Cucurbitane Glycosides from the Fruits of *Siraitia grosvenorii* and Their Inhibitory Effects on Epstein−Barr Virus Activation

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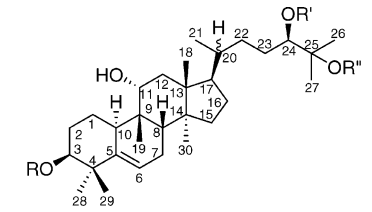
Six new cucurbitane glycosides, mogroside II B (**2**), 11-deoxymogroside III (**4**), 7-oxomogroside II E (**5**), 7-oxomogroside V (**6**), 11-oxomogroside II A_1 (**7**), and 11-oxomogroside IV A (**8**), and two known but new naturally occurring cucurbitane glycosides, mogroside II A_1 (**1**) and mogroside III A_2 (**3**), were isolated from an ethanol extract of the fruits of *Siraitia grosvenorii*. Upon evaluation of compounds **1**−**8** for inhibitory effects against the Epstein−Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), all compounds exhibited inhibitory effects with IC_{50} values of 346−400 mol ratio/32 pmol TPA. In addition, compounds **1**−**8** showed weak inhibitory effects on activation of (±)-(*E*)-methyl-2-[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexemide (NOR 1), a nitric oxide (NO) donor.

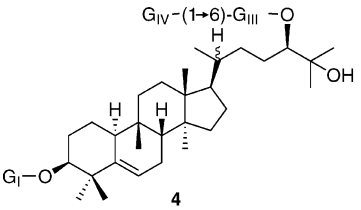
The fruits of *Siraitia grosvenorii* (Swingle) Lu & Zhang (*Momordica grosvenorii*; Cucurbitaceae) grow in Guangxi, People's Republic of China, and are used as an expectorant as well as a natural sweetener in that country. Many cucurbitane-type triterpene glycosides have been isolated and characterized from this species.[1−4] We have reported earlier the isolation and characterization of some cucurbitane glycosides from *S. grosvenorii* fruits and their inhibitory effects on the induction of Epstein−Barr virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells,[5] which is known to be a primary screening test for antitumor promoters (potential cancer chemopreventive agents).[6] The cucurbitane glycosides from *S. grosvenorii* fruits have been studied also by Takasaki et al. from the point of view of their cancer chemopreventive effects.[7] Inhibition of rat intestinal maltase and suppression of the rise in blood glucose levels in rats by oral administration of cucurbitane glycosides of *S. grosvenorii* have been reported recently.[8]

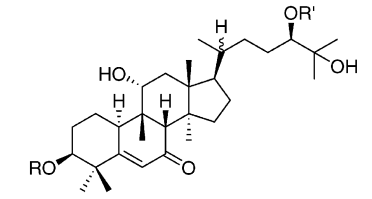
In a continuing study on the triterpene glycosides of *S. grosvenorii* fruit extract, we have isolated six new (**2**, **4**−**8**) and two known but new naturally occurring cucurbitane glycosides (**1**, **3**), and we now report their characterization as well as their inhibitory effects on the induction of EBV-EA induced by TPA and on activation of (±)-(*E*)-methyl-2-[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexemide (NOR 1), a nitric oxide (NO) donor, in Chang liver cells (normal human hepatic cells) as a primary screening test for antitumor initiators.[9]

Results and Discussion

Six new cucurbitane glycosides, mogroside II B (**2**), 11-deoxymogroside III (**4**), 7-oxomogroside II E (**5**), 7-oxomogroside V (**6**), 11-oxomogroside II A_1 (**7**), and 11-oxomogroside IV A (**8**), and two known cucurbitane glycosides, mogroside II A_1 (**1**)[3] and mogroside III A_2 (**3**),[3] were isolated from an ethanol (EtOH) extract of the fruits of *S. grosvenorii*. Compounds **1** and **3** were previously synthesized from mogroside V by partial acid hydrolysis,[3] but in the present study these compounds have been found as naturally occurring substances for the first time. The identification of compounds **1**−**8** was performed by spectroscopic comparison with structurally related compounds from the literature.







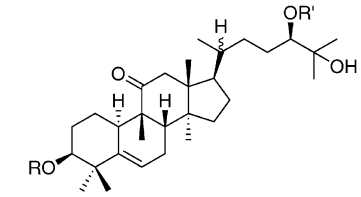


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Table 1. ^{13}C NMR Spectroscopic Data (δ values; 150 MHz, C_5D_5N) of Compounds **2** and **4**−**8**

carbon	2	4	5	6	7	8	carbon	2	4	5	6	7	8
aglycon moiety							sugar moiety						
1	26.7	22.7	27.0	27.1	21.2	22.2	G_I 1	107.3	107.4	107.1	106.8		106.9
2	29.5	29.1	29.0	28.9	29.7	28.6	G_I 2	75.5	75.5	75.4	75.5		75.4
3	87.9	87.7	87.8	87.3	75.6	86.6	G_I 3	78.7	78.6	78.7	78.5		78.6
4	42.4	41.8	43.9	43.8	41.9	42.0	G_I 4	71.8	71.9	71.8	71.7		71.8
5	144.2	143.2	170.6	170.8	141.4	141.3	G_I 5	78.1	78.2	78.5	78.0		77.3
6	118.4	118.8	125.2	125.2	119.0	118.5	G_I 6	63.1	63.2	63.0	70.4		70.5
7	24.6	24.6	201.2	201.2	24.2	24.1	G_{II} 1				105.5		105.5
8	43.5	43.9	60.0	60.1	44.1	44.0	G_{II} 2				75.3		75.3
9	40.1	34.7	40.7	40.8	49.1	49.0	G_{II} 3				78.1		78.6
10	36.8	38.6	39.3	39.2	36.1	36.0	G_{II} 4				71.8		71.8
11	77.8	32.6	76.7	76.9	213.9	213.8	G_{II} 5				78.6		78.5
12	41.1	30.8	40.7	40.8	48.8	48.8	G_{II} 6				62.8		62.8
13	47.4	46.5	47.0	47.2	49.1	49.0	G_{III} 1		106.3	105.9	103.7	106.3	106.3
14	49.8	49.4	48.6	48.7	49.7	49.7	G_{III} 2		75.1	75.4	82.5	75.1	75.1
15	34.6	35.1	34.6	34.6	34.6	34.6	G_{III} 3		78.5	78.2	78.7	78.5	78.5
16	28.4	28.1	28.3	28.6	28.0	28.0	G_{III} 4		72.1	71.8	71.5	72.1	72.1
17	51.1	51.2	50.6	50.9	50.0	50.0	G_{III} 5		76.4	78.6	77.4	76.4	76.4
18	17.0	15.7	16.9	16.9	17.0	17.0	G_{III} 6		70.5	62.7	70.2	70.4	70.4
19	26.3	28.2	26.6	26.6	20.2	20.3	G_{IV} 1		104.8		104.9	104.9	104.8
20	36.5	36.2	36.5	36.8	36.0	36.0	G_{IV} 2		75.5		75.3	75.5	75.5
21	18.9	18.9	18.8	19.0	18.5	18.5	G_{IV} 3		78.1		78.5	78.1	78.1
22	34.5	33.2	33.4	33.3	33.0	33.0	G_{IV} 4		71.5		71.8	71.5	71.5
23	29.1	29.8	29.6	29.8	29.8	29.7	G_{IV} 5		78.6		78.4	78.6	78.6
24	75.7	92.8	90.8	92.2	92.7	92.7	G_{IV} 6		62.6		63.6	62.6	62.6
25	80.6	72.7	72.0	72.6	72.7	72.7	$G_{V/VI}$ 1	97.6			105.7		
26	22.5	24.3	25.3	24.5	24.3	24.3	$G_{V/VI}$ 2	75.5			76.4		
27	23.0	27.0	27.0	27.1	27.0	27.0	$G_{V/VI}$ 3	78.4			78.3		
28	27.7	28.4	27.6	27.5	27.9	28.3	$G_{V/VI}$ 4	71.8			72.7		
29	26.3	26.0	25.6	25.5	26.3	25.8	$G_{V/VI}$ 5	78.9			78.5		
30	19.3	18.0	19.5	19.7	18.3	18.3	$G_{V/VI}$ 6	62.8			62.6		

The molecular formula of **2** was determined to be $C_{42}H_{72}O_{14}$ on the basis of HRESIMS (positive-ion mode) ([M + Na]$^+$, *m*/*z* 823.4810). The ^{13}C (Table 1) and ^1H NMR spectra (Table 2) of **2** showed the presence of seven tertiary methyls, of which two are attached to oxygen-bearing carbons [δ_C 80.6 (s)], a secondary methyl, three oxymethines, and an olefinic methine. The ^1H NMR spectrum also showed two anomeric proton signals [δ_H 4.89 (1H, d, J = 8.0 Hz) and 5.21 (1H, d, J = 7.7 Hz)], along with other resonances due to two glucose moieties. The ^{13}C NMR spectrum of the aglycon moiety of **2** was very similar to that of mogrol [(24*R*)-cucurbit-5-ene-3β,11α,24,25-tetrol][10] except for the glycosylation shifts[4] of the C-3 and C-25 signals. HMBC experiments showed cross-correlations between H-3 (δ_H 3.67) and the C-1 (δ_C 107.3) of the glucose (G_I) moiety attached at C-3, between H-1 (δ_H 4.89) of the glucose (G_I) and C-3 (δ_C 87.9) of the aglycon, and between the H-1 (δ_H 5.21) of the glucose (G_{VI}) moiety and C-25, which supported the proposed structure of **2** [3,25-diglucosylmogrol (mogrol 3,25-di-*O*-β-D-glucopyranoside)], which we named mogroside II B. Analysis of the IR, ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, NOESY, and ESIMS^n spectra of **2** confirmed this structure.

Compound **4** was assigned a molecular formula of $C_{48}H_{82}O_{18}$, as determined from its [M + Na]$^+$ ion at *m*/*z* 969.5403 in the HRESIMS. In the ^{13}C (Table 1) and ^1H NMR spectra (Table 2), compound **4** showed the presence of seven tertiary methyls, of which two are attached to an oxygen-bearing carbon [δ_C 72.7 (s)], a secondary methyl, two oxymethines, and an olefinic methine. The ^1H NMR spectrum of **4** further displayed three anomeric proton signals at δ_H 4.85 (1H, d, J = 7.6 Hz), 4.88 (1H, d, J = 7.9 Hz), and 4.91 (1H, d, J = 7.9 Hz). The close similarity of the ^{13}C NMR data to those of mogroside III [3-*O*-β-D-glucopyranosylmogrol 24-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside],[4] except for the lack of signals due to an oxymethine, suggested **4** to be a dehydoxylated analogue of mogroside III, most probably at C-11α. HMBC experiments (Tables S1 and S2, Supporting Information) showed cross-correlations between H-3 (δ_H 3.68) and the C-1 (δ_C 107.4) of the glucose (G_I) moiety attached at C-3, between H-1 (δ_H 4.88) of the glucose (G_I) and C-3 (δ_C 87.7) of the aglycon, between H-24 (δ_H 3.78) and the C-1 (δ_C 106.3) of the glucose (G_{III}) moiety attached at C-24 of the aglycon, between H-1 (δ_H 4.91) of the glucose (G_{III}) and C-24 (δ_C 92.8) of the aglycon, and between H-1 (δ_H 4.85) of the glucose (G_{IV}) and the C-6 (δ_C 70.5) of the glucose (G_{III}). The above evidence, coupled with the analysis of ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, TOCSY, NOESY, and ESIMS^n spectra, confirmed that **4** possesses the structure 3-*O*-β-D-glucopyranosyl 11-deoxymogrol 24-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside and has been named 11-deoxymogroside III.

Compound **5** exhibited a [M + Na]$^+$ ion at *m*/*z* 837.4588 in the HRESIMS, compatible with the molecular formula $C_{42}H_{70}O_{15}$. The ^{13}C and ^1H NMR spectra of **5** exhibited the presence of seven tertiary methyls (with two attached to oxygen-bearing carbons), a secondary methyl, three oxymethines, an olefinic methine, and a carbonyl. The NMR spectra also showed the presence of two glucose moieties [anomeric protons: δ_H 4.88 (1H, d, J = 7.9 Hz) and 4.99 (1H, d, J = 7.9 Hz)]. The carbonyl group was shown to be present as an α,β-unsaturated ketone (λ_{max} 248 nm; ν_{max} 1639 cm^{-1}), most probably as a Δ^5-7-one group.[11,12] The above evidence, coupled with the spectroscopic comparison with 3β-acetoxy-11α-hydroxycucurbit-5-en-7-one[11] and 3β-hydroxycucurbita-5,24-dien-7-one,[12] suggested that **5** possesses the structure 7-oxomogrol 3,24-di-*O*-β-D-glucopyranoside or 7-oxomogroside II E. Analysis of the ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, NOESY, and ESIMS^n spectra of **5** confirmed the proposed structure.

Compound **6** gave a [M + Na]$^+$ ion in the HRESIMS at *m*/*z* 1323.6194, consistent with a molecular formula of $C_{60}H_{100}O_{30}$. The ^{13}C and ^1H NMR spectra for the aglycon moiety of **6** were almost superimposable on those of **5**. Compound **6** showed also the presence of five glucose moieties [anomeric protons: δ_H 4.80 (1H, d, J = 7.9 Hz), 4.86 (1H, d, J = 7.5 Hz), 4.92 (1H, d, J = 7.9 Hz),

Table 2. ^{1}H NMR Spectroscopic Data (δ values; 600 MHz, C_5D_5N) of Compounds **2** and **4**−**8**[a]

proton(s)	**2**	**4**	**5**	**6**	**7**	**8**
aglycon moiety						
1	2.00, 2.92	1.49, 1.83	2.10, 3.02	2.11, 3.10	1.64, 2.07	1.63, 1.94
2	2.03, 2.42	1.92, 2.47	2.09, 2.43	2.10, 2.49	1.86, 1.95	1.92, 2.47
3	3.67 (br s)	3.68 (br s)	3.79 (br s)	3.81 (br s)	3.72 (br s)	3.69 (br s)
6	5.50 (br d, 5.7)	5.48 (br d, 5.8)	6.29 (s)	6.28 (s)	5.69 (d, 5.5)	5.51 (d, 5.8)
7	1.69, 2.34	1.70, 2.24			1.86, 2.33	1.76, 2.17
8	1.66 (br d, 7.7)	1.65	2.54 (s)	2.53 (s)	1.87	1.79
10	2.79 (br d, 11.2)	2.26	3.21	3.24	2.54	2.41
11	4.15	1.36, 1.59	4.13 (dd, 6.2, 10.6)	4.14		
12	2.09, 2.12	1.42, 1.56	2.10 (2H)	2.20, 2.24	2.53, 2.96	2.49, 2.91
15	1.07, 1.17	1.10, 1.17	1.17, 1.69	1.16, 1.70	1.17, 1.29	1.15, 1.26
16	1.55, 1.99	1.44, 2.02	1.46, 2.02	1.46, 2.09	1.46, 2.11	1.45, 2.09
17	1.67	1.55	1.65	1.79	1.74	1.73
18	0.91 (s)	0.85 (s)	0.92 (s)	0.93 (s)	0.77 (s)	0.74 (s)
19	1.32 (s)	0.87 (s)	1.27 (s)	1.29 (s)	1.27 (s)	1.18 (s)
20	1.53	1.52	1.50	1.50	1.43	1.43
21	1.01 (d, 6.3)	0.94 (d, 6.2)	0.96 (d, 6.5)	1.09 (d, 6.5)	0.87 (d, 6.5)	0.86 (d, 6.5)
22	1.66, 1.72	1.75, 1.90	1.82, 1.89	1.79, 1.90	1.74, 1.87	1.74, 1.88
23	1.56, 1.72	1.72, 1.77	1.69, 1.81	1.58, 1.90	1.55, 1.72	1.53, 1.73
24	4.03 (br d, 8.3)	3.78 (br d, 10.0)	3.86 (br d, 8.6)	3.76 (br d, 9.2)	3.77 (br d, 9.6)	3.76 (br d, 10.0)
26	1.53 (s)	1.45 (s)	1.46 (s)	1.46 (s)	1.46 (s)	1.45 (s)
27	1.53 (s)	1.34 (s)	1.40 (s)	1.34 (s)	1.34 (s)	1.33 (s)
28	1.15 (s)	1.11 (s)	1.23 (s)	1.14 (s)	1.16 (s)	1.08 (s)
29	1.55 (s)	1.53 (s)	1.57 (s)	1.52 (s)	1.43 (s)	1.51 (s)
30	0.92 (s)	0.75 (s)	0.88 (s)	0.98 (s)	0.99 (s)	0.92 (s)
sugar moiety						
G_I 1	4.89 (d, 8.0)	4.88 (d, 7.9)	4.88 (d, 7.9)	4.80 (d, 7.9)		4.81 (d, 7.5)
G_I 2	3.94	3.96	3.97	3.91		3.91
G_I 3	4.18	4.20	4.20	4.13		4.13
G_I 4	4.17	4.18	4.19	4.00		4.01
G_I 5	3.93	3.95	3.93	4.07		4.07
G_I 6	4.33, 4.49	4.38, 4.56	4.36, 4.52	4.30, 4.80		4.31, 4.81
G_{II} 1				5.15 (d, 7.6)		5.14 (d, 7.9)
G_{II} 2				4.04		4.04
G_{II} 3				4.24		4.24
G_{II} 4				4.24		4.24
G_{II} 5				3.95		3.96
G_{II} 6				4.37, 4.52		4.37, 4.53
G_{III} 1		4.91 (d, 7.9)	4.99 (d, 7.9)	4.92 (d, 7.9)	4.90 (d, 8.2)	4.89 (d, 7.5)
G_{III} 2		4.04	4.03	4.16	4.04	4.04
G_{III} 3		4.17	4.20	4.22	4.17	4.15
G_{III} 4		3.97	4.19	3.91	3.97	3.96
G_{III} 5		4.16	4.00	4.07	4.16	4.16
G_{III} 6		3.95, 4.96	4.32, 4.54	3.96, 4.90	3.96, 4.97	3.96, 4.97
G_{IV} 1		4.85 (d, 7.6)		4.86 (d, 7.5)	4.85 (d, 7.9)	4.85 (d, 7.5)
G_{IV} 2		4.06		4.05	4.06	4.06
G_{IV} 3		4.25		4.23	4.24	4.25
G_{IV} 4		4.24		4.23	4.26	4.25
G_{IV} 5		3.91		3.9	3.90	3.91
G_{IV} 6		4.36, 4.49		4.35, 4.48	4.38, 4.50	4.37, 4.50
$G_{V/VI}$ 1	5.21 (d, 7.7)			5.44 (d, 7.9)		
$G_{V/VI}$ 2	4.02			4.07		
$G_{V/VI}$ 3	4.23			4.18		
$G_{V/VI}$ 4	4.20			4.11		
$G_{V/VI}$ 5	3.93			3.93		
$G_{V/VI}$ 6	4.36, 4.50			4.31, 4.49		

[a] Figures in parentheses denote *J* values (Hz).

5.15 (1H, d, $J = 7.6$ Hz), and 5.44 (1H, d, $J = 7.9$ Hz)]. HMBC experiments (Tables S1 and S2, Supporting Information) showed cross-correlations between H-3 (δ_H 3.81) and the C-1 (δ_C 106.8) of the glucose (G_I) moiety attached at C-3, between H-1 (δ_H 4.80) of the glucose (G_I) and C-3 (δ_C 87.3) of the aglycon, between H-1 (δ_H 5.15) of the glucose (G_{II}) and C-6 (δ_C 70.4) of the glucose (G_I), between H-24 (δ_H 3.76) and C-1 (δ_C 103.9) of the glucose (G_{III}) moiety attached at C-24 of the aglycon, between H-1 (δ_H 4.92) of the glucose (G_{III}) and C-24 (δ_C 92.2) of the aglycon, between H-1 (δ_H 4.86) of the glucose (G_{IV}) and C-6 (δ_C 70.2) of the glucose (G_{III}), and between H-1 (δ_H 5.44) of the glucose (G_V) and C-2 (δ_C 82.5) of the glucose (G_{III}). This evidence, in combination with the spectroscopic comparison with compound **5** and mogroside V {3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl mogrol 24-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside},[2,10] suggested that **6** possesses 7-oxomogrol as an aglycon moiety, and the linkage of the sugar moiety is the same as that of mogroside V. Hence, **6** was formulated as 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl 7-oxomogrol 24-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside (7-oxomogroside V). The structure proposed for **6** was supported from the analysis of its ^{13}C DEPT, ^{1}H−^{1}H COSY, HMQC, HMBC, TOCSY, NOESY, and ESIMS^n spectra.

Table 3. Inhibitory Effets on the Induction of Epstein−Barr Virus Early Antigen and Inhibitory Ratio (I.R.) on NOR 1 Action of Compounds **1**−**8** and Reference Compounds

		percentage of EBV-EA induction[a] concentration (mol ratio/32 pmol TPA)						
	compound	1000		500	100	10	IC_{50}[b] (mol ratio/32 pmol TPA)	I.R. of NOR 1 activation[c]
1	mogroside IIA_1	9.5	(70)	26.9	81.3	100	358	1.6
2	mogroside IIB	7.2	(70)	26.0	76.0	100	359	1.5
3	mogroside $IIIA_2$	5.1	(70)	24.0	75.3	100	352	1.5
4	11-deoxymogroside III	10.6	(70)	25.7	81.9	100	357	1.5
5	7-oxomogroside IIE	7.7	(70)	24.4	80.8	100	343	1.5
6	7-oxomogroside V	13.7	(70)	31.0	84.7	100	400	1.4
7	11-oxomogroside IIA_1	8.5	(70)	25.0	80.6	100	346	1.4
8	11-oxomogroside IVa	10.3	(70)	27.3	82.5	100	367	1.4
	reference compounds							
	β-carotene	8.6	(70)	34.2	82.1	100	397	
	glycyrrhizin							2.2
	carboxy-PTIO							8.0

[a] Values represent percentage relative to the positive control value. TPA (32 pmol, 20 ng) = 100%. Values in parentheses are viability percentages of Raji cells. [b] IC_{50} represents the molar ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol TPA. [c] Determined at the concentration of 350 nmol. Inhibitory ratio of NOR 1 (positive control; 350 nm) was taken as 1.0.

Compound **7** was assigned the molecular formula $C_{42}H_{70}O_{14}$ (HRESIMS *m*/*z* 821.4656 $[M + Na]^+$). The ^{13}C and ^1H NMR spectra of **7** showed the presence of seven tertiary methyls (with two attached to an oxygen-bearing carbon), a secondary methyl, two oxymethines, an olefinic methine, and a carbonyl. The spectra also showed the presence of two glucose moieties [anomeric protons: δ_H 4.85 (1H, d, J = 7.9 Hz) and 4.90 (1H, d, J = 8.2 Hz)]. With the above evidence, coupled with the spectroscopic comparison with those of compound **1**[3] and 11-oxomogrol,[10] compound **7** was suggested as possessing 11-oxomogrol as the aglycon moiety and a β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside group linked to C-24 of the aglycon as a sugar moiety. Compound **7** was, therefore, assigned as 11-oxomogrol 24-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (11-oxomogroside II A_1). Analysis of the ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, NOESY, and ESIMS^n spectra of **7** confirmed the structure proposed.

Compound **8** exhibited a $[M + Na]^+$ ion at *m*/*z* 1145.5698 in the HRESIMS, corresponding to a molecular formula of $C_{54}H_{90}O_{24}$. The ^{13}C and ^1H NMR spectra for the aglycon moiety of **8** were superimposable on those of **7**, whereas these showed the presence of four glucose moieties [anomeric protons: δ_H 4.81 (1H, d, J = 7.5 Hz), 4.85 (1H, d, J = 7.5 Hz), 4.89 (1H, d, J = 7.5 Hz), and 5.14 (1H, d, J = 7.9 Hz)]. HMBC experiments showed cross-correlations between H-3 (δ_H 3.69) and C-1 (δ_C 106.9) of the glucose (G_I) moiety attached at C-3, between H-1 (δ_H 4.81) of the glucose (G_I) and C-3 (δ_C 86.6) of the aglycon, between H-1 (δ_H 5.14) of the glucose (G_{II}) and C-6 (δ_C 70.5) of the glucose (G_I), between H-24 (δ_H 3.76) and C-1 (δ_C 106.3) of the glucose (G_{III}) moiety attached at C-24 of the aglycon, between H-1 (δ_H 4.89) of the glucose (G_{III}) and C-24 (δ_C 92.7) of the aglycon, and between H-1 (δ_H 4.85) of the glucose (G_{IV}) and C-6 (δ_C 70.4) of the glucose (G_{III}). On comparison of the spectroscopic data with those of mogroside IV A,[3] compound **8** could be suggested to possess a β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside moiety at both C-3 and C-24 of 11-oxomogrol, its aglycon. Hence, the structure of **8** was formulated as 11-oxomogrol 3,24-di-*O*-β-both-glucopyranosyl-(1→6)-β-both-glucopyranoside (11-oxomogroside IV A), and this assignment was supported from the analysis of the ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, TOCSY, NOESY, and ESIMS^n spectra.

The inhibitory effect on EBV-EA activation induced by TPA was examined as a preliminary evaluation of the potential anti-tumor-promoting effects[6] of the eight compounds, **1**−**8**. The results are shown in Table 3, together with comparable data for β-carotene (a vitamin A precursor studied widely in cancer chemoprevention animal models[13]). All of the compounds tested showed inhibitory effects with IC_{50} values of 343−400 mol ratio/32 pmol TPA, which were almost comparable with that of β-carotene (397 mol ratio/32 pmol TPA). In addition, using an in vitro screening model for NO scavenging, the inhibitory effects of compounds **1**−**8** were evaluated for their scavenging activity against NO generation by NOR 1 in a cultured cell system. Table 3 shows the inhibitory ratios (I.R.) of the eight compounds and two reference compounds, the natural product glycyrrhizin and the synthetic NO scavenger carboxy-PTIO, on NOR 1 action. All of the cucurbitane glycosides tested showed only a weak inhibitory effects (I.R. 1.4−1.6).

Although not evaluated quantitatively, compounds **1**−**8** all exhibited a sweet taste in water, which suggests that these compounds participate in the overall sweetness of the fruits of *S. grosvenorii*.

Experimental Section

General Experimental Procedures. Crystallizations were performed in ethyl acetate (EtOAc), and melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 polarimeter in MeOH at 25 °C. IR spectra were recorded in KBr disks. NMR spectra were recorded with a JEOL ECA-600 (^1H, 600 MHz; ^{13}C, 150 MHz) or with a JEOL LA-500 (^1H, 500 MHz; ^{13}C, 125 MHz) spectrometer in C_5D_5N with tetramethylsilane as an internal standard. ESIMS and HRESIMS were recorded on an Agilent 1100 LC/MSD TOF (time-of-flight) system [ionization mode: positive; nebulizing gas (N_2) pressure: 35 psi; drying-gas (N_2): flow, 12 L/min, temp, 325 °C; capillary voltage: 3000 V; fragmentor voltage: 225 V] and on an Agilent 1100 LC/MSD Trap XCT plus system [ionization mode: positive; nebulizing gas (N_2) pressure: 50 psi; drying-gas (N_2): flow, 10 L/min, temp, 350 °C; capillary voltage: 3000 V; fragmentor voltage: 225 V]. Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan) and C_{18} silica (Chromatorex-ODS, 100−200 mesh; Fuji Silysia Chemical, Ltd., Aichi, Japan) were used for open column chromatography. Reversed-phase preparative HPLC (with refractive index detector) was carried out on C_{18} silica columns (25 cm × 10 cm i.d.) at 25 °C at a flow rate of 2.0 mL/min of the eluent, on a TSK ODS-120A 5 μm column (Toso Co., Tokyo, Japan) [eluent: MeOH−H_2O−acetic acid (AcOH) (50:50:1) (HPLC system I)] and on a Pegasil ODS II 5 μm column (Senshu Scientific Co., Ltd., Tokyo, Japan) [eluent: MeOH−H_2O−AcOH (70:30:1) (system II), MeOH−H_2O−AcOH (60:40:1) (HPLC system III), MeOH−H_2O−AcOH (40:60:1) (HPLC system IV), or MeCN−H_2O (7:13) (HPLC system V)].

Chemicals and Materials. Oven-dried fruits of *Siraitia grosvenorii* Swingle (Cucurbitaceae), cultivated in the southwestern Chinese province of Guangxi, People's Republic of China, were obtained in

September 2004. The plant material was authenticated by one (N.B.) of the authors, and a voucher specimen (No. KO0008-1H-27-4) has been deposited in the Research Laboratory, Ichimaru Pharcos Co. Ltd. The following chemicals were purchased: TPA from ChemSyn Laboratories (Lenexa, KS), glycyrrhizin and β-carotene from Sigma Chemical Co. (St. Louis, MO), the EBV cell culture reagents and *n*-butanoic acid from Nacalai Tesque, Inc. (Kyoto, Japan), and NOR 1 and carboxy-PTIO [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt] from Dojindo Laboratories (Kumamoto, Japan). Mogrol, 11-oxomogrol, mogroside II E, mogroside III, mogroside IV A, and mogroside V used for the NMR spectroscopic comparison were isolated from the fruits of *S. grosvenorii*.[5]

Extraction and Isolation. Air-dried and powdered *S. grosvenorii* fruits (2 kg) were extracted with 99% ethanol (EtOH) by soaking for one week at room temperature three times to give an extract (75.5 g). The extract was partitioned between EtOAc and H_2O (1:1, v/v) mixture. The H_2O phase was then extracted with *n*-butanol (*n*-BuOH), which gave *n*-BuOH and H_2O phases. Removal of the solvent under reduced pressure from the EtOAc, *n*-BuOH, and H_2O phases yielded EtOAc (16.0 g), *n*-BuOH (12.0 g), and H_2O (40.5 g) fractions, respectively.

***n*-Butanol-Soluble Fraction.** A portion (11.2 g) of the *n*-BuOH fraction was subjected to chromatography on a Diaion HP-20 (600 g) column. Step gradient elution was conducted with H_2O−MeOH (4:1 → 0:1) to give fractions BA (2.3 g), BB (1.6 g), BC (4.5 g), BD (0.7 g), and BE (0.3 g), listed in decreasing order of polarity. Fraction BC was then separated by ODS (220 g) column chromatography [eluent: H_2O−MeOH (1:1 → 0:1)] to give fractions BC1 (1.03 g), BC2 (2.77 g), and BC3 (0.47 g). On further ODS (50 g) column chromatography [eluent: H_2O−MeOH (7:3→0:1)], fraction BC1 afforded fractions BC1a (196 mg), BC1b (489 mg), BC1c (252 mg), and BC1d (82 mg). Preparative HPLC (HPLC system I) of fraction BC1c gave **5** [6.9 mg; retention time (t_R) 20.6 min]. Fraction BC2 was further separated by HPLC system III to give **8** (5.6 mg; t_R 17.6 min) and a mixture (23.0 mg; t_R 41.6 min) of **2** and **3**. Preparative HPLC (HPLC system V) of the mixture afforded **2** (2.2 mg; t_R 12.4 min) and **3** (4.7 mg; t_R 10.8 min). In turn, preparative HPLC (HPLC system II) of fraction BC3 yielded **1** (25.1 mg; t_R 25.6 min), **4** (40.3 mg; t_R 43.4 min), and **7** (5.6 mg; t_R 28.8 min).

Water-Soluble Fraction. A portion (25.8 g) of the H_2O fraction was subjected to Diaion HP-20 (1.2 kg) column chromatography using H_2O−MeOH (1:0 → 0:1) to give fractions WA (18.2 g), WB (2.5 g), WC (1.1 g), and WD (1.0 g), listed in increasing order of polarity. Fraction WC was further separated by ODS (100 g) column chromatography [eluent: H_2O−MeOH (4:1 → 0:1)] to give fractions WC1 (731 mg), WC2 (220 mg), WC3 (190 mg), and WC4 (157 mg). Fraction WC3 was subjected to HPLC system IV and yielded **6** (7.3 mg; t_R 37.0 min).

Mogroside II A_1 {mogrol [(24*R*)-cucubit-5-ene-3β,11α,24,25-tetrol] 24-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside} (1). The ^{1}H and ^{13}C NMR data of **1** are shown in Tables S3 and S4, respectively, since the assigned NMR data of this compound are unavailable in the literature; ESIMS *m/z* 823 $[M + Na]^+$.

Mogroside II B (mogrol 3,25-di-*O*-β-D-glucopyranoside) (2): fine needles, mp 183−186 °C (EtOAc); $[\alpha]^{25}_D$ +16.2 (*c* 0.46, MeOH); IR (KBr) ν_{max} 3400 (OH), 2931, 1647, 1459, 1379, 1078, 1030 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 823.4810 (calcd for $C_{42}H_{72}O_{14}Na$ $[M + Na]^+$, 823.4819); ESIMS *m/z* 823 $[M + Na]^+$, MS^2 *m/z* 661 $[823 - C_6H_{10}O_5]^+$, MS^2 *m/z* 643 $[823 - C_6H_{12}O_6]^+$, MS^3 *m/z* 463 $[643 - C_6H_{12}O_6]^+$.

Mogroside III A_2 [3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl mogrol 24-*O*-β-D-glucopyranoside] (3). The ^{1}H and ^{13}C NMR data of **3** are shown in Tables S3 and S4, respectively, since the assigned NMR data of this compound are unavailable in the literature; ESIMS *m/z* 985 $[M + Na]^+$.

11-Deoxymogroside III [3-*O*-β-D-glucopyranosyl 11-deoxymogrol 24-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside] (4): fine plates, mp 189−192 °C (EtOAc); $[\alpha]^{25}_D$ +5.0 (*c* 0.54, MeOH); IR (KBr) ν_{max} 3398 (OH), 2935, 1647, 1460, 1377, 1171, 1076, 1034 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 969.5403 (calcd for $C_{48}H_{82}O_{18}Na$ $[M + Na]^+$, 969.5398); ESIMS *m/z* 969 $[M + Na]^+$, MS^2 *m/z* 807 $[969 - C_6H_{10}O_5]^+$, MS^2 *m/z* 789 $[969 - C_6H_{12}O_6]^+$, MS^3 *m/z* 645 $[807 - C_6H_{10}O_5]^+$, MS^3 *m/z* 627 $[807 - C_6H_{12}O_6]^+$.

7-Oxomogroside II E (7-oxomogrol 3,24-di-*O*-β-D-glucopyranoside) (5): fine needles, mp 196−199 °C (EtOAc); $[\alpha]^{25}_D$ +22.9 (*c* 0.94, MeOH); UV λ_{max} (log ε) 248 (4.03) nm; IR (KBr) ν_{max} 3392 (OH), 2929, 1639 (>C=O), 1460, 1381, 1171, 1076, 1034 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 837.4588 (calcd for $C_{42}H_{70}O_{15}Na$ $[M + Na]^+$, 837.4612); ESIMS *m/z* 815 $[M + H]^+$, MS^2 *m/z* 653 $[815 - C_6H_{10}O_5]^+$, MS^2 *m/z* 635 $[815 - C_6H_{12}O_6]^+$, MS^2 *m/z* 491 $[815 - 2C_6H_{10}O_5]^+$, MS^2 *m/z* 455 $[815 - 2C_6H_{12}O_6]^+$.

7-Oxomogroside V {3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-7-oxomogrol 24-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranoside-(1→6)]-β-D-glucopyranoside} (6): fine needles, mp 196−200 °C (EtOAc); $[\alpha]^{25}_D$ +3.8 (*c* 0.60, MeOH); UV λ_{max} (log ε) 248 (4.04) nm; IR (KBr) ν_{max} 3398 (OH), 2927, 1641 (>C=O), 1460, 1381, 1169, 1074 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 1323.6194 (calcd for $C_{60}H_{100}O_{30}Na$ $[M + Na]^+$, 1323.6197); ESIMS *m/z* 1323 $[M + Na]^+$, MS^2 *m/z* 1161 $[1323 - C_6H_{10}O_5]^+$, MS^3 *m/z* 999 $[1161 - C_6H_{10}O_5]^+$, MS^4 *m/z* 837 $[999 - C_6H_{10}O_5]^+$, MS^4 *m/z* 675 $[837 - C_6H_{10}O_5]^+$, MS^4 *m/z* 513 $[675 - C_6H_{10}O_5]^+$.

11-Oxomogroside II A_1 [11-oxomogrol 24-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside] (7): fine plates, mp 144−147 °C (EtOAc); $[\alpha]^{25}_D$ +48.3 (*c* 0.69, MeOH); IR (KBr) ν_{max} 3398 (OH), 2929, 1687 (>C=O), 1647, 1461, 1379, 1171, 1074, 1038 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 821.4656 (calcd for $C_{42}H_{70}O_{14}Na$ $[M + Na]^+$, 821.4663); ESIMS *m/z* 821 $[M + Na]^+$, MS^2 *m/z* 669 $[821 - 152]^+$, MS^2 *m/z* 659 $[821 - C_6H_{10}O_5]^+$, MS^3 *m/z* 507 $[669 - C_6H_{10}O_5]^+$.

11-Oxomogroside IV A [11-oxomogrol 3,24-di-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside] (8): fine plates, mp 184−188 °C (EtOAc); $[\alpha]^{25}_D$ +17.3 (*c* 0.44, MeOH); IR (KBr) ν_{max} 3398 (OH), 2925, 1684 (>C=O), 1457, 1379, 1171, 1074, 1031 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 1145.5719 (calcd for $C_{54}H_{90}O_{24}Na$ $[M + Na]^+$, 1145.5719); ESIMS *m/z* 1145 $[M + Na]^+$, MS^2 *m/z* 669 $[1145 - 152 - 2C_6H_{10}O_5]^+$, MS^3 *m/z* 507 $[669 - C_6H_{10}O_5]^+$.

In Vitro EBV-EA Activation Experiment. For the protocol for this in vitro assay, refer to previous articles.[14,15]

In Vitro NOR 1 Inhibition Experiment. For the protocol for this in vitro assay, refer to previous articles.[9,15]

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Supporting Information Available: ^{13}C and ^{1}H NMR and HMBC NMR data for **4**−**6** and ^{13}C and ^{1}H NMR data for **1** and **3**. This information is available free of charge via the Internet at http://pubs.acs.org.

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