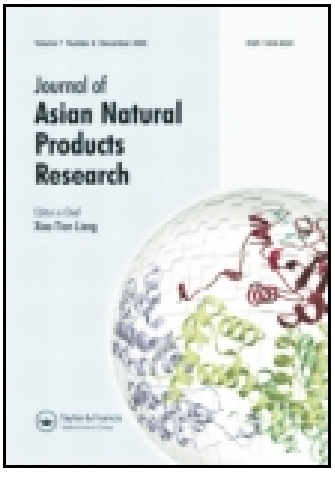

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Two new cucurbitane triterpenoids from the seeds of *Momordica charantia*

Lin Ma[a], Ai-Hua Yu[ab], Li-Li Sun[a], Wan Gao[a], Meng-Meng Zhang[a], Ya-Lun Su[a], Hua Liu[b], Teng-Fei Ji[a]* and Di-Zao Li[c]*

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Two new cucurbitane triterpenoids **1** and **2** were isolated, together with six known compounds, from the seeds of *Momordica charantia* L. The structures of new compounds were determined to be 3-*O*-{[β-D-galactopyranosyl(1 → 6)]-*O*-β-D-galactopyranosyl}-23(*R*), 24(*R*), 25-trihydroxycucur-bit-5-ene (**1**), 3-*O*-[β-D-galacto-pyranosyl]-25-*O*-β-D-galactopyranosyl-7(*R*), 22(*S*), 23(*R*), 24(*R*), 25-pentahydroxy-cucurbit-5-ene (**2**), respectively. Their structures were elucidated by the combination of mass spectrometry, one- and two-dimensional NMR experiments and chemical reactions.

Keywords: *Momordica charantia*; seeds; cucurbitane triterpenoids; Cucurbitaceae

1. Introduction

The fruit, seeds, aerial parts, and roots of *Momordica charantia* L. (Cucurbitaceae) have been used to treat diabetes. Over 100 compounds have been isolated from the fruits, seeds, leaves, canes, and roots of this genus, mainly cucurbitane- and oleanene-type triterpenes, Recent studies have discovered many new cucurbitane triterpenoids from the fruits and the roots of *M. charantia* L. [1–3], and cucurbitane triterpenoids from the fruits of this genus showed a significant enhancement of glucose disposal and increases in fatty acid oxidation. The cucurbitane triter-penoids from *M. charantia* may provide novel leads for the development of a new class of AMPK-activating agents [4]. But a little research on the seeds of *M. charantia* was reported [5,6]. To search for the hypoglycemic principles, we have exam-ined the ethanolic extracts of *M. charantia* purchased from Anguo of Hebei Province, China. We report the isolation and structural elucidation of two new cucurbi-tane triterpenoids from the seeds of *M. charantia* (Figure 1).

2. Results and discussion

We have examined the ethanol extract of the seed of *M. charantia* and have isolated two new cucurbitane triterpenoids **1** and **2**, together with six known compounds momocharaside A (**3**), momocharaside B (**4**) [5], goyasaponin II (**5**), goyasaponin I (**6**) [7], momordicoside C (**7**), and momordicoside E (**8**) [6]. Compounds **3**–**6** were isolated for the first time from the seeds of *M. charantia* L., whose structures were determined by comparing their

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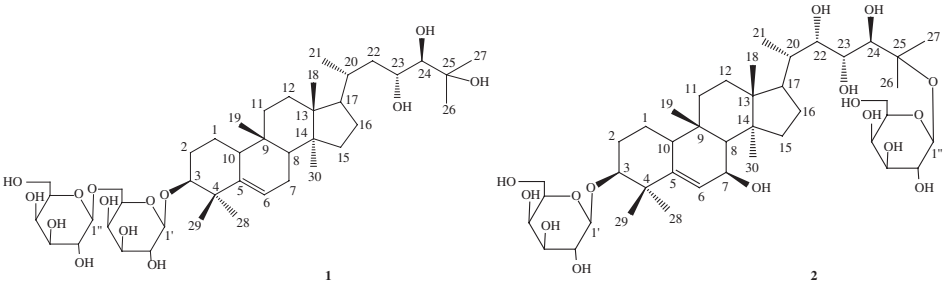

Figure 1. Structures of compounds **1** and **2** from the seeds of *M. charantia*.

physical properties and the spectral data with those reported in the literature.

Compound **1** was obtained as a white powder. The positive-ion quasimolecular ion peak was observed at *m/z* 823 $[M + Na]^+$ and the molecular formula $C_{42}H_{72}O_{14}$ was determined by positive-ion HR-ESI-MS measurement (*m/z* 823.4814 $[M + Na]^+$; calcd 823.4813). The ^1H NMR spectrum of **1** (Table 1) showed signals for seven tertiary methyl groups at δ 0.73, 0.81, 0.82, 1.07, 1.45, 1.57, and 1.58 (each 3H, s), a secondary methyl at δ 1.25 (3H, d, $J = 6.6$ Hz), and one olefinic proton at δ 5.41 (1H, d, $J = 5.5$ Hz). Acid hydrolysis of **1** furnished galactose, which was identified by HPLC comparison with an authentic sample. In the ^{13}C NMR spectrum of **1** (Table 1), 30 aglycon carbon signals and 12 sugar signals were found, indicating that **1** was a triterpene saponin. The ^1H and ^{13}C NMR (Table 1) spectra of compound **1** exhibited two sugar anomeric protons assignable to two β-D-galactopyranosyl moiety (δ_H 5.25, 1H, d, $J = 8.0$ Hz; δ_H 4.86, 1H, d, $J = 8.0$ Hz). The identities of the sugar chain sequence were determined by a combination of DEPT and two-dimensional NMR experiments (such as HMQC and HMBC). The sequence of the sugar chain was deduced from the HMBC correlations of the anomeric proton signal H-1′ at δ_H 5.25 (1H, d, $J = 8.0$ Hz) and C-3 at δ_C 87.3, H-1″ at δ_H 4.86 (1H, d, $J = 8.0$ Hz) and C-6′ at δ_C 70.7. The ^{13}C NMR data of the aglycone of compound **1** were very similar to those of 23(*R*), 24(*R*), 25-trihydroxycucurbit-5-ene 3-*O*-{[β-glucopyranosyl(1 → 6)]-*O*-β-glucopyranosyl}-25-*O*-β-gluco-pyranoside [1]. The analysis of HMBC spectrum (Figure 2) confirmed that **1** was 3-*O*-{[β-D-galactopyranosyl(1 → 6)]-*O*-β-D-galactopyranosyl}-23(*R*), 24(*R*), 25-trihydroxycucurbit-5-ene.

Compound **2** was obtained as a white powder. The positive-ion quasimolecular ion peak was observed at *m/z* 855 $[M + Na]^+$ and the molecular formula $C_{42}H_{72}O_{16}$ was determined by positive-ion HR-ESI-MS measurement (*m/z* 855.4713 $[M + Na]^+$; calcd 855.4716). The ^1H NMR spectrum of **2** (Table 1) showed signals for seven tertiary methyl groups at δ 0.78, 0.81, 0.83, 1.03, 1.44, 1.71, and 1.82 (each 3H, s), a secondary methyl at δ 1.37 (3H, d, $J = 6.5$ Hz), and one olefinic proton at δ 5.42 (1H, d, $J = 4.2$ Hz). Acid hydrolysis of **2** furnished galactose, which was identified by HPLC comparison with an authentic sample. In the ^{13}C NMR spectrum of **2** (Table 1), 30 aglycone carbon signals and 12 sugar signals were found, indicating that **2** was a triterpene saponin. The ^1H and ^{13}C NMR (Table 1) spectra of compound **2** exhibited two sugar anomeric protons assignable to two β-D-galactopyranosyl moiety (δ_H 5.21, 1H, d, $J = 8.0$ Hz; δ_H 4.91, 1H, d, $J = 7.5$ Hz). The identities of the sugar chain sequence were determined by a combination of DEPT and two-dimensional NMR exper-

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** (pyridine-d_5, δ in ppm and J in Hz).

	1		**2**	
No.	^{13}C NMR (DEPT)	^{1}H NMR	^{13}C NMR (DEPT)	^{1}H NMR
1	22.7 (CH_2)	1.59–1.61, 1.82-1.84 (2H, 2m)	24.6 (CH_2)	1.45–1.47, 1.68–1.70 (2H, 2m)
2	29.1 (CH_2)	2.56–2.60 (2H, m)	28.0 (CH_2)	2.37–2.39 (2H, m)
3	87.3 (CH)	3.72 (1H, brs)	86.9 (CH)	3.66 (1H, brs)
4	41.7 (C)		42.2 (C)	
5	143.2 (C)		140.6 (C)	
6	118.7 (CH)	5.41 (1H, d, $J = 5.5$)	122.7 (CH)	5.42 (1H, d, $J = 4.2$)
7	24.5 (CH_2)	1.62–1.79 (2H, 2m)	88.6 (CH)	4.00–4.02 (1H, m)
8	43.8 (CH)	1.62 (1H, overlap)	45.7 (CH)	1.66 (1H, overlap)
9	34.7 (C)		32.8 (C)	
10	38.5 (CH)	2.22 (1H, overlap)	45.0 (CH)	2.22 (1H, overlap)
11	32.5 (CH_2)	1.30–1.33, 1.52–1.57 (2H, 2m)	71.6 (CH_2)	1.32–1.36, 1.54–1.56 (2H, 2m)
12	30.8 (CH_2)	1.30–1.44, 1.52–1.57 (2H, 2m)	30.8 ($CH_{2)}$	1.52–1.60 (2H, 2m)
13	46.5 (C)		47.1 (C)	
14	49.6 (C)		49.2 (C)	
15	35.2 (CH)	1.13 (1H, overlap)	35.5 (CH)	1.12 (1H, overlap)
16	27.4 (CH_2)	1.91–1.97, 1.40–1.45 (2H, 2m)	30.2 (CH_2)	1.53–1.55, 2.41–2.44 (2H, 2m)
17	47.8 (CH)	2.04 (1H, q, $J = 10.0$)	48.0 (CH)	2.00 (1H, q, $J = 10.5$)
18	15.6 (CH_3)	0.81 (3H, s)	15.5 (CH_3)	0.83 (3H, s)
19	28.2 (CH_3)	0.82 (3H, s)	32.1 (CH_3)	0.81 (3H, s)
20	43.4 (CH)	2.15–2.17 (1H, m)	43.2 (CH)	2.18–2.21 (1H, m)
21	13.4 (CH_3)	1.25 (3H, d, $J = 6.5$)	15.2 (CH_3)	1.37 (3H, d, $J = 6.5$)
22	33.3 (CH_2)	2.14–2.16, 1.92–1.94 (2H, 2m)	71.8 (CH)	4.66 (1H, d, $J = 4.5$)
23	70.1 (CH)	4.84 (1H, s)	75.5 (CH)	4.24 (1H, s)
24	75.8 (CH)	4.39 (1H, overlap)	75.8 (CH)	4.36 (1H, overlap)
25	72.7 (C)		80.1 (C)	
26	26.3 (CH_3)	1.57 (3H, s)	24.5 (CH_3)	1.82 (3H, s)
27	26.4 (CH_3)	1.58 (3H, s)	23.5 (CH_3)	1.71 (3H, s)
28	28.5 (CH_3)	1.07 (3H, s)	29.4 (CH_3)	1.44 (3H, s)
29	25.9 (CH_3)	1.45 (3H, s)	25.4 (CH_3)	1.03 (3H, s)

(*Continued*)

Table 1 – *continued*

	1		**2**	
No.	^{13}C NMR (DEPT)	^{1}H NMR	^{13}C NMR (DEPT)	^{1}H NMR
30	18.1 (CH_3)	0.73 (3H, s)	19.1 (CH_3)	0.78 (3H, s)
1′	107.5 (CH)	5.25 (1H, d, $J = 8.0$)	105.4 (CH)	5.21 (1H, d, $J = 8.0$)
2′	73.3 (CH)	4.05 (1H, overlap)	73.9 (CH)	4.01 (1H, overlap)
3′	75.7 (CH)	4.16 (1H, overlap)	74.2 (CH)	3.87 (1H, overlap)
4′	72.2 (CH)	4.35 (1H, overlap)	72.8 (CH)	4.13 (1H, overlap)
5′	75.9 (CH)	4.00 (1H, overlap)	77.1 (CH)	4.19 (1H, overlap)
6′	70.7 (CH_2)	4.95 (1H, overlap), 4.00 (1H, overlap)	61.5 (CH_2)	4.46 (1H, dd, $J = 12.0$, 2.5), 4.30 (1H, overlap)
1″	105.8 (CH)	4.86 (1H, d, $J = 8.0$)	96.3 (CH)	4.91 (1H, d, $J = 7.5$)
2″	72.3 (CH)	4.05 (1H, overlap)	73.9 (CH)	3.99 (1H, overlap)
3″	73.3 (CH)	4.36 (1H, overlap)	74.2 (CH)	3.86 (1H, overlap)
4″	70.4 (CH)	4.35 (1H, overlap)	70.2 (CH)	4.11 (1H, overlap)
5″	76.3 (CH)	4.16 (1H, overlap)	76.9 (CH)	4.16 (1H, overlap)
6″	63.3 (CH_2)	4.53 (1H, overlap), 4.40 (1H, overlap)	61.3 (CH_2)	4.58 (1H, dd, $J = 12.0$, 2.0), 4.37 (1H, overlap)

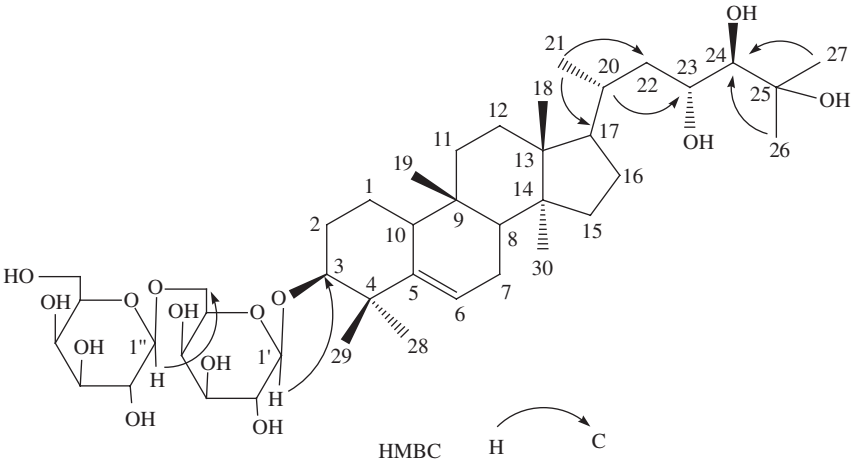

Figure 2. Key HMBC correlations of compound **1**.

iments (such as HMQC and HMBC). The sequence of the sugar chain was deduced from the HMBC correlations of the anomeric proton signal H-1′ at δ_H 5.21 (1H, d, $J = 8.0$ Hz) and C-3 at δ_C 86.9, H-1″ at δ_H 4.91 (1H, d, $J = 7.5$ Hz) and C-25 at δ_C 80.1 (Figure 3). The ^{13}C NMR data of the aglycone of compound **2** were very similar to those of 3-*O*-[β-D-glucopyranosyl(1/6)-β-D-glucopyranosyl]-25-*O*-β-D-glucopyranosyl-22(*S*), 23(*R*), 24(*R*), 25-tetrahydroxycucurbit-5-ene [4]. The relative configuration of C-7 in compound **2** was assigned on the basis of NOESY correlation of H-7 and CH_3-30, so 7-OH was β-configuration. The absolute configuration of C-7 was *R*. So the structure of compound **2** was determined as 3-*O*-β-D-galactopyranosyl-25-*O*-β-D-galactopyranosyl-7(*R*), 22(*S*), 23(*R*), 24(*R*), 25-pentahydroxyl cucurbit-5-ene.

The structures of compounds **3**–**8** were identified by comparison of their spectral data with those reported in the literature.

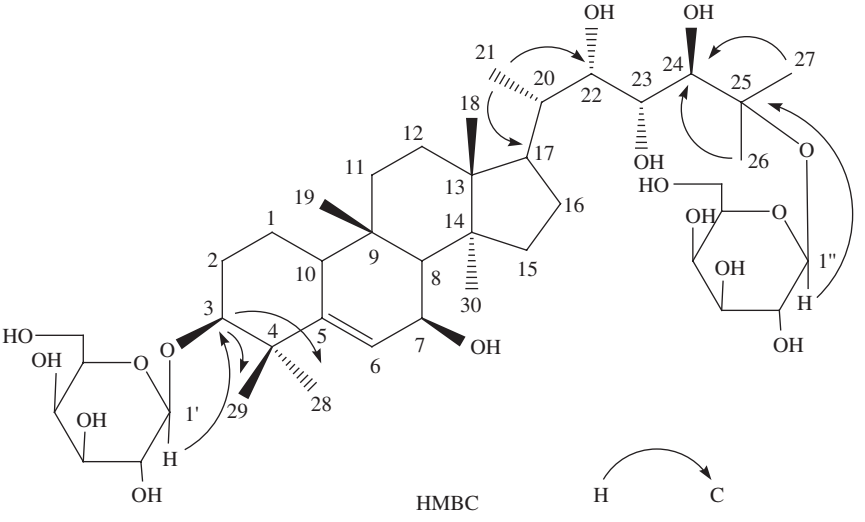

Figure 3. Key HMBC correlations of compound **2**.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter (Perkin-Elmer, Foster City, CA, USA). UV spectra were recorded with a UV-2401 spectrometer (Shimadzu Corp., Kyoto, Japan). IR spectra were obtained on a Nicolet 5700 IR spectrometer (Thermo corp., West Palm Beach, FL, USA). NMR spectra were recorded on a VARIAN INOVA 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer (Varian, Palo Alto, CA, USA). ESI-MS was carried out with Angilent 1100 LC/MSD (Angilent Technologies Ltd, Santa Clara, CA, USA). For column chromatography, silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China), ODS (40–60 μm; YMC Co. Ltd, Kyoto, Japan), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used. The analytical HPLC was carried out on Angilent 1200 LC with DAD, and the preparative HPLC was carried out on Shimadzu LC-20A (Shimadzu Corp., Kyoto, Japan) with YMC-Pack ODS column (20 mm × 250 mm, 10 μm; YMC Co. Ltd).

3.2 Plant material

The seeds of *M. charantia* were purchased from Anguo of Hebei Province in 2011, and identified by Associate Professor Lin Ma at Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (No. ID-S-2547) has been deposited at our laboratory in the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

3.3 Extraction and isolation

The seeds of *M. charantia* (15.0 kg) were defatted three times by petroleum ether (90 l each time), then extracted three times under reflux in 95% ethanol (90 l each time), and the combined solution was concentrated under reduced pressure to yield an extract (1.6 kg). The alcohol extract was partitioned successively with $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble extract (175 g) was subjected to silica gel column chromatography with gradient elution [$CHCl_3$–MeOH 20:1 (2 l), $CHCl_3$–MeOH 9:1 (2 l), $CHCl_3$–MeOH 4:1 (2 l), $CHCl_3$–MeOH–H_2O 7:3:0.5 (2 l), $CHCl_3$–MeOH–H_2O 6:4:0.5 (2 l), MeOH (2 l)] to give 11 fractions (Fr1–11). Compounds **1** (16 mg) and **2** (20 mg) from Fr8 (9.2 g) were purified by repeated column chromatography over silica gel column chromatography with gradient elution [$CHCl_3$–MeOH–H_2O 7:3:0.5], ODS (MeOH–H_2O, 65:35), and Sephadex LH-20 (MeOH). Further fractionation of Fr6 by silica gel column chromatography with gradient elution [$CHCl_3$–MeOH–H_2O 7:3:0.5 (4 l), $CHCl_3$–MeOH–H_2O 6:4:0.5 (4 l)] gave six subfractions (Fr1′–6′). Compounds **3** (15 mg), **4** (460 mg), **5** (24 mg), and **6** (17 mg) from Fr6′ (11.4 g) were isolated by repeated column chromatography over silica gel with $CHCl_3$–MeOH–H_2O 6:4:0.5, ODS (MeOH–H_2O, 60:40), and Sephadex LH-20 (MeOH). Compound **7** (21 mg) from Fr5′ (4.9 g) was separated by repeated column chromatography over silica gel with $CHCl_3$–MeOH–H_2O 7:3:0.5, Sephadex LH-20 (MeOH), and preparative HPLC (MeOH–H_2O, 65:35; flow rate: 7 ml/min; t_R: 20.03 min; detection wavelengths: 210 nm). Compound **8** (12 mg) from Fr5 (6.4 g) was isolated by repeated normal phase silica gel with $CHCl_3$–MeOH–H_2O 6:4:0.5, Sephadex LH-20 (MeOH), and preparative HPLC (MeOH–H_2O, 60:40; flow rate: 7 ml/min; t_R: 26.80 min; detection wavelengths: 210 nm).

3.3.1 Compound **1**

White powder. $[\alpha]_D^{25} + 100.0$ ($c = 1.0$, MeOH); UV (MeOH) λ_{max} nm (log ε): 233 (0.41), 327 (0.57); IR (KBr) ν_{max}:

3402, 2954, 1695, 1603, 1281, 1170, 1020, 817 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; HR-ESI-MS: *m/z* 823.4814 $[M + Na]^+$ (calcd for $C_{42}H_{72}O_{14}Na$, 823.4813).

3.3.2 *Compound 2*

White powder. $[\alpha]_D^{25} + 84.0$ ($c = 1.0$, MeOH); UV (MeOH) λ_{max} nm ($\log \varepsilon$): 232 (0.39), 329 (0.53); IR (KBr) ν_{max}: 3413, 2948, 1696, 1601, 1283, 1168, 819 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; HR-ESI-MS: *m/z* 855.4713 $[M + Na]^+$ (calcd for $C_{42}H_{72}O_{16}Na$, 855.4716).

3.4 *Acid hydrolysis of compounds 1 and 2*

About 80 μl of D-glucose, D-galactose, L-rhamnose, D-xylose, and L-arabinose aqueous solutions (each 2 mg/ml) was mixed with 80 μl of 0.5-mol/l PMP CH_3OH solution and 80 μl of 0.3-mol/l NaOH aqueous solution. The mixtures were heated at 70°C for 30 min and then cooled to room temperature to which 80 μl of 0.3-mol/l HCl aqueous solution was added. The resulted mixture was extracted with $CHCl_3$ (0.5 ml, 3 times), and the water fractions were identified by HPLC analysis (Phenomenex C18, 250 mm × 4.6 mm, 5 μm); flow phase: A: CH_3CN–20 mmol/l NH_4OAc aqueous solution (15:85), B: CH_3CN–20 mmol/l NH_4OAc aqueous solution (40:60); flow rate: 1.2 ml/min; gradient elution, 0 → 20 min, volume fraction of B from 0% to 60%; detection wavelengths: 245 nm; sample volume: 20 μl.

Compounds **1** (5 mg) and **2** (4 mg) were heated in an ampule with 2 ml of aqueous 2-M HCl-1,4-dioxane (1:1) at 80°C for 6 h. The aglycone was extracted with chloroform, and the aqueous layer was evaporated under reduced pressure and taken as preparations of the normal sugar derivatives. Then, compounds **1** and **2** only furnished D-galactose ($t_R = 13.20$ min), which were identified by HPLC analysis of the derivatives [8] with standard D-galactose derivative, the absolute configurations of the galactose units in compounds **1** and **2** were determined as D.

Acknowledgments

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