Octanorcucurbitane Triterpenoids Protect against *tert*-Butyl Hydroperoxide-Induced Hepatotoxicity from the Stems of *Momordica charantia*

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Four novel octanorcucurbitane triterpenes, octanorcucurbitacins A—D (1—4), together with one known octanorcucurbitane triterpene, kuguacin M (5), were isolated from the methyl alcohol extract of the stems of *Momordica charantia*. Their structures were elucidated on the basis of extensive spectroscopic analyses. Compound 3 inhibited *tert*-butyl hydroperoxide (*t*-BHP)-induced hepatotoxicity against HepG2 cells.

Key words *Momordica charantia*; Cucurbitaceae; octanorcucurbitane; *tert*-butyl hydroperoxide; cytotoxic

Bitter gourd, *Momordica charantia* L. (Cucurbitaceae) is a slender-stemmed tendril climber and is wildly cultivated as a vegetable crop in tropical and subtropical areas, including Asia, East Africa, and South America. Tissues of this plant have extensively been used in the treatment of diabetes and diseases of liver. Several pharmacological and phytochemical studies have revealed that the extracts or constituents of tissues of *M. charantia* possess anti-diabetic and anti-inflammatory activities.[1—3] In addition, more than sixty cucurbitane-type triterpenoids have been isolated from the fruits,[3—10] seeds,[11,12] roots,[13] leaves and vines[14,15] of *M. charantia*. As part of our program aimed at the discovery of the cucurbitane-type triterpenes from *M. charantia* originated in Taiwan, we reported the isolation and structural elucidation of fourteen cucurbitane-type triterpenoids from the MeOH extract of the stems of this plant.[16,17] In our continuing investigation on *M. charantia*, we further purified four novel octanorcucurbitane triterpenes, 20,21,22,23,24,25,26,27-octanorcucurbit-5-ene-3,7,17-trione (**1**), 20,21,22,23,24,25,26,27-octanorcucurbit-5-ene-3,7,16-trione (**2**), 5β,19-epoxy-20,21,22,23,24,25,26,27-octanorcucurbit-6-ene-3,17-dione (**3**), and (19*R*)-5β,19-epoxy-19-methoxy-20,21,22,23,24,25,26,27-octanorcucurbit-6-ene-3,17-dione (**4**), along with one known octanorcucurbitane triterpene, kuguacin M (**5**),[15] from the same part of the plant. This manuscript deals with the isolation and structural elucidation of the octanorcucurbitacins **1**—**5** and the protective effects on *tert*-butyl hydroperoxide (*t*-BHP)-induced hepatotoxicity and cytotoxic activities against human hepatoma HepG2 cells of octanorcucurbitacins **1**—**3** and **5** (Fig. 1).

Results and Discussion

Compound **1** was obtained as a white amorphous powder. Its high resolution electron impact mass spectrum (HR-EI-MS) gave a molecular ion at *m*/*z* 342.2192, corresponding to the molecular formula, $C_{22}H_{30}O_3$, which indicated eight degrees of unsaturation. A significant UV absorption maximum at 248 nm suggested the presence of an α,β-unsaturated ketone. The IR spectrum showed absorption bands at 1654 cm^{-1} due to a conjugated ketone unit and 3050, 1654 and 890 cm^{-1} due to a conjugated double bond. In addition, the IR absorption bands at 1713 and 1742 cm^{-1} indicated two isolated ketone moieties located at six-membered and five-membered rings, respectively. The ^{1}H- and ^{13}C-NMR spectra of **1** (Tables 1, 2) displayed signals characteristic for the presence of five methyl singlets [δ_H 0.85, 0.99, 1.09, 1.31, 1.34 (3H each, s)] and one set of α,β-unsaturated carbonyl system [δ_H 6.17 (1H, d, J=2.0 Hz); δ_C 124.9 (d), 167.9 (s), 200.7 (s)]. The ^{13}C-NMR spectrum of **1** revealed 22 carbon signals, which were sorted by the distortionless enhancement by polarization transfer (DEPT) experiments into five methyl, six methylene, two methine, four quaternary, one olefinic methine, one quaternary olefinic, and three ketone carbonyl carbons. On the basis of the fact that the major tetracyclic triterpenoids presenting in the genus *Momordica* plants are cucurbitane-type compounds, compound **1** was tentatively proposed to be a 20,21,22,23,24,25,26,27-octanorcucurbitacin derivative. Furthermore, comparison of the ^{1}H- and ^{13}C-NMR data with those of the known compound, (23*E*)-25-hydroxycucurbita-5,23-diene-3,7-dione,[16] indicated that both compounds exhibited identical structure in rings A—C and the NMR signals of side chain (C20—27) were absent in **1**. Thus, compound **1** was considered as a 20,21,22,23,24,25,26,27-octanorcucurbitacin triterpene with an isolated ketone in the ring D. The heteronuclear multiple bond coherence (HMBC) correlations between H-16 (δ_H 2.13, 2.46)/C-17 (δ_C 217.4), Me-18 (δ_H 1.09)/C-12 (δ_C 23.1), Me-18/C-13 (δ_C 53.1), Me-18/C-14 (δ_C 43.8), and Me-18/C-17 (Fig. 2) confirmed that the isolated ketone was located at C-17 posi-

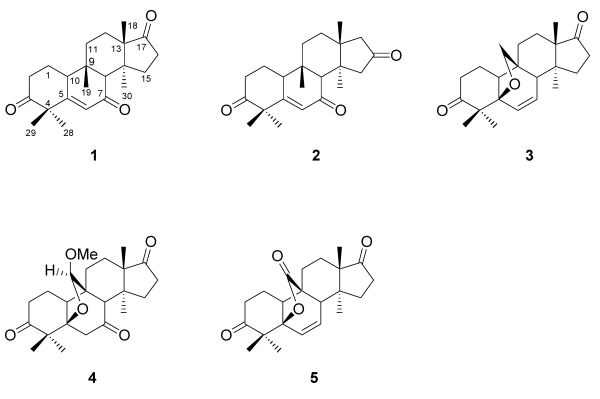

Fig. 1. Structures of Compounds **1**—**5** from *M. charantia*

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Table 1. ^{1}H-NMR Data for **1**—**5** (400 MHz in $CDCl_3$)

Position	1	2	3	4	5
1	1.66 m, 2.16 m	1.68 m, 2.18 m	1.54 m, 1.96 m	1.58 m, 2.12 m	1.46 m, 2.16 m
2	2.54 m, 2.64 m	2.55 m, 2.63 m	2.29 m, 2.72 m	2.31 m, 2.72 m	2.34 m, 2.75 m
5					
6	6.17 d (2.0)	6.17 d (2.0)	6.13 dd (2.4, 10.0)	2.47 m, 2.74 m	6.30 dd (2.0, 9.6)
7			5.76 dd (4.0, 10.0)		5.87 dd (3.2, 9.6)
8	2.47 s	2.56 s	2.45 dd (2.4, 10.0)	3.27 br s	2.63 br s
10	2.86 ddd (2.0, 4.0, 11.6)	2.95 ddd (2.0, 4.4, 11.2)	2.54 m	2.82 m	2.95 dd (5.6, 12.0)
11	1.52 m, 1.94 m	1.67 m, 1.95 m	1.46 m, 1.85 m	1.56 m, 1.85 m	1.81 m, 2.26 m
12	1.56 m, 1.73 m	1.52 m, 2.01 m	1.49 m, 1.68 m	1.50 m, 1.60 m	1.62 m
15	1.53 m, 2.06 m	1.79 d (18.0), 2.50 m	1.74 m, 1.86 m	1.88 m, 2.22 m	1.78 m, 1.88 m
16	2.13 m, 2.46 m		2.21 m, 2.50 m	2.16 m, 2.50 m	2.24 m, 2.52 m
17		1.93 d (18.1), 2.30 d (18.1)			
18	1.09 s	1.13 s	1.08 s	1.07 s	1.14 s
19	0.99 s	1.00 s	3.52 d (8.8), 3.60 d (8.8)	4.61 s	
28	1.31 s	1.33 s	1.11 s	1.11 s	1.18 s
29	1.34 s	1.35 s	1.11 s	1.09 s	1.22 s
30	0.85 s	1.00 s	0.93 s	0.93 s	0.91 s
19-OC$\underline{H}_3$				3.37 s	

Table 2. ^{13}C-NMR Data for **1**—**5** (100 MHz in $CDCl_3$)

Position	1	2	3	4	5
1	24.2	23.7	24.8	24.5	25.3
2	38.0	38.0	35.9	35.5	35.0
3	210.8	210.9	213.9	212.7	210.6
4	51.6	51.5	48.4	49.5	47.7
5	167.9	168.1	89.8	89.5	88.0
6	124.9	125.0	133.0	48.9	131.8
7	200.7	201.3	129.4	211.5	131.6
8	58.1	57.1	51.0	52.2	43.6
9	37.4	36.9	46.3	49.8	51.6
10	41.8	41.7	40.1	42.9	40.9
11	30.6	30.7	22.9	22.6	20.8
12	23.1	27.9	23.9	23.5	22.9
13	53.1	41.8	52.2	52.7	52.0
14	43.8	44.4	43.8	43.7	43.1
15	31.8	50.6	30.1	31.9	30.0
16	33.6	217.8	33.4	33.4	33.0
17	217.4	49.6	217.5	217.6	217.0
18	17.3	22.7	16.9	17.5	16.7
19	27.6	27.6	79.9	110.0	180.2
28	28.6	28.5	24.8	25.0	23.3
29	22.8	23.0	16.7	17.2	16.9
30	19.1	19.1	19.7	20.3	19.3
19-O$\underline{C}H_3$				57.6	

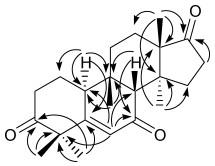

Fig. 2. Main HMBC Correlations of **1**

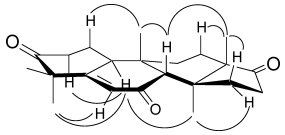

Fig. 3. Main NOESY Correlations of **1**

tion. The electron impact mass spectrum (EI-MS) spectrum of **1** showed the fragment ion at *m*/*z* 286 $[M-CO-C_2H_4]^+$ derived from the loss of ring D by the cleavage of C-13/C-17 and C-14/C-15 position. In addition, the fragment ion at *m*/*z* 164 $[M-C_{12}H_{18}O]^+$ coresopnded to the loss of rings C and D by the cleavage of C-9/C-10 and C-7/C-8 bonds. The relative configurations of sterogenic carbon atoms in the tetracyclic rings were determined by significant nuclear Overhauser effect (NOE) correlations between H-10 (δ_H 2.86)/H-2 (δ_H 2.64), H-10/Me-28 (δ_H 1.31), H-10/Me-30 (δ_H 0.85), H-8 (δ_H 2.47)/Me-18 (δ_H 1.09), and H-8/Me-19 (δ_H 0.99) in the nuclear Overhauser enhancement exchange spectroscopy (NOESY) spectrum (Fig. 3). From the above evidence, compound **1** was characterized as 20,21,22,23,24,25,26,27-octanorcucurbit-5-ene-3,7,17-trione, namely octanorcucurbitacin A. Complete ^{1}H- and ^{13}C-NMR chemical shift assignments were established by ^{1}H–^{1}H correlated spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), HMBC, and NOESY spectra.

The HR-EI-MS of **2** showed a molecular ion at *m*/*z* 342.2191, corresponding to the molecular formula, $C_{22}H_{30}O_3$, which indicated eight degrees of unsaturation. The IR spectrum displayed absorptions for a conjugated ketone (1650 cm^{-1}), a conjugated double bond (3045, 1650, 886 cm^{-1}) and two isolated ketones (1718, 1741 cm^{-1}). The significant UV absorption at 249 nm also suggested the presence of an α,β-unsaturated ketone. The ^{1}H- and ^{13}C-NMR spectra of **2** (Tables 1, 2) revealed resonances for five methyl singlets [δ_H 1.00 (3H×2, s), 1.13, 1.33, 1.35 (3H each, s)], one set of α,β-unsaturated carbonyl system [δ_H 6.17 (1H, d, J=2.0 Hz); δ_C 125.0 (d), 168.1 (s), 201.3 (s)]. Altogether, 22 carbon signals were observed in the ^{13}C-NMR spectrum of **2**, and were assigned by DEPT experiments as five methyl, six methylene, two methine, four quaternary, one olefinic methine, one quaternary olefinic, and three ketone carbonyl carbons. Compound **2** was tentatively proposed to exhibit a basic skeleton of 20,21,22,23,24,25,26,27-octanorcucurbitacin. The ^{1}H- and ^{13}C-NMR data were similar to those of **1**, except for the signals of the rings C and D. Two set of doublet coupling signals at δ_H 1.79 (d, J=18.0 Hz, H-15)/2.50 (m, H-15) and 1.93 (d, J=18.1 Hz, H-17$_a$)/2.30 (d, J=18.1 Hz, H-17$_b$) as well as the

HMBC correlations between H-8 (δ_H 2.56)/C-15 (δ_C 50.6), Me-30 (δ_H 1.00)/C-15, H-15 (δ_H 1.79, 2.50)/C-16 (δ_C 217.8), Me-18 (δ_H 1.13)/C-17 (δ_C 49.6), H-17 (δ_H 1.93, 2.30)/C-16, confirmed that the carbonyl group was located at C-16 position. The EI-MS fragment ions at *m/z* 299 $[M-CH_3]^+$, 286 $[M-CO-C_2H_4]^+$, and 164 $[M-C_{12}H_{18}O]^+$ were similar to those of **1**. Compound **2** was thus elucidated as 20,21,22,23,24,25,26,27-octanorcucurbit-5-ene-3,7,16-trione, namely octanorcucurbitacin B.

The molecular formula of compound **3** was assigned as $C_{22}H_{30}O_3$ based on the molecular ion at *m/z* 342.2205 in the HR-EI-MS. The IR spectrum of **3** showed bands that were attributable to isolated ketones (1732 cm^{-1}) and a *cis*-disubstituted double bond (3032, 1651, 765 cm^{-1}). The ^{1}H- and ^{13}C-NMR data (Tables 1, 2) indicated the presence of four tertiary methyls [δ_H 0.93, 1.08 (3H each, s), 1.11 (3H×2, s)], an oxymethylene [δ_H 3.52 (1H, d, J=8.8 Hz), 3.60 (1H, d, J=8.8 Hz); δ_C 79.9 (t)], and an allylic ABX system of *cis*-oriented cyclohexene [δ_H 6.13 (1H, dd, J=2.4, 10.0 Hz, H-6), 5.76 (1H, dd, J=4.0, 10.0 Hz, H-7), 2.45 (1H, dd, J=2.4, 10.0 Hz, H-8); δ_C 129.4 (d), 133.0 (d), 51.0 (d)]. The ^{13}C-NMR spectrum displayed 22 resonances, which were differentiated by DEPT experiments into four methyl, six methylene, two methine, four quaternary, one oxygenated methylene, two olefinic methine, one oxygenated quaternary, and two ketone carbonyl carbons. The ^{13}C-NMR data of **3** were closely similar to that of the known compound (23*E*)-5β,19-epoxycucurbita-6,23-diene-3β,25-diol,[16] except for the absence of the NMR signals of side chain (C20—27) and the ketone group at C-3 in **3** instead of the secondary carbinol group. The HMBC correlations between H-6 (δ_H 6.13)/C-4 (δ_C 48.4), C-5 (δ_C 89.8), C-8 (δ_C 51.0), and C-10 (δ_C 40.1); between H-7 (δ_H 5.76)/C-5, C-8, C-9 (δ_C 46.3), and C-14 (δ_C 43.8); and between H-19 (δ_H 3.52, 3.60)/C-8, C-9, C-10 (δ_C 40.1), and C-11 (δ_C 22.9) confirmed that the oxymethylene (C-19) was linked *via* an oxygen atom to C-5. The NOESY correlation of H-19 (δ_H 3.52) with H-8 (δ_H 2.45) was also found. Therefore, compound **3** was determined as 5β,19-epoxy-20,21,22,23,24,25,26,27-octanorcucurbit-6-ene-3,17-dione, namely octanorcucurbitacin C.

Compound **4** was isolated as a colorless amorphous solid. Its HR-EI-MS revealed a molecular ion peak at *m/z* $[M]^+$ 388.2240, and the molecular formula was determined to be $C_{23}H_{32}O_5$, representing eight degrees of unsaturation. The IR spectrum displayed absorptions for isolated ketone moieties (1708, 1742 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **4** (Tables 1, 2) exhibited four methyl singlets [δ_H 0.93, 1.07, 1.09, 1.11 (3H each, s)], a methoxy [δ_H 3.37 (3H, s)], a hemiacetal methine [δ_H 4.61 (1H, s); δ_C 110.0 (d)]. 23 carbon signals were observed in the ^{13}C-NMR spectrum of **4**, and were resolved by DEPT experiments as four methyl, seven methylene, two methine, four quaternary, one hemiacetal methine, three ketone carbonyl, and one oxygenated quaternary, one methoxy carbons. The ^{13}C-NMR data of **4** showed close resemblance with those of **3**, except for the carbon signals of ring B (C-6—C-10) and C-19. The oxymethylene group of C-19 in **3** (δ_C 79.9) was replaced by a hemiacetal group (δ_C 110.0) linking to C-5 through an oxygen atom. The EI-MS fragment ion at *m/z* 328 $[M-HCO_2CH_3]^+$ indicated the presence of hemiacetal moiety with a methoxy substituent. The HMBC correlations between H-19 (δ_H 4.61)/C-5 (δ_C 89.5), C-8 (δ_C 52.2), C-9 (δ_C 49.8), 19-O<u>C</u>H$_3$ (δ_C 57.6) and between H-8 (δ_H 3.27)/C-6 (δ_C 48.9), C-9, C-10 (δ_C 42.9) suggested that the hemiacetal group located at C-19 and linked to C-5. The *R* configuration of C-19 was confirmed by the NOE correlations between H-19 (δ_H 4.61)/H-1β (δ_H 1.60), and H-11β (δ_H 1.85); between 19-OCH$_3$ (δ_H 3.37)/H-18 (δ_H 1.07). Accordingly, compound **4** was established as (19*R*)-5β,19-epoxy-19-methoxy-20,21,22,23,24,25,26,27-octanorcucurbit-6-ene-3,17-dione, namely octanorcucurbitacin D.

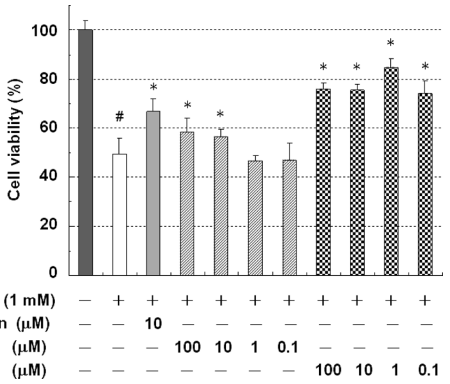

Fig. 4. Cytoprotective Effect of **1** and **3** on *t*-BHP-Toxicified HepG2 Cells

Statistically significant compared with normal data ($p<0.05$). * Statistically significant compared with *t*-BHP control data ($p<0.05$).

Compound **5** was assigned as $C_{22}H_{28}O_4$ by the molecular ion of HR-EI-MS at *m/z* $[M]^+$ 356.1982. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **5** with those of **3** revealed that these two compounds are structurally very similar in rings A, C, and D. The only difference was that the oxymethylene [δ_H 3.52 (1H, d, J=8.8 Hz), 3.60 (1H, d, J=8.8 Hz); δ_C 79.9 (t)] in **3** was replaced by a lactone carbonyl [δ_C 180.2 (s)]. This proposed structure was further suggested by the base peak at *m/z* 312 $[M-CO_2]^+$ in the EI-MS spectrum of **5** and the HMBC correlations between H-10 (δ_H 2.95)/C-19 (δ_C 180.2) and H-11 (δ_H 2.26)/C-19. Thus, compound **5** was formulated as 20,21,22,23,24,25,26,27-octanorcucurbit-6-ene-3,17-dioxo-19,5β-olide, kuguacin M, which was reported presenting in the vines and leaves of *M. charantia* by Chen *et al.* and recorded the NMR data in deuterium-pyridine solvent.[15] We also isolated compound **5** from the stems of the same plant. In this paper, we showed the complete ^{1}H- and ^{13}C-NMR chemical shift assignments of compound **5** detected by using deuterium-chloroform solvent.

These cucurbitane-type triterpenes, except for **4**, were evaluated for their cytotoxic activity against human hepatoma HepG2 cells with fluorouracil (5-FU) as a positive control (IC_{50}=1.6 μM).[18] Forty-eight hours after culture, they did not show significant growth inhibitory activity against the HepG2 cell line at a dose of 100 μM. The protective activities of compounds **1**—**3** and **5** on HepG2 cells injured by *t*-BHP was tested as reported previously.[19] The pretreatment of compounds **1** and **3** on HepG2 cells inhibited *t*-BHP-induced cytotoxicity. Compound **1** at a dose of 10 and 100 μM protected the *t*-BHP-induced cytotoxicity of HepG2 to 11.2% and 14.4% of control group, respectively. Meanwhile, compound **3** at a dose of 1 and 10 μM protected the *t*-BHP-induced cytotoxicity of HepG2 to 58.2% and 42.7% of control group, respectively (Fig. 4). The protective effect of com-

pound **3** was stronger than that of silybin, a commercial agent, which protected the *t*-BHP-induced cytotoxicity of HepG2 to 37.5% of control group at a dose of 10 μM (Fig. 4).

Experimental

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 obtained in $CDCl_3$ at a constant temperature controlled and adjusted to around 300 K on a Varian Mercury plus 400 NMR spectrometer, and the residual proton resonance ($CHCl_3$) of $CDCl_3$ was used as internal shift reference. The 2D NMR spectra were recorded by using standard pulse sequences. EI-MS and HR-EI-MS 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 stems of *M. 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) has been deposited.

Extraction and Isolation The sliced, air-dried stems (18 kg) of *M. charantia* were extracted with MeOH (3×30 l) at room temperature (7 d each). The combined MeOH extract was evaporated under reduced pressure to afford a black residue, which was suspended in H_2O (3 l), and then partitioned sequentially, using EtOAc and *n*-BuOH (3×3 l) as solvent. The EtOAc fraction (386 g) was passed through a Si gel column (120×10 cm), using a setpwise gradient mixture of hexane and EtOAc as eluent. Eleven fractions were collected as follows: 1 [5000 ml, hexane], 2 [4000 ml, hexane–EtOAc (49 : 1)], 3 [4000 ml, hexane–EtOAc (19 : 1)], 4 [4000 ml, hexane–EtOAc (9 : 1)], 5 [4000 ml, hexane–EtOAc (17 : 3)], 6 [4000 ml, hexane–EtOAc (8 : 2)], 7 [4000 ml, hexane–EtOAc (7 : 3)], 8 [3000 ml, hexane–EtOAc (5 : 5)], 9 [3000 ml, hexane–EtOAc (4 : 6)], 10 [3000 ml, hexane–EtOAc (2 : 8)], and 11 (6000 ml, EtOAc). Fraction 7 was further chromatographed on a Si gel column (5×45 cm), eluted with CH_2Cl_2–EtOAc (8 : 1 to 0 : 1) to obtain seven fractions (each about 600 ml), 7A—7G. Fr. 7D was subjected to column chromatography over Si gel eluted with hexane–CH_2Cl_2–EtOAc (3 : 3 : 1) and semipreparative HPLC eluted with hexane–EtOAc (7 : 3) to yield **3** (6 mg). Fraction 8 was further purified through a Si gel column (5×45 cm), eluted with CH_2Cl_2–EtOAc (7 : 1 to 0 : 1) to obtain six fractions (each about 500 ml), 8A—8F. Si gel column chromatography of fr. 8C eluted with a CH_2Cl_2–EtOAc gradient (100 : 1 to 0 : 1) to yield **1** (5 mg). Fr. 8E was subjected to column chromatography over Si gel eluted with hexane–CH_2Cl_2–EtOAc (3 : 3 : 1) and semipreparative HPLC eluted with CH_2Cl_2–EtOAc (3 : 2) to yield **2** (10 mg), **4** (1 mg) and **5** (15 mg).

Octanorcucurbitacin A (**1**): Amorphous white powder; $[\alpha]_D^{25}$ +96.6 (c=0.29, MeOH); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; IR (KBr) cm^{-1}: 3050, 2965, 2882, 1742, 1713, 1654, 1616, 1460, 1377, 1294, 1104, 997, 890, 734; UV (MeOH) λ_{max} (log ε) 203 (4.20), 248 (3.53) nm; EI-MS *m*/*z* 342 $[M]^+$ (100), 327 (25), 314 (30), 286 (25), 285 (42), 243 (41), 205 (72), 187 (21), 164 (35), 136 (40), 121 (25); HR-EI-MS *m*/*z* $[M]^+$ 342.2192 (Calcd for $C_{22}H_{30}O_3$ 342.2195).

Octanorcucurbitacin B (**2**): Amorphous white powder; $[\alpha]_D^{25}$ +93.0 (c=0.12, MeOH); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; IR (KBr) cm^{-1}: 3045, 2960, 2872, 1741, 1718, 1650, 1611, 1460, 1377, 1299, 1182, 1129, 1051, 886, 734; UV (MeOH) λ_{max} (log ε) 205 (4.20), 249 (3.49) nm; EI-MS *m*/*z* 342 $[M]^+$ (15), 327 (18), 314 (100), 299 (89), 286 (13), 285 (15), 271 (16), 243 (29), 215 (20), 205 (14), 164 (14), 136 (34), 121 (32); HR-EI-MS *m*/*z* $[M]^+$ 342.2191 (Calcd for $C_{22}H_{30}O_3$ 342.2195).

Octanorcucurbitacin C (**3**): Amorphous white powder; $[\alpha]_D^{25}$ −35.3 (c=0.17, MeOH); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; IR (KBr) cm^{-1}: 3032, 2955, 2877, 1732, 1651, 1460, 1382, 1270, 1099, 992, 934, 861, 765; EI-MS *m*/*z* 342 $[M]^+$ (13), 327 (3), 297 (3), 279 (3), 269 (10), 157 (22), 145 (32), 131 (53), 117 (70), 105 (52), 91 (100), 79 (53); HR-EI-MS *m*/*z* 342.2205 (Calcd for $C_{22}H_{30}O_3$ 342.2195).

Octanorcucurbitacin D (**4**): Amorphous white powder; $[\alpha]_D^{25}$ +230.8 (c=0.17, MeOH); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; IR (KBr) cm^{-1}: 3081, 2960, 2936, 2877, 1742, 1708, 1659, 1460, 1387, 1138, 1104, 1016, 973, 866, 778, 734; EI-MS *m*/*z* 388 $[M]^+$ (1), 356 (15), 328 (100), 313 (96), 285 (15), 271 (27), 243 (86), 185 (14), 91 (10); HR-EI-MS *m*/*z* 388.2240 (Calcd for $C_{23}H_{32}O_5$ 388.2250).

Kuguacin M (**5**): Amorphous white powder; $[\alpha]_D^{25}$ −34.6 (c=0.23, MeOH); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; IR (KBr) cm^{-1}: 2960, 2877, 1752, 1737, 1650, 1465, 1382, 1168, 1026, 910, 764; EI-MS *m*/*z* 356 $[M]^+$ (4), 341 (12), 312 (100), 297 (65), 279 (35), 255 (85), 227 (35), 211 (12); HR-EI-MS *m*/*z* $[M]^+$ 356.1982 (Calcd for $C_{22}H_{28}O_4$ 356.1988).

Cytotoxicity Assay The cytotoxicity of compounds **1**—**3** and **5** was measured by using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method based on the described procedures with some modifications.[18] Briefly, a volume of HepG2 cells 100 μl at a density of 1×10^5 cells/ml was incubated 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.

Protective Effect on Cytotoxicity Induced by *t*-BHP in HepG2 Cells The protective effect of compounds **1**—**3** and **5** on HepG2 cells injured by *t*-BHP was measured using the MTT colorimetric assay based on the described procedures with some modifications.[19] Briefly, HepG2 cells were plated on a 96-well plate with 1×10^4 cells per well. The cells were treated with different concentrations of test compounds. After preincubated for 2 h, the cultured media were changed to the media containing *t*-BHP (100 μM), incubated for 3 h and then rinsed with phosphate-buffered saline. 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. Absorbance at 550 nm was measured to estimate survived cells. The significance of various treatments was determined by the Student's *t*-test. The results were expressed as mean±S.E.M. Differences were considered significant if $p<0.05$.

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