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Cucurbitane-type triterpenoids from the stems and leaves of *Momordica charantia*

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ABSTRACT

Six new cucurbitane-type triterpenoids, karavilagenin F (**1**), karavilosides XII and XIII (**2**, **3**), momordicines VI, VII, and VIII (**4**, **5** and **6**), along with four known ones, 5β,19-epoxy-25-methoxycucurbita-6,23-diene-3β,19-diol (**7**), 5β,19-epoxycucurbita-6, 23-diene-3β,19,25-triol (**8**), kuguacin R (**9**), and (19*R*,23*E*)-5β,19-epoxy-19-methoxycucurbita-6,23,25-trien-3β-ol (**10**), were isolated from the stems and leaves of *Momordica charantia* L. Their chemical structures were elucidated by extensive 1D NMR and 2D NMR (HSQC, HMBC, COSY, and ROESY), MS experiments, and CD spectrum. Compound **6** showed weak cytotoxicity against five human cancer cells lines with IC_{50} values of 14.3–20.5 μmol/L.

1. Introduction

Momordica charantia L. (Cucurbitaceae), also called "kugua" in Chinese and "bitter gourd" in English, is widely cultivated as a favorable vegetable crop in China, India, Japan and other Asian countries. It has also been extensively used in folk medicine to prevent and treat fever polydipsia, diarrhea, colic and diabetes in China [1]. Many phytochemical researches on the fruits, vines, leaves, stems, and seeds of *M. charantia* have been reported, which cause isolation of many cucurbitane-type triterpenoids [2–4], few of sterols [5], alkaloids and others [6]. Meanwhile, numerous studies have also confirmed different pharmacological activities of crude extracts and partial triterpenoids, such as antidiabetic [7–9], antitumor [10–12], antioxidant [2,13], antiviral [14] and antiobesity [15,16]. In recent years, anti-cancer activities of *M. charantia*, such as mouse skin cancer [10], prostate cancer [11,12], breast cancer [17], and human bladder carcinoma [18] have been concerning, suggesting that compounds possessing antitumor effects may be present in this species.

Therefore, on the basis of an interest in the isolation and identification of compounds with potent anticancer from *M. charantia*, firstly, we documented the extraction, isolation, purification, and structural elucidation of six new cucurbitane-type triterpenoids (**1**–**6**) and four known compounds (**7**–**10**) (Fig. 1) from the stems and leaves of *M. charantia*; subsequently, the inhibitory activities of compounds **3**, **4** and **6** against five human cancer cell lines, HL-60, SMMC-7721, A-549, MCF-7, and SW480 by using the MTT method *in vitro* were also reported.

2. Experimental

2.1. General experimental procedures

Optical rotations were obtained with a Jasco P-1020 polarimeter. UV spectra were run on a UV 210A spectrophotometer. The CD spectra were recorded on a Chirascan instrument. ^{1}H and ^{13}C NMR spectra were measured on Bruker AV-400 and DRX-500 instruments (Bruker, Zurich,

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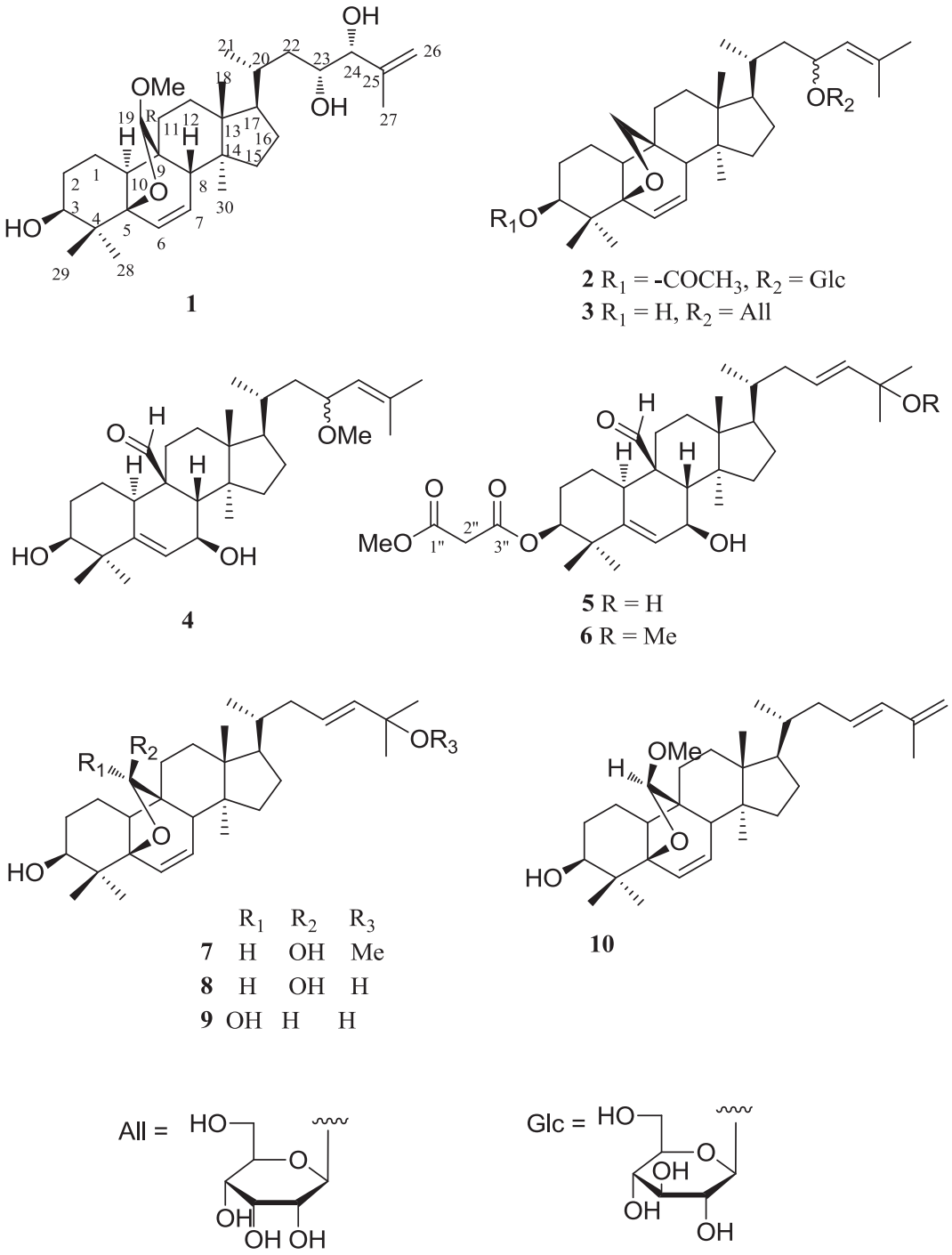

Fig. 1. The structures of **1**–**10**.

Switzerland) with Me_4Si (TMS) as internal standard. ESIMS and HREIMS data were recorded on an API QSTAR Pulsar spectrometer and infrared spectra were recorded on a Bruker Tensor-27 instrument by using KBr pellets. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a ZORBAX SB-C18 (5 μm, 9.4 × 250 mm) column. TLC was performed on precoated TLC plates (200–250 μM thickness, F254 Silica gel 60, Qingdao Marine Chemical, Inc.) with compounds visualized by spraying the dried plates with 10% aqueous H_2SO_4 followed by heating until dryness. Silica gel (200–300) mesh, Qingdao Marine Chemical, Inc., Lichroprep RP-18 (40–63 μm, Merck) was used for column chromatography. Methanol, dichloromethane, ethyl acetate, acetone, petroleum ether, n-butanol and methyl cyanides were purchased from Tianjin Chemical Reagents Co. (Tianjin, China).

2.2. Plant material

The mature stems and leaves of *M. charantia* were collected in Guangzhou, in June 2010. The plant material was identified by Prof. Dai Hao-fu, and a voucher specimen (no. KIB 2010-6) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sciences.

2.3. Extraction and isolation

Dried stems and leaves 9 kg of *M. charantia* were cut into small pieces and then extracted three times with methanol under reflux. The solvent was concentrated under vacuum to obtain the MeOH extract 550 g, which was suspended in H_2O and extracted with petroleum ether, $CHCl_3$ and n-BuOH, respectively. The $CHCl_3$ and n-BuOH mixed extract 350 g was chromatographed on silica gel ($CHCl_3$/MeOH, 80:1, 20:1, 0:1) to afford the $CHCl_3$/MeOH (20:1) fraction 110 g, which was subjected to RP-18 column, eluted with MeOH/H_2O (45:55, 50:50, 60:40, 65:35, 70:30, 80:20, 100:0) to give five fractions (Fr.1–Fr.5). Fr.4 (23.1 g) was subjected to silica gel CC and eluted with a gradient system of $CHCl_3$/MeOH (100:1, 50:1, 30:1, 5:1) to yield ten subfractions (Fr.4-1–Fr.4-10) by TLC. Fr.4-1 (4 g) was applied to silica gel CC using $CHCl_3$/$(Me)_2CO$ (120:1, 60:1, 40:1) to afford compound **6** (63.3 mg). Fr.4-3 (362.9 mg) was repeatedly isolated by semi-preparative HPLC with 70–80% $CNCH_3/H_2O$ (flow rate 3.0 mL/min; UV detector, 205 nm) and P-TLC (petroleum ether-acetone, 4:1) to yield compounds **1** (5.0 mg), **4** (3.5 mg), **5** (2.3 mg), **7** (4.0 mg), **8** (3.0 mg), **9** (3.7 mg), and **10** (6.0 mg). Fr.4-8 (3.5 g) was submitted to repeated chromatography over silica gel ($CHCl_3$/MeOH, 50:1 and 5:1) and semi-preparative HPLC ($CNCH_3/H_2O$, 60–70%; flow rate 3.0 mL/min; UV detector, 205 nm) to give compound **3** (t_R = 16.2 min, 8.5 mg). Fr.4-10 (5.9 g) was isolated by semi-preparative HPLC ($CNCH_3/H_2O$, 50–70%; flow rate 3.0 mL/min; UV detector, 205 nm) to afford compound **2** (t_R = 21.8 min, 1.8 mg).

Karavilagenin F (1): white amorphous powder; [α]24.9 D-60.9° (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.62) nm; IR (KBr) ν_{max} 3441, 2925, 2875, 1631, 1465, 1412, 1382, 1272, 1115, 1082 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 525 $[M + Na]^+$; HREIMS, *m/z* 502.3647 (calcd for $C_{31}H_{50}O_5$ $[M]^+$, 502.3658).

Karaviloside XII (2): white amorphous powder; [α]24.9 D-16.5° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.09) nm; IR (KBr) ν_{max} 3441, 2923, 2871, 1725, 1630, 1445, 1382, 1251,1155, 1080, 1038 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 683 $[M + Na]^+$; HREIMS, *m/z* at 660.4249 (calcd for $C_{38}H_{60}O_9$ $[M]^+$, 660.4237).

Karaviloside XIII (3): white amorphous powder; [α]25.1 D-11.2° (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.23) nm; IR (KBr) ν_{max} 3441, 2926, 2873, 1632, 1448, 1382, 1083, 1034 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 641$[M + Na]^+$; HREIMS, *m/z* at 618.4123 (calcd for $C_{36}H_{58}O_8$ $[M]^+$, 618.4132).

Momordicine VI (4): white amorphous powder; [α]24.9 D + 28.1° (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.71) nm; IR (KBr) ν_{max} 3440, 2934, 2877, 1710, 1631, 1465, 1449, 1381, 1085 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 509$[M + Na]^+$; HREIMS, *m/z* at 486.3715 (calcd for $C_{31}H_{50}O_4$ $[M]^+$, 486.3709).

Momordicine VII (5): white amorphous powder; [α]25.0 D + 51.0° (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.89) nm; IR (KBr) ν_{max} 3440, 2954, 2933, 2877, 1728, 1629, 1463, 1442, 1382, 1273, 1153, 1075, 1019, 977 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS, *m/z* at 595 $[M + Na]^+$; HREIMS, *m/z* at 572.3703 (calcd for $C_{34}H_{52}O_7$ $[M]^+$, 572.3713).

Momordicine VIII (6): white amorphous powder; [α]24.9 D-8.2° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.51) nm; IR (KBr)ν_{max} 3441, 2951, 2924, 2875, 2854, 1733, 1631, 1462, 1442, 1381, 1272, 1153, 1078, 1021, 980 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS, *m/z* at 609 $[M + Na]^+$; HREIMS, *m/z* at 586.3862 (calcd for $C_{35}H_{54}O_7$ $[M]^+$, 586.3870).

2.4. The CD spectrum

The CD spectrum was obtained at room temperature in DMSO and step scans were collected at 1 nm/step with an integration time of 1 s over the range 250–600 nm. For the CD standard measurements the chiral *vic*-diols (compound **1**, 1 mg) was dissolved in DMSO (concentration: 0.6667 mg/mL), and then added in suitable $[Mo_2(OAc)_4]$ so that situ formed Mo-complexes of **1** at ligand to metal molar rations was about 1.0:1.2. Due to the real complex structure as well as the concentration of the chiral complex formed in solution was not known, the CD data are presented as the $\Delta\varepsilon'$ values. These $\Delta\varepsilon'$ values are calculated in the usual way as $\Delta\varepsilon' = \Delta A / c \times d$, where *c* is the molar concentration of the chiral ligand, assuming 100% complexation (A = absorption; d = path length of the cell). $\Delta\varepsilon'$ is expressed in $[M^{-1} \times cm^{-1}]$ units.

2.5. Cytotoxicity assay

The cytotoxic activities of compounds **3**, **4** and **6** were evaluated against five human cancer cell lines: HL-60, SMMC-7721, A-549, MCF-7, and SW480, which were acquired from ATCC (Manassas, VA, USA) and were cultured in RPMI-1640 or DMEM medium (Hy-clone, Logan, UT, USA) by means of 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H-tetra-zolium (MTS) assay, an analog of MTT. Briefly, cells in RPMI-1640 or DMEM medium supplemented with 10% fetal bovine serum (FBS, Hyclone) were seeded into a 96-well cell culture plate in the presence of various concentrations of test compounds at 37 °C in a 5% CO_2 humidified incubator for 48 h. Reduced MTS crystals were dissolved in DMSO, and the optical density (OD) was measured at 490 nm in a 96-well microtiter plate reader (Bio-Rad 680) to determine cell growth inhibition. Cisplatin (MW 300) and paclitaxel were included as a positive control. The IC_{50} value of each compound was calculated by Reed and Muench's method.

3. Results and discussion

The methanol extracts of the stems and leaves of *M. charantia* were partitioned into petroleum ether, $CHCl_3$, and n-BuOH fractions. The $CHCl_3$ and n-BuOH mixed extract fraction was repeated subjected to silica gel, RP-18 column

Table 1
^{1}H NMR data (δ) of compounds **1–6** (*J* in Hz).

Position	1 [b, d]	2 [b, d]	3 [a, d]	4 [b, d]	5 [b, d]	6 [a, c]
Aglycon moiety						
1	1.50 m, 1.69 m	1.38 m, 1.46 m	1.40 m, 1.49 m	1.77 m, 2.10 overlapped	1.57 overlapped, 1.66 m	1.34 overlapped, 1.71 overlapped
2	1.82 m, 1.91 m	1.76 m, 1.85 m	1.80 m, 1.86 m	2.10 overlapped, 1.99 m	1.17 m, 1.86 m	1.34 overlapped, 1.89 m
3	3.58 brs	5.00 brs	3.54 m	3.85 brs	5.02 t	4.80 brs
6	5.65 dd (3.6, 9.6)	6.17 d (9.8)	6.11 d (9.7)	6.31 d (4.2)	6.16 d (3.9)	5.85 d (3.5)
7	6.11 dd (1.8, 9.6)	5.58 dd (3.5, 9.8)	5.58 dd (3.6, 9.7)	4.39 d (5.4)	4.31 d (5.3)	3.96 d (4.3)
8	3.07 brs	2.30 brs	2.25 brs	2.40 s	2.33 s	1.99 brs
10	2.49 dd (5.9, 12.2)	2.29 dd (5.4, 12.3)	2.29 dd (5.5, 12.1)	2.72 m	2.60 dd (4.2, 12.7)	2.50 d (11.7)
11	1.65 m, 1.76 m	1.39 m, 1.67 m	1.33 m, 1.61 m	1.61 m, 2.77 m	1.50 m, 2.68 m	1.54 m, 2.21 m
12	1.64 m	1.57 m	1.53 m, 1.59 m	1.66 m, 1.73 m	1.49 overlapped, 1.56 overlapped	1.22 m, 1.64 m
15	1.23 m	1.17 m, 1.26 m	1.15 m, 1.20 m	1.34 overlapped	1.27 m, 1.32 m	1.34 overlapped
16	1.46 m, 1.96 m	1.50 m, 1.97 m	1.46 m, 1.91 m	1.92 overlapped, 1.34 overlapped	1.29 m, 1.92 m	1.71 overlapped, 1.86 m
17	1.56 dd (8.9, 18.3)	1.50 m	1.46 m	1.50 m	1.49 overlapped	1.45 m
18	0.94 s	0.82 s	0.78 s	0,92 s	0.82 s	0.84 s
19	4.78 s	3.61 d (8.0) 3.73 d (8.0)	3.51 d (8.4) 3.60 d (8.4)	10.71 s	10.51 s	9.72 s
20	2.18 m	2.17 m	2.10 m	1.93 overlapped	1.43 m	1.49 m
21	1.13 d (6.3)	1.20 d (6.8)	1.13 d (6.3)	1.10 d (6.4)	0,94 d (6.3)	0.87 d (5.7)
22	1.62 m, 2.06 m	1.24 m, 2.12 m	1.20 m, 2.06 m	1.07 m, 1.87 m	1.83 m, 2.20 m	1.75 m, 2.14 m
23	4.28 dd (5.3, 9.6)	4.98 m	4.88 m	4.15 td (2.8, 9.8)	5.91 m	5.45 dd (6.6, 14.4)
24	4.57 d (5.3)	5.66 d (8.1)	5.64 d (8.7)	5.22 d (8.8)	5.93 m	5.35 d (15.7)
26	5.13 s, 5.48 s	1.70 s	1.66 s	1.75 s	1.53 s	1.21 s
27	2.04 s	1.78 s	1.69 s	1.71 s	1.54 s	1.21 s
28	0.87 s	1.21 s	1.36 s	1.51 s	1.11 s	1.07 s
29	1.40 s	0.95 s	0.88 s	1.20 s	1.24 s	1.13 s
30	0.89 s	0.90 s	0.85 s	0.88 s	0.74 s	0.71 s
19-OMe	3.38 s					
23-OMe				3.29 s		
25-OMe						3.10 s
3-$COCH_3$		2.09 s				
1″-OMe					3.68 s	3.69 s
2″					3.59 s, 3.60 s	3.31 s
sugar moiety						
1′		5.02 d (7.8)	5.40 d (7.8)			
2′		4.09 m	4.70 t			
3′		4.30 m	3.97 dd (2.2, 5.4)			
4′		4.28 m	4.22 dd (2.5, 9.3)			
5′		3.94 m	4.40 m			
6′		4.40 dd (5.0, 11.6) 4.53 dd (2.0, 11.6)	4.33 dd (4.7, 11.0) 4.45 dd (2.3, 11.0)			

[a] Recorded at 500 MHz.
[b] Recorded at 600 MHz.
[c] Data were determined in $CDCl_3$.
[d] Data were determined in C_5D_5N.

and semi-preparative HPLC to yield six new cucurbitane-type triterpenoids (**1**–**6**), in addition to four known ones (**7**–**10**).

Compound **1** was isolated as a white powder. Its molecular formula, $C_{31}H_{50}O_5$, was determined by HREIMS (*m/z* 502.3647, $[M]^+$, calcd 502.3658). The IR spectrum showed characteristic bands of hydroxy group (3441 cm^{-1}) and double bonds (1631 cm^{-1}). The ^1H and ^{13}C NMR (Tables 1 and 2) in combination with DEPT and HSQC spectra of **1** showed 31 carbon signals, indicating that it was a cucurbitane-type triterpene skeleton with an methoxyl [δ_H 3.38 (3H, s), δ_C 58.0, q]. In addition, the downfield region in ^{13}C-DEPT of **1** showed a disubstituted double bond [δ_C 133.2 (d), 132.1 (d)], an oxygenated quaternary carbon at δ 87.4 (s), and a hemiacetal signal at δ_C 112.6 (d) (proton resonance at δ_H 4.78, s), explicated the typical 6-en, 5, 19-hemiacetal ring. As mentioned above, the ^{13}C-NMR data of four rings in **1** were similar to those of in (19*R*,23*E*)-5β,19-epoxy-19-methoxycucurbita-6,23,25-trien-β-ol [19] and of the side chain in **1** were virtually identical to those of in (3β,7β)-3,7,22,23-tetrahydroxycucurbita-5,24-dien-19-al [4]. The OH group was at C-3 was further elucidated by HMBC experiment, in which the cross peak of H_3-28 (δ_H 0.87) with C-3 (δ_C 76.6), C-4 (δ_C 38.1) and C-5 (δ_C 87.4) was observed. The presence of a *vic*-diol system at C-23 and C-24 was indicated by the ^1H-^1H COSY coupling between H-23 (δ_H 4.28) and H-24 (δ_H 4.57) and was supported by the HMBC correlations

Table 2
^{13}C NMR data (δ) of compounds **1**–**6**.

Position	1 [b, d]	2 [b, d]	3 [a, d]	4 [b, d]	5 [b, d]	6 [a, c]
Aglycon moiety						
1	18.2 t	19.1 t	18.0 t	22.2 t	22.2 t	21.5 t
2	28.3 t	26.1 t	28.0 t	30.3 t	28.1 t	27.3 t
3	76.6 d	77.3 d	76.3 d	76.1 d	80.3 d	79.3 d
4	38.1 s	38.1 s	37.6 s	42.2 s	40.5 s	39.7 s
5	87.4 s	85.2 s	87.6 s	146.1 s	144.0 s	144.3 s
6	133.2 d	134.1 d	132.4 d	124.7 d	125.2 d	123.1 d
7	132.1 d	130.6 d	131.4 d	66.1 d	65.7 d	65.9 d
8	42.5 d	52.6 d	52.2 d	51.1 d	51.2 d	48.1 d
9	48.9 s	45.7 s	45.6 s	51.0 s	50.8 s	49.4 s
10	41.4 d	40.3 d	39.3 d	37.3 d	36.6 d	36.7 d
11	23.9 t	24.4 t	23.9 t	23.1 t	22.9 t	23.1 t
12	31.4 t	31.6 t	31.2 t	30.0 t	29.7 t	28.8 t
13	45.9 s	46.0 s	45.6 s	46.3 s	46.1 s	45.1 s
14	48.7 s	49.3 s	48.8 s	48.7 s	48.6 s	47.6 s
15	34.1 t	33.8 t	33.4 t	35.3 t	35.2 t	34.4 t
16	28.9 t	28.7 t	28.3 t	28.5 t	26.6 t	25.9 t
17	52.1 d	51.7 d	51.3 d	51.5 d	50.5 d	49.7 d
18	15.3 q	15.4 q	15.0 q	15.4 q	15.4 q	14.7 q
19	112.6 d	80.4 t	79.9 t	208.4 d	208.0 d	207.7 d
20	33.1 d	33.1 d	32.7 d	33.3 d	36.9 d	35.9 d
21	19.3 q	19.7 q	19.4 q	19.5 q	19.3 q	18.6 q
22	39.8 t	44.2 t	43.7 t	43.8 t	39.9 t	39.2 t
23	70.3 d	75.7 d	75.2 d	75.1 d	124.6 d	128.1 d
24	80.4 d	129.5 d	129.3 d	128.3 d	142.2 d	136.7 d
25	148.2 s	132.7 s	132.0 s	135.0 s	70.1 s	74.7 s
26	112.3 t	26.3 q	25.8 q	26.3 q	31.3 q	25.9 q
27	19.7 q	18.7 q	18.2 q	18.6 q	31.3 q	25.6 q
28	24.6 q	20.7 q	21.0 q	26.7 q	27.3 q	26.9 q
29	21.3 q	25.3 q	24.6 q	27.7 q	25.5 q	24.7 q
30	20.4 q	20.4 q	20.2 q	18.6 q	18.4 s	17.7 q
3-$COCH_3$		171.4 s				
3-$COCH_3$		21.8 q				
19-OMe	58.0 q					
23-OMe				56.0 q		
25-OMe						50.1 q
1″-OMe					52.8 q	52.4 q
1″					168.0 s	166.8 s
2″					42.3 t	41.4 t
3″					166.9 s	165.8 s
Sugar moiety						
1′		104.7 d	101.6 d			
2′		76.1 d	73.2 d			
3′		79.4 d	72.8 d			
4′		72.2 d	69.3 d			
5′		78.8 d	75.7 d			
6′		63.3 t	63.3 t			

[a] Recorded at 125 MHz.
[b] Recorded at 150 MHz.
[c] Data were determined in $CDCl_3$.
[d] Data were determined in C_5D_5N.

observed from H-23 to C-24 (δ_C 80.4) and from H-24 to C-26 (δ_C 112.3) and C-27 (δ_C 19.7).

Numerous researches recently indicated a simple and unequivocal method which mixed a transition metal chelate reagent dimolybdenum tetraacetate [$Mo_2(OAc)_4$] and chiral *vic*-diols in DMSO and record the CD spectrum in the 250–600 nm spectral range to determine the absolute configuration of acyclic chiral *vic*-diols [20–24]. The resulting CD spectrum suggested that the absolute configuration of *vic*-diols is correlated the sign of the O–C–C–O torsional angle with the sign of Cotton effects (CEs) occurring in the 300–400 nm spectral range (helicity rule [22], determined the molecular stereostructure of *vic*-diols). According to the rule, a positive (negative) sign of CEs occurring on around 310 and 400 nm is related to a positive (negative) sign of O–C–C–O torsional angle of the diol unit. According to the proposed method, the CD spectrum of *vic*-diols in **1** was acquired (Fig. 2). In the CD spectrum, two positive CD bands at around 300 and 400 nm can be seen and the positive sign of these CD bands corresponds to a positive torsion angle of the O–C–C–O moiety in its preferred antiperiplanar conformation, as presented in the bottom of Fig. 2. Therefore, the configuration at C-23 and C-24 was deduced as *S*, *S*.

The stereochemistry at C-19 of **1** was deduced to be *R* by comparing the ^{13}C-NMR chemical shifts of C-8 (δ_C 42.5), C-10 (δ_C 41.4), and C-19 (δ_C 112.6) with those of (19*R*,23*E*)-5β,19-epoxy-19-methoxycucurbita-6,23,25-trien-β-ol [19] [C-8 (δ_C 41.7), C-10 (δ_C 40.5), and C-19 (δ_C 112.1)] and (19*S*,23*E*)-5β,19-epoxy-19-methoxycucurbita-6,23-diene-3β,25-diol [25] [C-8 (δ_C 49.8), C-10 (δ_C 37.9), and C-19 (δ_C 114.7)]. The *R* configuration of C-19 was also supported by the ROE correlation between H-1β and H-19 [10]. The orientation of 3β-OH was also testified by ROESY experiment, following the ROE correlation of H-3 and H_3-28. Thus, compound **1** was concluded

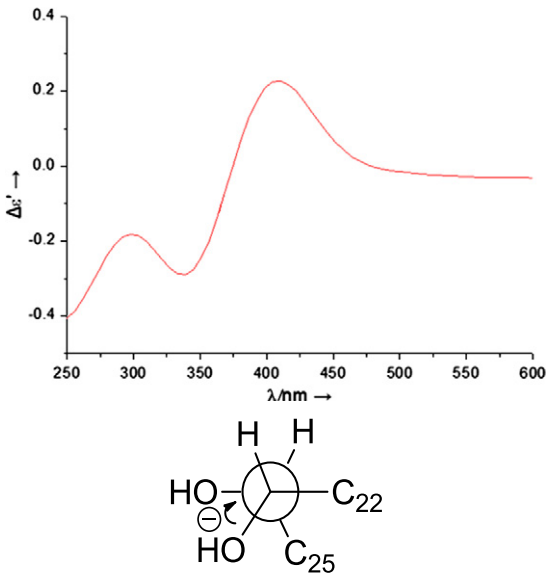

Fig. 2. CD spectra of *in situ* formed Mo-complexes of *vic*-diol **1** recorded in DMSO and preferred conformation of the diols in the chiral Mo-complexes (bottom).

to be (19*R*,23*S*,24*S*)-5β,19-epoxy-19-methoxycucurbita-6,25-dien-3β,23,24-triol (karavilagenin F).

Compound **2**, a white powder, has a molecular formula of $C_{38}H_{60}O_9$ as determined by the positive HREIMS ion at *m/z* $[M]^+$ (calcd 660.4237). The IR spectrum displayed absorptions at 3441, 1725, and 1630 cm^{-1}, suggesting the presence of hydroxy group, ester, and double bonds, respectively. The ^{1}H and ^{13}C NMR spectra of **2** showed 38 carbon signals, which were attributed to a triterpene skeleton, a glucosyl moiety [δ_C 104.7 (d), 76.1 (d), 79.4 (d), 72.2 (d), 78.8 (d), 63.3 (t); δ_H 5.02 (1H, d, J = 7.8 Hz), H-1′] [10,26], and an acetyl group [δ_H 2.09 (3H, s), δ_C 21.8 (q), 171.4 (s)]. The ^{1}H and ^{13}C NMR data of triterpene aglycone were closely similar to those of karavilagenin E [27]. By detailed analysis of its HSQC and HMBC spectra, a proton signal at δ 5.00 attributing to H-3 correlated with the acetyl carbon at δ_C 171.4 in the HMBC spectrum, which suggested that the acetyl group was located at C-3. Meanwhile, conjugation at C-23 of the aglycon with the glucosyl moiety was supported from the HMBC experiment, which showed cross correlations between H-23 (δ_H 4.98) and C-1′ (δ_C 104.7) of the glucose moiety, C-20 (δ_C 33.1), C-22 (δ_C 44.2), C-24 (δ_C 129.5), C-25(δ_C 132.7), and between H-1′ (δ_H 5.02) and C-23 (δ_C 75.7). The configuration of the glucosyl moiety was further determined to be β based on the coupling constant (δ_H 5.02 d, J = 7.8 Hz) of the anomeric proton. The 3β-OAc and 5β,19-oxygen bridge were explicated by ROESY correlations of H-3/H_3-28, of H-8/H_3-18 and of H-8/H_2-19. Consequently, compound **2** was assumed to be 5β,19-epoxycucurbita-6,24-dien-3β-acetoxy-23-ol 23-O-β-D-glucopyranoside (karaviloside XII).

Compound **3**, a white powder, has an elemental composition of $C_{36}H_{58}O_8$ (calcd 618.4132), based on the result of HREIMS at *m/z* (618.4123) $[M]^+$. Its IR spectrum showed the presence of hydroxy group (3441 cm^{-1}) and double bands (1632 cm^{-1}). The 1D-NMR spectrum resolved 36 carbon signals due to a triterpene skeleton and a β-D-allopyranosyl moiety [δ_C 101.6 (d), 73.2 (d), 72.8 (d), 69.3 (d), 75.7 (d), 63.3 (t); δ_H 5.40 (1H, d, J = 7.8 Hz), H-1′] [7,10]. Comparison of the ^{1}H and ^{13}C NMR data of **3** with those of **2** showed similarities except that the β-D-glucopyranosyl moiety at C-23 and the acetoxyl group at C-3 in **2** were replaced by a β-D-allopyranosyl functionality and a hydroxy group in **3**, respectively. Above deduction were further determined by following ^{1}H-^{1}H COSY and HMBC correlations: 1) the ^{1}H-^{1}H COSY correlation of olefinic proton signal at δ 5.64 attributing to H-24 with H_3-26 (δ_H 1.66) suggested that the trisubstituted double bond was located C-23; 2) the HMBC correlations from H-23 (δ_H 4.88) to C-1′ (δ_C 101.6), C-20 (δ_C 32.7), C-22 (δ_C 43.7), C-24 (δ_C 129.3), C-25(δ_C 132.0), and from H-1′ (δ_H 5.40) to C-23 (δ_C 75.2) demonstrated the presence of β-D-allopyranosyl function at C-23; 3) the HMBC correlation from the signal of H_3-28 (δ_H 1.36) and H_3-29 (δ_H 0.88) to C-3 (δ_C 76.3) revealed that there existed a hydroxy group at C-3. Therefore, compound **3** was assigned as 5β,19-epoxycucurbita-6,24-dien-3β,23-diol 23-O-β-D-allopyranoside (karaviloside XIII).

Compound **4** was obtained as white powder. It exhibited a $[M]^+$ ion at *m/z* 486.3715 (calcd 486.3709) in the HREIMS, compatible with the molecular formula of $C_{31}H_{50}O_4$. The IR spectrum showed characteristic absorptions for hydroxy group (3440 cm^{-1}), aldehyde group (1710 cm^{-1}) and double

bonds (1631 cm^{-1}). The 1D-NMR spectra of **4** revealed seven methyls [including a secondary methyl (δ_H 1.10, 3H, d, J = 6.4 Hz)], a methoxyl [δ_H 3.29 (3H, s), δ_C (56.0, q)], two trisubstituted double bond [δ_C 146.1 (s), 124.7 (d), 128.3 (d), 135.0 (s)] and an aldehyde carbon at δ_C 208.4 (d). These suggested that the 1D-NMR data of **4** were similar to those of momordicine I [28] except that the hydroxy group at C-23 in momordicine I was replaced by a methoxyl group in **4**, which was established by the carbon signal at C-23 in which **4** was shifted downfield to δ 75.1 (Δδ 8.5) by comparison with that of in momordicine I. Furthermore, β-orientation of 7-OH, 3-OH, and 19-CHO were carried out by ROESY correlations of H-3/H_3-28, H-7/H_3-30, H-19/H-1, and H-19/H-8. The above evidence established that **4** possesses the structure 3β,7β-dihydroxy-23-methoxycucurbita-5,24-dien-19-al (momordicine VI).

Compound **5** was isolated as white powder, and was analyzed for molecular formula $C_{34}H_{52}O_7$ based on its HREIMS (m/z, 572.3703 $[M]^+$, calcd 572.3713). The IR spectrum displayed diagnostic absorptions at hydroxy group (3440 cm^{-1}), ester (1728 cm^{-1}) and trans double bands (1629 and 977 cm^{-1}). The 1D-NMR spectra of **5** exhibited the presence of seven methyls (including a secondary methyl), an oxymethyl, two di- and trisubstituted double bonds, two esterified carbons and an aldehyde carbon. Detailed analysis of the ^{1}H- and ^{13}C-NMR data associated with HSQC and HMBC spectra suggested that compound **5** was similar to 3β,7β,25-trihydroxycucurbita-5,(23*E*)-dien-19-al [26] except that the hydroxy group at C-3 in the above known compound was replaced by the $-OCOCH_2COOCH_3$ fragment in **5**. The HMBC correlations of H-2″ (δ_H 3.59, 3.60) with C-1″ (δ_C 168.0) and C-3″ (δ_C 166.9), and of OMe-1″ (δ_H 3.68) with C-1″ (δ_C 168.0) confirmed the presence of $-OCOCH_2COOCH_3$ fragment, and the HMBC correlations of H-3 (δ_H 5.02) to C-3″ (δ_C 166.9), C-4, Me-28, and Me-29 further suggested that the fragment was connected to C-3. The correlations of H-3/H_3-28, H-7/H_3-30, H-19/H-1, and H-19/H-8 were observed in the ROESY experiments of **5**, suggesting 3-OH, 7-OH and C-19 as β-oriented. Thus, compound **5** was elucidated as 3β,7β,25-trihydroxycucurbita-5, 23(*E*)-dien-19-al 3-propanedioic acid, monomethyl ester (momordicine VII).

Compound **6**, a white powder, was determined as $C_{35}H_{54}O_7$ from its HREIMS m/z 586.3862, $[M]^+$, calcd 586.3870). Comparison of the 1D-NMR spectroscopic data of **6** with those of **5** (Tables 1 and 2) indicated strong resemblance in all signal except that the C-23, C-24, and C-25 signals in the spectra of **6** were shifted downfield to δ_C 128.1 (Δδ 3.5) and 74.7 (Δδ 4.6) and upfield to δ_C 136.7 (Δδ 3.5), respectively, which suggested that the extra methoxyl group was revealed at C-25. And this was confirmed by the HMBC correlations of 25-OMe (δ_H 3.10) and C-25 (δ_C 74.7, s). The ROESY spectrum showed that compounds **6** and **5** possessed the similar configurations. Furthermore, in the ^{1}H NMR spectra, H-23 at δ_H 5.45 (1H, dd, J = 6.6, 14.4 Hz) and H-24 at δ_H 5.35 (1H, d, J = 15.7 Hz) confirmed that the configuration of Δ^{23} was *E*. Thus, compound **6** was established to be 3β,7β-dihydroxy-25-methoxycucurbita-5,23(*E*)-dien-19-al-3-propanedioic acid, monomethyl ester (momordicine VIII).

The cytotoxic activities of compounds **3**, **4** and **6** were evaluated against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480). Compounds **3** and **4** showed all the IC_{50} > 40.0 μmol/L; compound **6** exhibited a weak cytotoxic effect against all the above five cell lines with the IC_{50} values at 16.2, 20.3, 20.5, 16.9, and 14.3 μmol/L, respectively.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.03.005.

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