Two New Cucurbitane Triterpenoids from Immature Fruits of *Momordica charantia*

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Two new cucurbitane triterpenoids, kuguacin X (**1**) and kuguaglycoside I (**2**), together with three known analogs, were isolated from immature fruits of *Momordica charantia*. By detailed analysis of IR, NMR, and MS data, acid hydrolysis, and comparison with spectroscopic data of known compounds, the new compounds were determined to be (23*E*)-5*β*,19-epoxycucurbita-6,23-diene-3*β*,22*ξ*,25-triol (**1**) and (23*E*)-5*β*,19-epoxycucurbita-6,23-dien-19-on-3*β*,25-diol 3-*O*-*β*-D-allopyranoside (**2**).

Introduction. – *Momordica charantia* (bitter melon) is a cucurbitaceous plant cultivated in Asia, Africa, and South America. Its fruit is a popular vegetable in the south of Asia. Fruits, vines, leaves, and roots of *M. charantia* have been used to treat toothache, diarrhea, furuncle, and diabetes in China. Some cucurbitane compounds from *M. charantia* showed bioactivities, such as antidiabetic [1][2], anticancer [3], anti-inflammatory [4], antiulcer [5], antifeedant [6], free-radical-scavenging [7], and antigluconeogenic activities [8]. In our previous studies, some cucurbitane triterpenoids were isolated and identified from *M. charantia* [9–13]. In the course of our search for potential bioactive cucurbitacins from *M. charantia*, two new ones, named kuguacin X (**1**) and kuguaglycoside I (**2**), along with three known compounds, goyaglycoside-d (**3**) [14], karaviloside II (**4**) [15], and momordicacoside G (**5**) [16], were isolated and identified (*Fig. 1*) on the basis of spectroscopic analysis and acid hydrolysis.

Results and Discussion. – Compound **1** was obtained as colorless powder. The molecular formula was determined as $C_{30}H_{48}O_4$ by HR-ESI-MS (*m*/*z* 473.3619 ([*M* + H]$^+$; calc. 473.3625)), ^{13}C-NMR, and DEPT spectra. The IR spectrum showed the presence of OH (3468 and 3402 cm^{-1}) and C=C (1644 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** (*Table*) exhibited the presence of seven Me groups at *δ*(H) 0.73 (*s*), 0.78 (*s*), 0.88 (*s*), 1.22 (*d*, *J* = 8.0), 1.35 (*s*), 1.53 (*s*), and 1.54 (*s*). The ^{13}C-NMR spectrum of **1** revealed 30 C-atom signals, which were assigned by DEPT experiments as seven Me, seven CH_2, and ten CH groups including four olefinic C-atoms, and six C_q-atoms. These signals indicated that **1** was a typical triterpenoid. The C-atom signals at *δ*(C)

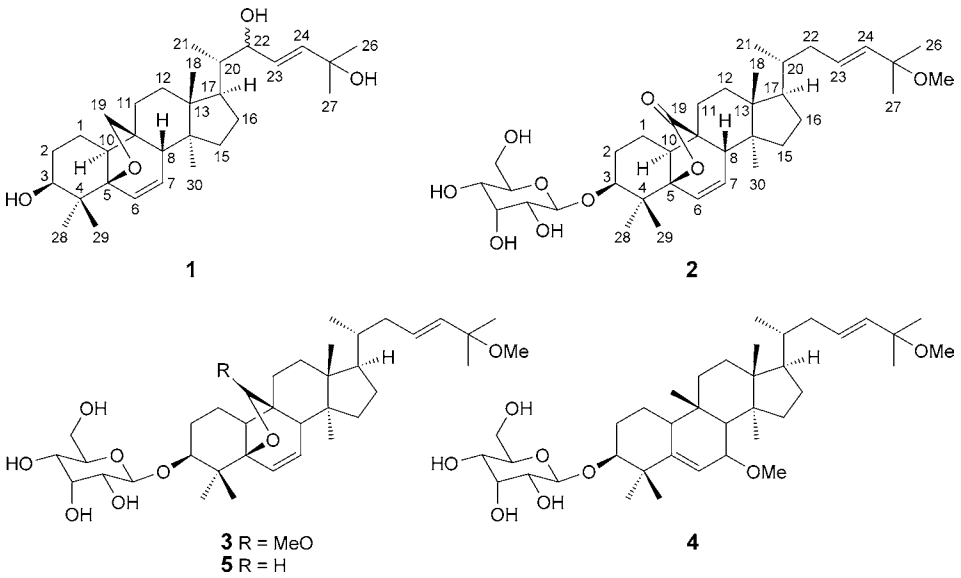

Fig. 1. *Structures of* **1**–**5**

132.2 (*d*, C(6)), 131.2 (*d*, C(7)), 125.9 (*d*, C(23)), 140.9 (*d*, C(24)), 87.4 (*s*, C(5)), and 79.6 (*t*, C(19)) further indicated that **1** belonged to the group of cucurbitane-type triterpenoids [17][18]. Comparison of the NMR data of **1** with those of (23*E*)-5*β*,19-epoxycucurbita-6,23-diene-3*β*,25-diol [18], indicated that the two compounds were very similar except for one OH group at C(22) (*δ*(C) 73.8 (*d*)) in **1**. The signal of C(22) was shifted downfield from *δ*(C) 39.1 (*t*) in (23*E*)-5*β*,19-epoxycucurbita-6,23-diene-3*β*,25-diol to 73.8 (*d*) in **1**, which indicated that the OH group in **1** was linked to C(22). The HMB correlations of the H-atom at *δ*(H) 4.55 (*dd*, *J* = 3.60, 3.55, H–C(22)) with the C-atoms at *δ*(C) 43.0 (C(20)), 13.0 (C(21)), and 125.9 (C(23)) in **1** further confirmed the above deduction (*Fig. 2*). The signals at *δ*(H) 3.54 (br. *s*, H–C(3)) and *δ*(C) 76.1 (*d*, C(3)) suggested that the relative configuration of the OH group at C(3) was *β* [11][17]. ROE correlations between *δ*(H) 3.54 (br. *s*, H–C(3)) and 2.24–2.26

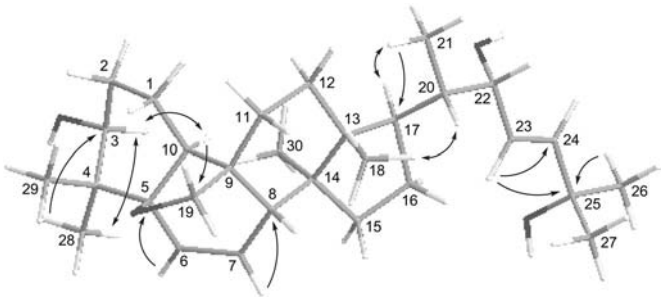

Fig. 2. *Key HMBC* (H → C) *and ROESY* (H ↔ H) *correlations of* **1**

Table. *^{1}H- and ^{13}C-NMR Data* (in (D_5)pyridine) *of* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.50–1.54 (*m*), 1.43–1.47 (*m*)	17.8 (*t*)	1.46–1.49 (*m*), 1.69–1.74 (*m*)	19.8 (*t*)
2	1.94–1.97 (*m*), 1.86–1.91 (*m*)	27.5 (*t*)	1.71–1.74 (*m*), 2.43–2.45 (*m*)	26.4 (*t*)
3	3.54 (br. *s*)	76.1 (*d*)	3.57–3.61 (*m*)	85.3 (*d*)
4		37.4 (*s*)		38.3 (*s*)
5		87.4 (*s*)		84.3 (*s*)
6	6.10 (*d*, *J* = 10.0)	132.2 (*d*)	6.30 (*d*, *J* = 9.7)	130.0 (*d*)
7	5.58 (*dd*, *J* = 9.7, 3.7)	131.2 (*d*)	5.61 (*dd*, *J* = 9.6, 2.6)	132.6 (*d*)
8	2.24 (*m*)	51.9 (*d*)	2.54 (br. *s*)	45.2 (*d*)
9		45.4 (*s*)		50.4 (*s*)
10	2.24–2.26 (*m*)	38.9 (*d*)	2.63–2.66 (*m*)	40.7 (*d*)
11	1.58–1.63 (*m*), 1.32–1.34 (*m*)	23.7 (*t*)	1.67–1.69 (*m*), 2.37–2.41 (*m*)	21.9 (*t*)
12	1.55–1.58 (*m*), 1.51–1.53 (*m*)	30.9 (*t*)	1.47–1.50 (*m*), 1.52–1.55 (*m*)	30.0 (*t*)
13		45.5 (*s*)		44.9 (*s*)
14		48.2 (*s*)		47.9 (*s*)
15	1.23–1.25 (*m*), 1.20–1.22 (*m*)	33.3 (*t*)	1.15–1.19 (*m*), 1.22–1.26 (*m*)	33.4 (*t*)
16	1.96–2.00 (*m*), 1.46–1.50 (*m*)	27.8 (*t*)	1.24–1.28 (*m*), 1.87–1.90 (*m*)	27.6 (*t*)
17	1.58–1.63 (*m*)	47.5 (*d*)	1.47–1.50 (*m*)	50.4 (*d*)
18	0.78 (*s*)	14.9 (*q*)	0.84 (*s*)	14.6 (*q*)
19	3.60 (*d*, *J* = 8.4), 3.51 (*d*, *J* = 8.3)	79.6 (*t*)		182.2 (*s*)
20	2.10–2.15 (*m*)	43.0 (*d*)	1.43–1.47 (*m*)	36.6 (*d*)
21	1.22 (*d*, *J* = 8.0)	13.0 (*q*)	0.92 (*d*, *J* = 4.7)	18.8 (*q*)
22	4.55 (*dd*, *J* = 3.60, 3.55)	73.8 (*d*)	1.80–1.84 (*m*), 2.22–2.24 (*m*)	39.5 (*t*)
23	6.23–6.31 (*m*)	125.9 (*d*)	5.92–5.94 (*m*)	124.2 (*d*)
24	6.20–6.28 (*m*)	140.9 (*d*)	5.92–5.94 (*m*)	141.8 (*d*)
25		69.6 (*s*)		69.8 (*s*)
26	1.54 (*s*)	30.7 (*q*)	1.54 (*s*)	30.9 (*q*)
27	1.53 (*s*)	30.8 (*q*)	1.54 (*s*)	30.9 (*q*)
28	1.35 (*s*)	20.8 (*q*)	0.89 (*s*)	19.3 (*q*)
29	0.88 (*s*)	24.6 (*q*)	0.77 (*s*)	23.9 (*q*)
30	0.73 (*s*)	20.1 (*q*)	1.53 (*s*)	20.8 (*q*)
Allo				
1′			5.30 (*d*, *J* = 7.9)	105.0 (*d*)
2′			3.90 (*d*, *J* = 7.8)	73.2 (*d*)
3′			4.63 (br. *s*)	72.4 (*d*)
4′			4.12 (*d*, *J* = 9.4)	69.2 (*d*)
5′			4.45–4.48 (*m*)	76.0 (*d*)
6′			4.37 (*dd*, *J* = 11.5, 6.0), 4.53 (*d*, *J* = 11.5)	63.3 (*t*)

[a]) Recorded at 500 (^{1}H) and 125 MHz (^{13}C). [b]) Recorded at 400 (^{1}H) and 100 MHz (^{13}C).

(*m*, H–C(10)) and 1.35 (*s*, Me(28)) also supported β-orientation of the OH group at C(3) (*Fig. 2*). Hence, **1** was determined to be (23*E*)-5β,19-epoxycucurbita-6,23-diene-3β,22ξ,25-triol.

Compound **2** was obtained as colorless powder. The molecular formula was determined as $C_{36}H_{56}O_9$ by HR-ESI-MS (*m*/*z* 655.3810 $[M+Na]^+$; calc. 655.3816), ^{13}C-NMR, and DEPT experiments. The IR spectrum showed the presence of OH (3450

and 3372 cm^{-1}) and C=C (1644 cm^{-1}) groups. The signals at δ(C) 105.0 (*d*), 73.2 (*d*), 72.4 (*d*), 69.2 (*d*), 76.0 (*d*), 63.3 (*t*), and δ(H) 5.30 (*d*, $J = 7.9$) (*Table*) suggested a β-allopyranosyl moiety in **2** [2]. After acid hydrolysis of **2** with 3% HCl/MeOH, D-allose was detected by GC analysis. In the ^{1}H-NMR spectrum, the aglycone moiety in **2** showed resonances for seven Me groups at δ(H) 0.77 (*s*), 0.84 (*s*), 0.89 (*s*), 0.92 (*d*, $J =$ 4.7), 1.53 (*s*), and 1.54 (*s*, 6 H). In the ^{13}C-NMR and DEPT spectra, the aglycone moiety in **2** revealed 30 C-atom signals including seven Me, seven CH_2, and nine CH groups, and seven C_q-atoms. These signals indicated that **2** was a typical triterpenoid [17][19][20]. Comparison of the spectroscopic data of **2** with those of karavilagenin D [21] indicated that the two compounds were very similar except for one additional sugar unit in **2**. The signal of C(3) was shifted downfield from δ(C) 75.2 (*d*) in karavilagenin D to 85.3 (*d*) in **2**, which indicated that the sugar unit was linked to C(3) in **2**. The HMB correlations of the anomeric H-atom at δ(H) 5.30 (*d*, $J = 7.9$, H–C(1′)) with δ(C) 85.3 (*d*, C(3)) in **2** further confirmed the above deduction. The correlations between δ(H) 3.57 – 3.61 (*m*, H–C(3)) and 0.89 (*s*, Me(28)) in the ROESY spectrum indicated that the relative configuration of H–C(3) was α (*Fig. 3*). Hence, **2** was determined to be (23*E*)-5β,19-epoxycucurbita-6,23-dien-19-on-3β,25-diol 3-*O*-β-D-allopyranoside.

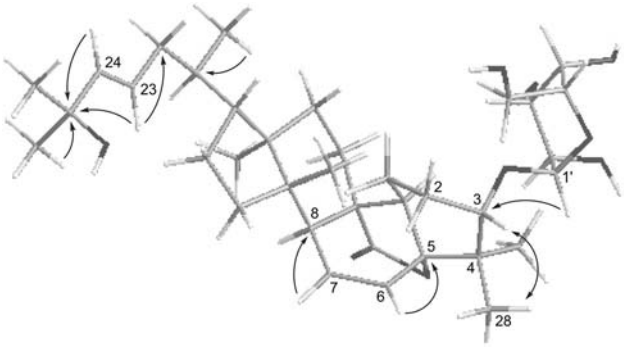

Fig. 3. *Key HMB* (H → C) *and ROESY* (H ↔ H) *correlations of* **2**

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Experimental Part

General. D-Allose was purchased from *Sigma* (USA). Fractions were monitored by thin layer chromatography (TLC) and spots were visualized by heating and spraying with 10% H_2SO_4. Column chromatography (CC): silica gel (SiO_2; 200 – 300 mesh, *Qingdao Marine Chemical*, P. R. China), *Lichroprep RP-18* (40 – 63 μm; *Merck*, Darmstadt, Germany), and *Sephadex LH-20* (*Pharmacia Fine Chemical Co., Ltd.*). GC: *Shimadzu GC-17A* gas chromatograph; *TC-1* capillary column (30 m × 0.25 mm); detector, H_2 FID. Optical rotations: *JASCO DIP-370* digital polarimeter. IR Spectra: *Shimadzu IR-450* instrument; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AV-400* or *DRX-500* instruments; in (D_5)pyridine; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS and HR-ESI-MS: *VG-AUTOSPEC-3000* spectrometer; in *m*/*z* (rel. %).

Plant Material. Fresh immature fruits of *M. charantia* were collected from Chengjiang County, Yunnan Province, P. R. China, in August 2008. The sample was identified by Prof. *Shu-Kun Chen* and a voucher specimen (No. KIB20080826) was deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

Extraction and Isolation. The dried and powdered fruits of *M. charantia* (37.49 kg) were extracted by maceration with 70% acetone. After filtration and evaporation of the solvent under reduced pressure, a residue (4.78 kg) was obtained. This residue was dissolved in H_2O (20 l) and then extracted successively with AcOEt (3 × 25 l). The AcOEt layer was concentrated to dryness to give an AcOEt extract (1.15 kg). The AcOEt extract was subjected to CC (SiO_2; $CHCl_3$/MeOH 1:0, 50:1, 20:1, and 0:1) to yield four fractions, *Frs. 1–4*. *Fr. 2* (93.60 g) was separated by CC (SiO_2; $CHCl_3$/acetone 6:1 to 4:1) to afford **1** (12 mg), **3** (900 mg), and **4** (670 mg). Compounds **2** (12 mg) and **5** (119 mg) were isolated from *Fr. 3* (84.45 g) by CC (SiO_2, $CHCl_3$/actone 4:1; *RP-18*, MeOH/H_2O 78:22 to 90:10; and *Sephadex LH-20*, MeOH).

Kuguacin X (=*(23*E*)-5β,19-Epoxycucurbita-6,23-diene-3β,22ξ,25-triol*=*(3*E*,6*S*)-6-[(1*R*,4*S*,5*S*,8*R*,9*R*,12*S*,13*S*,16*S*)-16-Hydroxy-5,9,17,17-tetramethyl-18-oxapentacyclo[10.5.2.0^{1,13}.0^{4,12}.0^{5,9}]nonadec-2-en-8-yl]-2-methylhept-3-ene-2,5-diol*; **1**). Colorless amorphous powder (MeOH). $[\alpha]_D^{21} = -83.9$ ($c = 1.7$, MeOH). IR: 3468, 3402, 2969, 2943, 2824, 1734, 1644, 1380, 1154, 1083, 974, 913. ^{1}H-NMR (500 MHz): see *Table*. ^{13}C-NMR (125 MHz): see *Table*. ESI-MS (pos.): 473 ($[M+H]^+$). HR-ESI-MS (pos.): 473.3619 ($[M+H]^+$, $C_{30}H_{49}O_4^+$; calc. 473.3625).

Kuguaglycoside I (=*(23*E*)-5β,19-Epoxycucurbita-6,23-dien-19-on-3β,25-diol 3*-O-β-D-*Allopyranoside*=*(1*R*,4*S*,5*S*,8*R*,9*R*,12*S*,13*S*,16*S*)-8-[(2*R*,4*E*)-6-Methoxy-6-methylhept-4-en-2-yl]-5,9,17,17-tetramethyl-18-oxapentacyclo[10.5.2.0^{1,13}.0^{4,12}.0^{5,9}]nonadec-2-en-19-one 16*-O-β-D-*Allopyranoside*; **2**). Colorless amorphous powder (MeOH). $[\alpha]_D^{21} = -69.2$ ($c = 1.6$, MeOH). IR: 3450, 3372, 2971, 2880, 1689, 1651, 1428, 1387, 1079, 1028. ^{1}H-NMR (400 MHz): see *Table*. ^{13}C-NMR (100 MHz): see *Table*. ESI-MS (neg.): 655 ($[M+Na]^+$). HR-ESI-MS (neg.): 655.3810 ($[M+Na]^+$, $C_{36}H_{56}NaO_9^+$; calc. 655.3816).

Acid Hydrolysis of **2** *for Sugar Analysis.* The experiment was implemented using the method previously described [22]. Compound **2** (2 mg) was treated with 3% HCl/MeOH (5 ml) at 92° for 3 h. $CHCl_3$/H_2O 1:1 (5 ml) was used for extraction. The aq. phase was neutralized with Ag_2CO_3. The filtrate was concentrated to dryness under reduced pressure. Neutralized hydrolysates of **2** were dissolved in 0.6 ml of pyridine, then, 0.4 ml $(Me_3Si)_2NH$ and 0.2 ml Me_3SiCl were added successively. The mixture was kept at 60° for 10 min under water bath condition. Next, the mixture was centrifuged for 20 min at $1.0 \cdot 10^4$ r min^{-1}. The supernatant was subjected to GC analysis under the following conditions: column temp., 200°/260°; programmed increase, 3°/min; carrier gas, N_2 (1 ml min^{-1}); injector and detector temp., 260°; injection volume, 1 µl; split ratio, 1/50. GC Analysis showed the presence of β-D-allose (t_R 13.54 min) in **2**.

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