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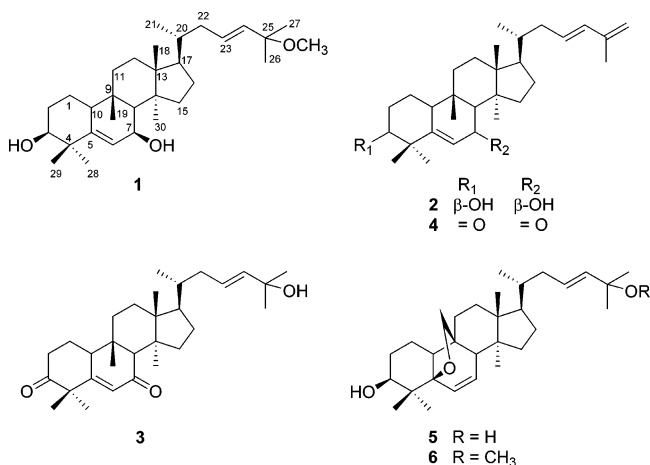
Cucurbitane-Type Triterpenoids from *Momordica charantia*Chi-I Chang,<sup>†</sup> Chiy-Rong Chen,<sup>‡</sup> Yun-Wen Liao,<sup>†</sup> Hsueh-Ling Cheng,<sup>†</sup> Yo-Chia Chen,<sup>†</sup> and Chang-Hung Chou<sup>\*,§</sup>

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Five new cucurbitane-type triterpenes, (23*E*)-25-methoxycucurbit-23-ene-3 $\beta$ ,7 $\beta$ -diol (**1**), (23*E*)-cucurbita-5,23,25-triene-3 $\beta$ ,7 $\beta$ -diol (**2**), (23*E*)-25-hydroxycucurbita-5,23-diene-3,7-dione (**3**), (23*E*)-cucurbita-5,23,25-triene-3,7-dione (**4**), and (23*E*)-5 $\beta$ ,19-epoxycucurbita-6,23-diene-3 $\beta$ ,25-diol (**5**), together with one known triterpene, (23*E*)-5 $\beta$ ,19-epoxy-25-methoxycucurbita-6,23-dien-3 $\beta$ -ol (**6**), have been isolated from the methanol extract of the stems of *Momordica charantia*. The structures of the new compounds were elucidated by spectroscopic methods.

*Momordica charantia* L. (Cucurbitaceae), a slender-stemmed tendril climber, is widely cultivated as a vegetable crop and has been used extensively in folk medicine as a remedy for diabetes in Asia. Previous investigations have shown that crude extracts of the fruit of *M. charantia* possess antidiabetic activity,<sup>1,2</sup> and many cucurbitane-type triterpenoids have been isolated from the fruits,<sup>3–9</sup> seeds,<sup>10–12</sup> and leaves and vines<sup>13</sup> of *M. charantia*. On the basis of an interest in the discovery of bioactive natural products, we have continued with our efforts to elucidate the antidiabetic components from Taiwanese *M. charantia*. We have examined the methanolic extract of the stems of this plant and have isolated five new cucurbitane-type triterpenoids (compounds **1–5**), together with one known cucurbitane-type triterpene (compound **6**).<sup>14</sup> In this paper, we report the extraction, isolation, purification, and structural elucidation of those new constituents based on spectroscopic analysis.



## Results and Discussion

Compound **1** gave a positive Liebermann-Burchard test. The HREIMS of the ion peak at  $m/z$  472.3916  $[M]^+$  was consistent with the molecular formula  $C_{31}H_{52}O_3$ . The IR spectrum showed the presence of hydroxyl ( $3360\text{ cm}^{-1}$ ) and double-bond ( $1625$  and  $700\text{ cm}^{-1}$ ) functionalities. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **1** (Table 1)

indicated the presence of seven tertiary methyls [ $\delta_{\text{H}}$  0.69, 0.89, 1.01, 1.04, 1.19 (3H each, s), 1.23 (3H  $\times$  2, s)], one secondary methyl [ $\delta_{\text{H}}$  0.87 (3H, d,  $J = 6.6\text{ Hz}$ )], a methoxyl [ $\delta_{\text{H}}$  3.12 (3H, s)], and two oxymethines [ $\delta_{\text{H}}$  3.52 (1 H, br s), 3.92 (1H, d,  $J = 5.6\text{ Hz}$ )]. In addition, olefinic NMR signals of a trisubstituted double bond [ $\delta_{\text{H}}$  5.80 (1H, d,  $J = 5.6\text{ Hz}$ );  $\delta_{\text{C}}$  122.6 (d), 146.8 (s)] and a *trans*-oriented disubstituted double bond [ $\delta_{\text{H}}$  5.36 (1H, d,  $J = 15.6\text{ Hz}$ ), 5.48 (1H, ddd,  $J = 15.6, 8.4, 5.6\text{ Hz}$ );  $\delta_{\text{C}}$  128.5 (d), 136.7 (d)] coupling to a neighboring methylene [ $\delta_{\text{H}}$  1.76 m, 2.16 m;  $\delta_{\text{C}}$  39.4 (t)] were also found. The  $^{13}\text{C}$  NMR spectrum of **1** revealed 31 carbon signals, which were assigned by DEPT experiments as nine methyls, seven methylenes, four methines, five quaternary carbons, four olefinic carbons, and two oxygenated carbons. By comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data with the known compound (23*E*)-3 $\beta$ -hydroxy-7 $\beta$ ,25-dimethoxycucurbita-5,23-dien-19-al,<sup>9</sup> compound **1** was considered as a cucurbitane-type triterpene with a  $\Delta^{23}$ -unsaturated C-8 side-chain moiety. The downfield shift of H-7 [ $\delta_{\text{H}}$  3.92 (1H, d,  $J = 5.6\text{ Hz}$ )] in **1** indicated the presence of a hydroxyl group at C-7 instead of 7 $\beta$ -methoxy group. Moreover, the disappearance of the aldehyde signal of C-19, along with the presence of one additional methyl carbon signal ( $\delta_{\text{C}}$  29.5), led to the proposal that the aldehyde group of (23*E*)-3 $\beta$ -hydroxy-7 $\beta$ ,25-dimethoxycucurbita-5,23-dien-19-al was reduced to a methyl group in **1**. The HMBC spectrum of **1** showed long-range correlations between H-3 ( $\delta_{\text{H}}$  3.52) and C-1 ( $\delta_{\text{C}}$  20.9) and C-5 ( $\delta_{\text{C}}$  146.8) and between H-7 ( $\delta_{\text{H}}$  3.92) and C-5 ( $\delta_{\text{C}}$  146.8), C-6 ( $\delta_{\text{C}}$  122.6), C-8 ( $\delta_{\text{C}}$  53.2), and C-9 ( $\delta_{\text{C}}$  33.9), and this suggested that two hydroxyl groups were located at C-3 and C-7, respectively. The olefinic proton H-6 ( $\delta_{\text{H}}$  5.80) also exhibited HMBC correlations with C-4 ( $\delta_{\text{C}}$  41.5), C-7 ( $\delta_{\text{C}}$  68.2), C-8 ( $\delta_{\text{C}}$  53.2), and C-10 ( $\delta_{\text{C}}$  38.5). Furthermore, the  $\alpha$ -orientation of H-7 was determined by the cross-peaks between H-7 ( $\delta_{\text{H}}$  3.92) and H-30 ( $\delta_{\text{H}}$  0.69) observed in the NOESY spectrum of **1**. From the above evidence, compound **1** was characterized as (23*E*)-25-methoxycucurbit-23-ene-3 $\beta$ ,7 $\beta$ -diol. Complete  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts were established by  $^1\text{H}$ – $^1\text{H}$  COSY, HMQC, HMBC, and NOESY spectra.

The HREIMS of **2** showed a molecular ion peak at  $m/z$  440.3625, corresponding to the molecular formula  $C_{30}H_{48}O_2$  and indicating seven degrees of unsaturation. The IR spectrum displayed absorptions for hydroxyl ( $3350\text{ cm}^{-1}$ ) and terminal double-bond ( $3080, 1650, 875\text{ cm}^{-1}$ ) functionalities. The  $^1\text{H}$  NMR spectrum of **2** (Table 1) showed resonances for five tertiary methyls [ $\delta_{\text{H}}$  0.69, 0.90, 1.03, 1.06, 1.20 (3H each, s)], a secondary methyl [ $\delta_{\text{H}}$  0.89 (3H, d,  $J = 6.4\text{ Hz}$ )], a vinylic methyl [ $\delta_{\text{H}}$  1.83 (3H, s)], two secondary oxygenated methines [ $\delta_{\text{H}}$  3.52 (1 H, t,  $J = 2.4\text{ Hz}$ ), 3.92 (1H, d,  $J = 5.6\text{ Hz}$ )], and a terminal methylene [ $\delta_{\text{H}}$  4.83 (2H, br s)]. Olefinic proton signals attributed to a trisubstituted double bond [ $\delta_{\text{H}}$  5.80 (1H, d,  $J = 5.6\text{ Hz}$ )] and a *trans*-oriented disubstituted double bond

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**Table 1.**  $^1\text{H}$  NMR Data for **1–5** (400 MHz in  $\text{CDCl}_3$ )

position	1	2	3	4	5
1	1.48 m, 1.56 m	1.50 m, 1.60 m	1.62 m, 2.10 m	1.63 m, 2.10 m	1.45 m
2	1.86 m, 1.72 m	1.72 m, 1.88 m	2.50 m, 2.58 m	2.51 m, 2.59 m	1.82 m
3	3.52 br s	3.52 t (2.4)			3.36 m
6	5.80 d (5.6)	5.80 d (5.6)	6.14 d (2.4)	6.14 d (2.4)	6.02 dd (1.6, 9.6)
7	3.92 d (5.6)	3.92 d (5.6)			5.61 dd (9.6, 3.6)
8	1.98 br s	2.00 br s	2.41 s	2.41 s	2.32 br s
10	2.28 m	2.29 m	2.89 m	2.90 m	2.24 dd (2.8)
11	1.42 m, 1.62 m	1.46 m, 1.62 m	1.54 m, 1.77 m	1.56 m, 1.78 m	1.46 m, 1.80 m
12	1.47 m, 1.64 m	1.48 m, 1.62 m	1.60 m, 1.74 m	1.62 m, 1.78 m	1.64 m
15	1.28 m, 1.32 m	1.28 m, 1.33 m	1.08 m, 1.55 m	1.10 m, 1.56 m	1.35 m
16	1.32 m, 1.88 m	1.40 m, 1.90 m	1.38 m, 1.86 m	1.42 m, 1.90 m	1.42 m, 2.00 m
17	1.44 m	1.46 m	1.42 m	1.50 m	1.48 m
18	0.89 s	0.90 s	0.87 s	0.88 s	0.84 s
19	1.04 s	1.06 s	0.93 s	0.92 s	3.50 d (8.4), 3.65 d (8.4)
20	1.48 m	1.32 m	1.54 m	1.52 m	1.45 m
21	0.87 d (6.6)	0.89 d (6.4)	0.88 d (6.6)	0.89 d (6.6)	0.87 d (6.6)
22	1.76 m, 2.16 m	1.79 m, 2.22 m	1.72 m, 2.12 m	1.78 m, 2.21 m	1.80 m, 2.14 m
23	5.48 ddd (15.6, 8.4, 5.6)	5.60 ddd (15.6, 7.2, 7.2)	5.57 m	5.59 m	5.57 m
24	5.36 d (15.6)	6.08 d (15.6)	5.57 m	6.09 d (15.6)	5.57 m
26	1.23 s	1.83 s	1.28 s	1.81 s	1.29 s
27	1.23 s	4.83, br s	1.28 s	4.83 br s	1.29 s
28	1.19 s	1.20 s	1.33 s	1.33 s	1.18 s
29	1.01 s	1.03 s	1.31 s	1.31 s	0.87 s
30	0.69 s	0.69 s	0.86 s	0.86 s	0.84 s
OCH <sub>3</sub>	3.12 s				
OH					3.98 d (9.6)

$[\delta_{\text{H}} 6.08 (1\text{H}, d, J = 15.6 \text{ Hz}), 5.60 (1\text{H}, \text{ddd}, J = 15.6, 7.2, 7.2 \text{ Hz})]$  coupling to a neighboring methylene  $[\delta_{\text{H}} 1.79 \text{ m}, 2.22 \text{ m}; \delta_{\text{C}} 39.7 (t)]$  were also observed. Altogether, 30 carbon signals were observed in the  $^{13}\text{C}$  NMR spectrum of **2** and were sorted into seven methyls, seven methylenes, four methines, four quaternary carbons, six olefinic carbons, and two secondary oxygenated carbons. The  $^{13}\text{C}$  NMR data were very similar to those of **1**, except for the signals of C-17–C-23 of the side chain. A (23*E*)- $\Delta^{23,25}$ -conjugated diene C-8 moiety as the side chain of **2** was suggested by both the NMR data  $[\delta_{\text{H}} 4.83 (2\text{H}, \text{br s}, \text{H-27}), 5.60 (1\text{H}, \text{ddd}, J = 15.6, 7.2, 7.2 \text{ Hz}), 6.08 (1\text{H}, d, J = 15.6 \text{ Hz}); \delta_{\text{C}} 114.0 (C-27), 129.4 (C-23), 134.1 (C-24), 142.2 (C-25)]$  and the fragment ions in the EIMS at  $m/z$  81  $[\text{CH}_2\text{CHCHC}(\text{CH}_3)\text{CH}_2]^+$ ,  $m/z$  109  $[\text{side chain}]^+$ ,  $m/z$  331  $[\text{M} - \text{side chain}]^+$ . In a HMBC experiment, correlations between H-23 ( $\delta_{\text{H}} 5.60$ ) and C-20 ( $\delta_{\text{C}} 36.6$ ), C-22 ( $\delta_{\text{C}} 39.7$ ), and C-25 ( $\delta_{\text{C}} 142.2$ ) and between H-27 ( $\delta_{\text{H}} 4.83$ ) and C-23 ( $\delta_{\text{C}} 129.4$ ), C-25 ( $\delta_{\text{C}} 142.2$ ), and C-26 ( $\delta_{\text{C}} 18.7$ ) were observed. The relative configurations of methyl groups and other protons in the tetracyclic rings were determined by significant NOE correlations between H-3 ( $\delta_{\text{H}} 3.52$ ) and H-28 ( $\delta_{\text{H}} 4.83$ ), H-7 ( $\delta_{\text{H}} 3.92$ ) and H-30 ( $\delta_{\text{H}} 0.69$ ), and H-30 ( $\delta_{\text{H}} 0.69$ ) and H-17 ( $\delta_{\text{H}} 1.46$ ) in the NOESY spectrum. Thus, compound **2** was elucidated as (23*E*)-cucurbita-5,23,25-triene-3 $\beta$ ,7 $\beta$ -diol.

By HREIMS, compound **3** revealed a molecular formula of  $\text{C}_{30}\text{H}_{46}\text{O}_3$  from the molecular ion at  $m/z$   $[\text{M}]^+ 454.3423$ , indicating the presence of eight degrees of unsaturation. The IR spectrum showed absorption bands at  $3400 \text{ cm}^{-1}$  (hydroxyl),  $1710 \text{ cm}^{-1}$  (isolated ketone), and  $1655 \text{ cm}^{-1}$  (conjugated ketone). The UV spectrum displayed an absorption maximum at 256 nm. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **3** (Tables 1 and 2) exhibited seven tertiary methyls  $[\delta_{\text{H}} 0.86, 0.87, 0.93, 1.31, 1.33 (3\text{H each}, s) \text{ and } 1.28 (3\text{H} \times 2, s)]$  and a secondary methyl  $[\delta_{\text{H}} 0.88 (3\text{H}, d, J = 6.6 \text{ Hz})]$  and an  $\alpha$ -H of an  $\alpha,\beta$ -unsaturated carbonyl system  $[\delta_{\text{H}} 6.14 (1\text{H}, d, J = 2.4 \text{ Hz}); \delta_{\text{C}} 125.4 (d), 167.6 (s), 202.4 (s)]$ . Unambiguous comparison of  $^1\text{H}$  and  $^{13}\text{C}$  NMR data between **1** and **3** revealed that two oxymethines at C-3 and C-7 in **1** were absent. Instead, an isolated ketone and  $\alpha,\beta$ -unsaturated carbonyl carbons at  $\delta_{\text{C}} 211.6 (C-3)$  and  $202.4 (C-7)$  and a low-field-shifted carbon at  $\delta_{\text{C}} 38.1 (C-2)$  were observed, indicating that **3** is an oxidized derivative of **1**. The isolated ketone and  $\alpha,\beta$ -unsaturated carbonyl carbons were respectively assigned at C-3 and C-7, which were supported by the HMBC correlations between H-2 ( $\delta_{\text{H}} 2.50, 2.58$ ) and C-1 ( $\delta_{\text{C}}$

**Table 2.**  $^{13}\text{C}$  NMR Data for **1–5** (75 MHz in  $\text{CDCl}_3$ )

position	1	2	3	4	5
1	20.9	21.0	23.6	23.6	17.6
2	28.6	28.7	38.1	38.1	27.3
3	76.7	76.7	211.6	211.6	76.1
4	41.5	41.5	51.4	51.4	37.2
5	146.8	146.7	167.6	167.6	87.5
6	122.6	122.5	125.4	125.4	131.9
7	68.2	68.2	202.4	202.3	131.5
8	53.2	53.1	59.2	59.2	52.0
9	33.9	33.9	36.8	36.8	45.4
10	38.5	38.6	41.2	41.2	38.8
11	32.4	32.5	31.3	31.3	23.5
12	30.0	30.0	29.7	29.7	30.8
13	45.9	45.9	48.5	48.5	45.4
14	48.2	48.2	45.7	45.8	48.8
15	34.6	34.6	34.5	34.5	33.1
16	27.7	27.8	27.7	27.8	28.0
17	49.9	50.1	49.4	49.6	50.0
18	15.4	15.4	15.4	15.4	14.9
19	29.5	29.6	27.2	27.2	79.8
20	36.1	36.6	36.2	36.6	36.1
21	18.7	18.8	18.7	18.9	18.6
22	39.4	39.7	39.0	39.6	39.1
23	128.5	129.4	125.0	129.0	125.1
24	136.7	134.1	139.6	134.3	139.6
25	74.8	142.2	70.7	142.1	71.0
26	25.8	18.7	30.0	18.7	29.8
27	26.1	114.0	29.9	114.2	29.9
28	25.4	25.4	23.0	23.0	20.5
29	27.7	27.7	28.4	28.4	24.5
30	17.7	17.8	17.9	18.0	20.0
OCH <sub>3</sub>	50.2				

23.6), C-3 ( $\delta_{\text{C}} 211.6$ ), and C-10 ( $\delta_{\text{C}} 41.2$ ) and between H-6 ( $\delta_{\text{H}} 6.14$ ) and C-4 ( $\delta_{\text{C}} 51.4$ ), C-7 ( $\delta_{\text{C}} 202.4$ ), C-8 ( $\delta_{\text{C}} 59.2$ ), and C-10 ( $\delta_{\text{C}} 41.2$ ).  $^1\text{H}$ – $^{13}\text{C}$  COSY, HMQC, and HMBC analysis, together with the fragment ion at  $m/z$  355  $[\text{M} - \text{CH}_2\text{CHCHC}(\text{CH}_3)_2\text{OH}]^+$ , confirmed the side chain to be  $\text{CH}_2\text{CHCHC}(\text{CH}_3)_2\text{OH}$ . Compound **3** was accordingly determined as (23*E*)-25-hydroxycucurbita-5,23-diene-3,7-dione.

The molecular formula of compound **4** was assigned as  $\text{C}_{30}\text{H}_{44}\text{O}_2$ , on the basis of the HREIMS ( $[\text{M}]^+ m/z 436.3342$ ),  $^{13}\text{C}$  NMR, and DEPT spectra. Analysis of the IR spectrum of **4** suggested that it contains an isolated ketone ( $1715 \text{ cm}^{-1}$ ), a conjugated ketone ( $1665 \text{ cm}^{-1}$ ), and a conjugated terminal double bond (3085, 1655,

880  $\text{cm}^{-1}$ ). The UV spectrum exhibited an absorption maximum at 258 nm.  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Tables 1 and 2) of the tetracyclic part of the triterpene skeleton were almost the same as compound **3**, but the data in the side chain were very similar to that of **2**. The proposed structure was also supported by the fragment ion peaks in the EIMS at  $m/z$  81 [ $\text{CH}_2\text{CHCHC}(\text{CH}_3)\text{CH}_2$ ] $^+$ ,  $m/z$  109 [side chain] $^+$ ,  $m/z$  327 [ $\text{M}$  - side chain] $^+$ , and  $m/z$  355 [ $\text{M}$  -  $\text{CH}_2\text{CHCHC}(\text{CH}_3)\text{CH}_2$ ] $^+$ . Thus, compound **4** was elucidated as (23*E*)-cucurbita-5,23,25-triene-3,7-dione.

Compound **5** was deduced to be a triterpenoid due to a positive Liebermann-Burchard test and was assigned the molecular formula  $\text{C}_{30}\text{H}_{48}\text{O}_3$ , on the basis of molecular ion peak at  $m/z$  456.3588 in the HREIMS. The IR spectrum of **5** showed bands attributable to hydroxyl group (3360  $\text{cm}^{-1}$ ) and double-bond (1623 and 700  $\text{cm}^{-1}$ ) functionalities. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Tables 1 and 2) indicated the presence of six tertiary methyls [ $\delta_{\text{H}}$  0.87, 1.18 (3H each, s), 0.84, 1.29 (3H  $\times$  2, s)], a secondary methyl [ $\delta_{\text{H}}$  0.87 (3H, d,  $J$  = 6.6 Hz)], an oxomethylene [ $\delta_{\text{H}}$  3.50 (1H, d,  $J$  = 8.4 Hz), 3.65 (1H, d,  $J$  = 8.4 Hz);  $\delta_{\text{C}}$  79.8 (t)], and a multiplet oxymethine [ $\delta_{\text{H}}$  3.36 (1H,  $W_{1/2}$  = 6.6 Hz, H-3)] coupling to a hydroxyl group [ $\delta_{\text{H}}$  3.98 (1H, d,  $J$  = 9.6 Hz); disappeared on  $\text{D}_2\text{O}$  exchange]. In addition, the NMR signals for an allylic ABX system of *cis*-oriented cyclohexene [ $\delta_{\text{H}}$  6.02 (1H, dd,  $J$  = 9.6, 1.6 Hz, H-6), 5.61 (1H, dd,  $J$  = 9.6, 3.6 Hz, H-7), 2.32 (1H, br s);  $\delta_{\text{C}}$  131.9 (d), 131.5 (d), 52.0 (d)] were also found. The above NMR data of **5** were very similar to those of the known compound (23*E*)-5 $\beta$ ,19-epoxy-25-methoxycucurbita-6,23-diene-3 $\beta$ -diol (**6**),<sup>14</sup> except that the methoxy group was replaced by a hydroxyl group at C-25. The structure of the side chain of **5** was also supported by the base peak of EIMS at  $m/z$  109 [side chain -  $\text{H}_2\text{O}$ ] $^+$ . Thus, compound **5** was determined as (23*E*)-5 $\beta$ ,19-epoxycucurbita-6,23-diene-3 $\beta$ ,25-diol. Although compound **5** has been obtained by the hydrolysis of momordicoside I,<sup>3</sup> it was isolated for the first time as a natural product in the present investigation.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured using a JASCO DIP-180 digital spectropolarimeter. UV spectra were measured in MeOH on a Shimadzu UV-1601PC spectrophotometer. IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. NMR spectra were recorded in  $\text{CDCl}_3$  at room temperature on a Varian Mercury plus 400 NMR spectrometer, and the solvent resonance was used as internal shift reference (TMS as standard). The 2D NMR spectra were recorded using standard pulse sequences. EIMS and HREIMS were recorded on a Finnigan TSQ-700 and a JEOL SX-102A spectrometer, respectively. TLC was performed by using silica gel 60  $\text{F}_{254}$  plates (200  $\mu\text{m}$ , Merck). CC was performed on silica gel (230–400 mesh ASTM, Merck). HPLC was performed by using a Lichrosorb silica gel 60 (5  $\mu\text{m}$ ) column (250  $\times$  10 mm).

**Plant Material.** The stems of *Momordica charantia* were collected in Ping-Tung, Taiwan, in July 2003. The plant material was identified by Prof. Sheng-Zehn Yang, Department of Forestry, National Pingtung University of Science and Technology. A voucher specimen (no. 2013) has been deposited at the Herbarium of this same institution.

**Extraction and Isolation.** Air-dried pieces of the stems of *M. charantia* (18 kg) were extracted three times with methanol (30 L) at room temperature (7 days each). The MeOH extract was evaporated in vacuo to afford a black residue, which was suspended in  $\text{H}_2\text{O}$  (3 L) and then partitioned sequentially using EtOAc and *n*-BuOH (2 L  $\times$  3). The EtOAc fraction (386 g) was chromatographed over silica gel, using mixtures of *n*-hexane and EtOAc of increasing polarity as eluents. Eleven fractions were collected as follows: 1 [5000 mL, *n*-hexane], 2 [4000 mL, *n*-hexane–EtOAc (49:1)], 3 [4000 mL, *n*-hexane–EtOAc (19:1)], 4 [4000 mL, *n*-hexane–EtOAc (9:1)], 5 [4000 mL, *n*-hexane–EtOAc (17:3)], 6 [4000 mL, *n*-hexane–EtOAc (8:2)], 7 [4000 mL, *n*-hexane–EtOAc (7:3)], 8 [3000 mL, *n*-hexane–EtOAc (5:5)], 9 [3000 mL, *n*-hexane–EtOAc (4:6)], 10 [3000 mL, *n*-hexane–EtOAc (2:8)], 11 (6000 mL, EtOAc). Fraction 5 was further chromatographed on a silica gel column (7  $\times$  45 cm), eluted with *n*-hexane– $\text{CH}_2\text{Cl}_2$ –EtOAc (8:6:1) to yield eight fractions (each 700 mL), 5A–5H. HPLC of

fraction 5D with *n*-hexane–EtOAc (8:2) as eluent, 2 mL/min, afforded **6**<sup>14</sup> (16 mg,  $t_{\text{R}}$  = 12.2 min) and **5** (12 mg,  $t_{\text{R}}$  = 21.3 min), respectively. Fraction 6 was further purified on a silica gel column (5  $\times$  45 cm) eluted with  $\text{CH}_2\text{Cl}_2$ –EtOAc (8:1). Seven fractions (6A–6G) (each 700 mL) were obtained as follows: HPLC of fraction 6E with *n*-hexane– $\text{CH}_2\text{Cl}_2$ –EtOAc (8:6:1) as eluent, 2 mL/min, afforded **2** (10 mg,  $t_{\text{R}}$  = 15.6 min) and **1** (8 mg,  $t_{\text{R}}$  = 18.2 min), respectively. Fraction 7 was further chromatographed on a silica gel column (5  $\times$  45 cm), eluted with  $\text{CH}_2\text{Cl}_2$ –EtOAc (8:1), to generate seven fractions (each 600 mL), 7A–7G. HPLC of fraction 6F with *n*-hexane–EtOAc (17:3) as eluent, 2 mL/min, afforded **4** (9 mg,  $t_{\text{R}}$  = 13.5 min) and **3** (12 mg,  $t_{\text{R}}$  = 20.2 min), respectively.

**(23*E*)-25-Methoxycucurbit-23-ene-3 $\beta$ ,7 $\beta$ -diol (1):** amorphous, white powder; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +86.6 (c 0.4,  $\text{CHCl}_3$ ); IR (KBr)  $\nu_{\text{max}}$  3360, 1625, 1463, 1379, 1242, 1191, 1131, 1021, 957, 825, 700  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; EIMS  $m/z$  472 [ $\text{M}$ ] $^+$  (3), 454 (14), 422 (27), 389 (100), 185 (12), 171 (13), 109 (15); HREIMS  $m/z$  472.3916 (calcd for  $\text{C}_{31}\text{H}_{52}\text{O}_3$  472.3819).

**(23*E*)-Cucurbita-5,23,23-triene-3 $\beta$ ,7 $\beta$ -diol (2):** amorphous, white powder; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +81.5 (c 0.3,  $\text{CHCl}_3$ ); IR (KBr)  $\nu_{\text{max}}$  3350, 3080, 1650, 1463, 1379, 1264, 1241, 1151, 1081, 1062, 953, 875  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; EIMS  $m/z$  440 [ $\text{M}$ ] $^+$  (1), 422 (18), 407 (14), 389 (18), 325 (5), 135 (19), 109 (30), 81 (100); HREIMS  $m/z$  440.3625 (calcd for  $\text{C}_{30}\text{H}_{48}\text{O}_2$  440.3642).

**(23*E*)-25-Hydroxycucurbita-5,23-diene-3,7-dione (3):** amorphous, white powder; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +88.3 (c 0.3,  $\text{CHCl}_3$ ); IR (KBr)  $\nu_{\text{max}}$  3400, 1710, 1655, 1467, 1338, 1224, 1152, 1076, 958, 735  $\text{cm}^{-1}$ ; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 202 (4.30), 256 (3.76) nm;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; EIMS  $m/z$  454 [ $\text{M}$ ] $^+$  (1), 355 (22), 205 (13), 121 (100), 95 (82); HREIMS  $m/z$  454.3423 (calcd for  $\text{C}_{30}\text{H}_{46}\text{O}_3$  454.3435).

**(23*E*)-Cucurbita-5,23,25-triene-3,7-dione (4):** amorphous, white powder; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +97.6 (c 0.4,  $\text{CHCl}_3$ ); IR (KBr)  $\nu_{\text{max}}$  3085, 1715, 1665, 1655, 1554, 1458, 1363, 1128, 1068, 998, 880, 737  $\text{cm}^{-1}$ ; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 202 (4.35), 258 (3.76) nm;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; EIMS  $m/z$  436 [ $\text{M}$ ] $^+$  (5), 393 (5), 355 (25), 327 (8), 149 (37), 121 (100), 79 (75); HREIMS  $m/z$  436.3342 (calcd for  $\text{C}_{30}\text{H}_{44}\text{O}_2$  436.333).

**(23*E*)-5 $\beta$ ,19-Epoxycucurbita-6,23-diene-3 $\beta$ ,25-diol (5):** amorphous, white powder; [ $\alpha$ ]<sub>D</sub><sup>25</sup> –82.2 (c 0.3,  $\text{CHCl}_3$ );  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; IR (KBr)  $\nu_{\text{max}}$  3360, 1623, 1457, 1375, 1236, 1160, 1035, 983, 851, 778, 700  $\text{cm}^{-1}$ ; EIMS  $m/z$  456 [ $\text{M}$ ] $^+$  (8), 438 (45), 390 (80), 309 (100), 281 (65); HREIMS  $m/z$  456.3588 [ $\text{M}$ ] $^+$  (calcd for  $\text{C}_{30}\text{H}_{48}\text{O}_3$  456.3605).

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