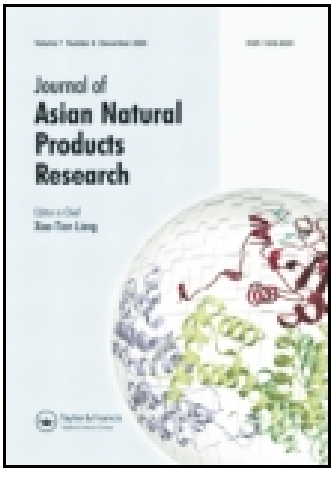

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Cucurbitane-type triterpenoids from the leaves of *Momordica charantia*

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Phytochemical investigation of the ethanol extract from the leaves of *Momordica charantia* L. led to the isolation of two new (**1**, **2**) and four known (**3**–**6**) cucurbitane-type triterpenoids. Their structures were elucidated on the basis of extensive analyses of spectroscopic data including IR, UV, MS, 1D, and 2D NMR. Also the absolute configurations of momordicines I (**3**) and II (**4**) were determined for the first time by application of the modified Mosher's method, acid hydrolysis, and GC analysis.

Keywords: *Momordica charantia*; Cucurbitaceae; cucurbitane-type triterpenoids; absolute configuration

1. Introduction

Momordica charantia L. (Cucurbitaceae), called bitter melon or 'kugua,' is a tropical or subtropical vine widely distributed in Asian, African, and Caribbean. It has been extensively used as a folk remedy in various Asian and African traditional medicine systems for a long time [1]. In China, it has been used to treat toothache, diarrhea, furuncle, and diabetes [2]. In recent decades, *M. charantia* has been widely investigated for its antidiabetic properties. There have been more than 70 cucurbitacins and cucurbitane glycosides isolated from the roots, stems, fruits, seeds, leaves, and vines of the plant [3]. As part of our ongoing search of chemical constituents for functional foods or traditional medicine, we carried out phytochemical investigations on *M. charantia*. As a result, we have obtained two new (**1**, **2**) and four known (**3**–**6**) cucurbitane-type triterpenoids from the ethanol extract of *M. charantia* leaves (Figure 1). Momordicines I (**3**) and II (**4**) were first isolated in 1984 [4] and, to the best of our knowledge, the absolute configuration at C-23 remains undetermined. We determined that the absolute configurations of momordicines I (**3**) and II (**4**) at C-23 were *R* by the modified Mosher's method and acid hydrolysis.

2. Results and discussion

Compound **1** was isolated as a white powder. The molecular formula was determined as $C_{31}H_{50}O_4$ based on its HR-ESI-MS at *m/z* 509.3615 $[M + Na]^+$. The IR spectrum exhibited absorption bands at 3444 and 1644 cm^{-1}, indicating the presence of hydroxyl and double bond. The ^1H NMR spectrum of **1** showed the presence of seven methyl groups [δ_H 0.85 (3H, s), 0.95 (3H, s), 1.01 (3H, d, $J = 6.4$ Hz), 1.09 (3H, s), 1.25 (3H, s), 1.67 (3H, d, $J = 1.0$ Hz), 1.70 (3H, d, $J = 0.8$ Hz)], a methoxyl [δ_H 3.26 (3H, s)], and two olefinic protons [δ_H 5.17 (1H, dt, $J = 8.4$, 1.4 Hz), 5.99 (1H, d, $J = 4.6$ Hz)].

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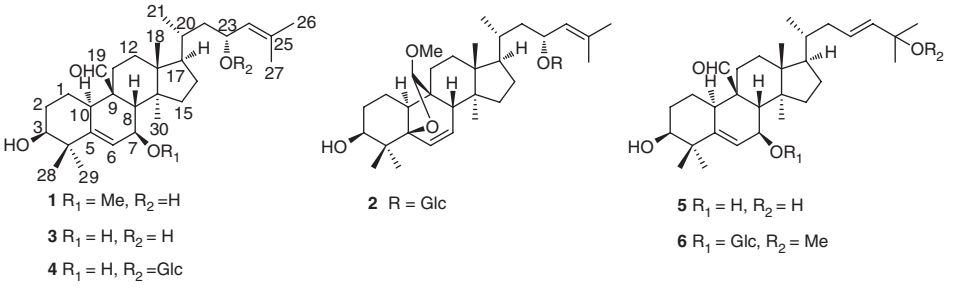

Figure 1. Structures of compounds **1**–**6**.

The ^{13}C NMR spectrum displayed an aldehyde group (δ_C 209.7), three oxymethine carbons (δ_C 66.7, 76.8, 77.2), and two double bonds (δ_C 122.2, 130.6, 133.6, 148.6). Comparison of the ^{1}H and ^{13}C NMR data of **1** (Table 1) with those of momordicine I [4] showed that they were similar, except for the presence of an additional methoxyl (δ_C 56.3) in **1**, and an oxy-methine at δ_C 65.6 (C-7) in momordicine I shifted to δ_C 76.8 in **1**, indicating that C-7 was substituted by a methoxyl. And this was confirmed by the HMBC correlation (Figure 2) between the methoxyl at δ_H 3.26 and C-7 at δ_C 76.8. According to the absolute configuration of momordicine I (**3**) determined by the modified Mosher's method as follows, C-23 in **1** was also assigned as *R*. Therefore, compound **1** was deduced and named as charantin A.

Compound **2** was also isolated as a white powder. Its molecular formula was determined as $C_{37}H_{60}O_9$ according to the HR-ESI-MS at *m/z* 671.4106 $[M + Na]^+$. The IR spectrum exhibited absorption bands at 3415 and 1648 cm^{-1}, indicating the presence of hydroxyl and double bond. The ^{1}H spectrum of **2** showed the presence of seven methyls [δ_H 0.88 (3H, s), 0.89 (3H, s), 0.90 (3H, s), 0.99 (3H, d, $J = 6.4$ Hz), 1.20 (3H, s), 1.70 (6H, s)], a methoxyl [δ_H 3.40 (3H, s)], and three olefinic protons [δ_H 5.24 (1H, d, $J = 8.8$ Hz), 5.58 (1H, dd, $J = 9.8$, 3.6 Hz), 5.96 (1H, dd, $J = 9.8$, 2.1 Hz)]. The ^{13}C NMR spectrum showed two oxymethine carbons at δ_C 76.9 and 77.8, and two C = C double bonds at δ_C 129.0, 132.1, 134.0, and 134.3 (Table 1). The NMR data were in good agreement with those of (19*R*,23*E*)-5β,19-epoxy-19-methoxycucurbita-6,23,25-trien-3β-ol [5], except for the signals of the side-chain moiety, suggesting that **2** had a 19-methoxy-5β,19-epoxy-3β-hydroxy-10α-cucurbitan-6-ene ring system [5]. The NMR data of **2** also showed the presence of a β-D-glucopyranosyl moiety [δ_H 4.26 (d, $J = 7.8$ Hz, H-1′)], and a long chain of C-atoms [δ_C 18.5 (C-26), 19.9 (C-21), 26.2 (C-27), 33.8 (C-20), 44.3 (C-22), 76.9 (C-23), 129.0 (C-24), 134.3 (C-25)], which

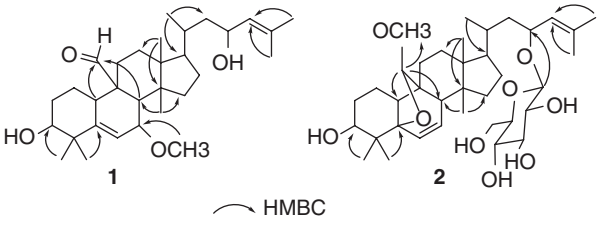

Figure 2. Key HMBC correlations of **1** and **2**.

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** (in CD_3OD, δ in ppm, J in Hz).

	1		**2**	
No.	δ_H	δ_C	δ_H	δ_C
1	1.49 (m), 1.56 (m)	22.2	1.51 (m), 1.61 (m)	18.5
2	1.72 (m), 1.97 (m)	30.0	1.66 (m), 1.83 (m)	28.3
3	3.54 (br s)	77.2	3.37 (m)	77.8
4	–	42.6	–	38.5
5	–	148.6	–	88.1
6	5.99 (d, 4. 6)	122.2	5.58 (dd, 9.8, 3.6)	132.1
7	3.52 (br d, 5.8)	76.8	5.96 (dd, 9.8, 2.1)	134.0
8	2.02 (br s)	46.9	2.90 (br s)	43.3
9	–	51.3	–	49.3
10	2.59 (m)	37.7	2.46 (t, 9.8)	42.1
11	1.45 (m), 2.37 (m)	23.4	1.65 (m), 1.80 (m)	24.4
12	1.26 (m), 1.72 (m)	30.4	1.65 (m), 1.65 (m)	32.1
13	–	47.1	–	46.5
14	–	48.9	–	49.7
15	1.38 (m), 1.38 (m)	36.0	1.35 (m), 1.35 (m)	34.8
16	1.41 (m), 1.94 (m)	28.7	1.43 (m), 1.96 (m)	29.2
17	1.53 (m)	52.2	1.46 (m)	52.3
18	0.95 (s)	15.4	0.90 (s)	15.3
19	9.77 (s)	209.7	4.70 (s)	113.4
20	1.73 (m)	33.9	1.81 (m)	33.8
21	1.01 (d, 6.4)	19.4	0.99 (d, 6.4)	19.9
22	0.97 (m), 1.63 (m)	45.7	1.00 (m), 1.80 (m)	44.3
23	4.42 (td, 9.5, 3.3)	66.7	4.53 (m)	76.9
24	5.17 (dt, 8.4, 1.4)	130.6	5.24 (d, 8.8)	129.0
25	–	133.6	–	134.3
26	1.67 (d, 1.0)	18.3	1.70 (s)	18.5
27	1.70 (d, 0.8)	26.0	1.70 (s)	26.2
28	1.09 (s)	27.9	1.20 (s)	20.9
29	1.25 (s)	26.1	0.88 (s)	24.6
30	0.85 (s)	19.0	0.89 (s)	20.5
7-OMe	3.26 (s)	56.3		
19-OMe			3.40 (s)	58.2
1′			4.26 (d, 7.8)	103.9
2′			3.12 (m)	75.7
3′			3.29 (m)	78.6
4′			3.29 (m)	71.8
5′			3.14 (m)	78.0
6′			3.65 (dd, 11.8, 5.3) 3.80 (dd, 11.8, 2.5)	63.0

Notes: Overlapped signals were reported without designating multiplicity. Spectra were measured at 400 MHz.

were almost superimposable on those of momordicine II (**4**) [4].

The HMBC correlation (Figure 2) between H-21 at δ_H 0.99 and C-17 at δ_C 52.3 suggested that the long chain was connected to C-17. Besides, the HMBC correlation between H-1′ at δ_H 4.26 and C-23 at δ_C 76.9 indicated that the β-D-glucopyranosyl moiety was connected to C-23. Consequently, the structure of **2** was determined and named as charantin B.

The known compounds were identified as momordicines I (**3**) and II (**4**) [4], 3β,7β,25-trihydroxycucurbita-5,(23*E*)-dien-19-al (**5**) [6], and momordicoside K (**6**) [7], by comparing their physical and spectroscopic data with the literature.

In addition, the absolute configurations of momordicines I (**3**) and II (**4**) at C-23 were determined as *R* configuration by the modified Mosher's method and acid hydrolysis.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined by a Jasco P-1020 digital polarimeter (Jasco, Shanghai, China). UV spectra were recorded using a Jasco V-550 UV/VIS spectrophotometer (Jasco). A Jasco FT/IR-480 plus FT-IR spectrometer was used to determine IR spectra (Jasco). ESI-MS data were obtained with a Finnigan LCQ Advantage Max mass spectrometer (Thermo Electron, Massachusetts, USA). HR-ESI-MS data were measured on an Agilent 6210 LC/MSD TOF mass spectrometer (Waters, Massachusetts, USA). 1D and 2D NMR spectra were performed on a Bruker AV-400 spectrometer (Bruker, Faellanden, Switzerland) using TMS as the internal standard, and chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Open column chromatography (CC) was performed using macroporous resin (Diaion HP-20, Shanghai, China), silica gel (200–300 mesh; Qingdao Marine Chemical Plant, Qingdao, China), ODS silica gel (50 μm; YMC, Tokyo, Japan), and Sephadex LH-20 (25–100 μm, Fluka, Buchs, Switzerland). Thin-layer chromatography (TLC) was performed using precoated silica gel plates (GF254, Yantai Jiangyou Silica Gel Technology Development Co., Ltd., Yantai, China). Analytical HPLC was carried out on a Waters chromatograph equipped with an evaporative light-scattering detector, a P680 pump, and a C_{18} reversed-phase column (Cosmosil, 5 μm, 4.6 mm × 250 mm; Nacalai Tesque, Kyoto, Japan). Preparative HPLC was performed on a Varian Prostar system equipped with UV detectors (Varian, California, USA) and a preparative Cosmosil C_{18} column (20 mm × 250 mm; Nacalai Tesque). All the reagents were purchased from Tianjin Damao Chemical Company (Damao, Tianjin, China).

3.2 Plant material

The leaves of *M. charantia* were collected in an experimental field of College of Resources and Environment, South China Agricultural University in August, 2005 and authenticated by Prof. Mao-Xin Zhang. A voucher specimen (2010110723) has been deposited at the Laboratory of Insect Ecology, College of Resources and Environment, South China Agricultural University.

3.3 Extraction and isolation

Air-dried leaves of *M. charantia* (1.0 kg) were macerated with 90% EtOH under room temperature. The combined extracts were evaporated *in vacuo* to afford a crude extract which was suspended in water and partitioned successively with petroleum ether (19 g), EtOAc (20 g), and *n*-BuOH (60 g). The EtOAc fraction was chromatographed on a silica gel column, using mixtures of petroleum ether, EtOAc, and MeOH with increasing polarity as eluents to yield nine fractions. Fraction 7 (0.54 g) was purified by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and then by preparative HPLC to yield compound **1** (MeOH/H_2O, 70:30, 8 ml/min, 210 nm; t_R 45.3 min, 8.7 mg) and compound **2** (MeOH/H_2O, 70:30, 8 ml/min, 210 nm; t_R 22.5 min, 6.8 mg), respectively. Fraction 6 (2.5 g) was subjected to repeated chromatograph on silica gel ($CHCl_3$/MeOH, 100:1 → 85:15) to yield **3** (50 mg) and **5** (25 mg). Fraction 8 (4.6 g) was applied to silica gel CC (CHCl3/MeOH, 100:1 → 80:20) to yield **4** (30 mg).

3.3.1 Compound *1*

White powder; $[\alpha]_D^{27.0} + 176.0$ ($c = 0.67$, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 210

(3.6); IR (KBr) ν_{max}: 3444, 2954, 1697, 1644, 1454, 1378, 1081 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS *m/z*: 509.3615 $[M + Na]^+$ (calcd for $C_{31}H_{50}O_4Na$, 509.3601).

3.3.2 *Compound 2*

White powder; $[\alpha]_D^{27.0}$ −52.2 ($c = 1.0$, MeOH); UV (MeOH) λ_{max} (nm) ($\log \varepsilon$): 208 (3.9); IR (KBr) ν_{max}: 3415, 2941, 1648, 1449, 1378, 1077 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS *m/z*: 671.4106 $[M + Na]^+$ (calcd for $C_{37}H_{60}O_9Na$, 671.4130).

3.4 *Preparation of (R)- and (S)-MTPA esters (3a and 3b) of momordicine I (3)*

The absolute configuration of momordicine I (**3**) was determined for the first time by the modified Mosher's method [8].

Compound **3** (1.5 mg) was dissolved in deuterated pyridine (0.5 ml) in a clean NMR tube under a gentle nitrogen stream and a ^1H NMR spectrum was recorded as a reference. Then (*S*)-α-methoxy-α-(trifluoromethyl) phenylacetic chloride (5 μl) was added into the NMR tube under the N_2 gas stream and immediately shaken until uniformly mixed. After sealing with parafilm, the reaction NMR tube was kept for 8 h at room temperature. The ^1H NMR spectrum, recorded directly from the reaction NMR tube, showed the production of the corresponding (*R*)-MTPA ester (**3a**). In an identical fashion, another portion of compound **3** (1.5 mg) was reacted in a second NMR tube with (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic chloride (5 μl) at room temperature for 8 h using deuterated pyridine (0.5 ml) as the solvent, to afford the (*S*)-MTPA ester of **3** (**3b**).

Compound **3a**: ^1H NMR (300 MHz, C_5D_5N): δ 5.262 (1H, br s, H-3), 6.056 (1H, overlapped, H-6), 5.676 (1H, d, $J = 5.3$ Hz, H-7), 2.040 (1H, s, H-8), 0.782 (3H, s, H-18), 9.939 (1H, s, H-19), 1.015 (3H, d, $J = 4.9$ Hz, H-21), 6.114 (1H, m, H-23), 5.409 (1H, m, H-24), 1.868 (3H, br s, H-26), 1.741 (3H, br s, H-27), 1.205 (3H, s, H-28), 1.205 (3H, s, H-29), 0.722 (3H, s, H-30).

Compound **3b**: ^1H NMR (300 MHz, C_5D_5N): δ 5.181 (1H, br s, H-3), 6.033 (1H, overlapped, H-6), 5.703 (1H, d, $J = 5.5$ Hz, H-7), 2.045 (1H, s, H-8), 0.886 (3H, s, H-18), 9.813 (1H, s, H-19), 1.067 (3H, d, $J = 6.2$ Hz, H-21), 6.068 (1H, m, H-23), 5.245 (1H, m, H-24), 1.866 (3H, br s, H-26), 1.712 (3H, br s, H-27), 1.292 (3H, s, H-28), 1.212 (3H, s, H-29), 0.810 (3H, s, H-30).

3.5 *Acid hydrolysis and GC analysis of 4*

Solution of compound **4** (1.5 mg) was hydrolyzed with 1.5 ml of 2 N HCl (MeOH) for 3 h at 80°C. The reaction mixture was dissolved in H_2O and extracted with CH_2Cl_2. The aglycone was elucidated as **3** by comparison of their optical rotations ($[\alpha]_D^{25.4} + 83.9$ ($c = 1.0$, MeOH) for the aglycone, $[\alpha]_D^{25.4} + 86.7$ ($c = 1.0$, MeOH) for compound **3**), TLC, HPLC, and NMR spectral data. The aqueous layer was concentrated and dried by N_2. Then, 1 ml of dry pyridine and 2 mg of L-cysteine methylester hydrochloride were added to the residue. The mixture was heated at 60°C for 2 h and concentrated to dryness with N_2. *N*-(trimethylsilyl) imidazole (0.2 ml) was added into the mixture and then kept at 60°C for 1 h. At last, the solution was diluted with H_2O (1 ml) and extracted with hexane (1 ml). The organic layer was analyzed using GC under the following conditions: AT-SE-30 (0.5 μm × 0.32 μm × 30 m), detector: FID, column temperature: 220°C, detector temperature: 270°C, injector temperature: 270°C, and carried gas: N_2. The standard D-glucose was subjected to the same reaction and GC analysis under the above conditions. As a result, D-glucose was detected from the hydrolysates of **4**.

Acknowledgments

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