Cucurbitane-Type Triterpenoids from the Fruits of *Momordica charantia* and Their Cancer Chemopreventive Effects

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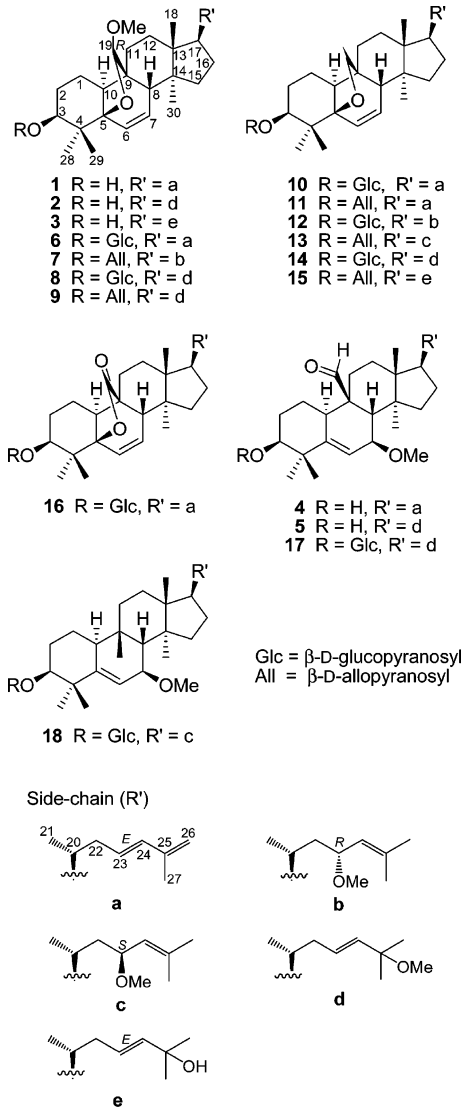
Thirteen cucurbitane-type triterpene glycosides, including eight new compounds named charantosides I (**6**), II (**7**), III (**10**), IV (**11**), V (**12**), VI (**13**), VII (**16**), and VIII (**17**), and five known compounds, **8**, **9**, **14**, **15**, and **18**, were isolated from a methanol extract of the fruits of Japanese *Momordica charantia*. The structures of the new compounds were determined on the basis of spectroscopic methods. On evaluation of these triterpene glycosides and five other cucurbitane-type triterpenes, **1**−**5**, also isolated from the extract of *M. charantia* fruits, for their inhibitory effects on the induction of Epstein−Barr virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells, these compounds showed inhibitory effects on EBV-EA induction with IC_{50} values of 200−409 mol ratio/32 pmol TPA. In addition, upon evaluation of compounds **1**−**5** for inhibitory effects against activation of (±)-(*E*)-methyl-2[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexemide (NOR 1), a nitrogen oxide (NO) donor, compounds **1**−**3** showed moderate inhibitory effects. Compounds **1** and **2** exhibited marked inhibitory effects in both 7,12-dimethylbenz[*a*]anthracene (DMBA)- and peroxynitrite ($ONOO^-$; PN)-induced mouse skin carcinogenesis tests.

The plant *Momordica charantia* L. (Cucurbitaceae) is commonly known as "bitter gourd" or "bitter melon" in English and is cultivated throughout the world for use as a vegetable as well as a medicine. *M. charantia* has been used traditionally medicinally in developing countries mostly for healing diabetes and as a carminative and in the treatment of colic.[1,2] Previous investigations have shown that crude extracts of the fruit of *M. charantia* possess antidiabetic activity,[3,4] and many cucurbitane-type triterpenoids have been isolated from the fruits,[5−12] seeds,[13−15] leaves and vines,[16] and stems[17] of *M. charantia*.

In the course of our search for potential antitumor promoters from natural sources,[18,19] we have previously isolated five cucurbitane-type triterpenoids, **1**−**5**, from the MeOH extract of the dried fruit of Japanese *M. charantia*, which is commonly called "nigauri" or "goya".[20] Our continuing study on the triterpenoid constituents of the MeOH extract of *M. charantia* fruit has now led to the isolation of eight new (**6**, **7**, **10**−**13**, **16**, and **17**) and five known (**8**, **9**, **14**, **15**, and **18**) cucurbitane-type triterpene glycosides. This paper describes the characterization of these compounds and the inhibitory effects on the activation of Epstein−Barr virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells by compounds **1**−**18** and on the activation of (±)-(*E*)-methyl-2[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexemide (NOR 1), a nitrogen oxide (NO) donor, by compounds **1**−**5**. In addition, we report the inhibitory effects of (19*R*,23*E*)-5*β*,19-epoxy-19-methoxycucurbita-6,23,25-trien-3*β*-ol (**1**) and (19*R*,23*E*)-5*β*,19-epoxy-19,25-dimethoxycucurbita-6,23-dien-3*β*-ol (**2**) in 7,12-dimethylbenz[*a*]anthracene (DMBA)- and peroxynitrite ($ONOO^-$; PN)-induced two-stage mouse skin carcinogenesis tests.



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Results and Discussion

Thirteen cucurbitane-type triterpene glycosides, including eight new compounds, charantosides I (**6**), II (**7**), III (**10**), IV (**11**), V (**12**), VI (**13**), VII (**16**), and VIII (**17**), and five known compounds, goyaglycoside-c (**8**),[10] goyaglycoside-d (**9**),[10] momordicoside F_1 (**14**),[5] momordicoside F_2 (**15**),[5] and karaviloside I (**18**),[11] were isolated from a MeOH extract of the fruits of *M. charantia*. Identification of the five known compounds was performed by MS and ^{1}H NMR spectroscopic comparison of the corresponding compounds with literature values.

All of the eight new compounds were suggested to be monoglycosides on the basis of the IR absorption bands due to a glycosidic function (e.g., **6**: ν_{max} 3421, 1068, 1036 cm^{-1})[10,11] and an anomeric proton signal of the glycosyl moiety observed in their ^{1}H NMR spectra. The proposed structures for all of the eight new compounds as described below were supported by analysis of the HMBC and NOESY data (Tables S1−S8, Supporting Information), in addition to ^{13}C DEPT, ^{1}H−^{1}H COSY, and HMQC data.

The molecular formula of compound **6** was determined to be $C_{37}H_{58}O_8$ on the basis of its HRESIMS (positive-ion mode) ([M + Na]$^+$, *m*/*z* 653.4017). The UV absorptions at λ_{max} 239 (log ϵ 4.09), 230 (4.28), and 225 (4.26) suggested the presence of a (23*E*)-$\Delta^{23,25}$-conjugated diene system in the side chain of the aglycon moiety.[20,21] The ^{13}C (Table 1) and ^{1}H NMR spectra (Table 2) of **6** showed the presence of four tertiary methyls, a secondary methyl, a vinylic methyl, an *O*-methyl, an oxymethine, two disubstituted double bonds, a terminal methylene, and an acetal methine. The ^{1}H NMR spectrum also showed signals assignable to a β-D-glucopyanosyl moiety [δ_H 4.99 (1H, d, J = 7.9 Hz; H-1′)].[10,11] The NMR data of the aglycon moiety of **6** were in good agreement with those of compound **1** except that the former exhibited a downfield glycosylation shift for the C-3 signal in the ^{13}C NMR spectrum.[12,22] Compound **6** showed a diagnostic NOE correlation between H-1β and H-19 in the NOESY experiment, which supported the 19*R*-stereochemistry.[6,23] The 19*R*-stereochemistry allows very close spatial orientation of H-19 to H-1β, which is consistent with the appearance of the NOE correlation between the ^{1}H NMR signals of H-1β and H-19 in the NOESY spectrum. In addition, conjugation at C-3 of the aglycon moiety with the glucosyl moiety was supported from the HMBC experiment, which showed cross-correlations between H-3 (δ_H 3.75) and the C-1′ (δ_C 105.3) of the glucose moiety and between H-1′ of the glucose (δ_H 4.99) and C-3 of the aglycon (δ_C 83.7). The above evidence suggested that **6** has a structure of (19*R*,23*E*)-5β,19-epoxy-19-methoxycucurbita-6,23,25-trien-3β-ol 3-*O*-β-D-glucopyranoside (charantoside I).

Compound **7** was assigned a molecular formula of $C_{38}H_{62}O_9$, as determined from its [M + Na]$^+$ ion at *m*/*z* 685.4290 in the HRESIMS. The ^{13}C (Table 1) and ^{1}H NMR spectra (Table 2) of **7** showed the presence of four tertiary methyls, a secondary methyl, two vinylic methyls, two *O*-methyls, both a di- and a trisubstituted double bond, an oxymethine, and an acetal methine, in addition to a β-D-allopyranosyl function.[10−12] The ^{13}C and ^{1}H NMR spectra of **7** were very similar to those of compound **9**,[10] except for the signals due to a side chain of the aglycon moiety. Compound **7** exhibited ^{1}H NMR signals for the side-chain protons at δ_H 1.08 (3H, d, J = 6.2 Hz; a secondary methyl), 1.71 and 1.74 (each 3H and br s; two vinylic methyls), 3.28 (3H, s; an *O*-methyl), 4.13 (1H, dt, J = 9.0, 3.1 Hz; an allylic oxymethine), and 5.22 (1H, d, J = 9.0 Hz; an olefinic methine). This permitted the positioning of the double bond at C-24 and the methoxyl group at C-23 in the side chain and was used to formulate a (23ξ)-23-methoxy-Δ^{24} side-chain structure.[24] The stereochemistry at C-23 of **7** was deduced to be *R* by comparison of its side-chain ^{13}C NMR signals with those of compound **13** and (23*R*)- and (23*S*)-cycloart-24-ene-3β,23-diols.[24] Thus, the $\Delta\delta_C$ values [δ_C(**7**) − δ_C(**13**)] for the side-chain signals were calculated as −0.9 (C-20), −0.9 (C-21), +0.4 (C-22), −1.6 (C-23), +0.5 (C-24), −1.4 (C-25), −0.1 (C-26), and −0.4 (C-27) from the ^{13}C NMR data of compounds **7** and **13** (Table 1), which were almost consistent with the $\Delta\delta_C$ values [δ_C(23*R*) − δ_C(23*S*)] of −0.5 (C-20), −1.0 (C-21), ±0 (C-22), −1.0 (C-23), +0.7 (C-24), −1.8 (C-25), ±0 (C-26), and ±0 (C-27) calculated from the ^{13}C NMR data of (23*R*)- and (23*S*)-cycloart-24-ene-3β,23-diols.[25] The above evidence suggested that **7** possesses the structure (19*R*,23*R*)-5β,19-epoxy-19,23-dimethoxycucurbita-6,24-dien-3β-ol 3-*O*-β-D-allopyranoside (charantoside II). As will be mentioned later, compound **13** (charantoside VI) was deduced to possess a 23*S*-stereochemistry.

Compound **10** exhibited a [M + Na]$^+$ ion at *m*/*z* 623.3914 in the HRESIMS, compatible with the molecular formula, $C_{36}H_{56}O_7$. The ^{13}C (Table 1) and ^{1}H NMR spectra (Table 2) of **10** showed the presence of four tertiary methyls, a secondary methyl, a vinylic methyl, an oxymethylene, a terminal methylene, two disubstituted double bonds, and a β-D-glucopyranosyl functionality. The UV absorptions at λ_{max} 238, 229, and 224 suggested that the aglycon moiety possesses a (23*E*)-$\Delta^{23,25}$ side chain.[20,21] The above evidence, coupled with the spectroscopic comparison with compounds **6** and **14**, led to the formulation of the structure of **10** as (23*E*)-5β,19-epoxycucurbita-6,23,25-trien-3β-ol 3-*O*-β-D-glucopyranoside (charantoside III).

Compound **11** was assigned the molecular formula $C_{36}H_{56}O_7$ (HRESIMS *m*/*z* 623.3919 [M + Na]$^+$) and exhibited ^{13}C (Table 1)

Table 1. ^{13}C NMR Spectroscopic Data (δ values; 150 MHz, C_5D_5N) for Eight Cucurbitane Glycosides from the Fruits of *Momordica charantia*

carbon	**6**	**7**	**10**	**11**	**12**	**13**	**16**	**17**[a]
aglycon moiety								
1	18.7	18.7	18.9	18.9	18.9	18.9	19.7	22.2
2	27.4	27.4	27.6	27.6	27.6	27.6	26.5	29.3
3	83.7	83.4	85.4	85.1	85.4	85.1	85.5	86.9
4	39.2	39.1	39.1	39.0	39.1	39.0	38.4	42.1
5	85.5	85.5	85.9	85.9	85.8	85.9	84.2	147.5
6	133.2	133.1	134.7	134.7	134.2	134.2	133.0	120.7
7	131.6	131.6	129.9	129.9	130.0	130.0	132.5	75.7
8	42.2	42.2	52.3	52.3	52.3	52.3	44.9	45.8
9	48.2	48.2	45.3	45.3	45.3	45.3	50.7	50.2
10	41.7	41.6	40.2	40.1	40.1	40.1	40.8	36.8
11	23.3	23.4	23.8	23.8	23.9	23.8	21.9	22.6
12	30.9	31.1	31.0	31.0	31.2	31.2	30.0	29.3
13	45.4	45.4	45.5	45.5	45.6	45.5	45.4	45.8
14	48.3	48.4	48.9	48.9	48.9	48.9	47.9	47.9
15	33.9	33.8	33.4	33.4	33.4	33.4	33.4	35.0
16	28.3	28.4	28.3	28.3	28.4	28.6	27.8	27.6
17	50.7	51.4	50.6	50.6	51.3	51.3	50.8	50.2
18	14.9	14.8	15.0	15.0	15.0	14.9	14.6	15.0
19	112.4	112.4	80.1	80.2	80.1	79.7	182.0	207.0
20	36.9	32.9	36.8	36.8	32.8	33.8	36.8	36.3
21	19.0	19.0	18.9	18.9	19.0	19.9	18.8	19.0
22	40.1	43.4	40.1	40.1	43.4	43.0	40.1	39.7
23	129.9	74.8	129.9	129.9	74.9	76.4	129.8	128.4
24	134.7	127.9	135.2	135.2	127.9	127.4	134.8	137.7
25	142.6	134.4	142.5	142.5	134.5	135.8	142.5	74.8
26	114.6	25.8	114.7	114.7	25.8	25.9	114.7	26.1
27	18.9	18.1	18.9	18.9	18.1	18.5	18.9	26.4
28	24.4	24.8	25.6	25.6	25.6	25.6	24.0	27.8
29	21.2	21.3	21.1	21.1	21.1	21.1	20.9	25.8
30	20.0	20.0	20.2	20.2	20.2	20.2	19.3	18.1
OMe-7								55.9
OMe-19	57.6	57.6						
OMe-23		55.6			55.6	55.3		
OMe-25								50.1
sugar moiety								
1′	105.3	102.3	106.7	103.8	106.7	103.7	107.7	107.3
2′	76.2	73.8	75.8	73.1	75.8	73.1	75.3	75.2
3′	78.0	71.7	78.3	72.4	78.3	72.4	78.4	78.8
4′	72.0	69.2	71.9	69.3	71.9	69.3	71.7	71.7
5′	78.7	76.5	78.4	76.2	78.4	76.2	78.8	78.3
6′	63.0	63.3	63.1	63.4	63.1	63.4	63.1	63.1

[a] Determined at 125 MHz.

Table 2. ^{1}H NMR Spectroscopic Data (δ values; 600 MHz, C_5D_5N) for Eight Cucurbitane Glycosides from the Fruits of *Momordica charantia*[a]

proton(s)	**6**	**7**	**10**	**11**	**12**	**13**	**16**	**17**[b]
aglycon moiety								
1α	1.44	1.44	1.34	1.34	1.35	1.35	1.47	1.57
1*β*	1.90	1.90	1.77	1.78	1.77	1.77	1.70	1.82
2α	1.78	1.74	1.78	1.80	1.78	1.78	1.77	1.92
2*β*	2.18 (dq, 13.5, 3.5)	2.13 (dq, 13.1, 3.7)	2.33	2.33	2.37	2.34	2.46 (dd, 3.5, 13.0)	2.44 (dq, 13.2, 2.6)
3α	3.75 (br s)	3.70 (br s)	3.71 (br s)	3.66 (br s)	3.70 (br s)	3.65 (br s)	3.68 (br s)	3.74 (br s)
6	6.18 (dd, 2.4, 9.6)	6.15 (dd, 2.4, 9.6)	6.21 (dd, 2.0, 9.6)	6.20 (dd, 2.1, 9.6)	6.20 (dd, 2.0, 9.6)	6.19 (d, 9.6)	6.33 (dd, 2.0, 9.7)	6.07 (d, 4.9)
7	5.63 (dd, 3.5, 9.6)	5.61 (dd, 3.8, 9.6)	5.58 (dd, 3.8, 9.6)	5.58 (dd, 3.5, 9.6)	5.56 (dd, 3.8, 9.6)	5.56 (dd, 3.8, 9.6)	5.63 (dd, 3.1, 9.7)	3.48 (br d, 5.4)
8*β*	3.13 (br s)	3.13 (br s)	2.32	2.34	2.31	2.31 (br s)	2.57 (t, 2.0)	2.19 (s)
10α	2.47 (dd, 5.9, 12.7)	2.47 (dd, 5.8, 12.7)	2.28	2.31	2.29	2.29	2.67 (dd, 5.5, 12.4)	2.57
11α	1.74	1.76	1.63	1.62	1.62	1.64	1.71	1.44
11*β*	1.63	1.67	1.33	1.33	1.34	1.34	2.41 (dt, 5.5, 15.2)	2.59
12α	1.61	1.66	1.56	1.54	1.61	1.61	1.51	1.58
12*β*	1.52	1.61	1.45	1.46	1.52	1.51	1.58	1.52
15α	1.31 (2H)	1.30 (2H)	1.28	1.30	1.26	1.27	1.28	1.34
15*β*			1.21	1.22	1.19	1.20	1.20	1.14
16α	1.94	1.96	1.94	1.95	1.95	1.98	1.91	1.94
16*β*	1.31	1.43	1.32	1.32	1.43	1.35	1.30	1.33
17α	1.51	1.48	1.47	1.47	1.45	1.53	1.49	1.55
18	0.90 (s)	0.95 (s)	0.78 (s)	0.78 (s)	0.79 (s)	0.78 (s)	0.87 (s)	0.94 (s)
19a	4.86 (s)	4.88 (s)	3.61 (d, 8.0)	3.61 (d, 8.3)	3.59 (d, 7.9)	3.58 (d, 8.3)		10.21 (s)
19b			3.74 (d, 8.0)	3.77 (d, 8.3)	3.73 (d, 7.9)	3.75 (d, 8.3)		
20	1.50	1.94	1.49	1.51	1.92	1.55	1.48	1.53
21	0.95 (d, 5.8)	1.08 (d, 6.2)	0.93 (d, 5.8)	0.94 (d, 6.2)	1.05 (d, 6.5)	1.04 (d, 5.5)	0.92 (d, 5.8)	0.98 (d, 5.4)
22a	1.84	1.07	1.84	1.84	1.05	1.57	1.81	1.85
22b	2.30 (br dd, 5.8, 13.4)	1.86	2.31	2.30	1.85	1.72	2.28 (br dd, 6.2, 13.4)	2.24 (br d, 5.4, 13.5)
23	5.77 (ddd, 5.8, 8.2, 15.1)	4.13 (dt, 9.0, 3.1)	5.77 (ddd, 6.2, 6.8, 14.8)	5.78 (ddd, 6.2, 8.1, 15.1)	4.13 (dt, 9.9, 3.1)	4.11 (dt, 8.9, 5.1)	5.76 (ddd, 6.2, 8.6, 15.1)	5.65
24	6.31 (d, 15.1)	5.22 (d, 9.0)	6.31 (d, 14.8)	6.31 (d, 15.1)	5.22 (dt, 8.6, 1.4)	5.16 (br d, 8.9)	6.30 (d, 15.1)	5.57 (d, 15.7)
26a	4.98 (s)	1.74 (br s)	4.98 (s)	4.98 (s)	1.75 (d, 1.1)	1.75 (s)	4.98 (br s)	1.72 (s)
26b	5.03 (s)		5.03 (s)	5.04 (s)			5.03 (br s)	
27	1.92 (s)	1.71 (br s)	1.92 (s)	1.92 (s)	1.71 (d, 1.0)	1.73 (s)	1.91 (s)	1.34 (s)
28	0.86 (s)	0.82 (s)	0.93 (s)	0.90 (s)	0.93 (s)	0.89 (s)	0.94 (s)	1.14 (s)
29	1.50 (s)	1.44 (s)	1.54 (s)	1.48 (s)	1.53 (s)	1.47 (s)	1.60 (s)	1.34 (s)
30	0.90 (s)	0.93 (s)	0.87 (s)	0.87 (s)	0.89 (s)	0.86 (s)	0.82 (s)	0.78 (s)
OMe-7								3.20 (s)
OMe-19	3.45 (s)	3.48 (s)						
OMe-23		3.28 (s)			3.30 (s)	3.30 (s)		
OMe-25								3.23 (s)
sugar moiety								
1′	4.99 (d, 7.9)	5.46 (d, 7.6)	4.92 (d, 7.9)	5.40 (d, 7.9)	4.91 (d, 7.9)	5.38 (d, 7.9)	4.86 (d, 7.9)	4.86 (d, 8.3)
2′	4.00 (t, 7.9)	3.88 (dd, 2.0, 7.6)	4.00 (t, 7.9)	3.94 (dd, 2.4, 7.9)	3.99 (t, 7.9)	3.92 (dd, 2.7, 7.9)	3.97 (dd 7.9, 8.9)	3.87 (t, 8.3)
3′	4.26 (t, 8.9)	4.71 (t, 2.0)	4.22 (t, 8.9)	4.69 (t, 2.4)	4.23 (t, 9.0)	4.68 (t, 2.7)	4.20 (t, 8.9)	4.20 (t, 8.9)
4′	4.22 (t, 8.9)	4.18 (dd, 2.0, 9.7)	4.20 (t, 8.9)	4.18 (br d, 9.6)	4.19 (t, 9.0)	4.17 (br dd, 2.4, 9.2)	4.13 (t, 8.9)	4.15 (t, 8.9)
5′	3.99	4.45	3.98	4.46	3.97 (ddd, 3.1, 5.5, 8.2)	4.45	3.95	3.94
6′a	4.42 (dd, 4.4, 11.3)	4.37 (dd, 4.8, 11.3)	4.41 (dd, 5.5, 11.7)	4.38 (dd, 5.1, 11.7)	4.40 (dd, 5.5, 11.7)	4.37 (dd, 5.1, 11.7)	4.38 (dd, 5.5, 12.1)	4.36 (dd, 5.1, 11.7)
6′b	4.59 (br d, 11.3)	4.52 (dd, 2.0, 11.3)	4.57 (br d, 11.7)	4.53 (br d, 11.7)	4.57 (dd, 2.4, 11.7)	4.52 (dd, 2.4, 11.7)	4.56 (dd, 2.4, 12.1)	4.54 (dd, 2.4, 11.7)

[a] Figures in parentheses denote *J* values (Hz). [b] Determined at 500 MHz.

Table 3. Inhibitory Effects on the Induction of Epstein−Barr Virus Early Antigen and Inhibitory Ratio (I.R.) on NOR 1 Action of Compounds Isolated from *Momordica charantia* and Reference Compounds

		concentration (mol ratio/TPA)[a]					
		1000	500	100	10	IC_{50}[b] (mol ratio/ 32 pmol TPA)	IR (%) for NOR1 action
	compound	% to control (% viability)					
1	(19*R*,23*E*)-5β,19-epoxy-19-methoxy-cucurbita-6,23,25-trien-3β-ol	0 (70)	18.5	60.2	82.7	203	2.0
2	(19*R*,23*E*)-5β,19-epoxy-19,25-dimethoxy-cucurbita-6,23-dien-3β-ol	0 (70)	19.0	42.4	83.9	200	1.9
3	(23*E*)-5β,19-epoxy-19-methoxycucurbita-6,23-diene-3β,25-diol	0 (70)	27.6	72.5	94.5	315	1.9
4	(23*E*)-3β-hydroxy-7β-methoxycucurbita-5,23,25-trien-19-al	0 (70)	22.5	75.4	95.3	291	1.6
5	(23*E*)-3β-hydroxy-7β,25-dimethoxy-cucurbita-5,23-dien-19-al	0 (70)	33.7	77.5	97.3	328	1.5
6	charantoside I	12.6 (70)	35.4	81.1	100	372	
7	charantoside II	12.3 (70)	35.0	82.1	100	373	
8	goyaglycoside-c	13.5 (70)	33.7	80.0	100	398	
9	goyaglycoside-d	11.2 (70)	34.2	80.3	100	358	
10	charantoside III	12.9 (70)	36.0	82.1	100	384	
11	charantoside IV	12.1 (70)	35.0	81.1	100	371	
12	charantoside V	10.5 (70)	30.6	77.0	100	376	
13	charantoside VI	12.7 (70)	35.9	82.0	100	380	
14	momordicoside F_1	11.3 (70)	31.5	78.1	100	385	
15	momordicoside F_2	9.1 (70)	29.7	76.9	100	362	
16	charantoside VII	9.9 (70)	28.1	75.1	100	361	
17	charantoside VIII	15.3 (70)	38.5	82.5	100	401	
18	karaviloside I	16.1 (70)	39.2	83.7	100	409	
	reference compounds						
	β-carotene	8.6 (70)	34.2	82.1	100	400	
	curcumin	0 (70)	22.8	81.8	100	341	
	glycyrrhizin	27.4 (>80)	63.3	83.3	100	572	2.2
	carboxy-PTIO						8.0

[a] Values represent percentages 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 mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol of TPA.

and ^{1}H NMR signals (Table 2) almost indistinguishable from those of **10** except for resonances due to a glycosyl moiety. Compound **11** exhibited NMR signals for a β-D-allopyranosyl function as the glycosyl moiety, and consequently, this was characterized as (23*E*)-5β,19-epoxycucurbita-6,23,25-trien-3β-ol 3-*O*-β-D-allopyranoside (charantoside IV).

Compound **12** exhibited a $[M + Na]^+$ ion at *m*/*z* 655.4194 in the HRESIMS, corresponding to a molecular formula of $C_{37}H_{60}O_8$. The ^{13}C (Table 1) and ^{1}H NMR spectra (Table 2) of **12** exhibited the presence of four tertiary methyls, a secondary methyl, an *O*-methyl, two vinylic methyls, an oxymethylene, both a di- and a trisubstituted double bond, and a β-D-glucopyranosyl moiety. The NMR data for the ring system of the aglycon and the glycosyl moieties of **12** were superimposable on those of **10**, whereas the NMR data for the side-chain moiety of **12** were almost indistinguishable from those of **7**. Therefore, the structure of compound **12** was assigned as (23*R*)-5β,19-epoxy-23-methoxycucurbita-6,24-dien-3β-ol 3-*O*-β-D-glucopyranoside (charantoside V).

Compound **13** gave a $[M + Na]^+$ ion in the HRESIMS at *m*/*z* 655.4167, consistent with a molecular formula of $C_{37}H_{60}O_8$. The ^{13}C (Table 1) and ^{1}H NMR spectra (Table 2) of **13** suggested it to be a stereoisomer of compound **12**. The NMR data showed that **13** possesses a β-D-allopyranosyl group as a glycosyl moiety. In addition, as mentioned above, comparison of the side-chain ^{13}C NMR data with those of compound **7** and (23*R*)- and (23*S*)-cycloart-24-ene-3β,23-diols[24,25] made it possible to assign the stereochemistry at C-23 of **13** as *S*. Consequently, the structure of **13** was characterized as (23*S*)-5β,19-epoxy-23-methoxycucurbita-6,24-dien-3β-ol 3-*O*-β-D-allopyranoside (charantoside VI).

Compound **16** possesses the molecular formula $C_{36}H_{54}O_8$ as determined from the HRESIMS ($[M + Na]^+$ *m*/*z* 637.3694). The ^{13}C (Table 1) and ^{1}H NMR spectra (Table 2) exhibited the presence of four tertiary methyls, a secondary methyl, a vinylic methyl, a terminal methylene, two disubstituted double bonds, a carbonyl, and a β-D-glucopyranosyl unit. The NMR spectra of **16** were quite similar to those of **6** except that the former showed a carbonyl signal (δ_C 182.0) instead of the *O*-methyl and an acetal methine signal observed for the latter. This suggested that C-19 of **16** is part of a 5β,19-γ-lactone ring, which was supported by observation of ^{13}C−^{1}H long-range couplings from H-8, H-10, and H-11β to C-19 in the HMBC spectrum (Table S7, Supporting Information). Thus, the structure of **16** was assigned as (23*E*)-3β-hydroxycucurbita-6,-23,25-trien-5β,19-olide 3-*O*-β-D-glucopyranoside (charantoside VII).

The molecular formula of compound **17** was determined as $C_{38}H_{62}O_9$ from its HRESIMS ($[M + Na]^+$ *m*/*z* 685.4294). The ^{13}C (Table 1) and ^{1}H NMR spectra (Table 2) of **17** showed the presence of six tertiary methyls, a secondary methyl, two *O*-methyls, both a di- and a trisubstituted double bond, an aldehyde, and a β-D-glucopyranosyl unit. The NMR data for the aglycon moiety of **17** were very similar to those of compound **5**[20] except that the former exhibited a downfield glycosylation shift for the C-3 signal in the ^{13}C NMR spectrum.[12,22] The above evidence suggested that **17** has the structure (23*E*)-3β-hydroxy-7β,25-dimethoxycucurbita-5,23-dien-19-al 3-*O*-β-D-glucopyranoside (charantoside VIII).

Acid hydrolysis of **6**, **10**, **12**, **16**, and **17** gave D-glucose as a sugar identified by HPLC, whereas **7**, **11**, and **13** yielded D-allose, which supported the proposed structures of the eight new glycosides.

The inhibitory effects on the induction of EBV-EA induced by TPA were examined as a preliminary evaluation of antitumor-promoting activity. Table 3 shows the inhibitory effects of 13 cucurbitane-type triterpene glycosides, **6**−**18**, isolated in this study, and five cucurbitane-type triterpenes, **1**−**5**, isolated recently from an *n*-hexane-soluble fraction of the MeOH extract of *M. charantia* fruits,[20] against TPA (32 pmol)-induced EBV-EA activation in Raji cells. All of the compounds tested caused high viability (70%) of Raji cells even at 32 nmol (mol ratio of compound to TPA = 1000:1), indicating their very low cytotoxicity at this high concentration. Each compound tested showed an inhibitory effect, with an IC_{50}

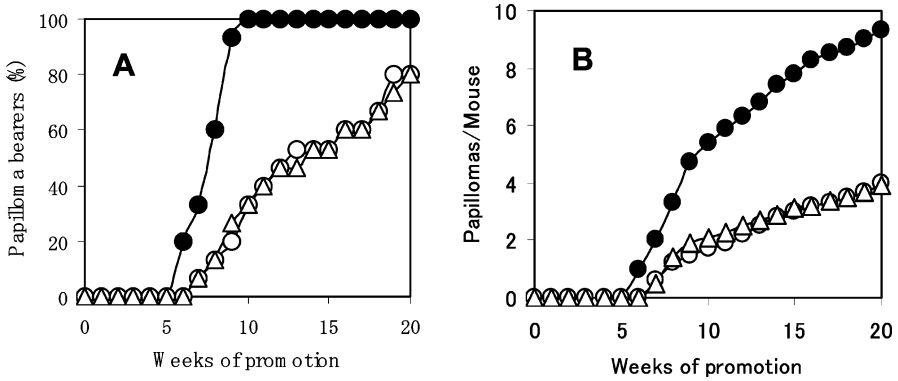

Figure 1. Inhibitory effect of (19*R*,23*E*)-5β,19-epoxy-19-methoxycucurbita-6,23,25-trien-3β-ol (**1**) and (19*R*,23*E*)-5β,19-epoxy-19-dimethoxycucurbita-6,23-dien-3β-ol (**2**) on mouse skin carcinogenesis induced by DMBA and TPA. (A) Percentage of mice bearing papillomas. (B) Average number of papillomas per mouse. ●, positive control, TPA (1.7 nmol) alone (group I); ○, TPA (1.7 nmol) + 85 nmol of **1** (group II); △, TPA (1.7 nmol) + 85 nmol of **2** (group III). At 20 weeks of promotion, the number of papillomas per mouse differed significantly ($p < 0.01$, using the Student's *t*-test) between the groups treated with compounds **1** and **2** and the control group. The number (standard deviations are shown in parentheses) of papillomas per mouse for each group was 9.3 (1.6), 4.0 (1.4), and 3.9 (1.3) for groups I, II, and III, respectively.

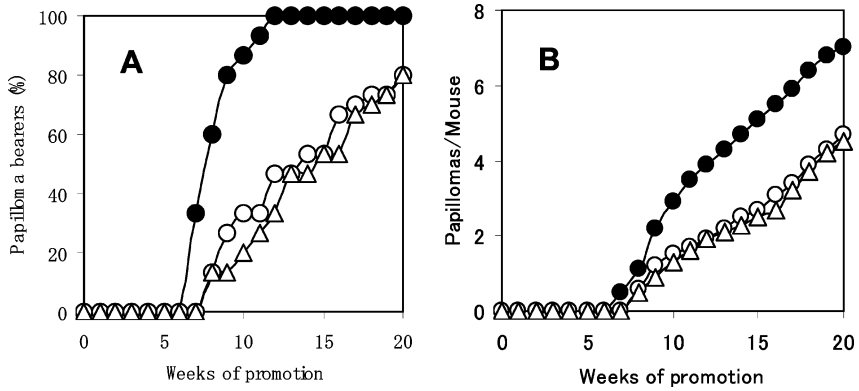

Figure 2. Inhibitory effect of (19*R*,23*E*)-5β,19-epoxy-19-methoxycucurbita-6,23,25-trien-3β-ol (**1**) and (19*R*,23*E*)-5β,19-epoxy-19-dimethoxycucurbita-6,23-dien-3β-ol (**2**) on mouse skin carcinogenesis induced by PN and TPA. (A) Percentage of mice bearing papillomas. (B) Average number of papillomas per mouse. ●, positive control, PN (390 nmol) + TPA (1.7 nmol) alone (group I); ○, PN (390 nmol) + 0.0025% of **1** (2 weeks) + TPA (1.7 nmol) (group II); △, PN (390 nmol) + 0.0025% of **2** (2 weeks) + TPA (1.7 nmol) (group III). At 20 weeks of promotion, the number of papillomas per mouse differed significantly between the groups treated with compounds **1** and **2** and the control group ($p < 0.01$, using the Student's *t*-test). The number (standard deviations are shown in parentheses) of papillomas per mouse for each group was 7.0 (1.9), 4.7 (1.8), and 4.5 (1.3) for groups I, II, and III, respectively.

value (concentration of 50% inhibition with respect to positive control) of 200−409 mol ratio/32 pmol TPA. As such, these compounds were comparable with or more potent than the reference compound, β-carotene (IC_{50} = 397 mol ratio/32 pmol TPA), a vitamin A precursor studied widely in cancer chemoprevention animal models. Of the compounds tested, five triterpenes without glycosyl moieties, **1**−**5**, exhibited more potent inhibitory effects (IC_{50} values 200−328 mol ratio/32 pmol TPA) than the triterpenes with glycosyl units, **6**−**18** (IC_{50} values 358−409 mol ratio/32 pmol TPA). Since inhibitory effects against EBV-EA induction have been demonstrated to correlate with those against tumor promotion in vivo,[26] compounds **1**−**5** are potential antitumor promoters.

The highly inhibitory compounds **1**−**5** were then evaluated for their scavenging activity against NO generation by NOR 1 in a cultured cell system using an in vitro screening model for NO scavenging.[27] Table 3 shows the inhibitory ratios (I.R.) of these compounds and two reference compounds, the natural product glycyrrhizin and the synthetic NO scavenger carboxy-PTIO, on NOR 1 activity. Among the isolates tested, three compounds, **1**−**3**, exhibited moderate inhibitory effects (I.R. 1.9−2.0) that were almost equivalent to the value for glyzyrrhizin (I.R. 2.2). NO is known to be involved in several potential toxic mechanisms and is a mutagen and can cause mutations in both microorganisms and mammalian cells.[28,29]

On the basis of the results of the in vitro assays described above, we evaluated subsequently the inhibitory effects of compounds **1** and **2** in two tumor models in mouse skin. The incidence (%) of papilloma-bearing mice and the average numbers of papillomas per mouse in a two-stage carcinogenesis test in mouse skin using DMBA as an inhibitor and TPA as a promoter are presented in Figures 1A and 1B, respectively. The incidence of the papilloma-bearing mice was high and 100% at 10 weeks promotion in group I (positive control). Further, more than five and nine papillomas were formed per mouse at 10 and 20 weeks of promotion, respectively. The formation of papillomas in mouse skin was delayed and the mean numbers of papillomas per mouse were reduced by treatment with **1** and **2**. Thus, in groups II (treated with **1**) and III (treated with **2**), the percentage ratios of papilloma-bearing mice were only 33% (II and III) at 10 weeks and 80% (II and III) at 20 weeks, and the mean papillomas per mouse were 1.7 (II) and 2.1 (III) at 10 weeks and 4.0 (II) and 3.9 (III) at 20 weeks.

Compounds **1** and **2** were then evaluated on their inhibitory effects against the initiation of PN, and the results are shown in Figure 2. In the positive control group (group I), the first papilloma appeared after 7 weeks and the incidence of the papilloma-bearing mice was 100% after 12 weeks (Figure 2A). Oral administration of **1** and **2** exhibited a significant inhibitory effect in the PN-induced and TPA-promoted experiment in mice. When **1** (group II) and **2**

(group III) (0.0025% each) were administrated in drinking water, from 1 week before to 1 week after the initiation treatment, whereas the first papilloma appeared after 8 weeks, the incidence of the papilloma-bearing mice was 80% even after 20 weeks of promotion in both groups II and III. In the average number of papillomas per mouse (Figure 2B), **1** and **2** reduced the number of papillomas compared to the control group. Whereas approximately 7 papillomas per mouse were observed after 20 weeks of promotion in group I, only 4.7 (group II) and 4.5 (group III) papillomas were observed after the same period of promotion. From these results, **1** and **2** appear to be effective for the inhibition of PN-initiated carcinogenesis on mouse skin. PN ($ONOO^-$), which is produced by the reaction of NO with superoxide, is a potent tumor-initiating agent[27] as well as an oxidant and nitrating and hydroxylating agent. Compounds **1** and **2** may be suggested as being able to intercept and neutralize potent chemical carcinogens, such as reactive oxygen species (ROS; superoxide, and peroxy and hydroxy radicals) and NO donors.

From the results of in vitro EBV-EA induction, in vitro NOR 1 inhibition, and in vivo two-stage carcinogenesis tests, the cucurbitane-type triterpenes from the MeOH extract of *M. charantia* fruits, especially, compounds **1** and **2**, may be useful as agents that inhibit chemical carcinogenesis.

Experimental Section

General Experimental Procedures. Crystallizations were performed in MeOH, 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. UV spectra on a Shimadzu UV-2200 spectrometer and IR spectra on a JASCO FTIR-300E spectrometer were recorded in EtOH and KBr disks, respectively. NMR spectra were recorded with a JEOL ECA-600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) or with a JEOL LA-500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer in C_5D_5N with tetramethylsilane as an internal standard. 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]. Silica gel (Kieselgel 60, 230−400 mesh, Merck) and octadecyl silica gel (Chromatorex-ODS, 100−200 mesh; Fuji Silysia Chemical, Ltd., Aichi, Japan) were used for open column chromatography. Reversed-phase preparative HPLC was carried out on an octadecyl silica column (Pegasil ODS II column, 25 cm × 10 mm i.d.; Senshu Scientific Co., Ltd., Tokyo, Japan) at 25 °C at a flow rate of 2.0 mL/min with the eluent MeCN−H_2O [9:1 (HPLC system I), 17:3 (HPLC system II), 4:1 (HPLC system III), and 3:1 (HPLC system IV)], and normal-phase analytical HPLC on an aminopropyl silica column (Senshu PAK NH2-1251-N, 25 cm × 4.6 mm i.d.; Senshu Scientific Co., Ltd.) at 25 °C at a flow rate of 1.0 mL/min with the eluent MeCN−H_2O [9:1 (HPLC system V)].

Chemicals and Materials. Sliced and dried fresh whole fruits of "nigauri" (*M. charantia*), cultivated in Okinawa prefecture, Japan, in the summer of 2002, used in this study was purchased from Taiyo Co., Ltd. (Osaka, Japan).[20] The following chemicals were purchased: TPA from ChemSyn Laboratories (Lenexa, KS), DMBA and glyzyrrhizin from Sigma Chemical Co. (St. Louis, MO), the EBV cell culture reagents and *n*-butanoic acid from Nacalai Tesque, Inc. (Kyoto, Japan), peroxynitrite (PN) solution, NOR 1, and carboxy-PTIO from Dojindo Laboratories (Kumamoto, Japan).

Extraction and Isolation. Sliced and dried fruit material of *M. charantia* (3.6 kg) was extracted with MeOH and yielded a MeOH extract (261 g) after evaporation of the solvent in vacuo. The extract was partitioned between H_2O and EtOAc, giving an EtOAc-soluble fraction (44 g). The EtOAc fraction was further partitioned between *n*-hexane−MeOH−H_2O (19:19:1), which yielded *n*-hexane (13.0 g) and MeOH−H_2O (27.4 g) soluble fractions. Column chromatography on silica gel (516 g) of the MeOH−H_2O fraction, eluted successively with solvents of increasing polarity [*n*-hexane−EtOAc, 1:1 (2.9 L), 1:4 (1.8 L), 0:1 (2.7 L); EtOAc−MeOH, 4:1 (2.1 L), 1:1 (3.6 L), 3:7 (4.5 L)], afforded 13 fractions, A−M, listed in increasing order of polarity. Fraction H (16.0 g), eluted with EtOAc−MeOH (4:1), was subjected to further chromatography on ODS (185 g), which was eluted successively with solvents of decreasing polarity [MeOH−H_2O, 7:3 → 1:0], and yielded 48 fractions, H1−H48, each of which was recovered from 100 mL of eluting solution. Isolation of the following 13 compounds was performed by preparative HPLC: compound **13** [3.5 mg; retention time (t_R) 32.8 min] from fraction H19 (95 mg) by HPLC system IV; compound **17** (1.7 mg; t_R 20.4 min) from fraction H20 (75 mg) by HPLC system III; compound **7** (2.2 mg; t_R 22.0 min) from fraction H21 (260 mg) by HPLC system II; compounds **8** (8.4 mg; t_R 27.6 min), **12** (10.0 mg; t_R 24.4 min), and **16** (15.9 mg; t_R 20.0 min) from fraction H22 (487 mg) by HPLC system III; compounds **9** (18.5 mg; t_R 45.6 min), **14** (30.3 mg; t_R 34.0 min), and **15** (18.7 mg; t_R 40.8 min) from fraction H23 (306 mg) by HPLC system IV; compounds **6** (7.1 mg; t_R 25.2 min) and **18** (3.4 mg; t_R 38.0 min) from fraction H25 (283 mg) by HPLC system I; and compounds **10** (7.7 mg; t_R 24.8 min) and **11** (7.2 mg; t_R 28.4 min) from fraction H27 (154 mg) by HPLC system I.

(19*R*,23*E*)-5β,19-Epoxy-19-methoxycucurbita-6,23,25-trien-3β-ol 3-*O*-β-D-glucopyranoside (6; charantoside I): amorphous solid; $[\alpha]^{25}_D$ −116.9 (*c* 0.47, MeOH); UV (EtOH) λ_{max} (log ϵ) 239 (4.09), 230 (4.28), 225 (4.26) nm; IR ν_{max} 3421, 1647, 1068, 1036 cm^{-1}; ^{13}C and ^{1}H NMR, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 653.4017 (calcd for $C_{37}H_{58}O_8Na$ $[M + Na]^+$, 653.4029).

(19*R*,23*R*)-5β,19-Epoxy-19,23-dimethoxycucurbita-6,24-dien-3β-ol 3-*O*-β-D-allopyranoside (7; charantoside II): amorphous solid; $[\alpha]^{25}_D$ −80.6 (*c* 0.32, MeOH); IR ν_{max} 3431, 1080, 1036 cm^{-1}; ^{13}C and ^{1}H NMR, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 685.4290 (calcd for $C_{38}H_{62}O_9Na$ $[M + Na]^+$, 685.4291).

(23*E*)-5β,19-Epoxycucurbita-6,23,25-trien-3β-ol 3-*O*-β-D-glucopyranoside (10; charantoside III): amorphous solid; $[\alpha]^{25}_D$ −85.2 (*c* 0.19, MeOH); UV (EtOH) λ_{max} (log ϵ) 238 (4.13), 230 (4.35), 224 (4.27) nm; IR ν_{max} 3406, 1645, 1080, 1036 cm^{-1}; ^{13}C and ^{1}H NMR, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 623.3914 (calcd for $C_{36}H_{56}O_7Na$ $[M + Na]^+$, 623.3923).

(23*E*)-5β,19-Epoxycucurbita-6,23,25-trien-3β-ol 3-*O*-β-D-allopyranoside (11; charantoside IV): colorless needles; mp 256−260 °C; $[\alpha]^{25}_D$ −152.8 (*c* 0.16, MeOH); UV (EtOH) λ_{max} (log ϵ) 238 (4.15), 229 (4.32), 225 (4.27) nm; IR ν_{max} 3421, 1645, 1086, 1034 cm^{-1}; ^{13}C and ^{1}H NMR, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 623.3919 (calcd for $C_{36}H_{56}O_7Na$ $[M + Na]^+$, 623.3923).

(23*R*)-5β,19-Epoxy-23-methoxycucurbita-6,24-dien-3β-ol 3-*O*-β-D-glucopyranoside (12; charantoside V): colorless needles; mp 235−240 °C; $[\alpha]^{25}_D$ −68.5 (*c* 0.76, MeOH); IR ν_{max} 3423, 1084, 1032 cm^{-1}; ^{13}C and ^{1}H NMR, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 655.4194 (calcd for $C_{37}H_{60}O_8Na$ $[M + Na]^+$, 655.4185).

(23*S*)-5β,19-Epoxy-23-methoxycucurbita-6,24-dien-3β-ol 3-*O*-β-D-allopyranoside (13; charantoside VI): amorphous solid; $[\alpha]^{25}_D$ −73.5 (*c* 0.48, MeOH); IR ν_{max} 3392, 1084, 1033 cm^{-1}; ^{13}C and ^{1}H NMR, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 655.4167 (calcd for $C_{37}H_{60}O_8Na$ $[M + Na]^+$, 655.4185).

(23*E*)-3β-Hydroxycucurbita-6,23,25-trien-5β,19-olide 3-*O*-β-D-glucopyranoside (16; charantoside VII): colorless needles; mp 258−262 °C; $[\alpha]^{25}_D$ −120.4 (*c* 0.79, MeOH); UV (EtOH) λ_{max} (log ϵ) 238 (4.07), 229 (4.34), 224 (4.26) nm; IR ν_{max} 3423, 1745, 1643, 1080, 1038 cm^{-1}; ^{13}C and ^{1}H NMR, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 637.3694 (calcd for $C_{36}H_{54}O_8Na$ $[M + Na]^+$, 637.3716).

(23*E*)-3β-Hydroxy-7β,25-dimethoxycucurbita-5,23-dien-19-al 3-*O*-β-D-glucopyranoside (17; charantoside VIII): amorphous solid; $[\alpha]^{25}_D$ +12.4 (*c* 0.20, MeOH); IR ν_{max} 3427, 1070, 1036 cm^{-1}; ^{13}C and ^{1}H NMR, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 685.4294 (calcd for $C_{38}H_{62}O_9Na$ $[M + Na]^+$, 685.4291).

Acid Hydrolysis of Compounds 6, 7, 10−13, 16, and 17. A solution of triterpene glycoside (2 mg each) in 1 M HCl−MeOH (5 mL) was heated under reflux for 1 h. The reaction mixture was partitioned between EOAc and H_2O, and the H_2O layer was neutralized by passing through Amberlite MB-3. The H_2O eluate was concentrated, and the residue was subjected directly to HPLC system V for the identification of the sugar moiety of the glycosides. The sugar from **6**, **10**, **12**, **16**, and **17** was identified as D-glucose (t_R 22.1 min), whereas that from **7**, **11**, and **13** as D-allose (t_R 17.2 min).

In Vitro EBV-EA Activation Experiment. For the protocol for this in vitro assay, refer to two previous articles.[26,30]

In Vitro NOR 1 Inhibition Experiment. For the protocol for this in vitro assay, refer to two previous articles.[27,30]

In Vivo Two-stage Mouse Skin Carcinogenesis Assay Initiated by DMBA. For the protocol for this in vivo assay, refer to a previous article.[30]

In Vivo Two-stage Mouse Skin Carcinogenesis Assay Initiated by PN. Tumors were induced with PN and promoted with TPA. The animals were divided into two experimental groups (15 mice each). The back of each mouse was shaved with surgical clippers, and the mice were topically treated with PN (390 nmol) in NaOH (1 mM, 0.1 mL) as an initiation treatment. For group I (positive control group), 1 week after the initiation, papilloma formation was promoted by the twice weekly application of TPA (1 μg, 1.7 nmol) in acetone (0.1 mL) on the skin (no papilloma formation was seen with topical application of the acetone solvent alone). For groups II and III (test groups), 0.0025% of test compounds (**1** and **2**; 2.5 mg/100 mL each in drinking water) were administered orally for 2 weeks before the promotion treatment (1 week both before and after the initiation) and subsequently promoted by the twice a week application with TPA (1.7 nmol) in acetone (0.1 mL). The incidence of papilloma bearers and numbers of papillomas per mouse were detected weekly for 20 weeks. Skin papillomas were first counted when they reached approximately 1 mm $\times$ 1 mm in size. The Student's *t*-test was used for statistical analyses of the numbers of papillomas per mouse. The animal weights were not statistically different between any of the groups during the in vivo assay period.

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Supporting Information Available: ^{13}C and ^{1}H NMR, HMBC, and NOESY NMR data for compounds **6**−**18**. This information is available free of charge via the Internet at http://pubs.acs.org.

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