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Structures of New Triterpenoids and Cytotoxicity Activities of the Isolated Major Compounds from the Fruit of *Momordica charantia* L.

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Supporting Information

ABSTRACT: Two new cucurbitane-type triterpene glycosides, charantagenins D (**1**) and E (**2**), and one new sterol, 7-oxo-stigmasta-5,25-diene-3-*O*-*β*-D-glucopyranoside (**3**), were isolated from the fruit of *Momordica charantia* L. together with another eight known compounds. Their structures were determined on the basis of spectral analysis. Cytotoxicity activities of the isolated major compounds were evaluated against lung cancer cell line A549, glioblastoma cell line U87, and hepatoma carcinoma cell line Hep3B by using a 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in vitro assay. Results showed compounds **1** and **7** (goyaglycoside d) with an −OMe substituent group in the side chain exhibited significant cytotoxic activities against cancer cells. Impressively, the IC_{50} values of the new compound **1** to A549, U87, and Hep3B were 1.07, 1.08, and 14.01 μmol/L, respectively, which were much lower than those of other tested compounds.

KEYWORDS: *Momordica charantia L, Cucurbitaceae, charantagenin D, charantagenin E, 7-oxostigmasta-5,25-diene-3-O-β- D-glucopyranoside, cytotoxicity*

INTRODUCTION

Momordica charantia L. (Cucurbitaceae), commonly called "kugua" in China, is known as not only a food but also a medicine, which exhibits various biological activities, including antidiabetic and antiviral activities.[1,2] Until now, many cucurbitane-type triterpenoids and other bioactive compounds have been isolated and identified from *M. charantia*.[3−10] Bioactive properties of *M. charantia* against numerous cancers (lymphoid leukemia, lymphoma, choriocarcinoma, melanoma, breast cancer, skin tumor, prostate cancer, squamous carcinoma of tongue and larynx, human bladder carcinomas) were reported in previous studies,[11−18] which suggested that it contains compounds that might have anticancer potential. However, most of the preceding studies have been conducted using crude preparations of *M. charantia* and without proper identification of the bioactive compounds. The main phytochemicals that have been documented with cytotoxic activity are a group of ribosome-inactivating proteins named *α*- and *β*-momorcharin, momordin, cucurbitacin B, and a chemical analogue of *M. charantia* protein named MAP-30.[19−22] It is therefore important to identify all of the potentially bioactive compounds, especially triterpenoids and their glycosides, that exhibit cytotoxicity against cancer cell lines from this plant.

Previously, three cucurbitane-type triterpenoids, charantagenins A, B, and C, were isolated from the alcohol extract of the dried fruit of Chinese *M. charantia*, which is commonly called "charantagenins".[10] The continued study on the triterpenoid constituents of the alcohol extract of *M. charantia* fruit now lead to the isolation of two new cucurbitane-type triterpenoids, named charantagenins D (**1**) and E (**2**) and one new sterol, 7-oxostigmasta-5,25-diene-3-*O*-*β*-D-glucopyranoside (**3**) together with eight known compounds [charantoside VI (**4**)[23] kuguaglycoside C (**5**),[24] momordicoside K (**6**),[25] goyaglycoside d (**7**),[26] goyaglycoside b (**8**)[26] stigmasta-7,25(27)-dien-3*β*-ol (**9**),[27] charantadiol A (**10**),[28] and 3*β*,25-dihydroxy-5*β*,19-epoxycucurbita-6(23*E*)-diene (**11**)[29]] (Figure 1). Besides the isolation and structure elucidation of the new compounds, the cytotoxic effects of the compounds (**1**−**3**, **5**−**7**, **9**−**11**) against three cancer cell lines (A549, U87, and Hep3B) were also evaluated using the MTT assay in the present study. The current data suggested that these compounds might have the potential to be developed as anticancer agents in future translational studies. Further studies on the in vivo antitumor activity and the underlying mechanisms are underway.

MATERIALS AND METHODS

General. Crystallizations were performed in MeOH. Optical rotations were measured on a P-E 241 MC polarimeter using methanol as the solvent. UV spectra on a UV-721W spectrometer and IR spectra on a Bruker IFS-55 infrared spectrophotometer were recorded in MeOH and KBr disks, respectively. NMR spectra were recorded with a Bruker ARX-600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) or with a Bruker ARX-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer in $CDCl_3$ or C_5D_5N with tetramethylsilane as internal standard (see the Supporting Information). High-resolution electrospray ionization mass specta (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]. Column chromatography was carried out on silica gel (Qingdao Haiyang Chemical Co., Ltd., 200−400 mesh), and octadecyl silica gel (Chromatorex-ODS, 100−200 mesh; Fuji Silysia Chemical, Ltd., Aichi,

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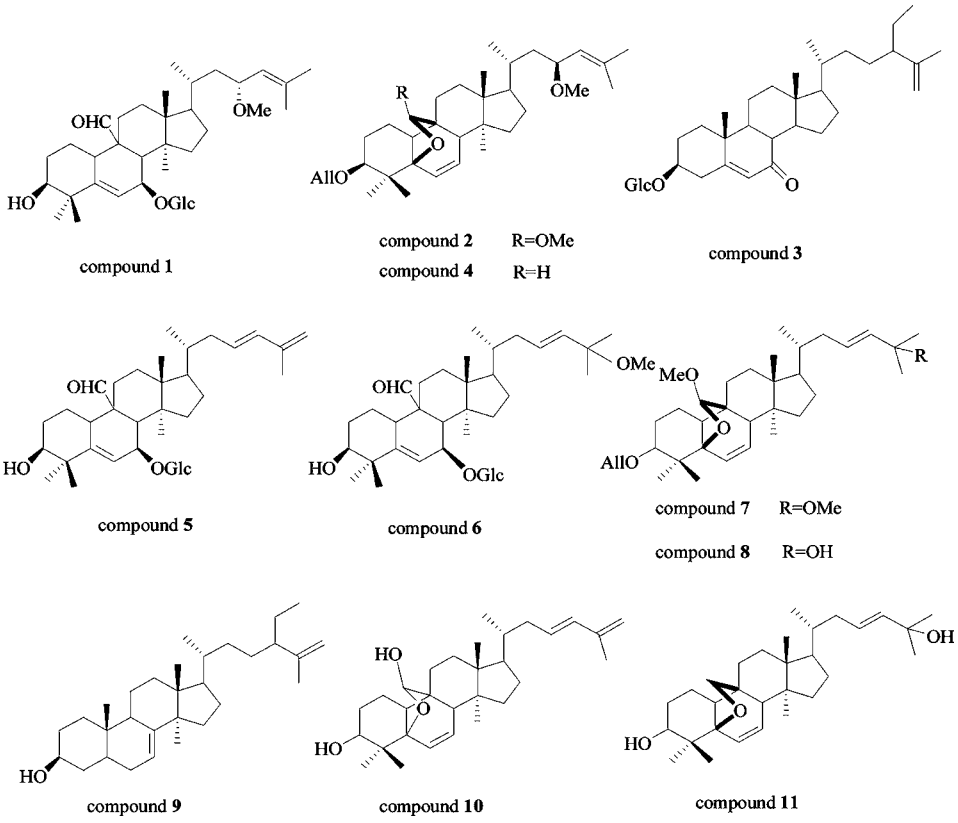

Figure 1. Structures of compounds **1**−**11**.

Japan) was used for open column chromatography. Reversed-phase preparative HPLC was carried out on an octadecyl silica column [YMC-Pack ODS A (250 mm × 10 mm, 5 μm) Beijing, China] at 25 °C at a flow rate of 10.0 mL/min with the eluent MeOH/H_2O 88:22 (HPLC system I), 89:11 (HPLC system II), 9:1 (HPLC system III), or 95:5 (HPLC system IV).

Materials and Chemicals. The dried immature fruits of *M. charantia* (10 kg), which were cultivated in Shanxi province, China, were bought from Shenyang Pharmaceutical University, China. A voucher specimen (No. 2007035) was identified by Prof. Sun Qi-shi and deposited at the herbarium department of Shenyang Pharmaceutical University, China.

All chemicals and solvents were of analytical or HPLC grade. Cell culture medium, fetal bovine serum (FBS), phosphate-buffered saline (PBS), sodium pyruvate, nonessential amino acids, penicillin−streptomycin, and other cell culture supplies were obtained from the Media Preparation Shared Facility of Shenyang Pharmaceutical University. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sino-American Biotechnology (Beijing, China)

Extraction and Isolation. Air-dried fruits of *M. charantia* (10 kg) were cut into small pieces (1−2 mm^2) and extracted three times with 70% EtOH (50 L total) under reflux. Evaporation of the solvent was performed under reduced pressure to provide the EtOH extract (950 g), and then the extract (950 g) was partitioned in H_2O/EtOAc (1:1), giving an EtOAc-soluble fraction (dry weight, 180 g). The fraction (180 g) was fractionated by silica gel column (10 × 200 cm, 1800 g) chromatography (eluted with acetone and ligarine in increasing polarity) to obtain eight fractions A−H, and compounds **9** (40 mg), **10** (80 mg), and **11** (201 mg). Fractions C (10 g), D (9 g), and E (11 g) were subjected to further chromatography on silica gel (5 × 100 cm, 350 g; eluted with methanol and chloroform in increasing polarity) respectively, yielding 15 fractions, C1−C15, 20 fractions, D1−D20, and 18 fractions, E1−E18. Fractions D10−12 (230 mg) and E3−4 (200 mg) were subjected to further chromatography on ODS (1 × 30 cm, 8 g), which was eluted successively with solvents of decreasing polarity (MeOH/H_2O, 5:5−7:3−8:2−9:1−1:0) and yielded 17 fractions, D10-12-1−D10-12-17, and 10 fractions, E3-4-1−E3-4-10. Isolation of the following eight compounds was performed by preparative HPLC: compound **8** [3.5 mg; retention time (t_R) 48.2 min] was from fraction C5-7 (200 mg) by HPLC system I; compound **4** (3.2 mg; t_R 50.2 min) was from fraction C10 (89 mg) by HPLC system I; compounds **6** (42 mg; t_R 17.5 min) and **5** (22 mg; t_R 28.9 min) were from fraction D9 (160 mg) by HPLC system III; compound **3** (8.3 mg; t_R 30.1 min) was from fraction D6 (128 mg) by HPLC system IV; compound **1** (18.5 mg; t_R 29.8 min) was from fraction D10-12-5 (98 mg) by HPLC system IV; compounds **2** (8.1 mg; t_R 35.4 min) and **7** (25 mg; t_R 48.3 min) were from fraction E3-4-9 (62 mg) by HPLC system III. The extraction and isolation schematic is shown in Figure 2.

In Vitro Cytotoxicity Assay. The cytotoxicity of compounds isolated from the fruit of *M. charantia* L. against A549 lung cancer cell line (ATCC No. CCL-185, RPMI-1640 medium), U87 glioblastoma cell line (ATCC No. HTB-14, DMEM with high glucose), and Hep3B hepatoma carcinoma cell line (ATCC No. HB-8064, RPMI-1640 medium) were assessed by MTT colorimetric method. Briefly, cells in RPMI-1640 or DMEM medium supplemented with 10% fetal bovine serum (FBS) were incubated in a 96-well plate in the presence of different concentrations of test compounds (0.1, 1, 10, 100 μmol/L) at 37 °C in a 5% CO_2 incubator for 72 h. Reduced MTT crystals were dissolved in DMSO, and then the OD value at 492 nm of each well was measured using a plate reader to determine cell growth inhibition. Etoposide and 5-FU (Sigma, 99% purity) were included as a positive control.

Statistical Analysis. Two-sided unpaired or paired Student's *t* tests were used to analyze the significance of differences. $p < 0.05$ was considered to be significant. The experiments were performed in triplicate independently. The data were expressed as the mean ± SD.

RESULTS AND DISCUSSION

Three new compounds and eight known compounds were isolated from an alcohol extract of the fruits of *M. charantia*. Identification of the eight known compounds was performed by nuclear magnetic resonance (NMR) and mass spectrometric (MS) analyses and by comparison of obtained values with literature values of the corresponding compounds.

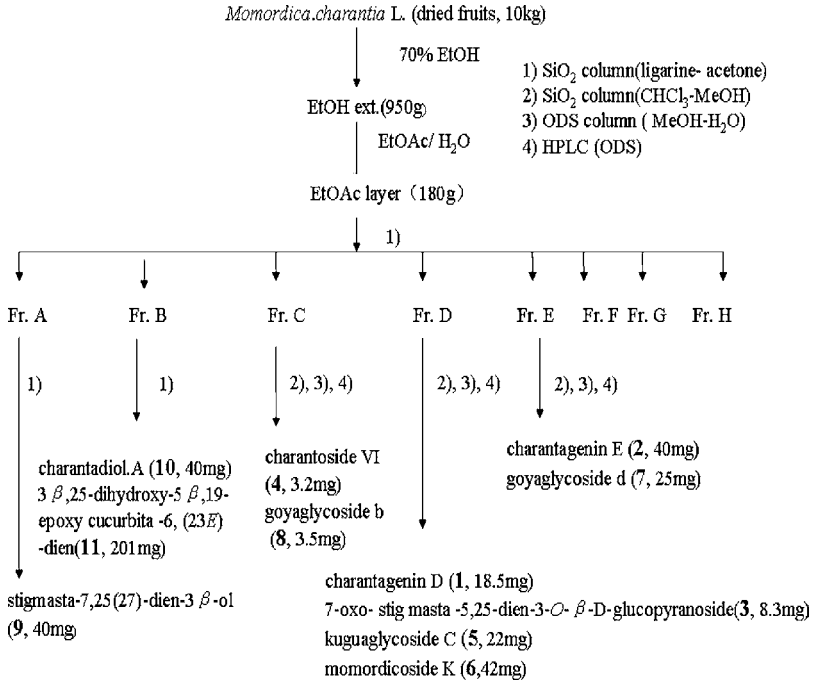

Figure 2. Extraction and isolation flow schematic.

Compound 1 was obtained as colorless needles. The molecular formula was determined as $C_{37}H_{60}O_9$ on the basis of its HRESIMS (negative-ion mode) ($[M + Cl]^-$, m/z 683.3932). Its IR spectrum showed absorptions for a hydroxyl group (3413 cm^{-1}) and an aldehyde group (2875 cm^{-1}). The ^1H NMR spectrum (Table 1) showed signals assignable to a β-D-glucopyranosyl moiety [δ 4.98 (1H, d, J = 7.8 Hz; H-1′)], seven methyl groups [δ 1.45 (3H, s), 1.15(3H, s), 0.75 (3H, s), 0.89 (3H, s), 1.75(3H, br s), 1.70 (3H , br s), 1.07 (3H, d, J = 6.0 Hz)], an *O*-methyl [δ 3.30 (br s, 3H)], two olefinic protons [δ 6.19 (1H, d, J = 4.8 Hz), 5.22 (1H, d, J = 9.0 Hz)], and an aldehyde proton [δ 10.49 (1H, s)]. The ^{13}C NMR (Table 2) showed 37 carbon signals. The DEPT spectrum exhibited 8 methyls, 8 methylenes, 15 methines, and 6 quaternary carbons. The ^{13}C NMR spectrum showed olefinic carbons appeared at δ 147.7, 122.4, 127.9, and 134.5 and an aldehyde carbon resonance at δ 207.0. The NMR data for the tetracyclic part of the aglycon and the glycosyl moieties of **1** were similar to those of compound **5**,[24] whereas the NMR data for the side-chain moiety of **1** were almost indistinguishable from those of charantoside II[23] and charantoside V.[23] The chemical structure of **1** was further elucidated by HMBC (Figure 3) experiment, in which the following cross peaks were observed: H-7 (δ_H 4.59, 1H, br d, J = 5.4 Hz) correlated with C-6 (δ_C 122.4), C-5 (δ_C 147.7), C-9 (δ_C 50.4), C-14 (δ_C 48.2), and C-1′ (δ_C 101.8); H-26 (δ_H 1.75, 1H, br s) and H-27 (δ_H 1.71, 1H, br s) correlated with C-25 (δ_C 134.5) and C-24 (δ_C 127.9); H-21 (δ_H 1.07, 1H, d, J = 6.0 Hz) correlated with C-17 (δ_C 51.3), C-20 (δ_C 33.0), and C-22 (δ_C 43.4). Therefore, the structure of compound **1** was deduced to be (23*R*)-3β-hydroxy-23-methoxycucurbita-5,24-dien-19-al-7-*O*-β-D-glucopyranoside (charantagenin D).

Compound 2 was assigned a molecular formula of $C_{38}H_{62}O_9$, as determined from its $[M + Na]^+$ ion at m/z 685.4286 in the HRESIMS. Its IR spectrum showed absorption at 1630 cm^{-1}, indicating the presence of a double bond. The ^1H (Table 1) and ^{13}C NMR spectra (Table 2) of **2** 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. Compound **2** exhibited ^1H NMR signals for the side-chain protons at δ 1.04 (3H, d, J = 5.5 Hz; a secondary methyl), 1.73 and 1.75 (each 3H and br s; two vinylic methyls), 3.30 (3H, s; an *O*-methyl), 4.11 (1H, dt, J = 8.9,5.1 Hz; an allylic oxymethine), and 5.17 (1H, d, J = 8.9 Hz; an olefinic methine). The NMR data of **2** were very similar to those of charantosides II[23] except for the signals due to the stereochemistry at C-23. Thus, the $\Delta\delta_C$ values [δ_C (charantosides II) − δ_C (2)] for the side-chain signals were calculated as −0.9 (C-20), −1.0 (C-21), +0.4 (C-22), −1.6 (C-23), +0.6 (C-24), −1.4 (C-25), −0.1 (C-26), and −0.5 (C-27) from the ^{13}C NMR data of charantosides II and 2 (Table 1), which were almost consistent with the $\Delta\delta_C$ values [δ_C (23R) − δ_C (23S)] 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.[30] The above evidence suggested that **2** possesses the structure (19*R*,23*S*)-5β,19-epoxy-19,23-dimethoxycucurbita-6, 24-dien-3β-ol-3-*O*-β-D-allopyranoside (charantagenin E).

Compound 3 was obtained as a gum. It was determined to have a molecular formula of $C_{35}H_{56}O_7$ based on a $[M + H]^+$ ion at m/z 589.4096 in the HRESIMS. Its IR spectrum showed strong absorption bands at 1670 cm^{-1} due to a conjugated carbonyl group, which was also confirmed by a strong absorption at 237 nm in the UV spectrum. In the ^1H NMR spectrum (Table 1), the signals of a β-D-glucopyranosyl moiety [δ 5.01 (1H, d, J = 7.8 Hz; H-1′)], five methyl groups [δ 0.66 (3H, s), 1.0 (3H, s),1.60 (3H, s), 0.96 (3H, d, J = 6.6 Hz), 0.85 (3H, t, J = 7.2 Hz)], and three olefinic protons [δ 5.78 (1H d, J = 1.8 Hz), 4.79 (1H, br s), 4.86 (1H, br s)] were detected. The ^{13}C NMR spectrum (Table 2) of **3** showed signals for 35 carbons due to five methyl groups, one carbonyl group, and two pairs of olefinic groups. The NMR data of compound **3** were very similar to those of 7-oxostigmasta-5,25-dien-3β-ol[31] except that the former exhibited a downfield glycosylation

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−3 (δ Values: 600 MHz, C_5D_5N)

proton	1	2	3
aglycon moiety			
1	1.89−1.92 (m)	1.90	1.70−1.72 (m)
	1.67−1.71 (m)	1.44	
2	1.90−1.95 (m)	1.74	1.71−1.73 (m)
	2.01−2.03 (m)	2.13	2.16−2.18 (m)
3	3.80 (br s)	3.70 (br s)	4.0−4.02 (m)
4			2.51−2.53 (m)
			2.80−2.82 (m)
5			
6	6.19 (d, 4.8)	6.15 (dd, 2.4, 9.6)	5.78 (d,1.8)
7	4.59 (br d, 5.4)	5.61 (dd, 3.8, 9.6)	
8	2.54 (br s)	3.13 (br s)	2.20 (like t)
9			1.38−1.41 (m)
10	2.64−2.66 (m)	2.47 (dd, 5.8, 12.7)	
11	2.60−2.63 (m)	1.76	1.39−1.43 (m)
	1.56−1.58 (m)	1.67	
12	1.64−1.69 (m)	1.66	1.03−1.05 (m)
		1.61	1.94−1.96 (m)
13			
14			1.39−1.42 (m)
15	1.48−1.56 (m)	1.30	2.77 (m)
16	1.91−1.94 (m)	1.96	1.87−1.90 (m)
	1.42−1.43 (m)	1.43	1.20−1.30 (m)
17	1.52 (overlapped)	1.48	1.04−1.05 (m)
18	0.89 (s)	0.95 (s)	0.66 (s)
19	10.49 (s)	4.88 (s)	1.0 (s)
20	1.90	1.55	0.96 (m)
21	1.07 (d, 6)	1.04 (d, 5.5)	0.96 (d, 6.6)
22	1.09	1.57	1.10
	1.88−1.90 (m)	1.72	1.30−1.38 (m)
23	4.13 (dt, 9.0, 3.0)	4.11 (dt, 8.9, 5.1)	1.34−1.41 (m)
			1.26−1.29 (m)
24	5.22 (d, 9.0)	5.16 (br d, 8.9)	1.88−1.90 (m)
25			
26	1.75 (br s)	1.75 (s)	1.6 (s)
27	1.71 (br s)	1.73 (s)	4.79 (br s)
			4.86 (br s)
28	1.45 (s)	0.82 (s)	1.32−1.40 (m)
29	1.15 (s)	1.44 (s)	0.85 (t,7.2)
30	0.75 (s)	0.93 (s)	
OMe-19		3.48 (s)	
OMe-23	3.30 (s)	3.30 (s)	
sugar moiety			
1′	4.98 (d, 7.8)	5.46 (d, 7.6)	5.01 (d, 7.8)
2′	3.98−4.02 (m)	3.88 (dd, 2.0, 7.6)	4.06 (t, 7.8)
3′	4.27−4.33 (m)	4.71 (t, 2.0)	4.30 (t, 9.0)
4′	4.24−4.27 (m)	4.18 (dd, 2.0, 9.7)	4.25 (t, 9.0)
5′	3.96−4.05 (m)	4.45	3.96−4.0 (m)
6′	4.62 (dd, 2.0, 11.0)	4.37 (dd, 4.8, 11.3)	4.59 (dd, 4.8, 12.0)
	4.41 (5.4, 11.1)	4.52 (dd, 2.0, 11.3)	4.40 (br d, 12.0)

Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1−3 (δ Values: 150 MHz, C_5D_5N)

carbon	1	2	3
aglycon moiety			
1	21.9	18.5	36.5
2	29.8	27.4	29.9
3	75.6	83.4	78.7
4	42.0	39.1	39.1
5	147.7	85.6	165.3
6	122.4	133.1	126.4
7	71.9	131.6	201.5
8	45.0	42.2	45.6
9	50.4	48.1	50.1
10	36.7	41.6	38.7
11	22.7	23.3	21.4
12	29.4	31.0	39.1
13	45.9	45.3	43.3
14	48.2	48.3	50. 5
15	34.8	33.8	26.8
16	27.9	30.0	28.9
17	51.3	51.4	55.0
18	15.1	14.7	12.1
19	207.0	112.4	17.2
20	33.0	33.8	35.8
21	19.3	20.0	19.1
22	43.4	43.0	34.1
23	74.9	76.4	29.7
24	127.9	127.3	49.8
25	134.5	135.8	147.8
26	25.9	25.9	18.0
27	18.2	18.6	112.1
28	26.3	24.9	26.9
29	27.4	21.3	12.4
30	18.2	20.0	
OMe-19		57.7	
OMe-23	55.6	55.3	
sugar moiety			
1′	101.8	102.3	102.9
2′	75.0	73.8	75.3
3′	78.7	71.8	77.2
4′	71.9	69.2	71.8
5′	78.9	76.4	78.7
6′	62.3	63.2	63.0

shift (+8.3) for the C-3 signal in the ^{13}C NMR spectrum, which suggested that compound **3** was the glucoside of the latter. The structure of compound **3** was confirmed also by HMBC and HSQC. Namely, long-range correlations were observed between H-6 (δ_H 5.78, 1H, d, J = 1.8 Hz) and C-8 (δ_C 45.6) and C-10 (δ_c 38.7), between H-3 (δ_H 4.00−4.02, 1H, m) and C-1′ (δ_C 102.9) and C-5 (δ_C 78.7), between H-17 (δ_H 1.04−1.05, 1H, m) and C-12 (δ_C 39.1), C-14 (δ_C 50.5), C-18 (δ_C 12.1), C-20 (δ_C 35.8), and C-21 (δ_C 19.1), between H-24 (δ_H 1.88−1.90, 1H, m) and C-23 (δ_C 29.7), C-25 (δ_C 147.8), C-26 (δ_C 18.0), C-27(δ_C 112.1), C-28 (δ_C 26.9), and between H-27 (δ_H 4.79, 4.86, 2H, br s) and C-24 (δ_C 49.8), C-25(δ_C 147.8), and C-26 (δ_C 18.0). Accordingly, compound **3** was established to be 7-oxostigmasta-5,25-dien-3-*O*-β-D-glucopyranoside.

Compound 1: (23*R*)-3β-hydroxy-23-methoxycucurbita-5,24-dien-19-al-7-*O*-β-D-glucopyranodide (charantagenin D); colorless needles; mp 185−187 °C; $[a]_D^{20}$ +31.3(c 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 204 (2.48) nm; IR ν_{max} 3413, 2934, 2875, 1709, 1381, 1079, 1042 cm^{-1}; ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS m/z 683.3932 (calcd for $[M + Cl]^-$, m/z 683.3931).

Compound 2: (19*R*,23*S*)-5β,19-epoxy-19,23-dimethoxycucurbita-6,24-dien-3β-ol-3-*O*-β-D-allopyranoside (charantagenin E); colorless needles; mp 145−148 °C; $[a]_D^{20}$ −95.7 (c 0.11, MeOH);

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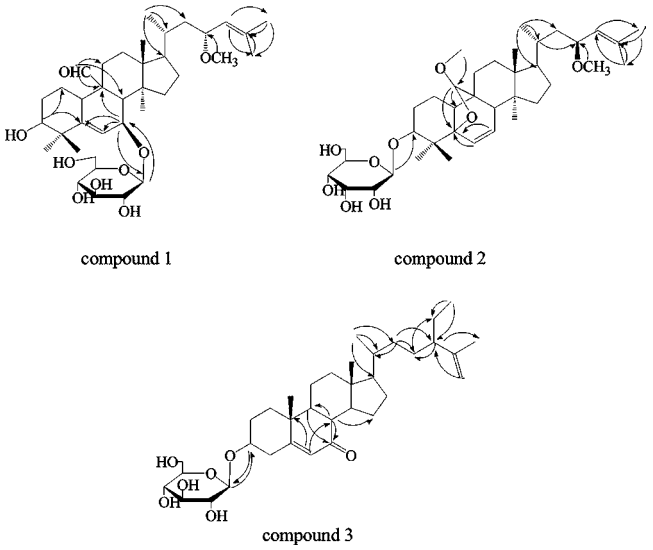

Figure 3. Major HMBC correlations of compounds **1**−**3**.

Table 3. ^{13}C NMR Spectroscopic Data of Compounds 1−3 (δ Values: 150 MHz, C_5D_5N)

	effects of compounds on survival rate IC_{50}[a] (μmol/L)		
compd	A549	U87	Hep3B
1	1.07	1.08	14.01
2	3.82	67.32	>100
3	>100	>100	>100
5	4.46	>100	>100
6	4.89	0.60	>100
7	5.32	0.19	19.30
9	15.10	8.65	>100
10	>100	>100	>100
11	>100	>100	>100
5-FU[b]	>100	>100	NT[c]
etoposide[b]	NT	NT	>100

[a] IC_{50} is defined as the concentration that resulted in a 50% decrease in cell growth. [b] Positive control. [c] Not tested.

UV (MeOH) λ_{max} (log ε) 205 (3.77) nm; IR ν_{max} 3424, 2933, 1630, 1381, 1085, 1033 cm^{-1}; ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 685.4286 (calcd for $[M + Na]^+$ ion at *m*/*z* 685.4286).

Compound 3: 7-oxo-stigmasta-5,25-diene-3-*O*-β-D-glucopyranoside; amorphous gum solid; decomposition point 201 °C; $[a]_D^{25}$ −64.2 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 237 (3.59), 210 (3.30), 303 (1.81) nm; IR ν_{max} 3422, 2939, 2872, 1670, 1379, 1076, 1030 cm^{-1}; ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 589.4096 (calcd for $C_{35}H_{56}O_7Na$ $[M + H]^+$, *m*/*z* 589.4099).

Compounds **1**−**3**, **5**−**7**, and **9**−**11** (purity > 99%) were tested for their cytotoxicities against three cancer cell lines by MTT assay (Table 3 and Figure 4). Test solutions were given to cells in various final concentrations as 0.1, 1, 10, or 100 μmol/L, and the cytotoxic potential of the isolated compounds was investigated by determining their concentrations required for 50% growth inhibition (IC_{50} value).

Most phytochemicals that have been reported to have cytotoxic activity are a group of ribosome-inactivated proteins named α-and β-momorcharin, momordin, cucurbitacin B, and a chemical analogue of *M. charantia* protein named MAP-30.[19−22] To the best of our knowledge, this is the first report of

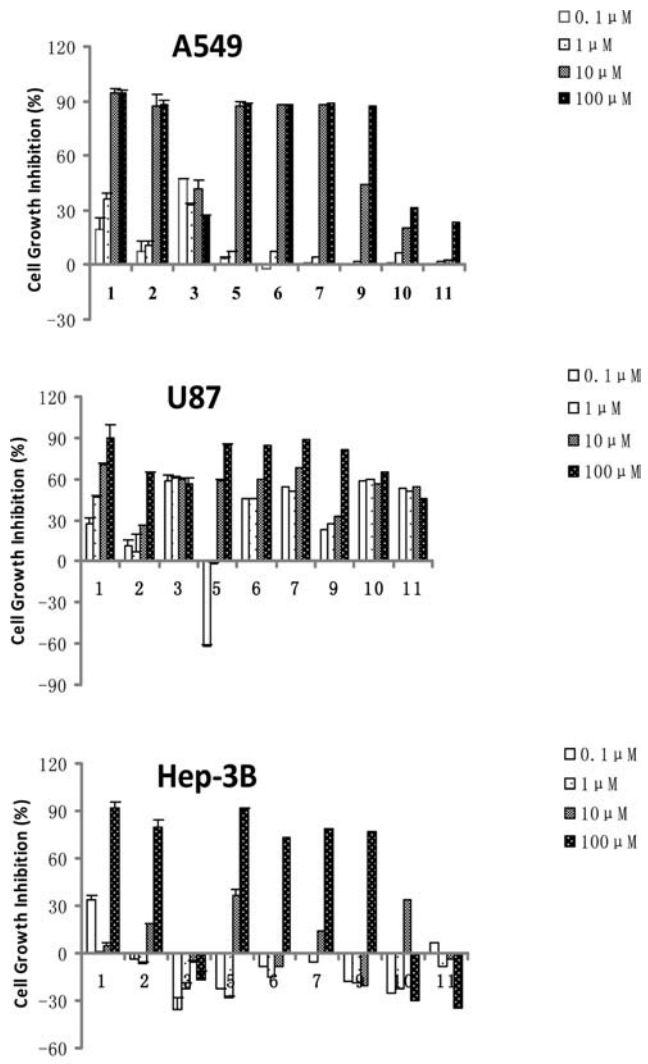

Figure 4. Cell growth inhibition of three cell lines (A549, Hep-3B, and U87) after incubation with test compounds (**1**−**3**, **5**−**7**, **9**−**11**) for 72 h: charantagenins D (**1**) and E (**2**) and one new sterol, 7-oxostigmasta-5, 25-diene-3-*O*-β-D-glucopyranoside (**3**), kuguaglycoside C (**5**), momordicoside K (**6**), goyaglycoside d (**7**), stigmasta-7,25(27)-dien-3β-ol (**9**), charantadiol A (**10**), and 3β,25-dihydroxy-5β,19-epoxycucurbita-6(23*E*)-diene (**11**).

the structure−function relationship between the cucurbitane-type triterpenoids and their cytotoxicity activity. In our previous study, we reported that 20(*R*)-dammarane-3β,12β,20,25-tetrol (25-OH-PPD), a dammarane triterpene sapogenin from *Panax ginseng*, exhibited significant cytotoxicity on cancer cells, and 20(*R*)-25-methoxydammarane-3β,12β-20-triol (25-OCH_3-PPD), a derivative of 25-OH-PPD (the difference is the variation in their side chains), showed a better antitumor activity. The cucurbitane-type triterpene glycosides studied currently share some common features with dammarane triterpene sapogenin in structure.[32−34] Our results showed that compounds **1** and **7** showed significant cytotoxicity, and compounds **2**, **6**, and **9** exhibited moderate cytotoxicity toward A549 lung cancer cell line and U87 glioblastoma cell line, whereas compound **5** showed cytotoxicity only toward A549 (IC_{50} = 4.46 μmol/L). Other compounds have no cytotoxicity at all. The current data suggested the activity of cucurbitane-type triterpenoid seems to be associated

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with the CHO in C-19 and OMe level in the side chain of the aglycone. This finding is consistent with our previous study in which we found, compared to 25-OH-PPD, 25-OCH_3-PPD exhibited higher activity against tumor cell lines mainly due to the methoxyl group in the side chain of C-25.[32] Further pharmacological studies are ongoing to assess the in vivo antitumor activities and to identify the underlying mechanisms of action. The data presented here may provide a basis for the development of new anticancer agents based on cucurbitane-type triterpenoids.

In summary, two new cucurbitacins and one new sterol were obtained from the fruit of *M. charantia*. Compound **1** exerted a stronger cytotoxic activity against three cell lines than any of the other compounds, whereas compound **2** showed moderate activity against A549 and U87 cell lines. Further biological studies on these compounds are still underway in our laboratory. Current data suggest the cucurbitane-type triterpenes from *M. charantia*, especially compound **1**, may have the potential to be developed as agents for future chemotherapy.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra data of the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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