Cucurbitane Glycosides from Unripe Fruits of *Siraitia grosvenori*

Dianpeng LI,*[,a,b] Tsuyoshi IKEDA,[a] Toshihiro NOHARA,*[,a] Jinlei LIU,[b] Yongxin WEN,[b] Tatsunori SAKAMOTO,[c] and Gen-Ichiro NONAKA[d]

[a] *Faculty of Medical and Pharmaceutical Science, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan:* [b] *Guanxi Institute of Botany, Chinese Academy of Science; Guilin 541006, China:* [c] *Sakamoto Limestone Industrial Co., Ltd.; 273–1 Tamana, Kumamoto 865–0013, Japan: and* [d] *Nonaka Usaien Pharmaceutical Co., Ltd.; 1–4–6 Zaimoto, Saga 840–0055, Japan.* Received February 3, 2007; accepted April 10, 2007

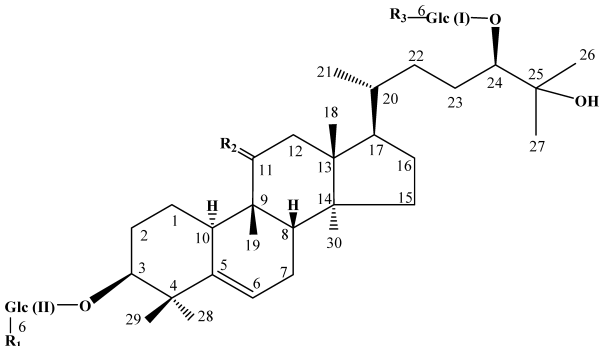
Studies on the constituents of the unripe fruits of *Siraitia grosvenori* led to the isolation of three new cucurbitane triterpene glycosides, 11-oxomogroside III (10), 11-dehydroxymogroside III (11), and 11-oxomogroside IV A (12). Their structures were determined on the basis of detailed analyses of 1D, 2D-NMR spectroscopic methods. All of the compounds isolated from the unripe fruits of *S. grosvenori* were tested for cytotoxic activities against tumor cells, HCT-116 and SMMC-7721.

Key words *Siraitia grosvenori*; Lo Han Kuo; cucurbitane-glycoside; unripe fruit; cytotoxic activity

Siraitia grosvenori SWINGLE (formerly *Momordica grosvenori* SWINGLE), a traditional Chinese fruit, belongs to the family cucurbitaceae and has been used as a pulmonary demulcent and emollient for the treatment of dry cough, sore throat, dire thirst, and constipation in folk medicine.[1)] A number of cucurbitane triterpene saponins from the ripe fruits were previously obtained.[2—10)] On the basis of its characteristic that the ripe fruit is very sweet, its extract is commercially utilized as a sweet component in sugar substitute; it is widely used as additive and ingredient in health foods and beverages. Meanwhile, owing to the influence of cold weather during winter, some fruits cannot mature naturally. The unripe fruits have a bitter taste, and at the place of cultivation, these may amount to one quarter of total production. We have isolated seven cucurbitane triterpene glycosides: 20-hydroxy-11-oxo- mogroside I A_1 (**1**), 11-oxomogroside II E (**2**), 11-oxomogroside I A_1 (**3**), mogroside II E (**4**), mogroside III (**5**), mogroside IV (**6**), mogroside V (**7**) and two flavonoid glycosides: kaempferol 7-α-L-rhamnopyranoside (**8**) and kaempferol 3,7-α-L-dirhamnopyranoside (**9**), from the unripe fruits of *S. grosvenori*.[11)] In our continuing study of this fruit, three new cucurbitane triterpene glycosides, 11-oxomogroside III (**10**), 11-dehydroxymogroside III (**11**), and 11-oxomogroside IV A (**12**), were isolated. This paper deals with the isolation and structure elucidation on the basis of detailed 1D, 2D-NMR spectroscopic analyses. All of the compounds isolated from the unripe fruits of *S. grosvenori* were tested for cytotoxic activities against HCT-116 and SMMC-7721 cell lines.

Fresh unripe fruits were extracted with methanol. A suspension of methanol-extract in water was subjected to a highly-porous polystyrene gel, Diaion HP-20, which was successively eluted with H_2O and 30%, 80%, and 100% methanol. The 80% methanolic eluate was chromatographed on silica gel, Sephadex LH-20, and reverse-phase silica gel to afford three glycosides, compounds **10**—**12** (Fig. 1), in yields of 0.0019%, 0.0014%, and 0.00019%, respectively.

Compound **10**, a white amorphous powder, $[\alpha]_D$ +56.7° (MeOH), showed a quasi-molecular ion peak at *m/z* 983.5261 $[M+Na]^+$ in the positive HR-FAB-MS, corresponding to the molecular formula $C_{48}H_{80}O_{19}Na$, which was supported by the ^{13}C-NMR spectrum and its distortionless enhancement by polarization transfer (DEPT) measurement (Table 1). The ^{13}C-NMR spectrum displayed signals due to eight methyls, eleven methylenes, twenty-two methines, and seven quaternary carbons. The ^{1}H-NMR spectrum (Table 1) of **10** exhibited signals due to eight tertiary methyls at δ 0.75, 0.86, 0.93, 1.12, 1.16, 1.33, 1.44, and 1.54 (each 3H), two oxygen-bearing methines at δ 3.61 (1H, br s, $W_{1/2}$=5.8 Hz) and 3.75 (1H, d, J=9.1 Hz), and one olefinic proton signal at δ 5.53 (1H, d, J=5.5 Hz), which correlated with the carbon signals at δ 16.9, 18.4, 18.2, 28.3, 20.2, 26.9, 24.2, 25.8, 87.1, 92.5, and 118.5, respectively, in the heteronuclear multiple quantum coherence (HMQC). In the ^{1}H-NMR spectrum (Table 1) of **10**, three anomeric protons at δ 4.94 (1H, d, J=7.9 Hz), 4.84 (1H, d, J=8.2 Hz), and 4.88 (1H, d, J=7.3 Hz) were observed along with other signals at δ 4.03, 4.15, 4.16, 3.92, 3.98, 4.86; 4.01, 4.18, 4.20, 4.15, 4.33, 4.52, and 4.02, 4.17, 4.23, 4.16, 4.30, 4.47 (each 1H), which correlated with the carbon signals at δ 106.2, 75.0, 78.0, 72.0, 76.4, 70.3; 104.8, 75.4, 78.7, 71.8, 78.4, 62.6, and 107.2, 75.5, 78.5, 71.5, 78.1, 63.2, respectively, in the HMQC (Table 1). Acid hydrolysis of **10** yielded only D-glucose. The J=7.9, 8.2, 7.3 Hz for



	R_1	R_2	R_3
11-Oxomogroside II E (**2**)	H	O	H
11-Oxomogroside III (**10**)	H	O	—Glc (III)
11-Dehydroxymogroside III (**11**)	H	H	—Glc (III)
11-Oxomogroside IVA (**12**)	—Glc (IV)	O	—Glc (III)

Fig. 1. The Structure of **2**, **10**—**12**

* To whom correspondence should be addressed. e-mail: none@gpo.kumamoto-u.ac.jp

Table 1. ^{13}C-, ^{1}H-NMR Spectral Data for Cucurbitane Glycosides **10**, **11** and **12** from the Unripe Fruits of *Siraitia grosvenori* (in C_5D_5N)

C no.	11-Oxomogroside III (**10**)		11-Dehydroxymogroside III (**11**)		11-Oxomogroside IV A (**12**)	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	22.0	1.60 (α), 1.96 (β)	22.7	1.58 (α), 1.95 (β)	22.2	1.58 (α), 1.98 (β)
2	29.6	1.91 (α), 1.71(β)	29.7	1.91 (α), 1.72 (β)	29.7	1.90 (α), 1.68 (β)
3	87.1	3.61 (br s, $W_{1/2}$=5.8)	87.7	3.68 (br s, $W_{1/2}$=5.8)	86.6	3.68 (br s, $W_{1/2}$=5.2)
4	41.9		41.8		42.0	
5	141.2		141.3		141.4	
6	118.5	5.53 (d, 5.5)	118.8	5.48 (d, 5.5)	118.5	5.52 (d, 5.2)
7	24.1	2.20 (α), 1.75 (β)	24.6	2.02 (α), 1.66 (β)	24.1	2.22 (α), 1.76 (β)
8	43.9	1.79	43.9	1.68	44.0	1.78
9	49.0		34.7		49.0	
10	36.0	2.46	38.6	2.25	36.0	2.40
11	213.7		32.6	1.69 (m)	213.7	
12	48.7	2.89 (α), 2.49 (β)	30.8	1.39	48.8	2.91 (α), 2.49 (β)
13	49.0		46.5		49.0	
14	49.6		49.5		49.7	
15	34.6	1.13 (α), 1.28 (β)	35.1	1.20	34.6	1.15 (α), 1.25 (β)
16	28.4	1.92 (α), 1.82(β)	28.4	1.92 (α),1.80 (β)	28.6	1.94 (α) , 1.82 (β)
17	49.9	1.63	51.2	1.56	50.1	1.68
18	16.9	0.75 (3H, s)	15.7	0.86 (3H, s)	17.0	0.74 (3H, s)
19	20.2	1.16 (3H, s)	28.1	0.87 (3H, s)	20.3	1.17 (3H, s)
20	36.0	1.45	36.2	1.47	36.0	1.40
21	18.4	0.86 (3H, d, 6.7)	18.9	0.94 (3H, d, 5.5)	18.3	0.87 (3H, d, 6.7)
22	32.9	1.69	33.2	1.75	33.0	1.68, 1.92
23	27.9	2.36	29.0	2.55	28.6	2.46
24	92.5	3.75 (d, 9.1)	92.7	3.77 (d, 9.8)	92.6	3.75 (d, 9.2)
25	72.6		72.7		72.7	
26	24.2	1.44 (3H, s)	24.2	1.45 (3H, s)	24.7	1.37 (3H, s)
27	26.9	1.33 (3H, s)	27.0	1.34 (3H, s)	27.0	1.33 (3H, s)
28	28.3	1.12 (3H, s)	28.2	1.11 (3H, s)	28.3	1.08 (3H, s)
29	25.8	1.54 (3H, s)	26.0	1.53 (3H, s)	25.8	1.48 (3H, s)
30	18.2	0.93 (3H, s)	18.1	0.76 (3H, s)	18.6	0.92 (3H, s)
Glc(I)-1	106.2	4.94 (d, 7.9)	106.3	4.87 (d, 8.0)	106.3	4.95 (d, 7.9)
-2	75.0	4.03[a)]	75.1	4.04[a)]	75.1	4.01 (t-like, 7.7)
-3	78.0	4.15[a)]	78.0	4.18[a)]	78.5	4.19[a)]
-4	72.0	4.16[a)]	72.1	4.20[a)]	72.2	4.16[a)]
-5	76.4	3.92[a)]	76.4	3.96[a)]	76.4	3.92[a)] (m)
-6	70.3	3.98, 4.86	70.4	3.97, 4.88	70.4	3.96, 4.89
Glc(II)-1	107.2	4.88 (d, 7.3)	107.3	4.89 (d, 7.9)	106.9	4.83 (d, 7.4)
-2	75.5	4.02[a)]	75.5	4.02[a)]	75.3	4.02[a)]
-3	78.5	4.17[a)]	78.5	4.16[a)]	78.6	4.16[a)]
-4	71.5	4.23[a)]	71.5	4.20[a)]	71.5	4.20[a)]
-5	78.1	4.16[a)]	78.1	4.18[a)]	77.3	4.18[a)]
-6	63.2	4.30, 4.47	63.2	4.34, 4.54	70.5	4.34, 4.94
Glc(III)-1	104.8	4.84 (d, 8.2)	104.7	4.83 (d, 8.0)	104.9	4.88 (d, 7.4)
-2	75.4	4.01[a)]	75.5	4.06[a)]	75.4	4.03[a)]
-3	78.7	4.18[a)]	78.7	4.22[a)]	78.7	4.21[a)]
-4	71.8	4.20[a)]	71.9	4.23[a)]	72.2	4.18[a)]
-5	78.4	4.15[a)]	78.6	4.16[a)]	78.4	3.95 (m)
-6	62.6	4.33, 4.52	62.6	4.36, 4.56	62.6	4.36, 4.51
Glc(IV)-1					105.5	5.14 (d, 7.9)
-2					75.5	4.04[a)]
-3					78.1	4.17[a)]
-4					71.9	4.22[a)]
-5					77.9	4.19[a)]
-6					62.9	4.33, 4.50

Numbers in parentheses denote *J* values (Hz). *a*) Overlapped.

three anomeric protons indicated all β-glycosidic linkages. When the ^{1}H- and ^{13}C-NMR spectra of **10** were compared with our reported compound 11-oxomogroside II E (Fig. 1),[11] signals ascribable to the aglycone were identical. Moreover, the ^{13}C-NMR data were analogous with the reported data of cucurbitane triterpenoid. The above observation led to the identification of the aglycone as 11-oxomogrol.[3,8] The ^{13}C-NMR glycosylation shift at δ 87.1 and 92.5 toward downfield suggested that the sugar moiety was attached to C-3 and C-24 of the aglycone. This was further confirmed by heteronuclear multiple bond connectivity (HMBC) correlation of anomeric protons (Fig. 2). By comparing the ^{1}H- and ^{13}C-NMR signals due to the sugar moieties of **10** with those of 11-oxomogroside II E, one more glucosyl signal increased in the sugar moiety of **10**, an anomeric proton at δ 4.84 (1H, d, J=8.2 Hz) and carbon signals at δ 104.8, 75.4, 78.7, 71.8,

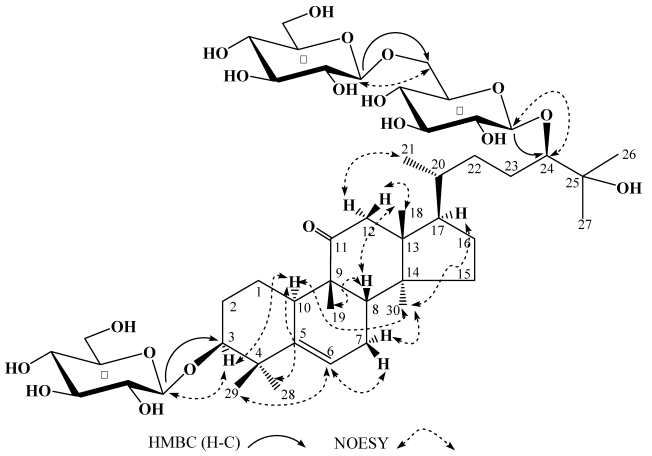


Fig. 2. Key HMBC and NOESY Correlations in **10**

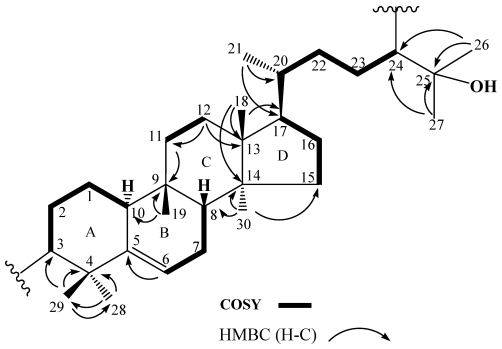


Fig. 3. ^{1}H–^{1}H COSY and Key HMBC Correlations in the Aglycone of **11**

78.4, 62.6, corresponding to a terminal β-glycopyranosyl group (Glc-III) in **10**. The linkage site of Glc-III was determined by downfield shift of C-6 at Glc-I from δ 62.8 to 70.3. This linkage was confirmed by HMBC experiment, which showed a long-range correlation between the signal at δ 4.84 (1H, d, J=8.2 Hz, Glc-III anomeric H) and 70.3 (Glc-I C-6), and also by the nuclear Overhauser effect spectroscopy (NOESY) correlation between the signal at δ 4.84 (1H, d, J=8.2 Hz, Glc-III anomeric H) and 3.98 (1H, Glc-I H-6). The NOESY of **10** (Fig. 2) showed correlations between H-6 and H_3-29; H_3-19 and H-8; H-8 and H-12; and H-12 and H_3-18 on the β-face of the molecule, and on the other hand, between H-3 and H_3-28; H-10 and H-3; H-7 and H_3-30; H-17 and H_3-21; and H-24 and Glc-I anomeric proton on the α-face. These observations accord with the skeleton of cucurbitane triterponoid. Hence **10** could be formulated as 3β,24,25-tri-hydroxy-(24*R*)-cucurbit-5-en-11-one 3-*O*-β-D-glucopyranoside-24-*O*-[β-D-glucopyranosyl(1—6)-β-D-glucopyranoside] (11-oxomogroside III).

Compound **11**, a white amorphous powder, $[\alpha]_D$ +7.5° (MeOH), showed a quasi-molecular ion peak at *m/z* 969.5486 $[M+Na]^+$ in the positive HR-FAB-MS, corresponding to the molecular formula $C_{48}H_{82}O_{18}Na$, which was supported by the ^{13}C-NMR spectrum and its DEPT measurement (Table 1). The ^{13}C-NMR spectrum displayed signals due to eight methyls, twelve methylenes, twenty-two methines, and six quaternary carbons. The ^{1}H-NMR spectrum (Table 1) of **11** exhibited signals due to eight tertiary methyls at δ 0.76, 0.86, 0.87, 0.94, 1.11, 1.34, 1.45, and 1.53 (each 3H), two oxygen-bearing methines at δ 3.68 (1H, br s, $W_{1/2}$=5.8 Hz) and 3.77 (1H, d, J=9.8 Hz), and one olefinic proton signal at δ 5.48 (1H, d, J=5.5 Hz), which correlated with the carbon signals at δ 18.1, 15.7, 28.1, 18.9, 28.2, 27.0, 24.2, 26.0, 87.7, 92.7, and 118.8, respectively, in the HMQC. When the ^{1}H- and ^{13}C-NMR spectra of **11** were compared with those of **10**, signals ascribable to the sugar moiety were identical (Table 1). The ^{13}C-NMR signals due to the A, B, D rings of the aglycone moiety of **11** were superimposed on those of **10**. On the other hand, in the ^{13}C-NMR spectrum of **11**, the C-11 carboxyl signal at δ 213.7 disappeared, and it was replaced by a methylene signal at δ 32.6, and the surrounding signals at C-9 and C-12 were shifted by −15.3 and −17.9 ppm, respectively, suggesting that **11** was the 11-deoxy compound of **10**, which was supported by the observation that the molecular ion of **11** was smaller by 14 mass units than that of **10**. The aglycone structure of **11** was further confirmed by HMBC and COSY correlations (Fig. 3). Consequently, the structure of **11** was characterized as 3β,24,25-tri-hydroxy-(24*R*)-cucurbit-5-en 3-*O*-β-D-glucopyranoside-24-*O*-[β-D-glucopyranosyl(1—6)-β-D-glucopyranoside] (11-dehydroxymogroside III).

Compound **12**, a white amorphous powder, $[\alpha]_D$ −16.9° (MeOH), showed a quasi-molecular ion peak at *m/z* 1144.1212 $[M+Na]^+$ in the positive HR-FAB-MS, corresponding to the molecular formula $C_{54}H_{90}O_{24}Na$, which was supported by the ^{13}C-NMR spectrum and its DEPT measurement (Table 1). The ^{13}C-NMR spectrum displayed signals due to eight methyls, twelve methylenes, twenty-seven methines, and seven quaternary carbons. The ^{1}H-NMR spectrum (Table 1) of **12** exhibited signals due to eight tertiary methyls at δ 0.74, 0.87, 0.92, 1.08, 1.17, 1.33, 1.37, and 1.48 (each 3H), two oxygen-bearing methines at δ 3.68 (1H, br s, $W_{1/2}$=5.2 Hz) and 3.75 (1H, d, J=9.2 Hz), and one olefinic proton signal at δ 5.52 (1H, d, J=5.2 Hz), which correlated with the carbon signals at δ 17.0, 18.3, 18.6, 28.3, 20.3, 27.0, 24.7, 25.8, 86.6, 92.6, and 118.5, respectively, in the HMQC. Moreover, it showed signals due to four anomeric protons at δ 4.95 (1H, d, J=7.9 Hz), 4.88 (1H, d, J=7.4 Hz), 4.83 (1H, d, J=7.4 Hz), and 5.14 (1H, d, J=7.9 Hz) along with signals at δ 4.01, 4.19, 4.16, 3.92, 3.96, 4.89; 4.03, 4.21, 4.18, 3.95, 4.36, 4.51; 4.02, 4.16, 4.20, 4.18, 4.34, 4.94, and 4.04, 4.17, 4.22, 4.19, 4.34, 4.50 (each 1H), which correlated with the carbon signals at δ 106.3, 75.1, 78.5, 72.2, 76.4, 70.4; 104.9, 75.4, 78.7, 72.2, 78.4, 62.6; 106.9, 75.3, 78.6, 71.5, 77.3, 70.5; and 105.5, 75.5, 78.1, 71.9, 77.9, 62.9, respectively, in the HMQC (Table 1). Acid hydrolysis of **12** yielded only D-glucose. The coupling constants J=7.9, 7.4, 7.4, 7.9 Hz of the respective anomeric protons indicated all β-glycosidic linkages. When the ^{1}H- and ^{13}C-NMR spectra of **12** were compared with those of **10**, signals ascribable to the aglycone were identical. On the other hand, one more glucosyl signal clearly occurred in the sugar moiety of **12**, that is, to bear an anomeric proton at δ 5.14 (1H, d, J=7.9 Hz) and the carbon signals at δ 105.5, 75.5, 78.1, 71.9, 77.9, 62.9, corresponding to a terminal β-glycopyranosyl moiety (Glc-IV) attached at Glc-II. The linkage site of Glc-IV was determined to be at C-6 of Glc-II, which was shifted toward downfield from δ 63.2 to 70.5. This linkage was confirmed by the HMBC (Fig. 4) experiment, which showed a long-range correlation between the signals at δ 5.14 (1H, d, J=7.9 Hz, Glc-IV anomeric H) and 70.5 (Glc-II C-6) , and also by the NOESY (Fig. 4) cor-

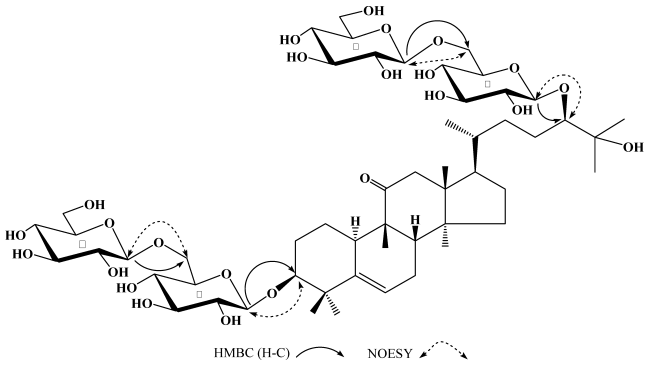


Fig. 4. Key HMBC and NOESY Correlations in the Sugar Moiety of **12**

Table 2. Cytotoxic Activities of Copound **1**—**12** against HCT-116 Colon Cancer Cells and SMMC-7721 Hepatoma Cells

Compounds	IC_{50} (μg/ml)[a]	
	SMMC-7721	HCT-116
20-Hydroxy-11-oxomogroside I A_1 (**1**)	295	624
11-Oxomogroside II E (**2**)	390	309
11-Oxomogroside I A_1 (**3**)	211	630
Mogroside II E (**4**)	226	657
Mogroside III (**5**)	263	401
Mogroside IV (**6**)	232	863
Mogroside V (**7**)	357	465
Kaempferol 7-α-L-rhamnopyranoside (**8**)	115	127
Kaempferol 3,7-α-L-dirhamnopyranoside (**9**)	250	331
11-Oxomogroside III (**10**)	290	260
11-Dehydroxymogroside III (**11**)	217	945
11-Oxomogroside IV (**12**)	288	n.d.[b]

a) Concentration inhibiting 50% of cell growth (IC_{50}). *b*) Not determined.

relation between the signals at δ 5.14 (1H, d, J=7.9 Hz, Glc-IV anomeric H) and δ 4.34, 4.94 (each 1H, Glc-II H-6). Hence **12** was formulated as 3β,24,25-tri-hydroxy-(24*R*)-cucurbit-5-en-11-one 3-*O*-[β-D-glucopyranosyl(1—6)-β-D-glucopyranoside]-24-*O*-[β-D-glucopyranosyl(1—6)-β-D-glucopyranoside] (11-oxomogroside IV).

Recently, physiological functions of *S. grosvenori* and its components have received considerable attention, and some interesting findings have been reported. For example, mogroside V has been shown to have inhibitory effects on the initiation and promotion of cancer. It might be valuable as a chemopreventive agent against chemical carcinogenesis.[12] 11-Oxomoroside V was found to have a strong inhibitory effect on low-density lipoprotein (LDL).[13] Its extract has antioxidant activity against free radicals generated by a hypocantine and xanthine oxidase system and Fe(II)-induced lipid peroxidation.[14] *S. grosvenori* appears useful as a noncaloric sugar substitute that has the added benefit of attenuation of postprandial glycemia *via* an inhibitory mechanism on maltase activity.[15] In our study, all cucurbitane triterpene glycosides and flavonol glycosides isolated from the fruits of *S. grosvenori* (compounds **1**—**12**) were tested for cytotoxic activities against HCT-116 colon cancer cells and SMMC-7721 hepatoma cells. The results of cytotoxicity assay are shown in Table 2. Although all of the compounds exhibited no apparent cytotoxic activities against cultured tumor cell lines, we think it is reasonable that a longer time-dependent relationship may yet be discovered, since the function of *S. grosvenori* extract as a food additive may need long-term absorbtion to be exhibited. This result also accords with the literature study.[16] Inhibitory effects against proliferation of HCT-116 and SMMC-7721 cells are suggested through a mast cell-dependent mechanism. The relationship of time-inhibition dependence needs further investigation.

Experimental

General Experimental Procedures Optical rotations were measured by P-1010 polarimeter (JASCO, Japan) at 25 °C. TLC was performed on precoated silica gel 60 F_{254} plate (Merck), and detection was by spraying 10% aq. H_2SO_4. Column chromatographies were carried out on Kiesel gel (40—100 mesh and 230—400 mesh, Kanto Chem.), Diaion HP-20 (Mitsubishi Chemical Ind.). Sephadex LH-20 (25—100 mm, Pharmacia Fine Chemicals), Wakogel 50 C18 (36—212 mm, Wako Pure Chemical Industuies, Ltd.), Chromatorex ODS (30—50 μm, Fuji Silysia Chemical Ltd.). FAB-MS were measured by JEOL JMS-DX303HF spectrometer (Xe atom beam, accel. voltage 2—3 kV, matrix glycerol), 200—300 mA. NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C by JNC-A500 NMR spectrometer and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as internal standard. Standard pulse sequences were employed for DEPT, HMQC, and HMBC experiments. NOESY spectra were measured with mixing times of 600 ms.

Plant Material Unripe fruits of *Siraitia grosvenori* (40—50 d of growing) were obtained from Lingui county, Guilin city of Guangxi province, China, in October 2004 and identified by Professor Wei Huanan. A voucher specimen (SG05820) of the plant is deposited at the Herbarium of Guangxi Institute of Botany, China.

Extraction and Isolation Fresh unripe fruits (5 kg) of *S. grosvenori* were extracted with methanol (8 l×3) at room temperature for 10 d. The extract was evaporated under reduced pressure to afford methanol extract (205 g). The extract was chromatographed on Diaion HP-20, with successive elution with H_2O and methanol 30%, 80%, and 100%. The 80% methanol eluate (30.5 g) was submitted to silica gel column and eluted with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2; 7 : 3 : 0.5; 6 : 4 : 1, v/v), gradiently, to afford ten fractions. Fraction 5 (940 mg) was repeatedly subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, v/v), followed by further purification with Sephadex LH-20 (30% MeOH) and to Wakogel C18 column chromatography (50—60% MeOH) to afford **10** (91.6 mg) and **11** (66.9 mg). Fraction 6 (320 mg) was repeatedly subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2; 7 : 3 : 0.5, v/v) followed by further purification with Chromatorex ODS (55—65% MeOH) to give **12** (9.4 mg).

11-Oxomogroside III (**10**): A white amorphous powder, $[\alpha]_D$ +56.7° (c=0.1, MeOH). Positive FAB-MS (*m/z*): 984 $[M+Na]^+$. Positive HR-FAB-MS (*m/z*): 983.5261 $[M+Na]^+$ (Calcd for $C_{48}H_{80}O_{19}Na$, 983.5192). ^{1}H- and ^{13}C-NMR (in pyridine-d_5) given in Table 1.

11-Dehydroxymogroside III (**11**): A white amorphous powder, $[\alpha]_D$ +7.5° (c=0.2, MeOH). Positive FAB-MS (*m/z*): 970 $[M+Na]^+$. Positive HR-FAB-MS (*m/z*): 969.5486 $[M+Na]^+$ (Calcd for $C_{48}H_{82}O_{18}Na$, 969.5399). ^{1}H- and ^{13}C-NMR (in pyridine-d_5) given in Table 1.

11-Oxomogroside IV (**12**): A white amorphous powder, $[\alpha]_D$ −16.9° (c=0.2, MeOH). Positive FAB-MS (*m/z*): 1144 $[M+Na]^+$. Positive HR-FAB-MS (*m/z*): 1144.1212 $[M+Na]^+$ (Calcd for $C_{54}H_{90}O_{24}Na$, 1144.1085). ^{1}H- and ^{13}C-NMR (in pyridine-d_5) given in Table 1.

Acid Hydrolysis of 10, 11, and 12 A solution of **10**, **11**, and **12** (5.0 mg, 4.5 mg and 2.5 mg, respectively) in 0.5 M HCl was heated under reflux for 2 h. The reaction mixture eluted with H_2O and MeOH successively was subjected to Amberlite IRA-400. The aqueous layer 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 with those of an authentic sample: D-glucose, t_R 13.5 min.

Assay for Cytotoxic Activity The cytotoxic assay was performed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method.[17,18] Human colon cancer cells HCT-116 and human liver cancer cells SMMC-7721 were precultured in RPMI 1640 medium (Nissui Co., Ltd.) supplemented with 10% heat-inactivated fresh fetal bovine serum (FBS) under humidified air containing 5% CO_2 at 37 °C. The cell suspension 150 μl was added to each well of a 96-microwell plate (flat bottom, poly-

styrene treated) and incubated for 24 h. Test compound solutions in 10% dimethyl sulfoxide (DMSO) in various concentrations (50, 100, 200, 400 μg/ml) were prepared and 50 μl of the test solution or 10% DMSO (control) was added to each well. The plate was kept in an incubator for 48 h. Then, 5% MTT was added and the plate was incubated for another 4 h. Thereafter, 150 μl of DMSO was added and the absorbency was read on a microplate reader (ELISA, Authos 2010, Anthos Labtec Instruments Inc.) at 492 nm. A dose–response course was plotted for each compound, and the concentrations giving 50% inhibition of cell growth (IC_{50}) were calculated (see Table 2).

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