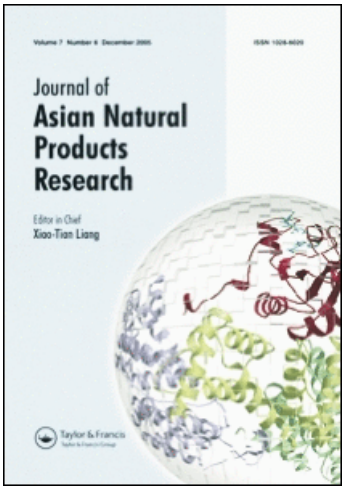


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

New phenolic glycosides from the seeds of *Cucurbita moschata*

Fa-Sheng Li[ab]; De-Qiang Dou[b]; Liang Xu[b]; Xiao-Feng Chi[a]; Ting-Guo Kang[b]; Hai-Xue Kuang[c]
[a] Department of Chemistry, Dalian Medical University, Dalian, China [b] College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, China [c] Heilongjiang University of Traditional Chinese Medicine, Harbin, China

To cite this Article Li, Fa-Sheng , Dou, De-Qiang , Xu, Liang , Chi, Xiao-Feng , Kang, Ting-Guo and Kuang, Hai-Xue(2009) 'New phenolic glycosides from the seeds of *Cucurbita moschata*', Journal of Asian Natural Products Research, 11: 7, 639 — 642
To link to this Article: DOI: 10.1080/10286020902942376
URL: http://dx.doi.org/10.1080/10286020902942376

Journal of Asian Natural Products Research
Vol. 11, No. 7, July 2009, 639–642

Taylor & Francis
Taylor & Francis Group

New phenolic glycosides from the seeds of *Cucurbita moschata*

Fa-Sheng Li[ab], De-Qiang Dou[b]*, Liang Xu[b], Xiao-Feng Chi[a], Ting-Guo Kang[b] and Hai-Xue Kuang[c]

[a]*Department of Chemistry, Dalian Medical University, Dalian 116044, China;* [b]*College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian 116600, China;* [c]*Heilongjiang University of Traditional Chinese Medicine, Harbin 140006, China*

(*Received 8 February 2009; final version received 1 April 2009*)

Two new phenolic glycosides were isolated from the seeds of *Cucurbita moschata*. Their structures were elucidated as (2-hydroxy)phenylcarbinyl 5-*O*-benzoyl-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranoside (**1**) and 4-β-D-(glucopyranosyl hydroxymethyl)phenyl 5-*O*-benzoyl-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranoside (**2**) on the basis of spectroscopic analysis and chemical evidence.

Keywords: *Cucurbita moschata*; Cucurbitaceae; phenolic glycosides

1. Introduction

Cucurbita moschata is an important crop in tropical areas. In Spain, the cultivation of this species is mainly based on landraces and maintained for centuries. Pumpkin, the fruit of *C. moschata*, is a herbaceous running plant, belonging to the melon family. The pumpkin seed of many species in *Cucurbita* genus is a herbal medicine and mainly used as a natural and safe de-worming agent, and the seeds are also able to get rid of all intestinal helminthes and parasites in the body when used properly [1]. Previous phytochemical investigations of the seeds of *C. moschata* yielded five acylated phenolic glycosides, cucurbitosides A−E [2]. In this paper, we report the isolation and structural elucidation of two new phenolic glycosides, (2-hydroxy)phenylcarbinyl 5-*O*-benzoyl-β-D-apiofuranosyl(1→2)-β-D-glucopyranoside (**1**) and 4-β-D-(glucopyranosylhydroxymethyl)phenyl 5-*O*-benzoyl-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranoside (**2**), on the basis of spectroscopic analysis and chemical evidence (Figure 1).

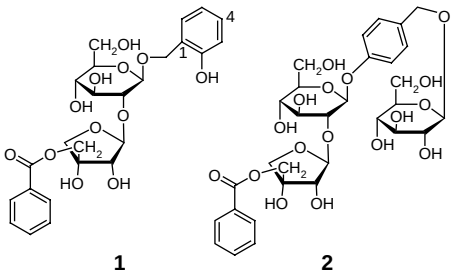
2. Results and discussion

Compound **1** was obtained as an amorphous powder, showing a molecular formula of $C_{25}H_{30}O_{12}$ by HR-ESI-MS. On acid hydrolysis, **1** afforded D-glucose and D-apiose as component sugars, which was identified by GC analysis of its trimethylsilyl thiazolidine derivative [3]. The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) showed the presence of signals assignable to β-glucopyranosyl and β-apiofuranosyl moieties [4]. Besides the signals due to sugars, the ^{1}H NMR spectrum of **1** also showed a set of proton signals due to 1,2-disubstituted aromatic ring at δ 7.26 (1H, dd, $J = 7.6$, 1.6 Hz), 7.00 (1H, td, $J = 7.6$, 1.6 Hz), 6.71 (1H, td, $J = 7.6$, 1.6 Hz), and 6.71 (1H, dd, $J = 7.6$, 1.6 Hz), as well as a methylene at δ 4.90 (1H, d, $J = 11.2$ Hz) and 4.65 (1H, d, $J = 11.2$ Hz), indicating the presence of a 2-hydroxyphenylcarbinyl

*Corresponding author. Email: doudeqiang2003@yahoo.com.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020902942376
http://www.informaworld.com



1 **2**

Figure 1. Structures of compounds **1** and **2**.

moiety. Furthermore, the signals attributable to a monosubstituted aromatic ring were observed at δ 8.02 (2H, dd, $J = 8.4$, 1.4 Hz), 7.60 (2H, tt, $J = 8.4$, 1.4 Hz), and 7.46 (1H, t, $J = 8.4$ Hz), in combination with the signal for a carbonyl carbon at δ 167.9 in the ^{13}C NMR spectrum, suggesting a benzoyl moiety in **1**. The connection of the aforementioned structural units was determined by the HMBC

Table 1. NMR spectroscopic data of compounds **1** and **2** (CD_3OD).[a]

	Compound **1**		Compound **2**	
Position	δ_H	δ_C	δ_H	δ_C
1		125.2		158.5
2		156.6	7.17 (d, 8.8)	117.1
3	6.71 (dd, 7.6, 1.6)	116.4	6.95 (dt, 8.8, 2.3)	130.7
4	7.00 (td, 7.6, 1.6)	130.1		134.4
5	6.71 (td, 7.6, 1.6)	120.5	6.95 (dt, 8.8, 2.3)	130.7
6	7.26 (dd, 7.6, 1.6)	130.9	7.17 (d, 8.8)	117.1
7	4.65 (d, 11.2),	67.8	4.51 (d, 11.4),	71.3
	4.90 (d, 11.2)		4.74 (d, 11.4)	
Glc-1′	4.48 (d, 7.6)	102.4	4.95 (d, 7.4)	100.6
2′	3.47 (dd, 9.2, 7.6)	78.9	3.66 (dd, 9.2, 7.4)	78.3
3′	3.48 (t, 9.2)	78.9	3.60 (t, 9.2)	78.9
4′	3.31 (m)	71.8	3.38 (m)	71.6
5′	3.27 (m)	78.0	3.40 (m)	78.1
6′	3.70 (dd, 12.1, 5.5),	62.8	3.68 (dd, 12.1, 5.1),	62.6
	3.88 (dd, 12.1, 2.1)		3.88 (dd, 12.1, 2.0)	
Api-1″	5.41 (d, 1.3)	110.5	5.51 (d, 1.2)	110.5
2″	4.01 (d, 1.3)	78.7	4.00 (d, 1.2)	78.7
3″		79.1		79.2
4″	3.70 (d, 9.9),	75.4	3.92 (d, 9.8),	75.5
	4.01 (d, 9.9)		4.31 (d, 9.8)	
5″	4.29 (d, 11.5),	68.6	4.28 (d, 11.5),	68.4
	4.37 (d, 11.5)		4.35 (d, 11.5)	
1‴		131.3		131.2
2‴ and 6‴	8.02 (dd, 8.4, 1.4)	130.8	7.93 (dd, 8.3, 1.5)	130.7
3‴ and 5‴	7.46 (t, 8.4)	134.3	7.42 (td, 8.3, 1.5)	134.4
4‴	7.60 (tt, 8.4, 1.4)	129.6	7.58 (tt, 8.3, 1.5)	129.6
7‴		167.9		167.7
Glc-1⁗			4.29 (d, 7.5)	103.1
2⁗			3.21 (t, 9.0)	75.2
3⁗			3.25 (t, 9.0)	78.0
4⁗			3.29 (m)	71.8
5⁗			3.32 (m)	78.2
6⁗			3.66 (dd, 12.1, 5.1),	62.9
			3.86 (dd, 12.1, 2.0)	

[a] δ values were established from the DQF-COSY, HMQC, and HMBC experiments.

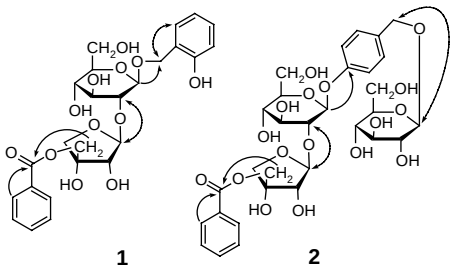


Figure 2. Key HMBC correlations of **1** and **2**.

experiment (Figure 2). The β-D-glucopyranosyl moiety was found to be connected to 7-OH of the phenylcarbinyl moiety by the HMBC correlation between Glc-H-1′ and C-7. The β-D-apiofuranosyl moiety was linked to the 2′-OH of β-D-glucopyranosyl moiety by the HMBC correlation between Api-H-1″ and Glc-C-2′. The connection of the benzoyl moiety at C-5″ of β-D-apiofuranosyl moiety was deduced from the HMBC correlations between Api-H-5″ and C-7‴. With all this evidence, the structure of compound **1** was established as (2-hydroxy)phenylcarbinyl 5-*O*-benzoyl-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranoside.

Compound **2** was obtained as an amorphous powder, showing a molecular formula of $C_{31}H_{40}O_{17}$ by HR-ESI-MS. On acid hydrolysis, **2** also afforded D-glucose and D-apiose as component sugar in the ratio of 2:1. In the ^{1}H NMR spectrum, the A_2B_2-type of aromatic proton signals at δ 7.17 (2H, d, $J = 8.8$ Hz) and 6.95 (2H, dt, $J = 8.8$, 2.3 Hz) and a benzylic hydroxymethyl at δ 4.74 (1H, d, $J = 11.4$ Hz) and 4.51 (1H, d, $J = 11.4$ Hz) suggested the presence of the 4-hydroxymethyl-phenol moiety in **1**, which was confirmed by the HMBC correlations between H-7 and C-3 and C-5. The presence of the benzoyl moiety in **2** was confirmed by the same method in **1**. The ^{1}H and ^{13}C NMR spectral data of **2** were similar to those of 4-hydroxybenzyl alcohol 4-*O*-(5-*O*-benzoyl)-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranoside (cucurbitoside C) [2], which was isolated from the same plant by Koike, except for one set of β-D-glucopyranosyl moiety signals. The additional β-D-glucopyranose moiety was linked to the 7-OH of the phenyl alcohol moiety by the HMBC correlation between Glc-H-1⁗ and C-7. Complete analyses of NMR spectral data indicated that compound **2** was a novel structure, which was established as 4-β-D-(glucopyranosyl hydroxymethyl)phenyl 5-*O*-benzoyl-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranoside.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Perkin-Elmer digital polarimeter. UV spectra were recorded on a Shimadzu UV-260 spectrometer. The IR spectra were measured with a Perkin-Elmer 683 infrared spectrometer (by a KBr disk method). The ESI-MS and HR-ESI-MS were taken on a Bruker FTMS Apex III spectrometer. The ^{1}H and ^{13}C NMR spectra were measured with a Bruker Avance 400 FT-NMR spectrometer. Column chromatography was performed on silica gel 60 (Marine Chemical Factory, Qingdao, China) and TLC was conducted on silica GF254 (Marine Chemical Factory). Detection was done by spraying 10% aqueous H_2SO_4, followed by heating. HPLC was performed using an ODS column (Phenomenex LUNA C18, 20 × 250 mm) with RI detector.

3.2 Plant material

The seeds of *C. moschata* were collected in Liaoning Province, China, in September 2007, and were identified by Prof. Kang Ting-Guo, College of Pharmacy, Liaoning University of Traditional Chinese Medicine. A voucher specimen (2007002) has been deposited at the identifier's University.

3.3 Extraction and isolation

The seeds (10 kg) were extracted with 70% EtOH three times for 24 h each at room temperature. The EtOH extract was concentrated (350 g), suspended in H_2O, and then partitioned successively with *n*-hexane, EtOAc, and *n*-BuOH. The *n*-BuOH layer (20 g) was subjected to silica gel column chromatography with a gradient of $CHCl_3$–MeOH to give 15 fractions. Further purification of fraction 9 (210 mg) by repeated preparative HPLC with 15% CH_3CN afforded two compounds, **1** (6 mg) ($t_R = 14.5$ min) and **2** (3 mg) ($t_R = 18.8$ min).

3.3.1 (2-Hydroxy)phenylcarbinyl 5-O-benzoyl-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranoside

An amorphous powder; $[\alpha]_D^{24}$ −76.0 ($c = 0.60$, CH_3OH); UV/vis λ_{max} (MeOH) 225 nm (log ε, 4.16); IR (KBr) (ν_{max}, cm^{-1}): 3389, 1723, 1633, 1599, 1455, 1382, 1262, 1071. ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectral data are shown in Table 1. Positive-ion ESI-MS *m/z*: 522 $[M]^+$. HR-ESI-MS *m/z*: 545.1635 $[M+Na]^+$(calcd for $C_{25}H_{30}O_{12}Na$, 545.1628).

3.3.2 4-β-D-(Glucopyranosyl hydroxymethyl)phenyl 5-O-benzoyl-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranoside

An amorphous powder; $[\alpha]_D^{24}$ −56.0 ($c = 0.20$, CH_3OH); UV/vis λ_{max} (MeOH) 222 nm (log ε, 4.02); IR (KBr) (ν_{max}, cm^{-1}): 3397, 1632, 1471, 1456, 1382, 1314, 1274, 1101, 1075, 1027. ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectral data are shown in Table 1. Positive-ion ESI-MS *m/z*: 684 $[M]^+$. HR-ESI-MS *m/z*: 707.2163 $[M+Na]^+$ (calcd for $C_{31}H_{40}O_{17}Na$, 707.2149).

3.4 Acid hydrolysis and determination of the absolute configuration of sugars in 1 and 2

Each solution of **1** and **2** (each 1 mg) in 1 M HCl (dioxane–H_2O, 1:1, 1 ml) was heated at 100°C for 2 h. The solution was extracted with EtOAc (1 ml × 3) to remove the aglycone. The aqueous layer was concentrated under reduced pressure to dryness. The residue was dissolved in pyridine (0.1 ml), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (0.15 ml) was added. The mixture was kept at 60°C for 1 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 ml) for 1 h. The mixture was partitioned between hexane and H_2O (0.5 ml each) and the hexane extract was analyzed by GC–MS. By comparison of the retention times of the derivatives from **1** and **2** with the standard compounds of D-glucose, L-glucose, and D-apiose derivatives prepared in a similar way, the presence of D-glucosyl and D-apiosyl moieties in compounds **1** and **2** were confirmed.

References

[1] Editorial Committee of Zhong Hua Ben Cao, State Administration of Traditional Chinese Medicine of the People's Republic of China, *Zhong Hua Ben Cao* (Shanghai Scientific Technological Publisher, Shanghai, 1999).

[2] K. Koike, W. Li, L.J. Liu, E. Hata, and T. Nikaido, *Chem. Pharm. Bull.* **53**, 225 (2005).

[3] S. Hara, H. Okabe, and K. Mihashi, *Chem. Pharm. Bull.* **35**, 501 (1987).

[4] L. Kitagawa, K. Hori, M. Sakagami, F. Hashiuchi, M. Yoshikawa, and J. Ren, *Chem. Pharm. Bull.* **41**, 1350 (1993).