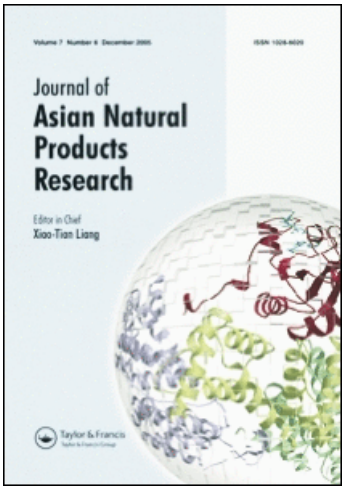

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Cucurbitane and hexanorcucurbitane glycosides from the fruits of *Cucurbita pepo* cv *dayangua*

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Phytochemical investigation of the fruits of *Cucurbita pepo* cv *dayangua* has led to the isolation of two cucurbitane glycosides: cucurbitacin L 2-*O*-β-D-glucopyranoside (**1**), cucurbitacin K 2-*O*-β-D-glucopyranoside (**2**) and two hexanorcucurbitane glycosides: 2,16-dihydroxy-22,23,24,25,26,27-hexanorcucurbit-5-en-11,20-dione 2-*O*-β-D-glucopyranoside (**3**) and 16-hydroxy-22,23,24,25,26,27-hexanorcucurbit-5-en-11,20-dione 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**4**). Compounds **1**, **2** and **3** were isolated from Cucurbita genus for the first time, while compound **4** is a new one. Their structures were determined on the basis of chemical and spectroscopic evidence.

Keywords: *Cucurbita pepo* cv *dayangua*; Cucurbitane glycoside; Hexanorcucurbitane glycoside; Cucurbita

1. Introduction

Cucurbita pepo cv *dayangua*, which is distributed in the Duo lun county of the autonomous region of Mongolia, has been employed in folk medicine to treat colds and alleviate aches [1,2]. Previous pharmacological tests showed that it possessed antibacterial, antiviral, anti-inflammatory and analgesic effects [3,4]. During our search for new anti-tumour agents, the ethanolic extract of fruits of the plant exhibited a significant dose-dependent inhibitory effect against HeLa and HepG cell growth. Phytochemical investigation of the ethanolic extract of the fruits has led to the isolation of two cucurbitane glycosides and two hexanorcucurbitane glycosides. The present paper deals with the isolation and structure elucidation of the four cucurbitacin saponins.

2. Results and discussion

Compounds **1**, **2** and **3** were identified as the previously known, cucurbitacin L 2-*O*-β-D-glucopyranoside, cucurbitacin K 2-*O*-β-D-glucopyranoside and 2,16-dihydroxy-

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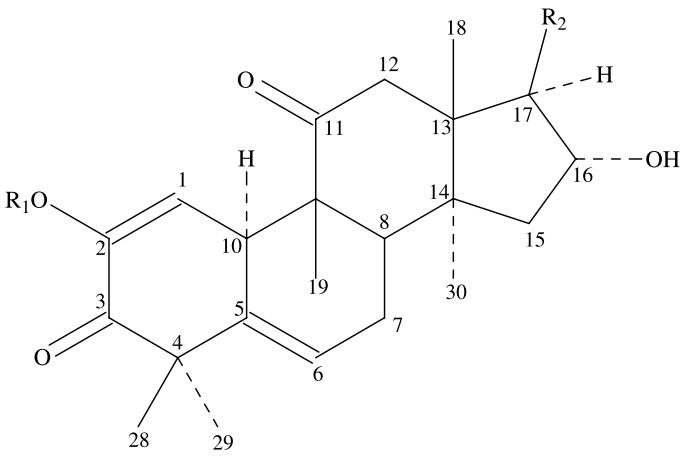
22,23,24,25,26,27-hexanorcucurbit-5-en-11,20-dione 2-*O*-β-D-glucopyranoside, respectively (figure 1) by comparison of their spectroscopic data with those reported in the literature [5].

Compound **4** was obtained as white amorphous powder (MeOH). It showed positivity to Molish and Liebermann–Burchard tests and the molecular formula was determined as $C_{36}H_{56}O_{13}$ by HRESI-MS (quasi-molecular ion peak at *m/z* 719.3643 $[M + Na]^+$). The sugar moieties were identified as glucose and rhamnose by TLC with authentic samples after acid hydrolysis. The ^{1}H NMR spectrum of **4** exhibited signals characteristic for six methyl protons at δ 0.76, 1.05, 1.38, 1.51, 1.15, 1.52, one trisubstituted olefinic proton at δ 5.94 and two anomeric protons at δ 4.89, 6.74. The ^{13}C NMR and DEPT spectra of **4** showed 36 carbons (table 2), including a pair of olefinic carbons at δ 139.6 and 119.7, two carbonyl carbons at δ 212.3 and 208.7, two anomeric carbons at δ 105.2 and 101.1. The ^{1}H NMR and ^{13}C NMR spectral data of **4** suggested that it was a hexanorcucurbitane glycoside [6,7]. Comparison of the ^{13}C NMR data with those of compound **3** (table 1) revealed the absence of two olefinic and one carbonyl carbon signals at δ 120.7, 146.9 and 196.9, due to C-1, C-2 and C-3 of compound **3** respectively, indicating the differences in ring A between the two compounds. In the HMBC spectrum, the two methyl protons at δ 1.05 and 1.51 showed



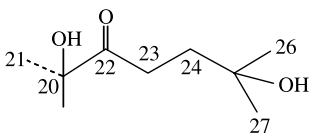
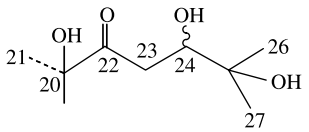
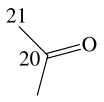
	R_1	R_2
1	β-glc	
2	β-glc	
3	β-glc	

Figure 1. The structures of compounds **1**, **2** and **3**.

Table 1. ^{13}C NMR spectral data of compounds **1**, **2** and **3** (150 MHz, C_5D_5N).

C	*1*	*2*	*3*	*C*	*1*	*2*	*3*
1	120.9	120.9	120.7	19	18.3	18.3	18.3
2	146.8	146.8	146.9	20	80.1	80.3	208.4
3	197.0	197.0	196.9	21	25.5	25.4	31.6
4	49.5	49.5	49.5	22	216.1	215.6	
5	137.0	137.0	137.0	23	32.8	40.9	
6	120.5	120.5	120.4	24	38.5	74.8	
7	23.9	23.9	24.0	25	69.0	72.2	
8	41.8	41.8	42.1	26	29.9	25.1	
9	50.9	51.1	50.2	27	30.1	27.6	
10	35.6	35.6	35.6	28	20.2	20.2	20.1
11	214.0	214.0	212.8	29	27.6	27.2	27.4
12	49.7	49.7	49.7	30	20.8	20.8	20.8
13	49.3	49.3	49.0	Glc-1′	100.6	100.6	100.6
14	48.7	48.6	47.8	2′	74.4	74.4	74.4
15	46.5	46.4	46.2	3′	78.5	78.5	78.5
16	70.6	70.6	71.4	4′	70.3	70.4	70.7
17	59.0	58.9	67.9	5′	78.8	78.8	78.7
18	20.4	20.5	19.9	6′	61.9	61.9	62.0

correlations to the methine carbon at δ 86.0, quarternary carbon at δ 42.1 and olefinic carbon at δ 139.6; the latter was characteristically due to C-5 of cucurbitane skeleton [5]. Thus the first two carbons were assigned to C-3 and C-4 respectively. The methylenic proton at δ 1.70 (δ 28.8) showed long-range correlation (HMBC) to carbons at δ 86.0 (C-3) and 42.1 (C-4), indicating that the methylene (δ 28.8) was connected to C-3. The long-range correlation between the methylenic proton at δ 1.56 (δ 22.5) and carbon at δ 86.0 (C-3) elucidated that the carbon at δ 22.5 was connected to the carbon at δ 28.8. Thus the two methylenic carbons at δ 22.5 and 28.8 were assigned to C-1 and C-2, respectively. Finally the carbons of the ring A of **4** were assigned as shown in table 2. From the comparison of ^{13}C NMR data of **4** with

Table 2. ^{1}H NMR and ^{13}C NMR spectral data and key HMBC correlations of compound **4** (600 Hz for ^{1}H and 150 MHz for ^{13}C, C_5D_5N).

C	δ_C	δ_H	*HMBC*	*C*	δ_C	δ_H	*HMBC*
1	22.5	1.56 m, 1.83 m	C-3	19	20.5	1.38 s	C-10, C-11 C-8, C-9
2	28.8	1.85 m, 1.70 m	C-3, C-10, C-4	20	208.7		
3	86.0	3.62 brs	C-1, C-5, C-1′	21	31.7	1.15 s	C-20, C-17
4	42.1			22	19.4	1.52 s	C-4, C-3, C-5
5	139.6			23	28.1	1.05 s	C-3, C-4, C-5
6	119.7	5.94 d 5.6	C-7, C-10, C-4, C-8	24	25.5	1.51 s	C-8, C-15, C-14, C-13
7	24.5	2.76 dd 18.8,8.1 1.93 m	C-6, C-5, C-9	1′	105.2	4.89 d 7.6	C-3
8	43.7	1.90 m		2′	76.2		
9	49.6			3′	80.5		C-2′, C-4′
10	35.8	2.51 m	C-9, C-11	4′	71.9	4.17 t 9.2	C-3′, C-5′, C-6′
11	212.3			5′	78.2	3.85 m	
12	47.5	2.54 d 14.4 3.30 d 14.4	C-11, C-13, C-14	6′	62.7		C-4′
13	50.5			1″	101.1	6.74 brs	C-5″, C-2′
14	49.2			2″	72.6	4.75 d 2.4	C-3″, C-4″
15	46.2	1.76 m, 1.93 m	C-16	3″	72.4		
16	71.4	3.50 m	C-20, C-14	4″	74.1		
17	68.1	3.48 d 6.5	C-20, C-13, C-16	5″	69.6		
18	20.0	0.76 s	C-12, C-14 C-17, C-13	6″	19.4	1.70 d 6.1	C-5″, C-4″

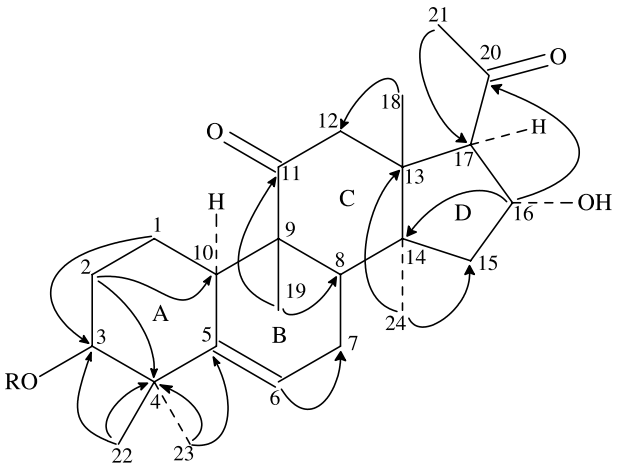


Figure 2. The structure and key HMBC correlations of compound **4**. R = -α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside.

those of **3** (table 1) and the analysis of HMQC and HMBC spectra of **4**, carbons of the ring B, C and D of the aglycone were also assigned (table 2).

The relative stereochemistry of **4** was determined by NOESY analysis. In the NOESY spectrum H_3-23 showed correlations to H-3 and H-10, while H_3-24 was correlated with H-10 and H-17. The H-10 was α orientated according to the study about cucurbitacin biogenesis [8]. So the H-3, H_3-23, H_3-24, H-17 should be α orientated. Because of undisplayed NOE correlations between H-16 and H-24, 17 in the NOESY spectrum, H-16 should be β orientated.

The direct and long-range connections between protons and carbons were assigned (table 2), and carbons at δ 105.2, 76.2, 80.5, 71.9, 78.2, 62.7 were assigned to the glucose unit, while carbons at δ 101.1, 72.6, 72.4, 74.1, 69.6, 19.4 were assigned to the rhamnose unit. The anomeric configuration of the sugar moieties were determined to be β for glucose on basis of the coupling constants ($J_{1',2'} = 7.6$ Hz), while α for rhamnose due to the chemical shift value of C-5″ (δ 69.6) [9]. The HMBC correlation was observed between an anomeric proton at δ 4.89 (H-1′) and carbon at δ 86.0 due to C-3 of the aglycone. Another anomeric proton at δ 5.38 (H-1″) was correlated with carbon at δ 76.2 (C-2′), together with the downfield shift of C-3′ (+2.0 ppm) and the upfield shift of C-4′ (−1.5 ppm), suggesting the rhamnopyranosyl unit was attached to C-2′of the glucopyranosyl moiety. Therefore, the structure of **4** was determined to be 16-hydroxy-22,23,24,25,26,27-hexanorcucurbit-5-en-11,20-dione 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (figure 2).

3. Experimental

3.1 General experimental procedures

^{1}H NMR (600 MHz, C_5D_5N) and ^{13}C NMR (150 MHz, C_5D_5N) spectra were recorded on a Bruker ARX-600 spectrometer with TMS as an internal standard. HRESI-MS data were measured with a Bruker AOEXIII 7.0 TESLA FT-MS. Column chromatography was carried out on silica gel (Qingdao Haiyang Chemical Co. Ltd., 200–300 mesh), Sephadex LH-20 (Amersham Pharmacia Biotech AB). Spots (on TLC) were visualised by spraying with vanillin (1%) and H_2SO_4 (10%) in EtOH and heating (110°C, 5 min).

3.2 Plant material

The fruits of *Cucurbita pepo* cv *dayangua* were collected from the planting base of Jilin University, Jilin Province of China in August 2002. The specimen was botanically identified by An-min Lu (Institute of Medicinal Plant Development of Chinese Academy of Medical Science, China). A voucher specimen has been deposited in the Herbarium of the Institute of Medicinal Plant Development of Chinese Academy of Medical Science, China.

3.3 Extraction and isolation

The air-dried fruits (15 kg) were extracted with 95% ethanol for three times under reflux. The combined solution was concentrated under vacuum and subjected to a column of macroporous absorption resin (AB-8) eluted with 70% EtOH, then the solution was evaporated to dryness under vacuum to give a residue (200 g). The residue was chromatographed on silica gel with $CHCl_3$/MeOH (in gradient) to give 15 fractions. Fractions 6 and 7 (13 g together) were repeatedly chromatographed on silica with a solvent system of $CHCl_3$/MeOH/H_2O (85:15:10) to give **1** (32 mg), **2** (40 mg) and **3** (25 mg). Fraction 13 (8 g) was further separated by Sephadex LH-20 eluting with 80% MeOH to give **4** (12 mg).

3.3.1 Compound 4. White amorphous powder (MeOH), HRESI-MS *m/z* 719.3643 $[M + Na]^+$ (calculated for $C_{36}H_{56}O_{13}Na$, 719.3627). ^1H NMR, ^{13}C NMR, and HMBC data: see table 2.

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