Cucurbitane-Type Triterpenoids from the Stems of *Momordica charantia*

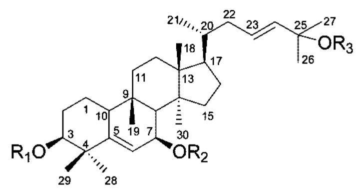
Chi-I Chang,[†,⊥] Chiy-Rong Chen,[‡,⊥] Yun-Wen Liao,[†] Hsueh-Ling Cheng,[†] Yo-Chia Chen,[†] and Chang-Hung Chou*[,§]

Graduate Institute of Biotechnology, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan, Republic of China, Department of Biological Science and Technology, Meiho Institute of Technology, Pingtung 91201, Taiwan, Republic of China, and Research Center for Biodiversity, China Medical University, Taichung 40402, Taiwan, Republic of China

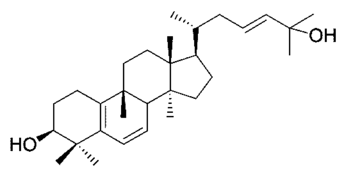
Received September 28, 2007

Four new cucurbitane-type triterpenes, cucurbita-5,23(*E*)-diene-3*β*,7*β*,25-triol (**1**), 3*β*-acetoxy-7*β*-methoxycucurbita-5,23(*E*)-dien-25-ol (**2**), cucurbita-5(10),6,23(*E*)-triene-3*β*,25-diol (**5**), and cucurbita-5,24-diene-3,7,23-trione (**6**), together with four known triterpenes, 3*β*,25-dihydroxy-7*β*-methoxycucurbita-5,23(*E*)-diene (**3**), 3*β*-hydroxy-7*β*,25-dimethoxy-cucurbita-5,23(*E*)-diene (**4**), 3*β*,7*β*,25-trihydroxycucurbita-5,23(*E*)-dien-19-al (**7**), and 25-methoxy-3*β*,7*β*-dihydroxycucurbita-5,23(*E*)-dien-19-al (**8**), were isolated from the methyl alcohol extract of the stems of *Momordica charantia*. The structures of the new compounds were elucidated by spectroscopic methods.

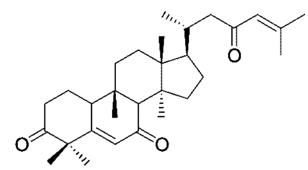
Momordica charantia L. (Cucurbitaceae), a slender-stemmed tendril climbing vegetable crop, has extensively been used in folk medicine as a remedy for diabetes in Asia. Previous investigations have shown that crude extracts of the fruit of *M. charantia* possess antidiabetic activity,[1,2] and many cucurbitane-type triterpenoids have been isolated from the fruits,[3–13] seeds,[14–16] and leaves and vines[17] of *M. charantia*. Recently we reported the isolation and structural elucidation of six cucurbitane-type triterpenoids from the methanolic extract of the stems of this plant.[18] We continued the study on the cucurbitane-type triterpenoid constituents from Taiwanese *M. charantia* and describe here the isolation and structural elucidation of four new cucurbitane-type triterpenes. These are cucurbita-5,23(*E*)-diene-3*β*,7*β*,25-triol (**1**), 3*β*-acetoxy-7*β*-methoxycucurbita-5,23(*E*)-dien-25-ol (**2**), cucurbita-5(10),6,23(*E*)-triene-3*β*,25-diol (**5**), and cucurbita-5,24-diene-3,7,23- trione (**6**). Four known triterpenes, 3*β*,25-dihydroxy-7*β* -methoxycucurbita-5,23(*E*)-diene (**3**),[10] 3*β*-hydroxy-7*β*,25-dimethoxycucurbita-5,23(*E*)-diene (**4**),[10] 3*β*,7*β*,25-trihydroxycucurbita-5,23(*E*)-dien-19-al (**7**),[5] and 25-methoxy- 3*β*,7*β*-dihydroxycucurbita-5,23(*E*)-dien-19-al (**8**),[19] come from the same part of the plant.



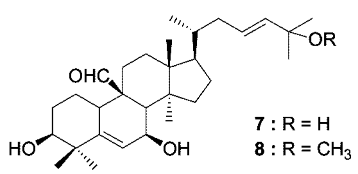
1 : R_1 = H, R_2 = H, R_3 = H
2 : R_1 = Ac, R_2 = Me, R_3 = H
3 : R_1 = H, R_2 = Me, R_3 = H
4 : R_1 = H, R_2 = Me, R_3 = Me



5



6



7 : R = H
8 : R = CH_3

Results and Discussion

Compound **1** gave a positive Liebermann-Burchard test, and its HREIMS spectrum showed an $[M - H_2O]^+$ ion at *m/z* 440.3649, indicating a dehydrated molecular formula of $C_{30}H_{48}O_2$. The IR spectrum indicated the presence of hydroxy (3550 cm^{-1}) and double-bond (3020, 1654 cm^{-1}) functionalities. The ^1H and ^{13}C NMR spectra of **1** (Table 1 and 2) displayed signals characteristic of the presence of seven methyl singlets [δ_H 0.66, 0.88, 1.01, 1.04, 1.18 (3H each, s), 1.28 (3H × 2, s)], one methyl doublet [δ_H 0.85 (3H, d, $J = 6.4$ Hz)], and two oxymethines [δ_H 3.53 (1 H, br s), 3.92 (1H, d, $J = 6.4$ Hz)]. In addition, olefinic protons of a trisubstituted double bond [δ_H 5.80 (1H, d, $J = 6.4$ Hz); δ_C 122.5 (d), 146.8 (s)] and a *trans*-oriented disubstituted double bond [δ_H 5.56 (2H, m); δ_C 125.3 (d), 139.4 (d)] coupling to a neighboring methylene [δ_H 1.70 (1H, m), 2.14 (1H, m); δ_C 39.1 (t)] were also found.[18] The ^{13}C NMR spectrum of **1** revealed 30 carbon signals, which were assigned by DEPT experiments as eight methyl, seven methylene, four methine, four quaternary, four olefinic, and two tertiary and one quaternary oxygenated carbons. The EIMS spectrum of **1** showed a base peak at *m/z* 389 $[M - 3H_2O - CH_3]^+$ and some fragment ions at *m/z* 440 $[M - H_2O]^+$, 422 $[M - 2H_2O]^+$, 407 $[M - 2H_2O - CH_3]^+$, 404 $[M - 3H_2O]^+$, and 109 [side chain $- H_2O]^+$, which closely resemble those of (23*E*)-cucurbita-5,23,25-triene-3*β*,7*β*-diol, with the molecular mass of 440.[18] Compound **1** had an 18 mass unit difference from (23*E*)-cucurbita-5,23,25-triene-3*β*,7*β*-diol and was proposed to be a hydrated derivative of (23*E*)-cucurbita-5,23,25-triene-3*β*,7*β*-diol. The downfield proton signals of two geminal methyl groups [δ_H 1.28 (3H × 2, s, H-26, 27)] suggested that the hydroxy group was attached to C-25 [δ_C 70.7 (s)]. Comparing the data of the ^1H and ^{13}C NMR of **1** and those of 3*β*,25-dihydroxy-7*β*-methoxycucurbita-5,23(*E*)-diene (**3**),[10] the data for the side chain were similar. Thus, compound **1** was assigned as cucurbita-5,23(*E*)-diene-3*β*,7*β*,25-triol. The structure of the side chain was confirmed by the HMBC correlations between H-27 (δ_H 1.28)/C-24 (δ_C 139.4) and H-22 (δ_H 1.70, 2.14)/C-24. The HMBC spectrum of **1** also showed long-range correlations between H-3 (δ_H 3.53)/C-1 (δ_C 20.9), C-5 (δ_C 146.8); H-7 (δ_H 3.92)/C-5, C-6 (δ_C 122.5), C-8 (δ_C 53.1), C-9 (δ_C 33.9); and H-6 (δ_H 5.80)/C-4 (δ_C 41.5), C-7 (δ_C 68.2), C-8 (δ_C 53.1), C-10 (δ_C 38.5), indicating that two hydroxy groups were attached to C-3 and C-7 (Figure 1). The relative configurations of stereogenic carbon atoms in the tetracyclic rings were determined by significant NOE correlations between H-3 (δ_H 3.53)/H-2 (δ_H 1.74, 1.94), H-3/H-28 (δ_H 1.01), H-3/H-29 (δ_H 1.18), H-7 (δ_H 3.92)/H-30 (δ_H 0.66), H-8 (δ_H 1.98)/H-18 (δ_H 0.88), H-8/H-19 (δ_H 1.04), H-10 (δ_H 2.28)/H-28, and

* To whom correspondence should be addressed. Tel: 886-4-2205-3366, ext. 1633. Fax: 886-4-2207-1500. E-mail: choumasa@mail.cmu.edu.tw.
[†] National Pingtung University of Science and Technology.
[⊥] C.-I.C. and C.-R.C. share equal contribution.
[‡] Meiho Institute of Technology.
[§] China Medical University.

10.1021/np070532u CCC: $40.75
Published on Web 07/19/2008

Table 1. ^{1}H NMR Data for **1**−**6** (400 MHz in $CDCl_3$)

position	1	2	3	4	5	6
1	1.52 m, 1.56 m	1.46 m, 1.60 m	1.46 m, 1.56 m	1.44 m, 1.56 m	2.10 m, 2.22 m	1.62 m, 2.10 m
2	1.74 m, 1.94 m	1.75 m, 1.84 m	1.70 m, 1.86 m	1.60 m, 1.84 m	1.90 m	2.50 m, 2.64 m
3	3.53 br s	4.73 br s	3.47 br s	3.48 t (3.2)	3.42 dd (10.0, 3.2)	
6	5.80 d (6.4)	5.75 d (5.2)	5.80 d (5.2)	5.81 d (5.2)	6.02 d (9.6)	6.15 d (2.0)
7	3.92 d (6.4)	3.40 d (5.2)	3.39 d (5.2)	3.39 d (4.4)	5.54 m	
8	1.98 s	2.04 s	2.08 s	2.02 s	2.17 m	2.41 s
10	2.28 dd (10.0, 6.0)	2.25 dd (9.2, 5.2)	2.23 dd (12.0, 4.8)	2.24 dd (12.0, 4.8)		2.88 ddd (11.2, 4.8, 2.0)
11	1.44 m, 1.62 m	1.48 m, 1.60 m	1.40 m, 1.60 m	1.40 m, 1.60 m	1.38 m, 1.76 m	1.60 m, 1.78 m
12	1.48 m, 1.62 m	1.48 m, 1.58 m	1.47 m, 1.64 m	1.45 m, 1.60 m	1.36 m, 1.48 m	1.62 m, 1.72 m
15	1.30 m, 1.34 m	1.32 m	1.30 m	1.28 m, 1.32 m	1.16 m, 1.24 m	1.12 m, 1.58 m
16	1.32 m, 1.88 m	1.30 m, 1.88 m	1.36 m, 1.88 m	1.36 m, 1.90 m	1.28 m, 1.86 m	1.32 m, 1.82 m
17	1.48 m	1.48 m	1.45 m	1.45 m	1.45 m	1.50 m
18	0.88 s	0.91 s	0.89 s	0.90 s	0.85 s	0.92 s
19	1.04 s	0.96 s	0.95 s	0.96 s	0.92 s	0.93 s
20	1.48 m	1.50 m	1.50 m	1.52 s	1.48 m	2.08 s
21	0.85 d (6.4)	0.87 d (6.0)	0.85 d (6.0)	0.87 d (6.0)	0.84 d (7.2)	0.90 d (5.6)
22	1.70 m, 2.14 m	1.73 m, 2.11 m	1.70 m, 2.12 m	1.76 m, 2.16 m	1.73 m, 2.13 m	2.10 m, 2.50 m
23	5.56 m	5.57 m	5.55 m	5.48 m	5.55 m	
24	5.56 m	5.57 m	5.55 m	5.36 d (16.0)	5.59 m	6.02 br s
26	1.28 s	1.29 s	1.26 s	1.22 s	1.28 s	1.86 s
27	1.28 s	1.29 s	1.26 s	1.22 s	1.28 s	1.86 s
28	1.01 s	1.04 s	1.00 s	1.00 s	1.02 s	1.31 s
29	1.18 s	1.09 s	1.17 s	1.18 s	0.95 s	1.33 s
30	0.66 s	0.69 s	0.66 s	1.00 s	0.70 s	0.87 s
7-OCH_3		3.34 s	3.31 s	3.31 s		
25-OCH_3				3.12 s		
$OCOCH_3$		1.98 s				

Table 2. ^{13}C NMR Data for **1**−**6** (100 MHz in $CDCl_3$)

position	1	2	3	4	5	6
1	20.9	21.6	21.0	21.2	23.5	23.5
2	28.7	26.4	28.6	28.6	29.5	38.1
3	76.7	78.6	76.7	76.9	75.7	211.5
4	41.5	39.9	41.7	41.7	37.9	51.4
5	146.8	146.8	146.8	146.8	131.8	167.7
6	122.5	119.2	120.8	120.8	125.4	125.4
7	68.2	77.3	77.3	77.2	125.7	202.6
8	53.1	47.7	47.8	47.9	45.9	59.1
9	33.9	33.9	33.9	33.9	36.4	36.7
10	38.5	38.6	38.6	38.6	135.7	41.2
11	32.5	32.3	32.6	32.6	27.1	31.2
12	30.0	30.0	30.0	34.6	32.0	29.7
13	45.8	46.0	46.0	46.0	48.7	48.6
14	48.2	47.8	47.8	47.8	45.5	45.9
15	34.6	34.6	34.6	34.6	32.1	34.5
16	27.7	27.6	27.6	27.6	27.9	27.7
17	49.9	49.9	49.9	49.9	50.4	50.0
18	15.4	15.4	15.4	15.4	14.9	15.4
19	29.5	28.5	28.7	28.7	29.8	27.2
20	36.2	36.2	36.2	36.1	36.3	33.2
21	18.7	18.6	18.7	18.7	18.6	19.8
22	39.1	39.0	39.1	39.4	39.1	51.6
23	125.3	125.2	125.2	128.5	125.4	201.2
24	139.4	139.4	139.5	136.7	139.4	124.2
25	70.7	70.6	70.7	74.9	70.7	155.0
26	29.8	29.8	29.9	25.7	29.9	20.7
27	29.9	29.9	29.8	26.1	29.8	27.7
28	27.8	27.9	27.7	27.7	25.6	28.4
29	25.4	24.8	25.4	25.3	21.2	23.1
30	17.8	17.9	17.9	17.9	16.1	18.0
7-OCH_3		56.3	56.2	56.2		
25-OCH_3				50.2		
O$COCH_3$		170.9				
OCOCH_3		21.2				

H-10/H-30 in the NOESY spectrum (Figure 2). ^{1}H and ^{13}C NMR chemical shifts were established by ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra.

The HREIMS of **2** showed a molecular ion at m/z 514.4028, corresponding to the molecular formula $C_{33}H_{54}O_4$, which indicated seven degrees of unsaturation. The IR spectrum displayed absorptions for hydroxy (3461 cm^{-1}), ester (1732 cm^{-1}), and a double bond (3052, 1659, 890 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) showed resonances for seven methyl singlets [δ_H 0.69, 0.91, 0.96, 1.04, 1.09 (3H each, s), 1.29 (3H × 2, s)], a methyl doublet [δ_H 0.87 (3H, d, J = 6.0 Hz)], an acetyl methyl [δ_H 1.98 (3H, s)], a methoxy [δ_H 3.34 (3H, s)], two oxygenated methines [δ_H 3.40 (1H, d, J = 5.2 Hz), 4.73 (1H, br s)], and three olefinic protons [δ_H 5.75 (1H, d, J = 5.2 Hz); δ_H 5.57 (2H, m)]. Altogether, 33 carbon signals were observed in the ^{13}C NMR spectrum of **2** and were sorted into eight methyl, one acetyl methyl, one methoxy, seven methylene, four methine, four quaternary, four olefinic, one carbonyl, and two tertiary and one quaternary oxygenated carbons. The ^{1}H and ^{13}C NMR data were similar to those of **3**,[10] except for the signals of the A-ring part of the tetracyclic skeleton. The downfield shift of H-3 [δ_H 4.73 (1H, br s)] and the HMBC correlations between H-3 and C-5 (δ_C 146.8) and the acetyl carbon (δ_C 170.9) confirmed that the acetyloxy group was located at C-3.[4] Moreover, H-7 (δ_H 3.40) showed HMBC correlation with the methoxy carbon (δ_C 56.3), suggesting that the methoxy group was attached to C-7. The EIMS fragment ions at m/z 436 [M − H_2O − AcOH]$^+$, 422 [M − CH_3OH − AcOH]$^+$, 404 [M − H_2O − CH_3OH − AcOH]$^+$, 389 [M − H_2O − CH_3OH − AcOH − CH_3]$^+$, and 109 [side chain − H_2O]$^+$ were similar to those of **3** and further confirmed that **2** was an acetylated derivative of **3**.[10] Thus,

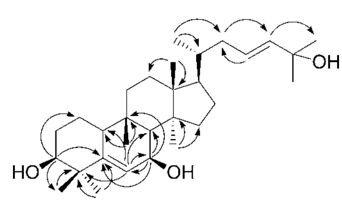


Figure 1. Main HMBC correlations of **1**.

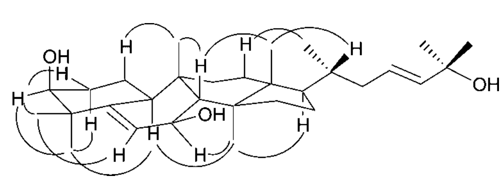


Figure 2. Main NOESY correlations of **1**.

compound **2** was elucidated as 3*β*-acetoxy-7*β*-methoxycucurbita-5,23(*E*)-dien-25-ol.

Compound **5** was deduced to be a triterpenoid due to a positive Liebermann-Burchard test, and the molecular formula was assigned as $C_{30}H_{48}O_2$ on the basis of the molecular ion at *m/z* 440.3649 in the HREIMS. The IR spectrum of **5** showed bands that were attributable to hydroxy (3383 cm^{-1}) and double-bond (1643 cm^{-1}) functionalities. The ^1H and ^{13}C NMR spectra of **5** (Tables 1 and 2) indicated the presence of seven methyl singlets [δ_H 0.70, 0.85, 0.92, 0.95, 1.02 (3H each, s), 1.28 (3H × 2, s)], a methyl doublet [δ_H 0.84 (3H, d, $J = 7.2$ Hz)], a *trans*-oriented disubstituted double bond [δ_H 5.55 (1H, m), 5.59 (1H, m)], a *cis*-olefin of a six-membered ring [δ_H 6.02 (1H, d, $J = 9.6$ Hz), 5.54 (1H, m); δ_C 125.4 (d), 139.4 (d)], and an axial oxymethine proton [δ_H 3.42 (1H, dd, $J = 3.2$, 10.0 Hz, H-3)].[18,20] Comparison of ^1H and ^{13}C NMR data of **1** and **5** (Tables 1 and 2) showed that the signals of the side-chain portion of **5** were almost the same as those of **1**. The tetracyclic skeleton of **5** exhibited a diene structure, which was proposed from the UV absorption band at 262 nm and the ^{13}C NMR signals [δ_C 131.8 (s), 125.4 (d), 125.7 (d), 135.7 (s)].[21] The HMBC correlations between H-1 (δ_H 2.10, 2.22)/C-5 (δ_C 131.8) and C-10 (δ_C 135.7); between H-6 (δ_H 6.02)/C-5, C-7 (δ_C 125.7), and C-10; and between H-7 (δ_H 5.54)/C-5, C-8 (δ_C 45.9), C-9 (δ_C 36.4), and C-14 (δ_C 45.5) suggested that the diene system was located at C-5, C-6, C-7, and C-10. Therefore, compound **5** was determined as cucurbita-5(10),6,23(*E*)-triene-3*β*],25-diol.

By HREIMS, compound **6** revealed a molecular formula of $C_{30}H_{44}O_3$ from the determination of the molecular ion at *m/z* $[M]^+$ 452.3297, indicating the presence of nine degrees of unsaturation. The IR spectrum showed absorption bands at 1718 cm^{-1} due to an isolated ketone moiety and at 1684 cm^{-1}, indicating a conjugated ketone unit. A significant UV absorption maximum at 249 nm also suggested the presence of an α,*β*-unsaturated ketone. The ^1H and ^{13}C NMR spectra of **6** (Tables 1 and 2) exhibited seven methyl singlets [δ_H 0.87, 0.92, 0.93, 1.31, 1.33 (3H each, s) and 1.86 (3H × 2, s)], a methyl doublet [δ_H 0.90 (3H, d, $J = 5.6$ Hz)], and two sets of α,*β*-unsaturated carbonyl systems [δ_H 6.15 (1H, d, $J = 2.0$ Hz); δ_C 125.4 (d), 167.7 (s), 202.6 (s) and δ_H 6.02 (1H, br s); δ_C 124.2 (d), 155.0 (s), 201.2 (s)]. These spectroscopic characteristics were similar to the known compound (23*E*)-25-hydroxycucurbita-5,23-diene-3,7-dione,[18] except for the signals of C-20−C-27. Thus, compound **6** was presumed to exhibit a cucurbit-5-ene-3,7-dione skeleton. Two geminal olefinic methyls [δ_H 1.86 (3H × 2, H-26, H-27)] on the side chain supported that the remaining double bond was located at C-24 (δ_C 124.2) and C-25 (δ_C 155.0). The α,*β*-unsaturated carbonyl carbon should be assigned at C-23 (δ_C 201.2). The proposed structure of the side chain was confirmed by the HMBC correlations between H-22 (δ_H 2.10, 2.50)/C-20 (δ_C 33.2), C-23 (δ_C 201.2) and between H-24 (δ_H 6.02)/C-23, C-26 (δ_C 20.7), C-27 (δ_C 27.7), together with the fragment ions at *m/z* 125 [side chain]$^+$ and 327 [M − side chain]$^+$. The base peak in the EIMS spectrum was at *m/z* 355 [M − $CH_2COCHC(CH_3)_2$]$^+$.[19] Accordingly, compound **6** was determined to be cucurbita-5,24-diene-3,7,23-trione.

Compounds **3** and **4**, which were first isolated from the stem of *M. charantia*, were also reported in the fruit.[10,11] In this paper, the complete ^1H and ^{13}C NMR assignments of **3** and **4** are presented.

These cucurbitane-type triterpenes were evaluated for their cytotoxic activity against human hepatoma SK-Hep 1 cells with etoposide as a positive control ($IC_{50} = 49.6$ μM). Forty-eight hours after culture, compounds **7** and **8** exhibited slight growth inhibitory activity against the SK-Hep 1 cell line with IC_{50} values of 98.3 and 91.6 μM, respectively. The other compounds showed no significant effect, with IC_{50} values of more than 100 μM.

Experimental Section

General Experimental Procedures. Optical rotations were measured by using a JASCO DIP-180 digital spectropolarimeter. UV spectra were measured in MeOH using a Shimadzu UV-1601PC spectrophotometer. IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. NMR spectra were recorded in $CDCl_3$ 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 by using standard pulse sequences. EIMS and HREIMS were recorded on Finnigan TSQ-700 and JEOL SX-102A mass spectrometers, respectively. TLC was performed by using Si gel 60 F_{254} plates (Merck). Column chromatography was performed on Si gel (230−400 mesh ASTM, Merck). HPLC was performed by using a Lichrosorb Si gel 60 (5 μm) column (250 × 10 mm).

Plant Material. The mature stems of *Momordica charantia* were collected in Pingtung County, Taiwan, in July 2003. The plant material was identified by Prof. Sheng-Zehn Yang, Curator of Herbarium, National Pingtung University of Science and Technology, where a voucher specimen (no. 2013) was deposited.

Extraction and Isolation. Air-dried pieces of the stems (18 kg) of *M. charantia* were extracted with MeOH (3 × 30 L) at room temperature (7 days each). The MeOH extract was evaporated in vacuo to afford a black residue, which was suspended in H_2O (3 L) and then partitioned sequentially, using EtOAc and *n*-BuOH (3 × 2 L) as solvent. The EtOAc fraction (386 g) was passed through a Si gel column (120 × 10 cm), using solvent mixtures of *n*-hexane and EtOAc with 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)], and 11 (6000 mL, EtOAc). Fraction 6 was further chromatographed on a Si gel column (5 × 45 cm), eluted with CH_2Cl_2−EtOAc (8:1), to obtain seven fractions (each about 700 mL), 6A−6G. HPLC of fraction 6C eluted with *n*-hexane−EtOAc (8:2) at 2 mL/min to yield **4** (10 mg, t_R = 17.4 min) and **3** (6 mg, t_R = 22.5 min), respectively. Fraction 7 was further purified through a Si gel column (5 × 45 cm), eluted with CH_2Cl_2−EtOAc (8:1), to obtain seven fractions (each about 600 mL), 7A−7G. HPLC fractionation of fraction 7E by elution with *n*-hexane−EtOAc (6:1) at 2 mL/min yielded **6** (8 mg, t_R = 19.6 min) and **5** (15 mg, t_R = 23.4 min), respectively. Fraction 8 was separated using a column packed with Si gel (5 × 45 cm) and eluted with CH_2Cl_2−EtOAc (7:1), to generate six fractions (each 500 mL), 8A−8F. HPLC of fraction 8D with *n*-hexane−acetone (7:3) as eluent, 2 mL/min, yielded **2** (3 mg, t_R = 15.7 min) and **1** (7 mg, t_R = 25.4 min). HPLC of fraction 8F eluted with *n*-hexane−acetone (7:3) at 2 mL/min yielded **8** (7 mg, t_R = 22.8 min) and **7** (52 mg, t_R = 29.2 min), respectively.

Cucurbita-5,23(*E*)-diene-3*β*,7*β*,25-triol (1): amorphous, white powder; $[\alpha]^{25}_D$ +13.5 (*c* 0.4, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3550, 3020, 2945, 2872, 1703, 1654, 1455, 1377, 1270, 1036, 973, 734 cm^{-1}; EIMS *m/z* 458 $[M]^+$ (1), 440 (14), 422 (35), 407 (18), 404 (21), 389 (100), 187 (30), 171 (36), 157 (29), 133 (37), 109 (50), 81 (35); HREIMS *m/z* 440.3649 (calcd for $C_{30}H_{48}O_2$ 440.3656).

3*β*-Acetoxy-7*β*-methoxycucurbita-5,23(*E*)-dien-25-ol (2): amorphous, white powder; $[\alpha]^{25}_D$ +71.6 (*c* 0.1, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3461, 3052, 2936, 2872, 2809, 1732, 1659, 1455, 1377, 1250, 1192, 1085, 977, 934, 890, 734 cm^{-1}; EIMS *m/z* 514 $[M]^+$ (2), 496 (3), 464 (20), 436 (18), 422 (8), 404 (31), 389 (93), 355 (15), 307 (14), 279 (17), 185 (46), 171 (56), 149 (100), 121 (47), 109 (75), 95 (72), 81 (70), 69 (66); HREIMS *m/z* 514.4028 (calcd for $C_{33}H_{54}O_4$ 514.4024).

3*β*,25-Dihydroxy-7*β*-methoxycucurbita-5,23(*E*)-diene (3): amorphous, white powder; ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3360, 3055, 2945, 2814, 1660, 1455, 1380, 1265, 1149, 1083, 1029, 977, 935, 736 cm^{-1}; EIMS *m/z* 472 $[M]^+$ (5), 454 (18), 439 (8), 422 (35), 404 (15), 389 (100), 351 (3), 171 (7), 109 (15), 81 (8); HREIMS *m/z* $[M]^+$ 472.3921 (calcd for $C_{31}H_{52}O_3$ 472.3923).

3*β*-Hydroxy-7*β*,25-dimethoxycucurbita-5,23(*E*)-diene (4): amorphous, white powder; ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3416, 2951, 2850, 1655, 1416, 1393, 1290, 1156, 1130, 1083, 976, 883 cm^{-1}; EIMS *m/z* 486 $[M]^+$ (7), 468 (20), 453 (16), 422 (26), 404 (21), 389 (100), 187 (20), 171 (24), 109 (25), 81 (18); HREIMS *m/z* $[M]^+$ 486.4077 (calcd for $C_{32}H_{54}O_3$ 486.4080).

Cucurbita-5(10),6,23(*E*)-triene-3*β*,25-diol (5): amorphous, white powder; $[\alpha]^{25}_{D}$ −97.3 (*c* 0.5, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3383, 2936, 2872, 1643, 1592, 1490, 1372, 1041, 937, 788, 734 cm^{-1}; UV (MeOH) λ_{max} (log ε) 204 (4.31), 262 (3.74) nm; EIMS *m*/*z* 440 (M^+, 1), 422 (9), 407 (12), 404 (13), 389 (100), 185 (25), 171 (43), 109 (54), 95 (40), 81 (49), 55 (34); HREIMS *m*/*z* $[M]^+$ 440.3649 (calcd for $C_{30}H_{48}O_2$ 440.3656).

Cucurbita-5,24-diene-3,7,23-trione (6): amorphous, white powder; $[\alpha]^{25}_{D}$ +38.2 (*c* 0.4, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 2950, 2870, 1718, 1684, 1645, 1611, 1455, 1377, 1266, 1041, 886 cm^{-1}; UV (MeOH) λ_{max} (log ε) 204 (4.21), 249 (3.50) nm; EIMS *m*/*z* 452 (M^+, 15), 412 (9), 397 (6), 379 (5), 355 (100), 327 (7), 328 (10), 205 (10), 187 (5), 175 (7), 125 (6), 121 (7), 98 (5), 83 (8); HREIMS *m*/*z* $[M]^+$ 452.3297 (calcd for $C_{30}H_{44}O_3$ 452.3292).

Cytotoxicity Assay. The cytotoxicity of compounds **1**−**8** was measured by using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method based on the described procedures.[22] SK-Hep 1 cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, L-glutamine 2 mM, 1% penicillin/streptomycin (penicillin 10000 U/mL, and streptomycin 10 mg/mL) in a humidified atmosphere of 5% CO_2 at 37 °C. A 100 μL volume of SK-Hep 1 cells at a density of 1×10^5 cells/mL was incubated under the same conditions for 24 h in a 96-well flat-bottomed microplate. Test samples dissolved in DMSO were added to the medium and incubated for 48 h. Subsequently, the wells were incubated with the MTT (100 μL/well concentrated at 5 mg/mL) at 37 °C for 4 h. After removing the supernatant, 200 μL of DMSO was added to redissolve the formazan crystals. The absorbance of the resulting formazan was measured by an enzyme-linked immunosorbent assay plate reader at 550 nm. The results were assayed in triplicate. The ratio of cell viability (%) was calculated by using the following formula: [(experimental absorbance − background absorbance)/(control absorbance − background absorbance)] × 100. The IC_{50} values of each compound were obtained from 50% inhibition of cell growth and were compared with that of the control.

Acknowledgment. This research was supported by grants to C.-H.C. from the National Science Council of Taiwan (NSC 94-2317-B-020-001 and NSC 96-2317-B-020-003). We thank Ms. S.-Y. Sun for the EIMS and HREIMS measurement in the Instrumentation Center of the College of Science, National Taiwan University. We are grateful to the National Center for High-Performance Computing for computer time and facilities. We also express our gratitude to Professor G. R. Waller, Prof. Emeritus, Dept. of Biochemistry and Molecular Biology, Oklahoma State University, for critical reading of language improvement.

References and Notes

(1) Rathi, S. S.; Grover, J. K.; Vats, V. *Phytother. Res.* **2002**, *16*, 236–243.
(2) Guevara, A. P.; Lim-Sylianco, C. Y.; Dayrit, F. M.; Finch, P. *Phytochemistry* **1989**, *28*, 1721–1724.
(3) Okabe, H.; Miyahara, Y.; Yamauchi, T. *Chem. Pharm. Bull.* **1982**, *30*, 3977–3986.
(4) Okabe, H.; Miyahara, Y.; Yamauchi, T. *Chem. Pharm. Bull.* **1982**, *30*, 4334–4340.
(5) Fatope, M. O.; Takeda, Y.; Yamashita, H.; Okabe, H.; Yamauchi, T. *J. Nat. Prod.* **1990**, *53*, 1491–1497.
(6) Murakami, T.; Emoto, A.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2001**, *49*, 54–63.
(7) Begum, S.; Ahmed, M.; Siddiqui, B. S.; Khan, A.; Saify, Z. S.; Arif, M. *Phytochemistry* **1997**, *44*, 1313–1320.
(8) El-Gengaihi, S.; Karawya, M. S.; Selim, M. A.; Motawe, H. M.; Ibrahim, N.; Faddah, L. M. *Pharmazie* **1995**, *50*, 361–362.
(9) Kimura, Y.; Akihisa, T.; Yuasa, N.; Ukiya, M.; Suzuki, T.; Toriyama, M.; Motohashi, S.; Tokuda, H. *J. Nat. Prod.* **2005**, *68*, 807–809.
(10) Harinantenaina, L.; Tanaka, M.; Takaoka, S.; Oda, M.; Mogami, O.; Uchida, M.; Asakawa, Y. *Chem. Pharm. Bull.* **2006**, *54*, 1017–1021.
(11) Nakamura, S.; Murakami, T.; Nakamura, J.; Kobayashi, H.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2006**, *54*, 1545–1550.
(12) Li, Q. Y.; Chen, H. B.; Liu, Z. M.; Wang, B.; Zhao, Y. Y. *Magn. Reson. Chem.* **2007**, *45*, 451–456.
(13) Akihisa, T.; Higo, N.; Tokuda, H.; Ukiya, M.; Akazawa, H.; Tochigi, Y.; Kimura, Y.; Suzuki, T.; Nishino, H. *J. Nat. Prod.* **2007**, *70*, 1233–1239.
(14) Miyahara, Y.; Okabe, H.; Yamauchi, T. *Chem. Pharm. Bull.* **1981**, *29*, 1561–1566.
(15) Dutta, P. K.; Chakravarty, A. K.; Chowdhury, U. S.; Pakrashi, S. C. *Indian J. Chem., Sect. B* **1981**, *20*, 669–671.
(16) Okabe, H.; Miyahara, Y.; Yamauchi, T.; Miyahara, K.; Kawasaki, T. *Chem. Pharm. Bull.* **1980**, *28*, 2753–2762.
(17) Yasuda, M.; Iwamoto, M.; Okabe, H.; Yamauchi, T. *Chem. Pharm. Bull.* **1984**, *32*, 2044–2047.
(18) Chang, C. I.; Chen, C. R.; Liao, Y. W.; Cheng, H. L.; Chen, Y. C.; Chou, C. H. *J. Nat. Prod.* **2006**, *69*, 1168–1171.
(19) Mulholland, D. A.; Sewram, V.; Osborne, R.; Pegel, K. H.; Connolly, J. D. *Phytochemistry* **1997**, *45*, 391–395.
(20) Lee, S. S.; Kishore, P. H.; Chen, C. H. *Helv. Chim. Acta* **2002**, *85*, 2403–2408.
(21) Moriarty, R. M.; Paaren, H. E. *J. Org. Chem.* **1981**, *46*, 970–977.
(22) Kuo, Y. H.; Li, S. Y.; Shen, Y. C.; Huang, H. C.; Hsu, Y. W.; Tseng, R. J.; Ou, J. C.; Chen, C. F. *Chin. Pharm. J.* **2001**, *53*, 257–263.

NP070532U