65 (3H, s, H_3-18), 0.84 (3H, d, J=6.9 Hz, H_3-21), 1.23 (3H, s, H_3-30), 1.32 (3H, d, J=6.8 Hz, H_3-28), 1.95 (3H, s, H_3-26),

3.62 (1H, d, J=7.6 Hz, H-19), 3.82 (3H, s, OCH_3), 4.70 (H, d, J=8.6 Hz, H′-19), 6.92 (1H, dd, J=6.8, 5.8 Hz, H-24). ^{13}C-NMR (in pyridine-d_5) δ: 19.9 (C-1) , 25.7 (C-2), 209.7 (C-3), 50.3 (C-4), 90.1 (C-5), 25.7 (C-6), 28.1 (C-7), 45.6 (C-8), 59.9 (C-9), 44.8 (C-10), 209.7 (C-11), 33.8 (C-12), 48.8 (C-13), 49.1 (C-14), 35.8 (C-15), 35.0 (C-16), 49.7 (C-17), 16.7 (C-18), 74.8 (C-19), 35.8 (C-20), 18.2 (C-21), 39.9 (C-22), 50.6 (C-23), 143.1 (C-24), 127.7 (C-25), 12.6 (C-26), 168.4 (C-27), 8.4 (C-28), 19.9 (C-30), 51.6 (OCH_3).

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