A New Cucurbitane Triterpene in Acid-treated Ethanol Extract from *Momordica charantia*

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Abstract: **Objective** To study the chemical constituents in the acid-hydrolyzed ethanol extract from *Momordica charantia*. **Methods** The ethanol extract from *M. charantia* was hydrolyzed by 36% HCl and the hydrolysate was isolated by silica gel column chromatography and preparative HPLC. The structures of the isolated compounds were identified by spectral analyses, physical constants, and chemical evidences. **Results** Two cucurbitane triterpenoids were isolated and identified as 5β,19-epoxy-cucurbita-6,22*E*,24-trien-3β-ol (**1**) and cucurbita-6,22(*E*),24-trien-3β-ol-19,5β-olide (**2**). **Conclusion** Compound **1** is a new compound.

Key words: cucurbitane; 5β,19-epoxy-cucurbita-6,22(*E*),24-trien-3β-ol; hydrolysate; *Momordica charantia*; triterpene
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Introduction

The plant *Momordica charantia* L. (Cucurbitaceae), commonly called "bitter melon" in English and "*kugua*" in Chinese, has long been used as a favorite vegetable as well as a folk medicine to treat dysmenorrhea, eczema, jaundice, leprosy, and leucorrhea (Grover and Yadav, 2004). Previous investigations had indicated that the cucurbitane triterpenes obtained from this species showed anticancer activity (Basch, Gabardi, and Ulbricht, 2003). With the aim to search for potential antitumor promoters, we recently studied *Panax ginseng* C. A. Meyer, *Gynostemma pentaphyllum* (Thunb.) Makino, *P. notoginseng* C. A. Meyer, and *P. japonicus* C. A. Meyer var. *major* (Burk) C. Y. Wu et K. M Feng, and found a triterpene, i.e. 25-OCH_3-PPD, from the hydrolysate of *P. ginseng*, which showed stronger anticancer activity (Zhao *et al*, 2007).

In the course of our study on the structure-activity relationship between *M. charantia* constituents and the anticancer activity, we phytochemically investigated the chemical constituents in the hydrolysate of ethanol extract from *M. charantia*, from which one new cucurbitane triterpene was isolated and identified as 5β,19-epoxy-cucurbita-6,22*E*,24-trien-3β-ol (**1**), along with one known compound, cucurbita-6,22(*E*),24-trien-3β-ol-19,5β-olide (**2**). This paper reports the isolation and structural elucidations of the two compounds.

Materials and methods

Materials and instruments

^{1}H-NMR and ^{13}C-NMR Spectra: Bruker AV—600 and ARX—300 Spectrometer; HR-TOF-ESI-MS: BIC Micro TOF-Q Mass Spectrometer, in *m/z*; IR Spectra: Bruker IFS — 55 Spectrophotometer, KBr pellets; Preparative HPLC (Beijing CXTH3000 system): P3000 Pump, UV3000 Spectrophotometric Detector at 203 nm, Daisogel C_{18} Reversed-phase Column (250 mm × 30 mm, 10 μm; flow rate 8.0 mL/min); Optical Rotations: Perkin-Elmer Polarimeter; Column chromatography: Silica gel (200 — 300 mesh, Qingdao Hailang Chemical Co.).

Plant material

The dried fruits of *Momordica charantia* L. were

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collected from Guangxi province, China, and authenticated by Prof. LU Jin-cai of Shenyang Pharmaceutical University. A voucher specimen (No. 20060102) of the plant was deposited at our laboratory.

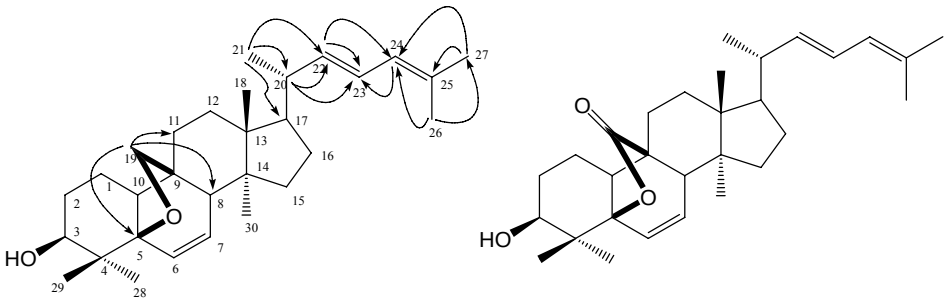
Extraction and isolation

The dried fruits of *M. charantia* (1.2 kg) were extracted for three times with 20-fold 70% EtOH on reflux. The EtOH extract (320 g) was separated by a Diaion D-101 (2 kg) column eluted with H_2O, 70% EtOH, and EtOH sequentially. 70% EtOH fraction (205 g) was dissolved in MeOH (200 L) and hydrolyzed with 36% HCl (100 L) at 60 ℃ for 6 h. The reaction mixture was washed with water, extracted with acetone, and then evaporated to give the acid hydrolyzed product (50 g). The hydrolyzed product was submitted to 30-fold silica gel chromatography eluted with petroleum ether-acetone (20:1, 10:1, 5:1, 3:1, 2:1, 1:1, acetone) and yielded 11 fractions (Frs. A—K). Fr. G was submitted to Sephadex LH-20 chromatography with $CHCl_3$-MeOH (1:1) and preparative HPLC with CH_3OH-H_2O (85:15) to provide compound **1** (6 mg), Fr. E was further separated by a preparative HPLC eluted with 87% MeOH and obtained compound **2** (12 mg). The structures of compounds **1** and **2** are shown in Fig. 1.

Results

Compound **1** was obtained as white amorphous powder, $[\alpha]_D^{20}$ −72.6 (*c* 0.01, MeOH). Its molecular formula, $C_{30}H_{46}O_2$, was deduced from the HR-TOF-ESI-MS: *m/z* 461.3389 $[M + Na]^+$ (Calcd. 461.3396). IR ν_{max}^{KBr} showed absorption at 3424 cm^{-1} (OH). The UV spectrum exhibited a conjugated double bond group based on the absorption at 235 nm. The ^{1}H-NMR data (Table 1) showed signals due to seven methyl groups at δ 0.80, 0.86, 0.89, 1.37, 1.74, 1.75 (s, each 3H), and 1.06 (brd, *J* = 7.1 Hz); five olefinic protons at δ 5.48 (dd, *J* = 15.0, 9.0 Hz), 5.59 (dd, *J* = 9.6, 3.6 Hz), 5.93 (d, *J* = 10.8 Hz), 6.14 (d, *J* = 9.6 Hz), and 6.36 (dd, *J* = 15.0, 10.8 Hz); three oxygen-bearing protons at δ 3.54 (brs), 3.55 (brd, *J* = 8.4 Hz), and 3.63 (brd, *J* = 8.4 Hz). ^{13}C-NMR spectrum showed signals (Table 1) assignable to the tetracylic part were very similar to those of 5β,19-epoxycucurbita-6,23*E*-diene-3β,25-diol (Harinantenaina *et al*, 2006), while the signals of side chain were different. The carbon signals at δ 138.9, 124.7, 126.2, 132.5 and their coupling constants in the ^{1}H-NMR spectrum suggested that a conjugated double bond existed in the side chain. The locations of the two double bonds were revealed to be Δ22, 24 by the 2D NMR experiments. In the HMBC spectrum, the following cross peaks could be found: from H-20 to C-22, C-23, from H-21 to C-17, C-20, C-22, from H-22 to C-21, C-22, C-24, from H-23 to C-22, from H-24 to C-22, C-23, from H-26 to C-24, C-25, and from H-27 to C-24, C-26. Thus, the structure of the side chain was deduced as nearly the same as those of 5β,19-epoxycucurbita-6,22(*E*),24-triene-3β,19-diol (Hsu *et al*, 2011). The 3-OH was β position according to the cross peak of H-3 (δ_H 3.54) and H-28 (δ_H 1.37) in NOESY spectra, moreover the correlations of H-8 (δ_H 2.30) and H-18 (δ_H 0.80), H-19 (δ_H 3.63, 3.55) could confirm the orientation of 5β,19-hemiacetal ring. Thus, the structure of compound **1** could be concluded as 5β,19-epoxy-cucurbita-6,22*E*,24-trien-3β-ol.

Compound **2** was isolated as white amorphous powder, $[\alpha]_D^{20}$ −67.5 (*c* 0.01, MeOH). IR ν_{max}^{KBr} showed absorption at 3435 and 1762 cm^{-1} indicating hydroxy and carbonyl groups. The absorption at 233 nm in the UV spectrum indicated a conjugated-bond group. By comparison, it could be found that the NMR data of compound **2** (Table 1) were in good agreement



1 **2**

Fig. 1 Structures of compounds 1 and 2 and key HMBC of compound 1

Table 1 ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz in C_5D_5N) data for compounds 1 and 2

Positions	Compound **1**		Compound **2**	
	δ_C	δ_H	δ_C	δ_H
1	18.7	1.45 (m), 1.75 (m)	18.5	1.64 (m), 1.32 (m)
2	28.1	1.90 (m, 2H)	26.5	1.88 (m, 2H)
3	76.2	3.54 (brs)	75.3	3.47 (brs)
4	37.6		37.1	
5	87.6		85.4	
6	131.3	6.14 (brd, *J* = 9.6 Hz)	131.1	6.20 (brdd, , *J* = 9.6, 1.8 Hz)
7	132.4	5.59 (dd, *J* = 9.6, 3.6 Hz)	133.4	5.72 (dd, *J* = 9.6, 3.0 Hz)
8	52.2	2.30 (m)	44.5	2.53 (1H, brs)
9	45.5		51.1	
10	39.3	2.31 (m)	40.0	2.66 (m)
11	23.9	1.66 (m), 1.42 (m)	21.7	1.79 (m), 2.27 (m)
12	31.1	1.61 (m), 1.50 (m)	29.9	1.71 (m), 1.26 (m)
13	45.7		45.1	
14	48.9		47.8	
15	33.4	1.21 (m), 1.19 (m)	33.2	1.32 (m, 2H)
16	28.7	1.87 (m, 2H)	27.8	1.82 (m), 1.31 (m)
17	50.5	1.53 (m)	50.4	1.56 (m)
18	15.3	0.80 (s, 3H)	14.8	0.96 (s, 3H)
19	80.0	3.63 (brd, *J* = 8.4 Hz), 3.55 (brd, *J* = 8.4 Hz)	181.5	
20	40.7	2.19 (m)	40.3	2.19 (1H, m)
21	21.0	1.06 (brd, *J* = 7.1 Hz)	20.5	1.04 (brd, *J* = 6.6 Hz)
22	138.9	5.48 (dd, *J* = 15.0, 9.0 Hz)	138.1	5.39 (dd, *J* = 15.0, 8.8 Hz)
23	124.9	6.36 (dd, *J* = 15.0, 10.8 Hz)	124.4	6.16 (dd, *J* = 15.0, 10.8 Hz)
24	126.2	5.93 (brd, *J* = 10.8 Hz)	125.1	5.75 (brd, *J* = 10.8 Hz)
25	132.5		133.2	
26	25.9	1.74 (s, 3H)	25.9	1.75 (s, 3H)
27	18.3	1.75 (s, 3H)	18.3	1.74 (s, 3H)
28	20.8	1.37 (s, 3H)	20.5	1.27 (s, 3H)
29	24.6	0.89 (s, 3H)	23.6	0.95 (s, 3H)
30	20.3	0.86 (s, 3H)	20.3	0.86 (s, 3H)

with those of compound **1** except that the C-19 signal (δ 80.0) of the compound **1** was replaced by δ 181.5 in the ^{13}C-NMR spectrum and the absence of an oxygenated methylene of the former in the ^{1}H-NMR spectrum. Therefore, according to the literature (Hsu *et al*, 2011), compound **2** could unambiguously be elucidated as cucurbita-6,22(*E*),24-trien-3β-ol-19,5β-olide.

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