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Inhibitory Effects of Cucurbitane Glycosides and Other Triterpenoids from the Fruit of *Momordica grosvenori* on Epstein−Barr Virus Early Antigen Induced by Tumor Promoter 12-*O*-Tetradecanoylphorbol-13-acetate

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Two new triterpene benzoates, 5-dehydrokarounidiol dibenzoate (**1**) and karounidiol dibenzoate (**2**), and two new triterpene glycosides, 5α,6α-epoxymogroside IE_1 (**8**) and 11-oxomogroside A_1 (**9**), along with 15 known triterpenoids (one triterpene benzoate, **3**; three triterpene mono-ols, **4**−**6**; one triterpene aglycon, **7**; and 10 triterpene glycosides, **10**−**19**), were isolated from the ethanol extract of the fruit of *Momordica grosvenori*. The structures of **1**, **2**, **8**, and **9** were determined on the basis of spectroscopic and chemical methods. Among the known triterpene glycosides, mogroside I E_1 (**12**) was a new naturally occurring compound. Eighteen triterpenoids (**2**−**19**) and 11-oxomogrol (**20**), a hydrolysis product of **9**, were evaluated with respect to their inhibitory effects on the induction of Epstein−Barr virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells, which is known to be a primary screening test for antitumor promoters. All of the compounds tested showed potent inhibitory effects on EBV-EA induction (70−100% inhibition at 1×10^3 mol ratio/TPA).

KEYWORDS: ***Momordica grosvenori*; fruit; Cucurbitaceae; sweet principle; triterpenoids; cucurbitane glycosides; antitumor promoter; Epstein−Barr virus early antigen**

INTRODUCTION

The fruit of *Momordica grosvenori* Swingle (Cucurbitaceae) grown in Kwangshi, China, is used as an expectorant as well as a natural sweet food in that country, and many cucurbitane-type triterpene glycosides have been isolated from it and characterized (*1*−*3*). We recently reported the isolation and characterization of some cucurbitane glycosides from the roots of *Bryonia dioica* (Cucurbitaceae) and their inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice and on the induction of Epstein−Barr virus early antigen (EBV-EA) (*4*). In continuing our study on the search for potent antitumor promoters in natural resources, we have investigated the triterpenoid constituents of the ethanol extract of *M. grosvenori* fruit. In this paper, we report the isolation and characterization of 19 compounds from the extract and inhibitory effects on the induction of EBV-EA by TPA of 19 compounds (**2**−**20**), evaluated in a preliminary screen for their potential cancer chemopreventive activities.

MATERIALS AND METHODS

General Methods. Crystallizations were performed in methanol (MeOH). Melting points were measured on a Yanagimoto micro-melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were determined on a Shimadzu UV-300 spectrometer in ethanol (EtOH). Infrared (IR) spectra were recorded on a JASCO IR-300 IR spectrometer in KBr disks. Nuclear magnetic resonance (NMR) spectra were recorded with a JEOL LA-500 spectrometer at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in C_5D_5N or in $CDCl_3$, and chemical shifts are expressed in δ (ppm) referred to tetramethylsilane (TMS). Fast atom bombardment mass spectra (FABMS) and high-resolution FABMS (HRFABMS) were obtained with a JEOL JMS-BU20 spectrometer using glycerol as the matrix. Electron-impact mass spectra (EIMS) and HREIMS were recorded on a JEOL JMS-GC mate spectrometer (70 eV) using a direct inlet system. Silica gel (silica gel 60, 230−400 mesh) (Merck), Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), and

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octadecyl silica (Chromatorex-ODS, 100−200 mesh) (Fuji Silysia Chemical, Ltd., Aichi, Japan) were used for open column chromatography. GLC was performed using a 30 m × 0.3 mm i.d. DB-17 fused-silica capillary column at 275 °C (J & W Scientific, Folsom, CA). Reversed-phase preparative high-performance liquid chromatography (HPLC) was carried out on a 25 cm × 1.0 cm i.d. Pegasil ODS II octadecyl silica gel column (Senshu Scientific Co., Ltd., Tokyo, Japan) at 25 °C, eluting with MeOH at 3 mL/min (HPLC I), MeOH−H_2O (7:3, v/v) at 2 mL/min (HPLC II), and MeOH−H_2O (6:4, v/v) at 2 mL/min (HPLC III) as mobile phase. Hydrolysis of the triterpene dibenzoate was performed with 5% (w/v) KOH in MeOH under reflux for 2 h. Acid hydrolysis of triterpene glycoside was performed with 1 M H_2SO_4−MeOH for 2 h under reflux on a water bath.

Chemicals and Materials. Samples of the fruit of *M. grosvenori* Swingle (Cucurbitaceae) were collected in China in September 1999. A voucher specimen has been deposited in the college of Science and Technology, Nihon University. Compounds were purchased as follows: TPA from ChemSyn Laboratories (Lenexa, KS); the EBV cell culture reagent and *n*-butyric acid from Nacalai Tesque, Inc. (Kyoto, Japan). Six compounds—karounidiol [D:C-friedo-oleana-7,9(11)-diene-3α,29-diol] and its 3-benzoate (**3**) (*5*), isomultiflorenol (D:C-friedo-olean-8-en-3*β*-ol) (**4**) and 5-dehydrokarounidiol [D:C-friedo-oleana-5,7,9(11)-triene-3α,29-diol] (*6*), *β*-amyrin (olean-12-en-3*β*-ol) (**5**) (*7*), and 10α-cucurbitadienol (10α-cucurbita-5,24-dien-3*β*-ol; **6**) (*8*)—were used as the reference specimens.

Extraction and Isolation. Air-dried and powdered *M. grosvenori* fruit (2 kg) was extracted with 99% ethanol (EtOH) under reflux. Evaporation of the solvent under reduced pressure yielded the EtOH extract (108 g), and the extract was partitioned into ethyl acetate (EtOAc)/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 (10.0 g), *n*-BuOH (16.7 g), and H_2O (80.1 g) fractions, respectively. The EtOAc fraction was further partitioned with *n*-hexane/MeOH/H_2O (95:95:10, v/v/v), which yielded *n*-hexane (4.9 g) and MeOH/H_2O (4.8 g) fractions.

n-Hexane Soluble Fraction. The *n*-hexane soluble fraction was chromatographed on a silica gel (200 g) column with a stepwise gradient of *n*-hexane/EtOAc [1:0 (0.4 L), 95:5 (2.0 L), 9:1 (2.6 L), 4:1 (1.4 L), 1:1 (1.2 L)] as eluants, which yielded fractions containing triterpene dibenzoates (62 mg; fraction HA), and triterpene monobenzoate and triterpene mono-ols (36 mg; fraction HB). Preparative HPLC (HPLC I) of fraction HA gave 5-dehydrokarounidiol dibenzoate (2.7 mg; **1**) and karounidiol dibenzoate (11.4 mg; **2**), and fraction HB yielded **3** (3.0 mg), **4** (3.2 mg), **5** (1.0 mg), and **6** (7.8 mg).

MeOH/H2O Soluble Fraction. The MeOH/H_2O fraction (4.8 g) was subjected to Diaion HP-20 column chromatography using H_2O−MeOH (1:1 → 0:1, v/v) to give fractions MA-MC. Fraction MB (80% MeOH eluate; 1.2 g) was further separated by Chromatorex-ODS column chromatography followed by preparative HPLC to give mogrol (61 mg; t_R = 62.8 min in HPLC II; **7**) and five triterpene monoglycosides, 5α,6α-epoxymogroside I E_1 (3.3 mg; t_R = 20.0 min; **8**), 11-oxomogroside I A_1(31 mg; t_R = 40.4 min; **9**), 11-oxomogroside I E_1 (6.5 mg; t_R = 28.8 min; **10**), mogroside I A_1 (218 mg; t_R = 37.6 min; **11**), and mogroside I E_1 (172 mg; t_R = 26.4 min; **12**).

n-BuOH Soluble Fraction. The *n*-BuOH fraction (16.7 g) was subjected to Diaion HP-20 column chromatography using H_2O−MeOH (1:1 → 0:1, v/v) to give fractions BA−BC. Fraction BB (80% MeOH eluate; 7.4 g) was further separated by preparative HPLC to give two triterpene glycosides, mogroside II E (917 mg; t_R = 53.2 min in HPLC III; **13**) and mogroside III (405 mg; t_R = 36.0 min; **14**).

H2O Soluble Fraction. The H_2O fraction (80.1 g) was subjected to Diaion HP-20 column chromatography using H_2O−MeOH (1:0 → 0:1, v/v) to give fractions WA−WD. Fraction WB (50% MeOH eluate; 9.8 g) was further separated by Chromatorex-ODS column chromatography followed by HPLC to give five triterpene glycosides, siamenoside I (90 mg; t_R = 23.1 min in HPLC III; **15**), mogroside IV A (408 mg; t_R = 28.0 min; **16**), mogroside IV E (352 mg; t_R = 30.8 min; **17**), 11-oxomogroside V (366 mg; t_R = 14.9 min; **18**), and mogroside V (2714 mg; t_R = 19.6 min; **19**).

Identification and Characterization. Identification of four triterpenoids, **3**−**6**, was performed by chromatographic (HPLC and GLC) and spectroscopic (MS and ^{1}H NMR) comparison with reference compounds. The following 11 compounds were identified by spectral comparison with literature: mogrol [(24*R*)-cucurbit-5-ene-3*β*,11α,24,-25-tetrol; **7**] (*2*), 11-oxomogroside I E_1 [(24*R*)-cucurbit-5-en-11-one-3*β*,24,25-triol 3-*O*-*β*-D-glucopyranoside; cabenoside D; **10**] (*3*), mogroside I A_1 [(24*R*)-cucurbit-5-ene-3*β*,11α,24,25-tetrol 24-*O*-*β*-D-glucopyranoside; **11**] (*3*), mogroside I E_1 [(24*R*)-cucurbit-5-ene-3*β*,11α,24,25-tetrol 3-*O*-*β*-D-glucopyranoside; **12**] (*3*), mogroside II E [(24*R*)-cucurbit-5-ene-3*β*,11α,24,25-tetrol 3,24-di-*O*-*β*-D-glucopyranoside; **13**] (*3*), mogroside III [3-*O*-*β*-D-glucopyranosyl (24*R*)-cucurbit-5-ene-3*β*,11α,24,25-tetrol 24-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside; **14**] (*9*), siamenoside I {3-*O*-*β*-D-glucopyranosyl (24*R*)-cucurbit-5-ene-3*β*,11α,24,25-tetrol 24-*O*-*β*-D-glucopyranosyl(1→2)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside; **15**} (*10*), mogroside IV A [(24*R*)-cucurbit-5-ene-3*β*,11α,24,25-tetrol 3,24-di-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside; **16**] (*3*), mogroside IV E [3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl (24*R*)-cucurbit-5-ene-3*β*,-11α,24,25-tetrol 24-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside; **17**] (*3*, *11*), 11-oxomogroside V {3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl (24*R*)-cucurbit-5-en-11-one-3*β*,24,25-triol 24-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranoside-(1→6)]-*β*-D-glucopyranoside; **18**} (*10*), and mogroside V {3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl (24*R*)-cucurbit-5-ene-3*β*,11α,24,25-tetrol 24-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranoside-(1→6)]-*β*-D-glucopyranoside; **19**} (*2*). Characterization of four new triterpenoids, **1**, **2**, **8**, and **9**, was performed by spectroscopic methods and, in part, by chemical correlation with known compounds. The MS and ^{1}H NMR data of **1** and **2**, the MS data of **8** and **9**, and the ^{1}H and ^{13}C NMR data of **12**, which was characterized for the first time as the natural product in this study, are described below. The ^{13}C and ^{1}H NMR spectral data along with heteronuclear multiple bond correlation (HMBC) spectroscopy data for **8** and **9** are shown in **Table 1**.

*5-Dehydrokarounidiol dibenzoate (**1**):* amorphous solid; UV λ_{max} 304, 314, 330 nm; IR υ_{max} 1720 and 1270 (OBz), 1600, 1582, and 709 (C_6H_5), 814 (>C═CH—) cm^{-1}; EIMS, *m/z* (relative intensity) 646 [M]$^+$ (4), 524 (10), 509 (6), 402 (2), 387 (6), 373 (8), 347 (6), 312 (41), 294 (30), 284 (26), 269 (28), 251 (32), 241 (31), 237 (21), 225 (28), 223 (30), 215 (26), 189 (26), 175 (29), 165 (32), 147 (42), 135 (38), 123 (52), 109 (70), 105 (100); HREIMS, *m/z* 646.4032 [M]$^+$ (calcd for $C_{44}H_{54}O_4$ 646.4022); ^{1}H NMR ($CDCl_3$) δ 0.88, 1.07, 1.11, 1.14, 1.17, 1.23, 1.29 (each 3H and s, unassigned), 4.12 and 4.17 (each 1H, d, J = 13.8 Hz, H-29), 4.99 (1H, br s, $W_{1/2}$ = 7.3 Hz, H-3α), 5.37 (1H, m, H-11), 5.66 (1H, d, J = 6.0 Hz, H-7), 5.91 (1H, d, J = 6.0 Hz, H-6), 7.28 (2H, ddt, J = 7.8, 7.8, 1.5 Hz), 7.42 (2H, ddt, J = 7.3, 7.3, 1.5 Hz), 7.44 (1H, ddt, J = 7.3, 7.8, 1.5 Hz), 7.52 (1H, ddt, J = 7.3, 7.3, 1.5 Hz), 7.90 (2H, dt, J = 7.3, 1.5 Hz), and 8.06 (2H, dt, J = 7.3, 1.5 Hz).

*Karounidiol dibenzoate (**2**):* amorphous solid; UV λ_{max} 230, 235, 245 nm; IR υ_{max} 1722 and 1270 (OBz), 1602, 1584, and 709 (C_6H_5), 814 (>C═CH) cm^{-1}; EIMS, *m/z* (relative intensity) 648 [M]$^+$ (13), 526 (79; [M]$^+$ − PhCOOH), 511 (19), 404 (10; [M]$^+$ − 2PhCOOH), 389 (24), 375 (3; ABC ring + C-16, C-27), 361 (3), 349 (2), 293 (5), 279 (6), 267 (6), 253 (35), 239 (11), 227 (17; *m/z* 375 − PhCOOH), 213 (10), 197 (10), 185 (13), 171 (14), 159 (17), 145 (14), 121 (15), 105 (100), 95 (27), 77 (28); HREIMS *m/z* 648.4179 [M]$^+$ (calcd for $C_{44}H_{56}O_4$ 648.4178); ^{1}H NMR ($CDCl_3$) δ 0.92, 0.92, 0.95, 0.99, 1.06, 1.11, and 1.13 (each 3H and s, unassigned), 4.13 and 4.16 (each 1H, d, J = 13.8 Hz, H-29), 4.91 (1H, br s, $W_{1/2}$ = 7.3 Hz, H-3α), 5.25 (1H, m, H-11), 5.54 (1H, m, H-7), 7.30 (2H, ddt, J = 7.6, 7.6, 1.5 Hz), 7.43 (2H, ddt, J = 7.6, 7.6, 1.5 Hz), 7.45 (1H, ddt, J = 7.6, 7.6, 1.5 Hz), 7.53 (1H, ddt, J = 7.6, 7.6, 1.5 Hz), 7.94 (2H, dt, J = 7.6, 1.5 Hz), and 8.08 (2H, dt, J = 7.6, 1.5 Hz).

*5α,6α-Epoxymogroside I E_1 [(24R)-5α,6α-epoxycucurbit-3β,11α,-24,25-tetrol 3-O-β-D-glucopyranoside] (**8**):* amorphous solid; IR υ_{max} 3423 (OH) cm^{-1}; HRFABMS *m/z* 677.4241 [M + Na]$^+$ (calcd for $C_{36}H_{62}O_{10}Na$, 677.4241); ^{1}H and ^{13}C NMR data, see **Table 1**.

*11-Oxomogroside I A_1 [(24R)-cucurbit-5-en-11-one-3β,24,25-triol 24-O-β-D-glucopyranoside] (**9**):* amorphous solid; IR υ_{max} 3409 (OH),

Table 1. ^{13}C, ^{1}H, and HMBC NMR Spectral Data for Two Cucurbitane Glycosides, **8** and **9**, from the Fruit of *Momordica grosvenori* (C_5D_5N)

	8			**9**		
C no.	δ_C	δ_H[a]	HMBC (H to C)	δ_C	δ_H[a]	HMBC (H to C)
1	20.5	2.40 (α), 3.08 (β)		21.3	1.64 (α), 2.06 (β)	
2	30.7	2.12 (α), 2.42 (β)		29.8	1.95 (α), 1.88 (β)	
3	87.1	3.74 (br s, $W_{1/2}$ = 7.2)	1, 5	75.6	3.72 (br s, $W_{1/2}$ = 7.2)	
4	41.3			41.9		
5	67.5			141.5		
6	53.0	3.19 (br d, 5.8)	4, 5, 7	119.0	5.69 (d, 5.8)	4, 7, 8, 10
7	23.6	1.67 (α), 2.20 (β)	9	24.2	2.35 (α), 1.86 (β)	
8	42.5	1.58	6, 7, 9, 10, 11, 14	44.1	1.89	7, 8, 10, 19, 30
9	40.0			49.2		
10	34.5	2.69 (br d, 9.0)	9, 11, 19	36.0	2.53	
11	78.9	4.02 (br d, 10.3)	8	213.9		
12	40.9	2.13 (α), 2.04 (β)	9, 11, 13, 14, 18	48.8	2.97 (α), 2.53 (β)	
13	46.4			49.1		
14	49.8			49.7		
15	34.4	1.18 (α), 1.06 (β)	9, 14, 16	34.6	1.18 (α), 1.32 (β)	
16	30.0	1.35 (α), 1.28 (β)	14, 20	29.5	1.78 (α), 1.70 (β)	
17	51.2	1.66	14, 20, 22	49.9	1.76	14, 20, 22
18	17.0	0.85 (s)	12, 13, 14, 17	17.0	0.76 (s)	12, 13, 14, 17, 21
19	25.0	1.51 (s)	8, 9, 10	20.2	1.28 (s)	1, 8, 9, 10, 11
20	36.4	1.59		36.6	1.45	
21	18.9	1.01 (d, 6.4)	17, 20, 22, 23	18.6	0.91 (d, 6.4)	17, 20, 22
22	34.1	1.65, 1.82	20, 21, 24, 26, 27	33.3	1.81, 1.92	
23	29.0	1.83 (2H)	20, 24, 25, 26. 27	28.1	2.15 (2H)	
24	79.1	3.77 (br d, 10.0)	25	90.7	3.89 (br d, 8.5)	22, 23, 25, 26, G1
25	72.7			71.8		
26	25.9	1.57 (s)	23, 24, 25, 27	25.4	1.47 (s)	24, 25, 27
27	26.2	1.53 (s)	22, 23, 24, 25, 26	27.0	1.42 (s)	23, 24, 25, 26
28	21.4	1.22 (s)	3, 4, 5, 29	28.7	1.17 (s)	4, 5, 29
29	25.8	1.21 (s)	3, 4, 5, 28	26.3	1.43 (s)	2, 3, 4, 5, 6, 28
30	20.7	0.91 (s)	8, 13, 14, 15	18.3	0.99 (s)	8, 9, 12, 13, 14, 15
G1	106.4	4.91 (d, 7.6)	3, G3, G5	105.9	5.00 (d, 7.8)	24, G3, G5
G2	75.8	4.01 (dd, 7.6, 7.6)	G3	75.4	4.05 (dd, 7.8, 7.8)	G1, G3
G3	78.4	4.22 (dd, 7.6, 7.6)	G4	78.6	4.24 (dd, 7.8, 7.8)	G2, G4, G5
G4	71.7	4.20 (dd, 7.6, 7.6)	G3, G5	72.0	4.22 (dd, 7.8, 7.8)	G2, G3, G5, G6
G5	78.4	3.93 (m)	G1	78.5	4.02 (m)	G1, G3
G6	63.0	4.37 (dd, 5.4, 12.4)	G5	62.7	4.35 (dd, 5.5, 12.0)	G5
		4.51 (dd, 2.5, 12.4)	G5		4.56 (dd, 2.5, 12.0)	G5

[a] Numbers in parentheses denote *J* values (hertz).

1691 (C═O) cm^{-1}; HRFABMS *m/z* 635.4120 $[M - H]^-$ (calcd for $C_{36}H_{59}O_9$, 635.4159); ^{1}H and ^{13}C NMR data, see **Table 1**.

Mogroside I E₁ (12): ^{1}H NMR (C_5D_5N) δ 0.92 (3H, s, H-28), 0.93 (3H, s, H-18), 0.99 (3H, d, *J* = 6.4 Hz, H-21), 1.22 (3H, s, H-19), 1.39 (3H, s, H-26), 1.40 (3H, s, H-30), 1.43 (3H, s, H-29), 1.46 (3H, s, H-27), 3.68 (1H, br s, H-3), 3.76 (1H, br d, *J* = 6.8 Hz, H-24), 4.19 (1H, m, H-11), 5.50 (1H, d, *J* = 5.1 Hz, H-6), 4.88 (1H, d, *J* = 8.0 Hz, H-G1), 4.01 (1H, m, H-G2), 4.21 (2H, m, H-G3, H-G4), 3.93 (1H, m, H-G5), 4.37 (1H, dd, *J* = 4.4, 9.2 Hz), and 4.51 (1H, dd, *J* = 2.0, 9.2 Hz) (H-G6); ^{13}C NMR (C_5D_5N) C-1 (δ_C 26.8), C-2 (29.5), C-3 (87.9), C-4 (42.4), C-5 (144.2), C-6 (118.5), C-7 (24.6), C-8 (43.5), C-9 (40.2), C-10 (36.9), C-11 (77.8), C-12 (41.1), C-13 (49.5), C-14 (49.8), C-15 (34.5), C-16 (28.5), C-17 (51.0), C-18 (17.0), C-19 (26.2), C-20 (36.3), C-21 (18.9), C-22 (34.2), C-23 (29.0), C-24 (79.0), C-25 (72.7), C-26 (25.9), C-27 (26.3), C-28 (27.7), C-29 (26.3), C-30 (19.3), C-G1 (107.3), C-G2 (75.5), C-G3 (78.7), C-G4 (71.8), C-G5 (78.1), C-G6 (63.1). The ^{1}H and ^{13}C NMR signal assignments of **12** were performed with the aid of ^{13}C DEPT NMR, $^{1}H-^{1}H$ correlation spectroscopy (COSY), ^{1}H-detected multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), and phase-sensitive nuclear Overhauser and exchange spectroscopy (NOESY) experiments.

In Vitro EBV-EA Activation Experiment. The inhibition of EBV-EA activation was assayed using Raji cells (EBV genome-carrying human lymphoblastoid cells; nonproducer type), cultivated in 10% fetal bovine serum (FBS) RPMI-1640 medium (Sigma, St. Louis, MO). The indicator cells (Raji cells; 1×10^6 cells/mL) were incubated in 1 mL of the medium containing 4 mM *n*-butyric acid as an inducer, 32 pM TPA [20 ng/mL in dimethyl sulfoxide (DMSO)], and a known amount (32, 16, 3.2, 0.32 nM) of the test compound at 37 °C in a CO_2 incubator. After 48 h, the cell suspensions were centrifuged at 1000 rpm for 10 min, and the supernatant was removed. The activated cells were stained with high-titer EBV-EA-positive sera from nasopharyngeal carcinoma patients, and the conventional indirect immunofluorescence technique was employed for detection. In each assay, at least 500 cells were counted, and the experiments were repeated three times. The average extent of EA induction was determined and compared with that on positive control experiments in which the cells were treated with *n*-butyric acid plus TPA, where the extent of EA induction was ordinarily more than around 40%. The viability of treated Raji cells was assayed by the Trypan blue staining method (*12*).

RESULTS AND DISCUSSION

Two new triterpene benzoates, **1** and **2**, and two new triterpene glycosides, **8** and **9**, along with 15 known triterpenoids (one triterpene benzoate, **3**; three triterpene monols, **4**−**6**; one triterpene aglycon, **7**; and 10 triterpene glycosides, **10**−**19**), were isolated from the EtOH extract of *M. grosvenori* fruit in this study. Compound **12** was previously synthesized from **19** by maltase hydrolysis (*3*), but this study found naturally occurring **12**. A pentaglycoside, **19**, the most relevant sweet principle of *M. grosvenori* fruit, followed by a diglycoside, **13**, constituted the predominant triterpenoid components of the extract. Characterization of two new triterpene dibenzoates (**1**, **2**) and two new triteprnene glycosides (**8**, **9**) is described below.

5-Dehydrokarounidiol Dibenzoate (1). Compound **1** ($C_{44}H_{54}O_4$) had two benzoyl groups [δ_H 7.28 (2H, t), 7.42 (2H, t), 7.44 (1H, dt), 7.52 (1H, dt), 7.90 (2H, dt), and 8.06 (2H, dt)],

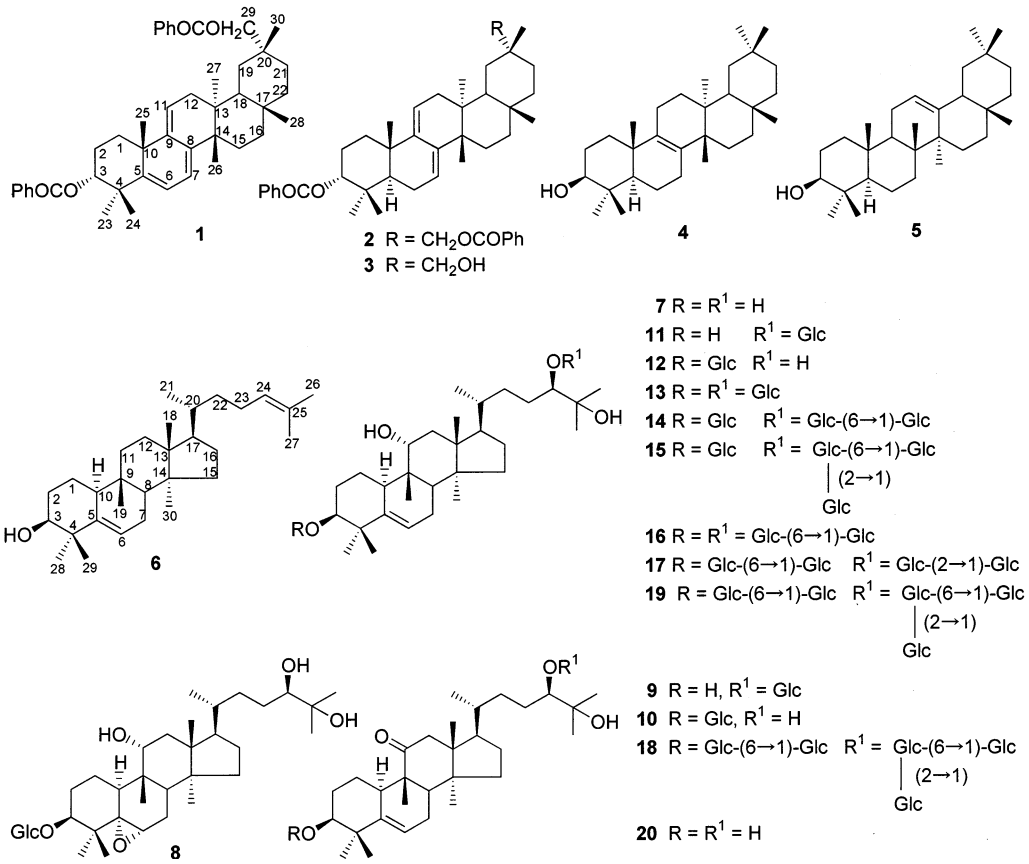

Figure 1. Structures of triterpenoids isolated from the fruit of *Momordica grosvenori*.

one equatorially oriented oxymethine [>C*H*O−; δ_H 4.99 (br s, $W_{1/2}$ = 7.3 Hz)], three olefinic methines [δ_H 5.37 (1H, m), 5.66 (1H, d), and 5.91 (1H, d)], one oxymethylene [−C*H*$_2$O−; δ_H 4.12 and 4.17 (each 1H, d, J = 13.8 Hz)], and seven tertiary methyls [δ_H 0.88, 1.07, 1.11, 1.14, 1.17, 1.23, and 1.29 (each 3H and s)]. The shifts of the olefinic methine ^{1}H signals and the UV absorptions (λ_{max} 304, 314, 330 nm) were consistent with a $\Delta^{5,7,9(11)}$-conjugated triene system (*6*). Close similarity of the ^{1}H NMR spectral data of **1**, with the exception of the ^{1}H signals of benzoyl groups, with those of 5-dehydrokarounidiol diacetate (*6*) suggested that compound **1** possesses the structure of 5-dehydrokarounidiol with two benzoyl groups at C-3 and C-29. This was supported by the diagnostic MS fragment ions at *m*/*z* 524 ([M]$^+$ − PhCOOH), 402 ([M]$^+$ − 2PhCOOH), 373 (ABC ring + C-26, C-27), and 225 (*m*/*z* 373 − PhCOOH). Alkaline hydrolysis of **1** yielded 5-dehydrokarounidiol (*6*); hence, compound **1** was assigned as 5-dehydrokarounidiol dibenzoate [D:C-friedo-oleana-5,7,9(11)-triene-3α,29-diol 3,29-dibenzoate].

Karounidiol Dibenzoate (2). Compound **2** ($C_{44}H_{56}O_4$) had two benzoyl groups, one equatorially oriented oxymethine, two olefinic methines, one oxymethylene, and seven tertiary methyls, as shown by the ^{1}H NMR spectral data (see the Identification and Characterization section). The shifts of the olefinic methine ^{1}H signals and the UV absorptions were consistent with a $\Delta^{7,9(11)}$-conjugated diene system (*5*). Comparison of the ^{1}H NMR data of **2** with those of karounidiol diacetate (*5*), together with the analyses of the MS fragmentations of **2**, led us to suggest that compound **2** is a dibenzoyl derivative at C-3 and C-29 of karounidiol. Alkaline hydrolysis of **2** yielded karounidiol (*5*) and karounidiol 3-benzoate (**3**); thus, the structure of **2** was established as karounidiol dibenzoate [D:C-friedo-oleana-7,9-(11)-diene-3α,29-diol 3,29-dibenzoate].

5α,6α-Epoxymogroside I E$_1$ (8). Compound **8** was assigned a molecular formula of $C_{36}H_{62}O_{10}$, as determined from its [M + Na]$^+$ ion at *m*/*z* 677.4241 in the HRFABMS (positive-ion mode) and ^{13}C DEPT NMR data. The ^{1}H NMR spectrum (**Table 1**) of **8** exhibited signals due to seven tertiary methyls [δ_H 0.85, 0.91, 1.22 (6H), 1.51, 1.53, and 1.57], one secondary methyl (δ_H 1.01, d, J = 6.4 Hz), and four oxymethines [δ_H 3.19 (br d), 3.74 (br s), 3.77 (br d), 4.02 (br d)]. It also showed a doublet signal at δ_H 4.91 (d, J = 7.6 Hz), ascribable to an anomeric proton, along with other ^{1}H signals due to a glucose moiety (δ_H 3.93, 4.02, 4.20, 4.22, 4.37, 4.51; each 1H) (**Table 1**) (*13*). Close simirality of the ^{1}H NMR and the ^{13}C NMR data (**Table 1**) with those of mogroside I E$_1$ (**12**) (see Materials and Methods), with the exception of the lack of the signals due to an olefinic bond for **8**, suggested **8** to be an oxygenated analogue of **12**, most probably epoxylated at C-5(6). On HMBC spectroscopy, the oxymethine H-6 of compound **8** provided cross-correlations with C-4, C-5, C-7, and C-8, along with the other correlations of H-28 (with C-3, C-4, C-5, and C-29) and H-29 (with C-3, C-4, C-5, and C-28) (**Table 1**), which supported the epoxy ring being located at C-5(6). The 5α,6α-stereochemistry of the epoxy ring and the α-orientation of the hydroxyl group at C-11 were established on the basis of the nuclear Overhauser effect (NOE) correlations observed between H-6β−H-29 (4β-Me)−H-19 (9β-Me)−H-8β, H-11β, and H-18 (13β-Me) on the β-face of the molecule in the NOESY experiment. Further NOE correlations were observed between H-3α−H-28 (4α-Me)−H-10α−H-30 (14α-Me)−H-17α] on the α-face of the molecule. Thus, we proposed that **8** is (24*R*)-5α,6α-epoxycucurbit-3β,-11α,24,25-tetrol 3-*O*-β-D-glucopyranoside (5α,6α-epoxymogroside I E$_1$). Analyses of the ^{13}C DEPT, ^{1}H−^{1}H COSY, and HMQC, in addition to HMBC and NOESY, spectra confirmed the final structure, as shown in **Figure 1**.

Table 2. Percentage of Epstein–Barr Virus Early Antigen Induction in the Presence of the Extracts of *Momoridica grosvenori* Fruit with Respect to a Positive Control (100%)[a]

extract	concentration (μg/mL)		
	100	10	1
ethanol extract	10.4 (70)	46.7	97.9
n-hexane soluble fraction	7.0 (70)	43.7	95.1
MeOH/H_2O soluble fraction	9.2 (70)	47.2	96.0
n-BuOH soluble fraction	19.5 (50)	59.0	100
H_2O soluble fraction	21.4 (60)	62.4	100

[a] Values represent relative percentages to the positive control value. TPA (32 pmol, 20 ng) = 100%. Values in parentheses are viability percentages of Raji cells.

11-Oxomogroside I A_1 (9). Compound **9** was assigned a molecular formula of $C_{36}H_{60}O_9$, as determined from its ^{13}C DEPT NMR data and $[M - H]^-$ ion at *m*/*z* 635.4120 in the HRFABMS (negative-ion mode). The ^1H NMR spectrum (**Table 1**) of **9** exhibited signals due to seven tertiary methyls [δ_H 0.76, 0.99, 1.17, 1.28, 1.42, 1.43, and 1.47], one secondary methyl (δ_H 0.91, d, J = 6.4 Hz), two oxymethines [δ_H 3.72 (br s) and 3.87 (d, J = 8.8 Hz)], an olefin methine (δ_H 5.69, d, J = 5.8 Hz), and an anomeric proton [δ_H 5.00 (d, J = 7.8 Hz)], along with other ^1H signals due to a glucose moiety. Comparison of the ^1H and ^{13}C NMR data of **9** with those of 11-oxomogrol [bryodulcosigenin; (24*R*)-cucurbit-5-en-11-one-3β,24,25-triol; **20**] (*14*) suggested **9** to be the 24-*O*-glucoside of **20**. The presence of a long-range $^3J_{C-H}$ correlation between the anomeric ^1H signal (H−G1, δ_H 5.00), together with the ^{13}C signal of C-24 (δ_C 90.7) observed in the HMBC spectrum of **9**, is consistent with a glucosidic linkage at C-24. Acid hydrolysis of **9** gave **20**; hence, **9** was formulated as (24*R*)-cucurbit-5-en-11-one-3β,-24,25-triol 24-*O*-β-D-glucopyranoside (11-oxomogroside I A_1).

Inhibitory Effect on EBV-EA Induction. The EtOH extract of *M. grosvenori* fruit, the *n*-hexane, MeOH/H_2O, *n*-BuOH, and H_2O soluble fractions obtained from the EtOH extract, 18 triterpenoids (**2**−**19**) isolated from the fractions of the EtOH extract, and an aglycon, **20**, obtained from **9** by acid hydrolysis, were evaluated for their inhibitory effects on the induction of EBV-EA by TPA in Raji cells as a primary screening test for antitumor promoters. The inhibitory effects of the EtOH extract and the four fractions are shown in **Table 2** and those of the 19 triterpenoids in **Table 3**, along with comparable data for β-carotene, a vitamin A precursor that has been studied intensively in cancer chemoprevention using animal models (*15*). The EtOH extract and the four fractions showed inhibitory effects against EBV activation with 79−93% inhibition at 100 μg/mL, and among the four fractions, the least polar *n*-hexane fraction exhibited the most potent inhibition (93% inhibition), whereas the most polar H_2O fraction showed the least inhibition (79% inhibition) (**Table 2**).

All of the triterpenoids from *M. grosvenori* fruit extract tested showed potent inhibitory effects on EBV-EA induction (70−100% inhibition at 1×10^3 mol ratio/TPA) while preserving the high viability (60−70%) of Raji cells, and most of them were equivalent to or more potent than β-carotene (**Table 3**). Among these, one triterpene monol (**6**), two triterpene aglycons (**7**, **20**), and six mono- and diglycosides (**8**−**13**) showed potent inhibitory effects on EBV-EA activation at 1×10 mol ratio/TPA (2−11% inhibition) and exhibited remarkably high inhibitory effects at a high concentration (97−100% inhibition at 1×10^3 mol ratio). Benzoylation of the hydroxyl group(s) of triterpene alcohols, and tri-, tetra-, and pentaglycosylation of cucurbitane aglycons led to a slight reduction of the inhibitory effects, as observed for two triterpene benzoates (**2**, **3**) (*16*) and six tri-, tetra-, and pentaglycosides (**14**−**19**), which exhibited 70−95% inhibition at 1×10^3 mol ratio. Free triterpene monools, **4**−**6**, and other uncharacterized components, but not the benzoates **1**−**3**, might be responsible for the strong inhibitory effect of the least polar *n*-hexane fraction (**Table 2**). The inhibitory effects against EBV-EA induction have been demonstrated to closely parallel those against tumor promotion in vivo (*17*, *18*), and the cucurbitane glycosides, the sweet principles of *M. grosvenori* fruit, and the other triterpenoid constituents in the extract of *M. grosvenori* fruit are, therefore, suggested to be valuable antitumor promoters (potential cancer chemopreventive agents).

Table 3. Percentage of Epstein–Barr Virus Early Antigen Induction in the Presence of **2**–**20** with Respect to a Positive Control (100%)[a]

	compound	concentration (mol ratio/TPA)			
		1000	500	100	10
2	karounidiol dibenzoate	29.7 (60)	63.7	89.0	100
3	karounidiol 3-benzoate	15.8 (70)	54.5	79.4	100
4	isomultiflorenol	2.6 (70)	45.2	74.6	100
5	β-amyrin	6.9 (70)	48.3	75.2	100
6	10α-cucurbitadienol	0 (70)	22.7	70.3	91.5
7	mogrol	0 (70)	27.3	71.6	90.2
8	5α,6α-epoxymogroside I E_1	0 (60)	25.2	68.1	92.0
9	11-oxomogroside I A_1	0 (70)	29.0	77.4	97.2
10	11-oxomogroside I E_1	0 (60)	26.1	69.3	94.1
11	mogroside I A_1	0 (70)	28.3	76.5	98.4
12	mogroside I E_1	3.1 (70)	29.0	73.2	96.7
13	mogroside II E	0 (70)	32.1	72.3	96.2
14	mogroside III	4.6 (60)	40.3	76.7	100
15	siamenoside I	10.0 (60)	50.2	81.3	100
16	mogroside IV A	8.5 (70)	33.7	76.7	100
17	mogroside IV E	9.2 (70)	31.6	75.6	100
18	11-oxomogroside V	12.6 (60)	51.3	83.0	100
19	mogroside V	5.1 (60)	42.3	78.9	100
20	11-oxomogrol	0 (70)	23.8	69.3	88.9
	β-carotene[b]	8.6 (70)	34.2	82.1	100

[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] Reference compound.

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