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Antiproliferative and Hypoglycemic Cucurbitane-Type Glycosides from the Fruits of *Momordica charantia*

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Supporting Information

ABSTRACT: This paper reports that bioassay-guided fractionations of EtOH extract of *Momordica charantia* fruits led to the isolation of 15 cucurbitane-type triterpene glycosides including 4 new compounds, kuguaosides A−D (**1**−**4**), along with 11 known ones, charantoside A (**5**), momordicosides I (**6**), F_1 (**7**), F_2 (**8**), K (**9**), L (**10**), and U (**11**), goyaglycosides-b (**12**) and -d (**13**), 7*β*,25-dihydroxycucurbita-5,23(*E*)-dien-19-al 3-*O*-*β*-D-allopyranoside (**14**), and 25-hydroxy-5*β*,19-epoxycucurbita-6,23-dien-19-on-3*β*-ol 3-*O*-*β*-D-glucopyranoside (**15**). Their structures were elucidated on the basis of spectroscopic analyses and chemical methods. This study also established the HPLC-ELSD fingerprinting profile of an antiproliferative fraction of which 11 main peaks were identified. Biological evaluation showed that several isolated cucurbitane-type triterpene glycosides had antiproliferative activities against MCF-7, WiDr, HEp-2, and Doay human tumor cell lines. In addition, compound **14** showed potent hypoglycemic activities by glucose uptake assay.

KEYWORDS: *Momordica charantia, cucurbitane, triterpene glycosides, kuguaoside, antiproliferative activity, hypoglycemic activity*

INTRODUCTION

Momordica charantia L. (Cucurbitaceae), also known as bitter melon, is widely cultivated as a vegetable crop in tropical and subtropical countries, and its fruits are called kugua in Taiwan.[1] The fruit of *M. charantia* has been used for hundreds of years in Asia, India, Africa, and South America as a traditional medicine and functional food to prevent and treat diabetes. Previous pharmacological investigations of *M. charantia* have shown various biological activities such as hypoglycemic,[2−4] anti-inflammatory,[5,6] and antitumor activities[7−10] and related bioactive constituents including cucurbitane-type triterpenoids, their glycosides, flavonoids, phenolic acids, and fixed oil, which were isolated from the fruits,[11−14] leaves and vines,[15] root,[16] and seeds.[17,18]

Previous studies have shown the extract of *M. charantia* inhibited lymphoma tumor formation in CBA/H mice and mammary tumor in SHN virgin mice.[10] Seed oil of *M. charantia* inhibits azoxymethane-induced colonic abervant crypt foci (ACF) in rats.[18] Isolated (19*R*,23*E*)-5*β*,19-epoxy-19-methoxycucurbita-6,23,25-trien-3*β*-ol and (19*R*,23*E*)-5*β*,19-epoxy-19,25-dimethoxycucurbita-6,23-dien-3*β*-ol exhibited remarkable inhibition in 7,12-dimethylbenz[*a*]anthracene and peroxynitrite-induced mouse skin carcinogenesis.[19] In addition, an aqueous extract of *M. charantia* caused inhibition of prostatic adenocarcinoma growth and exerted cytostatic as well as cytotoxic effects against human leukemic lymphocytes.[20] Recent literature has reported that *M. charantia* extracts possess a significant effect on inhibition of cancer cell proliferation and induction of apoptosis in breast cancer cells (MCF-7 and MDA-MB-231).[9]

A preliminary screening assay showed that the bioactive fraction (fraction 4) inhibited the proliferation of human breast adenocarcinoma [(MCF-7), IC_{50} = 33.19 *μ*g/mL], human medulloblastoma [(Doay), IC_{50} = 32.88 *μ*g/mL], human laryngeal carcinoma [(HEp-2), IC_{50} = 26.90 *μ*g/mL], and human colon adenocarcinoma [(WiDr), IC_{50} = 33.76 *μ*g/mL]. Therefore, we further investigated the bioactive constituents from this fraction of *M. charantia* fruits. Bioactivity-guided fractionation of Taiwanese *M. charantia* was used to determine the identity of bioactive compounds that inhibit tumor cell growth.

The objective of this research was to isolate and identify the bioactive compounds of *M. charantia* fruits with potent antiproliferative activity. We report here the isolation, structural elucidation, and antiproliferative activity of these cucurbitane-type triterpene glycosides from *M. charantia* fruits. The bioactive fraction was analyzes by high performance liquid chromatography−evaporative light scattering detection (HPLC-ESLD), and its 11 main peaks were isolated and characterized. In addition, owing to the traditional use of *M. charantia* for diabetes treatment, we were encouraged to evaluate the isolated cucurbitanes for their antidiabetes activity by glucose uptake assay.

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Table 1. ^{1}H NMR (400 MHz) Data of Compounds 1−4 and 15[a]

position	1	2	3	4	15
1	1.32 (m)	1.40 (m)	1.56 (m)	1.50 (m)	1.50 (m)
	1.74 (br d, 8.8)	1.84 (m)	1.90 (m)	1.82 (m)	1.82 (m)
2	1.76 (m)	1.75 (m)	1.93 (m)	1.90 (m)	1.77 (m)
	2.37 (br d,10.4)	2.21 (d, 10.8)	2.45 (br d, 10.0)	2.42 (br d, 10.4)	2.44 (br d, 12.8)
3	3.67 (br s)	3.71 (br s)	3.74 (br s)	3.71 (br s)	3.65 (br s)
6	6.17 (br d, 9.6)	6.14 (br d, 9.6)	6.21 (br d, 9.6)	6.02 (br d, 5.2)	6.30 (br d, 9.6)
7	5.53 (dd, 3.6, 9.6)	5.58 (br d, 9.6)	4.30 (br d, 9.6)	3.42 (br d, 5.2)	5.59 (dd, 9.6, 3.2)
8	2.27 (br s)	3.09 (br s)	2.32 (br s)	2.14 (br s)	2.52 (br s)
10	2.24 (m)	2.45 (dd, 5.2, 12.0)	2.56 (m)	2.51 (m)	2.62 (dd, 12.0, 5.2)
11	1.30 (m); 1.59 (m)	1.60 (m); 1.71 (m)	1.44 (m); 2.63 (m)	1.39 (m); 2.55 (m)	1.64 (m); 2.36 (m)
12	1.52 (m); 1.55 (m)	1.56 (m); 1.62 (m)	1.45 (m); 1.50 (m)	1.44 (m); 1.51 (m)	1.30 (m); 1.46 (m)
15	1.15 (m); 1.21 (m)	1.27 (m, 2H)	1.30 (m, 2H)	1.27 (m, 2H)	1.15 (m); 1.22 (m)
16	1.46 (m); 1.97 (m)	1.52 (m); 1.97 (m)	1.18 (m); 1.86 (m)	1.16 (m); 1.93 (m)	1.19 (m); 1.86 (m)
17	1.48 (m)	1.50 (m)	1.48 (m)	1.53 (m)	1.46 (m)
18	0.76 (s)	0.91 (s)	0.82 (s)	0.87 (s)	0.81 (s)
19	3.57 (d, 8.0)	4.83 (s)	10.54 (s)	10.18 (s)	
	3.71 (d, 8.0)				
20	2.11 (m)	2.10 (m)	1.44 (m)	1.53 (m)	1.44 (m)
21	1.09 (d, 6.4)	1.11 (d, 6.4)	0.94 (d, 6.0)	0.95 (d, 5.2)	0.91 (d, 6.4)
22	1.14 (m)	1.17 (m)	1.83 (m)	1.84 (m)	1.83 (m)
	1.96 (m)	1.93 (m)	2.20 (m)	2.23 (m)	2.20 (br d, 13.6)
23	4.79 (br t, 8.4)	4.79 (m)	5.92 (m)	5.93 (m)	5.89 (m)
24	5.59 (d, 8.4)	5.59 (d, 8.4)	5.89 (br d, 15.6)	5.89 (br d, 15.6)	5.90 (m)
26	1.69 (s)	1.68 (s)	1.52 (s)	1.52 (s)	1.53 (s)
27	1.67 (s)	1.67 (s)	1.52 (s)	1.53 (s)	1.53 (s)
28	0.90 (s)	0.83 (s)	1.13 (s)	1.10 (s)	0.91 (s)
29	1.50 (s)	1.46 (s)	1.65 (s)	1.63 (s)	1.58 (s)
30	0.86 (s)	0.90 (s)	0.75 (s)	0.69 (s)	0.74 (s)
OMe		3.42 (s)		3.16 (s)	
1′	4.88 (d, 7.6)	4.95 (d, 7.6)	4.90 (d, 7.6)	4.84 (d, 7.6)	4.83 (d, 7.6)
2′	4.03 (br d, 7.6)	4.03 (m)	3.89 (t, 8.4)	3.85 (t, 8.4)	3.96 (m)
3′	4.23 (m)	4.25 (m)	4.22 (m)	4.20 (m)	4.19 (m)
4′	4.19 (m)	4.20 (m)	4.16 (m)	4.18 (m)	4.12 (m)
5′	3.97 (m)	3.97 (m)	3.95 (m)	3.94 (m)	3.94 (m)
6′	4.39 (dd, 4.8, 11.2)	4.40 (m)	4.36 (br d, 11.6)	4.36 (br d, 11.6)	4.36 (dd, 5.2, 10.4)
	4.57 (br d, 11.2)	4.58 (br d, 10.8)	4.54 (dd, 5.6, 11.6)	4.54 (dd, 1.6, 11.6)	4.55 (br d, 10.4)

[a] δ in ppm, J in Hz. NMR solvent was pyridine-d_5.

MATERIALS AND METHODS

General Experimental Procedures. Infrared (IR) spectra were measured on a Mattson Genesis II spectrophotometer (Thermo Nicolet, Madison, WI, USA). Optical rotations were determined on a JASCO P-2000 polarimeter (Jasco Co., Tokyo, Japan). Electrospray ionization mass spectrometry (ESIMS) data were operated on a JOEL JMS-HX 110 mass spectrometer (JOEL, Tokyo, Japan). GC was performed on an Agilent Technologies 6890N Network GC System. High-resolution electronic ionization mass spectrometry (HREIMS) data were measured on a Finnigan MAT-95XL mass spectrometer (San Jose, CA, USA). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC-400 FT-NMR (Bruker BioSpin, Rheinstetten, Germany) using C_5D_5N (pyridine-d_5) as solvent for measurement. Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), Sephadex LH-20 (GE Healthcare, Uppsala, Sweden), and silica gel 60 (Merck 70−230 and 230−400 mesh, Merck, Darmstadt, Germany) were used for column chromatography, and precoated silica gel (Merck 60 F-254) plates were used for TLC. The spots on TLC were detected by spraying with an anisaldehyde−sulfuric acid solution and then heating at 100 °C. HPLC separations were performed on a Shimadzu LC-6AD series apparatus with an SPD-10A UV−vis spectrophotometric detector, equipped with a 250 × 10 mm i.d. preparative Cosmosil 5C_{18} AR-II column (Nacalai Tesque, Inc., Kyoto, Japan).

Plant Material. The fruits of Taiwanese *M. charantia* were purchased in Changhua County Taiwan in July 2009 and identified by Dr. I-Jung Lee. A voucher specimen (NRICM, No. 20090701) has been deposited in the herbarium of the National Research Institute of Chinese Medicine, Taipei, Taiwan.

Extraction and Isolation. The fresh fruits of Taiwanese *M. charantia* (2.4 kg) were extracted three times with 70% EtOH (5 L) at 50 °C for 24 h, and concentrated under reduced pressure. The EtOH extract (66 g) was subjected to Macroporous Adsorption Resin Diaion HP-20 column chromatography eluting with H_2O, 40% EtOH, 70% EtOH, 95% EtOH, and 100% EtOAc (each 2.5 L), respectively, to divide into five fractions, fraction 1 (45.0 g), fraction 2 (3.5 g), fraction 3 (3.4 g), fraction 4 (3.9 g), and fraction 5 (5.1 g). A preliminary screening assay showed that fraction 4 inhibited the proliferation of MCF-7, WiDr, HEp-2, and Doay human tumor cell lines. Therefore, bioactive fraction (fraction 4, 3.9 g) was further separated by chromatography on a Sephadex LH-20 column (4.5 × 80 cm) eluting with 100% MeOH (2 L) to afford three subfractions (fractions 4-1−4-3). Then, fraction 4-2 (2.7 g) was isolated to use reverse phase C_{18} gel column chromatography elution with gradient (from 20% MeOH to 100% MeOH) to give four fractions (fractions 4-2-1−4-2-4). Fraction 4-2-2 was further purified by semipreparative HPLC on a Cosmosil 5C_{18} AR-II column (250 × 10 i.d., mobile phase MeOH/H_2O = 80:20, flow rate = 3.0 mL/min, UV 210 nm) to yield compounds **1** (7.8 mg), **2** (3.1 mg), **6** (14.3 mg), and **11** (3.8 mg). Then fraction 4-2-3 was

Table 2. ^{13}C NMR (100 MHz) Data of Compounds 1−4 and 15[a]

position	1	2	3	4	15
1	18.8 (t)	18.6 (t)	22.4 (t)	22.2 (t)	19.7 (t)
2	27.5 (t)	27.3 (t)	28.6 (t)	28.5 (t)	26.4 (t)
3	85.5 (d)	83.9 (d)	86.9 (d)	86.9 (d)	85.4 (d)
4	39.0 (s)	39.1 (s)	41.9 (s)	42.0 (s)	38.4 (s)
5	85.8 (s)	85.4 (s)	145.5 (s)	147.4 (s)	84.2 (s)
6	133.9 (d)	133.0 (d)	123.7 (d)	120.7 (d)	132.9 (d)
7	130.0 (d)	131.8 (d)	65.5 (d)	75.7 (d)	132.4 (d)
8	52.1 (d)	42.1 (d)	50.8 (d)	45.8 (d)	44.8 (d)
9	45.1 (s)	48.0 (s)	50.4 (s)	50.5 (s)	50.7 (s)
10	40.0 (d)	41.5 (d)	36.8 (d)	36.7 (d)	40.6 (d)
11	23.8 (t)	23.3 (t)	22.6 (t)	22.5 (t)	21.8 (t)
12	31.1 (t)	30.9 (t)	29.3 (t)	29.1 (t)	29.9 (t)
13	45.5 (s)	45.4 (s)	45.6 (s)	45.7 (s)	45.2 (s)
14	48.8 (s)	48.3 (s)	48.2 (s)	47.8 (s)	47.8 (s)
15	33.3 (t)	33.7 (t)	34.8 (t)	34.9 (t)	33.3 (t)
16	28.4 (t)	28.4 (t)	27.6 (t)	27.5 (t)	27.6 (t)
17	51.2 (d)	51.3 (d)	50.0 (d)	50.5 (d)	50.3 (d)
18	14.9 (q)	14.8 (q)	14.9 (q)	14.9 (q)	14.5 (q)
19	80.0 (t)	112.4 (d)	207.8 (s)	207.1 (s)	182.1 (s)
20	32.7 (d)	32.8 (d)	36.5 (d)	36.5 (d)	36.5 (d)
21	18.7 (q)	18.9 (q)	18.9 (q)	18.9 (q)	18.7 (q)
22	45.3 (t)	45.4 (t)	39.5 (t)	39.5 (t)	39.4 (t)
23	65.1 (d)	65.1 (d)	124.1 (d)	124.1 (d)	124.1 (d)
24	131.8 (d)	131.6 (d)	141.7 (d)	141.7 (d)	141.7 (d)
25	130.8 (s)	130.7 (s)	69.7 (s)	69.7 (s)	69.7 (s)
26	25.7 (q)	25.7 (q)	30.8 (q)	30.8 (q)	30.8 (q)
27	18.0 (q)	18.0 (q)	30.8 (q)	30.8 (q)	30.8 (q)
28	25.6 (q)	24.9 (q)	27.8 (q)	27.8 (q)	23.9 (q)
29	20.9 (q)	21.1 (q)	25.8 (q)	25.7 (q)	20.8 (q)
30	20.2 (q)	19.9 (q)	18.1 (q)	18.1 (q)	19.3 (q)
OMe		57.6 (q)		55.9 (q)	
1′	107.0 (s)	105.5 (s)	107.2 (s)	107.3 (s)	107.7 (s)
2′	75.6 (d)	76.0 (d)	75.2 (d)	75.1 (d)	75.2 (d)
3′	78.2 (d)	77.9 (d)	78.8 (d)	78.7 (d)	78.4 (d)
4′	71.7 (d)	71.8 (d)	71.6 (d)	71.6 (d)	71.6 (d)
5′	78.3 (d)	78.6 (d)	78.3 (d)	78.3 (d)	78.7 (d)
6′	62.9 (t)	62.9 (t)	63.0 (t)	63.0 (t)	63.0 (t)

[a] δ in ppm, J in Hz. NMR solvent was pyridine-d_5.

purified by HPLC in the same solvent system to give compounds **8** (9.5 mg), **9** (15.6 mg), and **12** (7.1 mg). Using the same methods as for fraction 4-2-2 on HPLC with 75% MeOH, compounds **4** (8.2 mg), **7** (10.8 mg), **13** (9.7 mg), and **15** (10.7 mg) were obtained from fraction 4-2-4. The mobile phase with gradient elution of 40−60% acetonitrile/water in 0−60 min separated fraction 4-2-1 and obtained compounds **3** (9.3 mg), **5** (9.4 mg), **10** (9.6 mg), and **14** (12.3 mg).

Kuguaoside A ((23*R*)-5*β*,19-epoxycucurbita-6,24-diene-3*β*,23-diol 3-*O*-*β*-D-glucopyranoside, 1): white amorphous powder; $[\alpha]^{D}_{25}$ −82.6 (*c* 0.64, MeOH); IR (KBr) ν_{max} 3373, 2942, 2872, 1635, 1579, 1446, 1375, 1295, 1079, 1031 cm^{-1}; ESIMS *m*/*z* 641 [M + Na]$^+$; HRESIMS *m*/*z* 641.4064 [M + Na]$^+$ (calcd for $C_{36}H_{58}O_8Na$, 641.4032). ^{1}H (400 MHz, pyridine-d_5) and ^{13}C (100 MHz, pyridine-d_5) NMR spectral data are shown in Tables 1 and 2, respectively.

Kuguaoside B ((19*R*,23*R*)-5*β*,19-epoxy-19-methoxycucurbita-6,24-diene-3*β*,23-diol 3-*O*-*β*-D-glucopyranoside, 2): white amorphous powder; $[\alpha]^{D}_{25}$ −75.4 (*c* 0.24, MeOH); IR (KBr) ν_{max} 3383, 2931, 2874, 1635, 1577, 1446, 1375, 1260, 1112, 1079, 1032 cm^{-1}; ESIMS *m*/*z* 671 [M + Na]$^+$; HRESIMS *m*/*z* 671.4163 [M + Na]$^+$ (calcd for $C_{37}H_{60}O_9Na$, 671.4137). ^{1}H (400 MHz, pyridine-d_5) and ^{13}C (100 MHz, pyridine-d_5) NMR spectral data are shown in Tables 1 and 2, respectively.

Kuguaoside C (7*β*,25-dihydroxycucurbita-5,23(*E*)-dien-19-al 3-*O*-*β*-D-glucopyranoside, 3): white amorphous powder; $[\alpha]^{D}_{25}$ +27.8 (*c* 0.58, MeOH); IR (KBr) ν_{max} 3380, 2950, 2876, 1708, 1634, 1463, 1375, 1259, 1075, 1038 cm^{-1}; ESIMS *m*/*z* 657 [M + Na]$^+$; HRESIMS *m*/*z* 657.4002 [M + Na]$^+$ (calcd for $C_{36}H_{58}O_9Na$, 657.3981). ^{1}H (400 MHz, pyridine-d_5) and ^{13}C (100 MHz, pyridine-d_5) NMR spectral data are shown in Tables 1 and 2, respectively.

Kuguaoside D (3*β*,25-dihydroxy-7*β*-methoxycucurbita-5,23(*E*)-dien-19-al 3-*O*-*β*-D-glucopyranoside, 4): white amorphous powder; $[\alpha]^{D}_{25}$ +26.4 (*c* 0.50, MeOH); IR (KBr) ν_{max} 3384, 2953, 2875, 1712, 1615, 1463, 1383, 1260, 1076, 1038 cm^{-1}; ESIMS *m*/*z* 671 [M + Na]$^+$; HRESIMS *m*/*z* 671.4170 [M + Na]$^+$ (calcd for $C_{37}H_{60}O_9Na$, 671.4137). ^{1}H (400 MHz, pyridine-d_5) and ^{13}C (100 MHz, pyridine-d_5) NMR spectral data are shown in Tables 1 and 2, respectively.

Acid Hydrolysis of Compounds 1−4. Compounds **1**−**4** (1.0 mg) were each treated with 2 N methanolic HCl (2 mL) under conditions of reflux at 90 °C for 1 h, respectively. Each mixture was extracted with CH_2Cl_2 to afford the aglycone, and the aqueous layer was neutralized with Na_2CO_3 and filtered. To the evaporated filtrates were added 1-(trimethylsilyl)imidazole and pyridine (0.2 mL), with the whole stirred at 60 °C for 5 min. After the reaction mixture was dried under a stream of N_2, each residue was partitioned between CH_2Cl_2 and H_2O. Each CH_2Cl_2 fraction was subjected to gas chromatography by using a CP-Chirasil-L-Val column, according to a method previously reported.[21] The peaks of each hydrolysate from

compounds **1**–**4** were identified by comparison with authentic samples (D-glucose, t_R 30.60 min). All of the isolated glucoses from compounds **1**–**4** were identified as D-form.

HPLC Analysis. *Materials and Reagents.* The bioassay-guided fraction (fraction 4) was prepared as above-mentioned. The reference compounds, kuguaosides A–D (**1**–**4**), charantoside A (**5**), momordicosides I (**6**), F_2 (**8**), K (**9**), L (**10**), and U (**11**), goyaglycoside-b (**12**), 7*β*,25-dihydroxycucurbita-5,23(*E*)-dien-19-al 3-*O*-*β*-D-allopyranoside (**14**), and 25-hydroxy-5*β*,19-epoxycucurbita-6,23-dien-19-on-3*β*-ol 3-*O*-*β*-D-glucopyranoside (**15**), were isolated from fraction 4.

Apparatus and Conditions. The HPLC profile was performed on a Shimadzu liquid chromatography instrument equipped with an SCL-10AVP system controller, a Shimadzu LC-20AT pump, a 7715i manual injector with 20 μL sample loop, an evaporative light scattering detector (ELSD, Varian, 380-LC, England), a UV–vis detector (SPD-10A VP, Shimadzu, Japan) with detection at 210 nm, a four-channel vacuum degasser (Biotech, model 2003, Sweden), and SISC software (Scentific Information Service Corp., Taiwan) for data analysis. A separation column (Cosmosil $5C_{18}$-AR-II, 5 μm, 250 × 4.6 mm i.d., Japan) was employed eluting at a rate of 1.0 mL/min under room temperature. The mobile phase consisted of water (A) and acetonitrile (B) using a gradient program of 0–38% (B) in 0–10 min, 38–38% (B) in 10–20 min, and 38–45% (B) in 20–50 min. The flow rate of nebulizer gas (N_2) was maintained at 1.8 L/min, the nebulizer temperature was set at 30 °C, and the drift tube temperature was set at 40 °C. UV–vis detection was at 210 nm.

Preparation of Standards Solution. Each reference compound was accurately weighed and dissolved in MeOH; the terminate concentration was ca. 0.1 mg/mL.

Preparation of Sample Solution. Fraction 4 was dried under vacuum, then accurately weighed to about 10 mg, and dissolved in MeOH, in a 1.0 mL volumetric flask. The sample solutions were all filtered through a 0.45 μm filter (Millipore) before use, and the injection volume was 20 μL.

Antiproliferation Assay. The antiproliferation of compounds **1**–**15** (see Figure 1 for structures) was tested against MCF-7, Doay, HEp-2, and WiDr tumor cell lines by using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. The cell lines were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 units/mL streptomycin in 5% CO_2 and incubated at 37 °C. Test samples and control drug standard were prepared at concentrations of 1, 4, 10, 20, and 40 μg/mL. After seeding of 3×10^3 cells/well in a 96-well microplate for 4 h, 20 μL of sample or standard agent was placed in each well and incubated at 37 °C for 3 days, and then 20 μL of MTT (2 mg/mL) was added for 4 h. After removal of the medium and addition of DMSO (200 μL/well) into the microplate with shaking for 10 min, the formazan crystals (the product of MTT reacting with dehydrogenase existing in mitochondria) were redissolved, and their absorbance was measured on a model MR 7000 microtiter plate reader (Dynatech International Corp., Edgewood, NY, USA) at a wavelength of 550 nm. The IC_{50} values of each compound were obtained from 50% inhibition of cell growth and were compared with that of the control.

Glucose Uptake Assay. The method was analyzed by measuring the uptake of Ci 2-deoxy-[^{3}H]-D-glucose as described previously.[22] Mouse skeletal muscle cell line, C2C12 myoblasts (ATCC), were grown in DMEM supplemented with 10% FBS, 100 units/mL penicillin G, and streptomycin (37 °C; 5% CO_2). After 4–6 days, myoblasts had fused to form multinucleated myotubes. Cells were grown to 80–90% confluence before differentiation (initiated by replacing growth media with DMEM with 2% horse serum). C2C12 myoblasts cell grown in a 12-well plate were subjected to glucose uptake assay. Before treatment for 1 h, 10 μM sample was placed in each well, and then medium was removed and the samples were incubated with KRP buffer including 0.5 μCi/mL 2-deoxy-D-[^{3}H] glucose (GE Healthcare) at 37 °C. After 30 min, glucose uptake was terminated by washing the cells three times with cold PBS. Cells were lysed and radioactivity retained by the cell lysates determined by scintillation counting. Insulin was used as a positive control (100 μM).

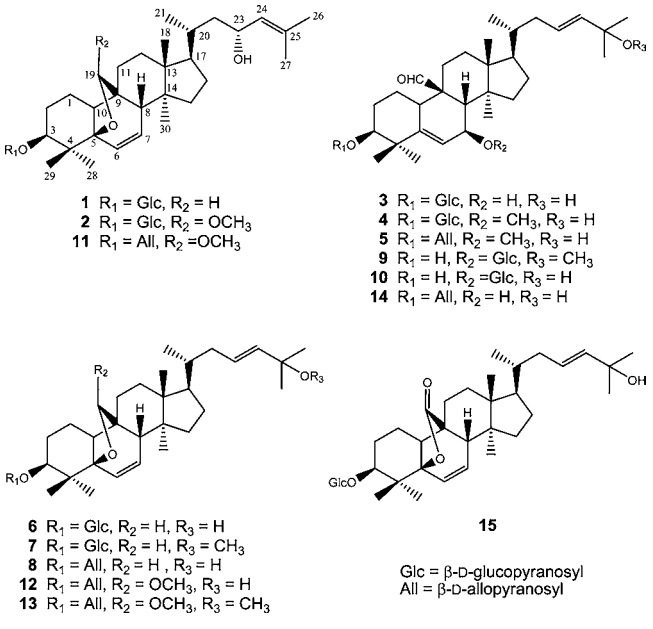

Figure 1. Structures of cucurbitane-type triterpene glycosides isolated from the fruits of *M. charantia*.

RESULTS AND DISCUSSION

Structural Elucidation. Compound **1** was obtained as a white powder, and its quasimolecular ion $[M + Na]^+$ at m/z 641.4064 (calcd for $C_{36}H_{58}O_8Na$, 641.4032) was found in the HRESIMS, suggesting that the molecular formula of **1** was $C_{36}H_{58}O_8$. The IR spectrum showed characteristic signals for the OH group (3373 cm^{-1}, strong) and double bond (1635 cm^{-1}, weak). The ^{1}H (Table 1) and ^{13}C NMR spectra of **1** (Table 2) showed the presence of four tertiary methyls [δ_H 0.76, 0.86, 0.90, 1.50 (each, 3H, s)], a secondary methyl [δ_H 1.09 (3H, d, J = 6.4 Hz)], two vinylic methyls [δ_H 1.67, 1.69 (each, 3H, s)], an oxymethylene [δ_H 3.57 (1H, d, J = 8.0 Hz),

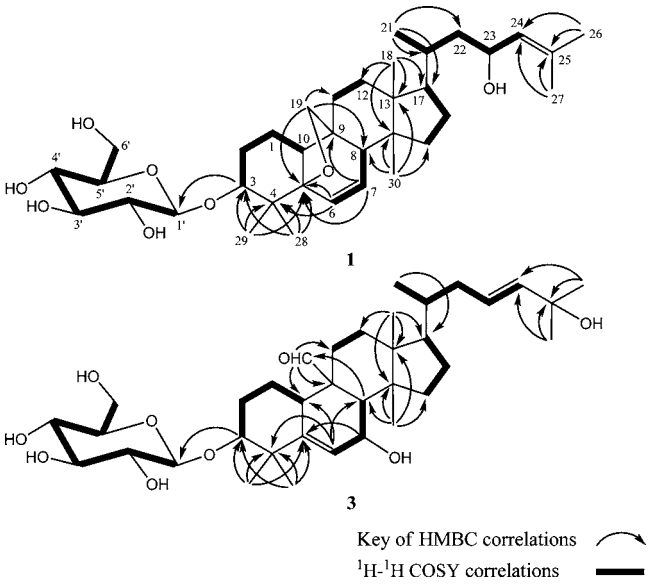

Figure 2. Key HMBC (→) and ^{1}H–^{1}H COSY (——) correlations of compounds **1** and **3**.

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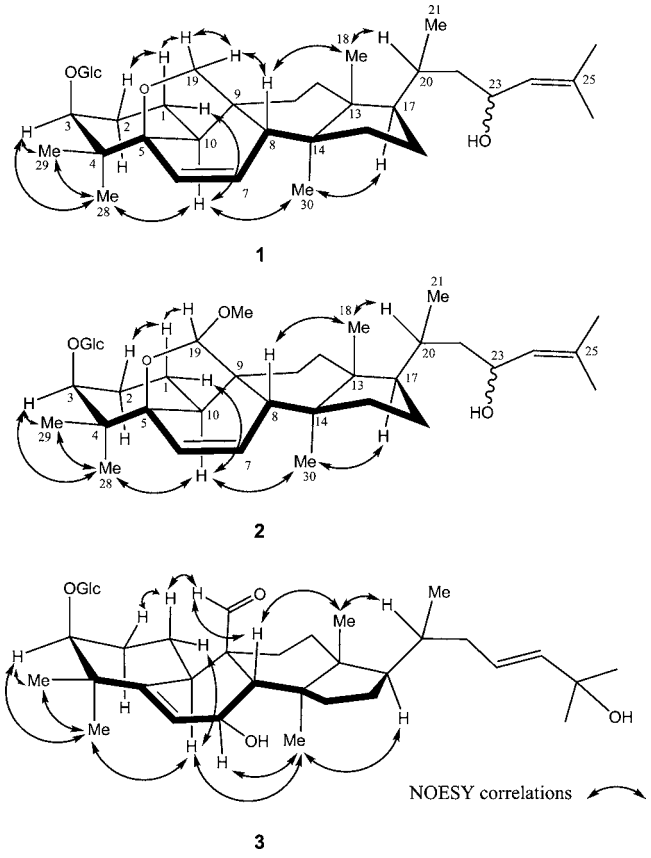

Figure 3. Key NOESY correlations of compounds **1**, **2**, and **3**.

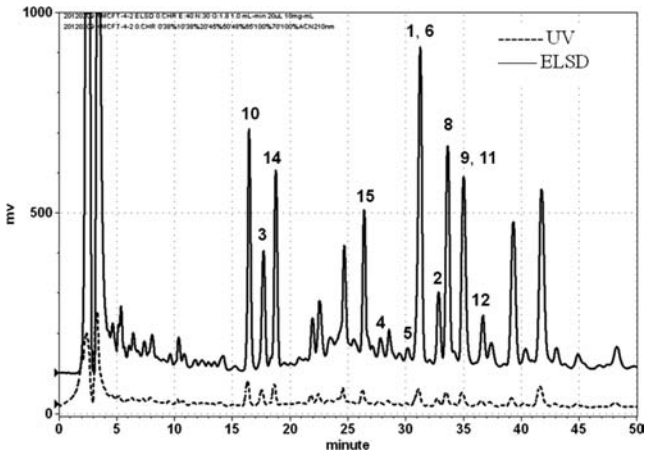

Figure 4. HPLC profile of the bioactive fraction 4 of *M. charantia*.

3.71 (1H, d, J = 8.0 Hz)], and two oxymethines [δ_H 3.67 (1H, br s), δ_H 4.79 (1H, br t, J = 8.4 Hz)], as well as one anomeric proton [δ_H 4.88 (1H, d, J = 7.6 Hz)] . Furthermore, olefinic NMR signals of a trisubstituted double bond [δ_H 5.59 (1H, d, J = 8.4 Hz); δ_C 131.8 (d), 130.8 (s)] and a cis double bond [δ_H 6.17 (1H, dd, J = 1.6, 9.6 Hz), 5.53 (1H, dd, J = 3.6, 9.6 Hz); δ_C 133.9 (d), 130.0 (d)] were also found. The ^{13}C NMR spectrum of **1** showed 36 carbon signals, which were attributed to a sugar moiety and a triterpene skeleton. A DEPT experiment permitted the definition of these 36 carbon resonances including 7 methyl, 9 methylene, 14 methine, and 6 quaternary carbons, which suggested that compound **1** is a 5,19-epoxycucurbitane-type triterpenoid with one sugar moiety.[11−16]

The assignments of the ^{1}H and ^{13}C signals of aglycone were established by ^{1}H−^{1}H COSY, HMQC, and HMBC (Figure 2) experiments.

The glucose was further confirmed to be β-glucose on the basis of coupling constant (d, J = 7.6 Hz) of anomeric proton in the ^{1}H NMR spectrum of **1**, and the D-form was identified by acid hydrolysis. In addition, the HMBC spectrum revealed a long-range correlation between δ_H 4.88 (Glc-H-1′) and δ_C 85.5 (C-3) of the triterpene, suggesting that the glucose moiety was attached to C-3 of the aglycone.

The relative stereochemistry of **1** was deduced by nuclear Overhauser enhancement spectroscopy (NOESY) experiments. In the ^{1}H NMR spectrum, the coupling constants of H-2 (δ_H 2.37, J = 10.4 Hz) were clearly observed, deducing its location at equatorial (β-orientation). Major cross-peaks of H-2β/H-1β (δ_H 1.74), H-1β/H$_2$-19, H$_2$-19/H-8, H-8/Me-18, Me-18/H-20, and H-1α (δ_H 1.32)/H-10, H-10/Me-28, H-3/Me-28, Me-29, Me-30/H-17 were observed in the NOESY spectrum of **1** (Figure 3), suggesting H-8, Me-18, and H-20 as β-oriented and then H-3, H-10, H-17, Me-28 and Me-30 as α-oriented. Thus, the structure and relative configuration of **1** were completely assigned as shown in Figure 1. In addition to using pyridine-d_5, we also employed $CDCl_3$ to measure the ^{13}C NMR of **1** and then decided the stereochemistry at C-23 of **1** to be R by comparing the ^{13}C NMR chemical shifts of the side chain [δ_C 32.6 (C-20), 44.4 (C-22), 65.8 (C-23), 129.0 (C-24), and 133.7 (C-25)] with reported (23R)-cycloart-24-ene-3β,23-diol [δ_C 33.0 (C-20), 44.5 (C-22), 66.1 (C-23), 129.1 (C-24), and 133.8 (C-25)] and (23S)-cycloart-24-ene-3β,23-diol [δ_C 33.5 (C-20), 44.5 (C-22), 67.3 (C-23), 128.4 (C-24), and 135.6 (C-25)].[23]

According to the above data, the complete structure of **1** was elucidated as a cucurbitane glycoside derivative, (23R)-5β,19-epoxycucurbita-6,24-diene-3β,23-diol 3-O-β-D-glucopyranoside, and has been named kuguaoside A.

Compound **2** was obtained as a white powder, which was assigned to be $C_{37}H_{60}O_9$ with eight degrees of unsaturation, and showed the quasimolecular ion peak $[M + Na]^+$ at m/z 671.4163 from the HRESIMS. The IR spectrum showed the absorption bands for hydroxyl (3383 cm^{-1}) and olefinic (1635 cm^{-1}) groups. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **2** with those of **1** suggested that both compounds had closely similar chemical shifts and possessed the same tetracyclic cucurbitane-type skeleton with a β-D-glucose. One of the different signals between compounds **1** and **2** was the functionality at C-19, including the absence of an oxygenated methylene and the appearance of oxygenated methine [δ_H 4.83 (1H, s) and δ_C 112.4] in **2**. The additional signals of δ_H 3.42 (3H, s) and δ_C 57.6 in **2** were characteristic of a methoxyl group, which connected to C-19 due to the HMBC (Figure 2) correlation between H-19 (δ_H 4.83) and methoxyl (δ_C 57.6), as well as C-5(δ_C 85.4), C-10(δ_C 41.5), and C-11(δ_C 23.3). This HMBC evidence also confirmed the epoxy bridge between C-5 and C-19. The NOESY spectrum (Figure 3) showed the correlations of Me-28/H-3, H-10, Me-29, Me-30/H-10, H-17, and Me-18/H-8, suggesting that H-8 and Me-18 were β-oriented and H-3, H-10, H-17, and Me-30 were α-oriented. In addition, the NOESY spectrum of **2** also showed correlations between H-19/H-1 and Me-18/H-21, suggesting that both C-19 and C-20 should be R conformations. Thus, the structure of **2** was determined as (23R)-5β,19R-epoxy-19-methoxycucurbita-6,24-

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Table 3. Antiproliferation Data of Compounds 1−15 from *M. charantia* Fruits

compound	IC_{50}[a] (μg/mL)			
	MCF-7	Doay	HEp-2	WiDr
1	12.60 ± 0.69	19.37 ± 1.73	15.89 ± 1.39	17.42 ± 0.79
2	29.77 ± 0.26	25.60 ± 1.32	28.07 ± 1.56	26.79 ± 1.09
3	27.58 ± 3.03	36.18 ± 3.5	–[b]	–
6	12.36 ± 0.13	12.04 ± 0.61	13.27 ± 1.31	16.63 ± 1.54
7	10.66 ± 0.63	11.68 ± 1.06	14.15 ± 0.41	11.55 ± 1.20
8	20.46 ± 0.97	30.68 ± 0.60	32.89 ± 0.56	21.21 ± 3.44
9	10.38 ± 0.98	20.12 ± 1.78	11.78 ± 1.05	17.49 ± 0.66
11	30.39 ± 1.96	29.47 ± 1.58	30.04 ± 0.89	16.16 ± 0.10
12	12.46 ± 0.21	12.73 ± 0.84	15.64 ± 1.60	14.64 ± 1.61
13	10.80 ± 0.85	10.12 ± 0.41	13.76 ± 0.32	17.02 ± 0.72

[a]IC_{50}, inhibitory concentration 50%. All values are presented as the mean ± SD (n = 3). Compounds **4**, **5**, **10**, **14**, and **15** are inactive at 40 μg/mL. [b]–, IC_{50} > 40 μg/mL.

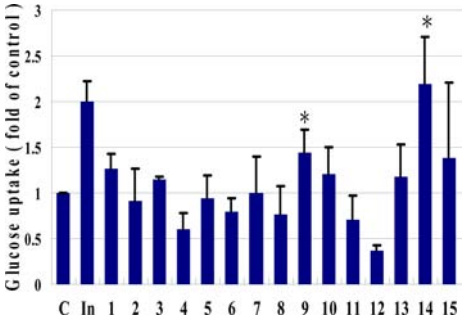

Figure 5. Glucose uptake activity of compounds **1**−**15**. All values are expressed as the mean ± SE of three experiments. (*) P < 0.05 as compared with the control group (n = 3).

diene-3β,23-diol 3-*O*-β-D-glucopyranoside and has been named kuguaoside B.

Compound **3** has an elemental composition of $C_{36}H_{58}O_9$, based on the result of HRESIMS at m/z 657.4002 $[M + Na]^+$. The IR spectrum showed absorptions at 3380 cm^{-1} (hydroxyl group), 1708 cm^{-1} (aldehyde group), and 1634 cm^{-1} (olefinic group). The ^1H NMR spectrum (Table 1) showed the presence of six singlet methyls (δ_H 0.82, Me-18; 1.52 × 2, Me-26, 27; 1.13, Me-28; 1.65, Me-29; 0.75, Me-30), one doublet methyl (0.94, d, J = 6.0, Me-21), a trisubstituted olefinic proton (6.21, br d, J = 9.6, H-6), trans double-bond protons (5.92, m, H-23; 5.89, br d, J = 15.6, H-24), and an aldehyde proton (10.54, s). The ^{13}C NMR and DEPT spectral data (Table 2) exhibited seven methyl carbons (δ_C 14.9, 18.9, 30.8 × 2, 27.8, 25.8, 18.1), seven methylene carbons (δ_C 22.4, 28.6, 22.6, 29.3, 34.8, 27.6, 37.5), three methine carbons (δ_C 50.8, 50.0, 36.5), four quaternary carbons (δ_C 41.9, 50.4, 45.6, 48.2), two oxymethine carbons (δ_C 86.9, 65.5), an oxygen-containing quaternary carbon (δ_C 69.7), a trisubstituted double-bond carbon (δ_C 145.5, 123.7), two olefinic methine carbons (δ_C 124.1, 141.7), and an aldehyde carbon (δ_C 207.8), along with six carbons of a sugar moiety (107.2, 75.2, 78.8, 71.6, 78.3, 63.0). The ^1H and ^{13}C NMR data of the triterpene moiety in compound **3** were found to be similar to those reported for 7β,25-dihydroxycucurbita-5,23(*E*)-dien-19-al 3-*O*-β-D-allopyranoside (**14**).[24] Furthermore, the HMBC spectrum (Figure 2) of compound **3** showed the correlation between H-3 and C-1′, directly helping to establish the location of glycosylation. The full structure of **3** was further deduced from the ^1H−^1H COSY, HMQC, and HMBC correlations (Figure 2). The NOESY correlations (Figure 3) of CHO-19/H-1β, H-8, and Me-18/H-8, H-20 in **3** suggested that the configurations of H-8, Me-18, and H-20 were in β-orientation. Moreover, correlations of Me-28/H-3, H-10, Me-29, Me-30/H-10, and H-17 agreed with α-configuration of H-3, H-10, H-17, and Me-30 for **3**. From the above corroboration, compound **3** was elucidated as 7β,25-dihydroxycucurbita-5,23(*E*)-dien-19-al 3-*O*-β-D-glucopyranoside and has been named kuguaoside C.

Compound **4** was isolated as a white amorphous powder, possessing a molecular formula of $C_{37}H_{60}O_9$ (m/z 671.4137, $[M + Na]^+$) calculated from the high-resolution ESIMS data. Similar to **3**, compound **4** showed absorptions of IR spectrum at 3384, 1712, and 1615 cm^{-1}, indicating hydroxyl, aldehyde carbonyl, and olefinic group functionalities, respectively. Detailed inspection of ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) indicated the presence of the key structural features of a 25-hydroxycucurbitane-5,23(*E*)-diene-19-al skeleton with a β-glucose and a methoxyl group (δ_H 3.16, s). Furthermore, comparisons of its ^1H and ^{13}C NMR data (Tables 1 and 2) with those of compound **3** revealed strong resemblance in all signals except that the C-7 and H-7 signals in the spectra of **4** were shifted downfield to δ_C 75.7 ($\Delta\delta$ 10.2) and upfield to δ_H 3.42 (br d, J = 5.2 Hz; $\Delta\delta$ −0.88), respectively, and the presence of a methoxyl group in **4**, which was suggested at position C-8. The planar structure and NMR assignments for compound **4** were confirmed by using 2D NMR data, including ^1H−^1H COSY, HMBC, and HMQC. The NOSEY experiments indicated that compound **4** has a configuration similar to that of compound **3**. Thus, the structure of compound **4** was determined as 3β,25-dihydroxy-7β-methoxycucurbita-5,23(*E*)-dien-19-al 3-*O*-β-D-glucopyranoside and has been named kuguaoside D.

Compounds **5**−**15** were identified as known cucurbitane-type triterpene glycosides, charantoside A (**5**),[11] momordicosides I (**6**),[12] F_1 (**7**),[12] F_2 (**8**),[12] K (**9**),[13] L (**10**),[13] and U (**11**),[14] goyaglycoside-b (**12**) and -d (**13**),[24] 7β,25-dihydroxycucurbita-5,23(*E*)-dien-19-al 3-*O*-β-D-allopyranoside (**14**),[25] and 25-hydroxy-5β,19-epoxycucurbita-6,23-dien-19-on-3β-ol 3-*O*-β-D-glucopyranoside (**15**)[26] (Figure 1), respectively, by comparing their NMR spectroscopic data with the literature values.

We have studied the HPLC fingerprinting profile (Figure 4) of the bioactive fraction (fraction 4) of *M. charantia* by HPLC-ELSD and identified the 11 main peaks by comparing the retention times (RT) with the reference compounds. The RTs of the isolated cucurbitanes **1**−**4**, **5**, **6**, **8**−**12**, **14**, and **15** were,

respectively, 31.24, 32.84, 17.67, 27.78, 30.16, 31.24, 33.61, 35.02, 16.44, 35.02, 36.65, 18.75, and 26.35 min.

All of the isolated cucurbitanes **1**−**15** was tested for antiproliferation against MCF-7 (human breast adenocarcinoma), Doay (human human medulloblastoma), HEp-2 (human laryngeal carcinoma), and WiDr (human colon adenocarcinoma) tumor cell lines in vitro. As summarized in Table 3, compounds **1**, **6**, **7**, **9**, **12**, and **13** showed moderate cytotoxic activities (IC_{50} values ca. 10−20 μg/mL) against the four human tumor cell lines, whereas the other compounds had no promising cytotoxicity. Further analysis of the relationships of these cucurbitane-type triterpenoid structures and bioactivities implied that the 5β,19-epoxy ring and C(25)-*O*-methyl functional groups were important for the antiproliferative activities of cucurbitane-type glycosides.

The fruits of *M. charantia* have been used in traditional Chinese medicine for patients suffering from diabetes and other metabolic disease. Furthermore, compounds **1**−**15** were also evaluated for hypoglycemic activity by glucose uptake assay on C2Cl2 myoblast cells. The result (Figure 5) revealed that compound **14** exhibited the most potent activity relative to the others, even beyond the positive control, insulin. Notably, most 5β,19-epoxy cucurbitanes with an allose moiety, such as compounds **8**, **11**, and **12**, may decrease the activities on glucose uptake assay.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1**−**4**. 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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