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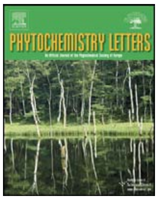

Phenolic glycosides from *Cucumis melo* var. *inodorus* seeds

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ABSTRACT

A new phenolic glycoside (*E*)-4-hydroxycinnamyl alcohol 4-*O*-(2′-*O*-β-D-apiofuranosyl)(1″ → 2′)-β-D-glucopyranoside (**1**) was isolated and identified from *Cucumis melo* seeds together with benzyl *O*-β-D-glucopyranoside (**2**), 3,29-*O*-dibenzoylmultiflor-8-en-3α,7β,29-triol (**3**) and 3-*O*-*p*-amino-benzoyl-29-*O*-benzoylmultiflor-8-en-3α,7β,29-triol (**4**). Their structures were elucidated by extensive NMR experiments including ^{1}H–^{1}H (COSY, TOCSY, ROESY) and ^{1}H–^{13}C (HSQC and HMBC) spectroscopy and chemical evidence. The multiflorane triterpene esters were identified as new melon constituents.

1. Introduction

Melon (*Cucumis melo* L. var. *inodorus*, Cucurbitaceae) is a commercially important crop in many countries. Fruits are consumed in the summer period and are popular because the pulp of the fruit is very refreshing and sweet, with pleasant aroma. A number of studies have investigated the relationship between a range of physical and chemical parameters of melons and their sensory evaluation (Wang et al., 1996; Hernandez-Gomez et al., 2005) and the compositional changes during ripening of fruits (Villanueva et al., 2004). The seeds of *C. melo* L. are used in Chinese folk medicine as antitussive, digestive, febrifuge and vermifuge (Duke and Ayensu, 1985) and melon seeds extract can be used as an antidiabetic and is beneficial in chronic eczema (Teotia and Ramakrishna, 1984; Lal and Lata, 1980). Many Cucurbitaceae seeds are rich in oil and protein. More recent studies were largely focused on fatty acids, tocopherols, sterols and phenolic profiles of seed oil (Abdalbasit and Bertrand, 2008) and on physicochemical properties (Yanty et al., 2008). As our current interest involves the chemistry of biologically active natural products, we investigated the chemical constituents of the seeds of *C. melo* L.

In this paper we report the isolation and structure elucidation of a new phenolic glycoside (**1**) together with three additional known compounds (**2**–**4**) from methanol extract of melon seeds.

2. Results and discussion

The MeOH extract of powdered seeds of *C. melo* was subjected to Kupchan's partitioning methodology (Kupchan et al., 1973) to give four extracts: *n*-hexane, $CHCl_3$, *n*-BuOH and the aqueous residue. The *n*-BuOH extract, after purification by droplet counter-current chromatography (DCCC) and reversed-phase HPLC, gave the new glycoside (**1**) and the known benzyl *O*-β-D-glucopyranoside (**2**). The $CHCl_3$ extract, purified by DCCC and HPLC, mainly contained multiflorane triterpene esters (**3**, **4**).

ESI-MS of compound **1** exhibited a quasi-molecular ion at *m*/*z* 467 $[M+Na]^+$, corresponding to a molecular formula $C_{20}H_{28}O_{11}$. This molecular formula was determined by HR-ESI-MS ($C_{20}H_{28}O_{11}$) $[M+Na]^+$ at *m*/*z* 467.1602, (calcd. for $C_{20}H_{28}O_{11}Na$ 467.1529) and confirmed by ^{1}H and ^{13}C NMR experiments. The structure of **1** was elucidated by detailed analyses of ^{1}H and ^{13}C NMR chemical shift, and by COSY, HSQC, and HMBC experiments (Table 1).

The ^{1}H NMR spectrum of **1** showed a carbinyl methylene group at δ 4.20 (2H, d, $J = 5.9$ Hz), two trans olefinic protons as ABX_3 type signal [δ_H 6.26 (1H, dq, $J = 5.9$, 16.3 Hz) and 6.55 (1H, d, $J = 16.3$ Hz)], aromatic protons as an A_2B_2 type pattern signals at δ_H 7.02 (2H, d, $J = 8.7$ Hz) and 7.35 (2H, d, $J = 8.7$ Hz) and two anomeric protons [δ_H 4.96 ($J = 7.4$ Hz) and 5.47 ($J = 1.4$ Hz)]. On acid hydrolysis **1** afforded D-glucose and D-apiose, which were identified by GC analysis (Lei et al., 2008). The chemical shift and coupling constants of H-1′ (δ_H 4.96 d, $J = 7.4$ Hz) and of H-1″ (δ_H 5.47 d, $J = 1.4$ Hz) and the chemical shift of C-1′ (δ_C 100.5) and C-1″ (δ_C 110.4) supported a β-configuration for the anomeric carbons (Ishii and Yanagisawa, 1998) (Fig. 1).

In the apiofuranoside ring when H-1″ and H-2″ are *trans*, $^3J_{1,2}$ is usually close to 1 Hz, indicating that both oxygen atoms take up

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Table 1
^{1}H and ^{13}C NMR (CD_3OD, 500 MHz) of compound **1**.

Position	δ_H[a] (*J* in Hz)	δ_C[a]	HMBC[b]
1	–	132.5	
2,6	7.35 d (8.7)	128.3	4, 7
3,5	7.02 d (8.7)	117.3	4, 1
4	–	157.4	
7	6.55 d (16.3)	130.9	2, 9
8	6.26 dq (5.9, 16.3)	128.3	1, 9
9	4.20 d (5.9)	63.4	7, 8
Glc			
1′	4.96 d (7.4)	100.5	4
2′	3.64 dd (7.4, 9.2)	78.2	1′, 1″
3′	3.59 t (9.1)	78.4	
4′	3.39	71.1	
5′	3.43 m	77.7	
6′	3.69 dd (5.4, 11.9), 3.89 dd (1.9, 11.9)	62.4	
Api			
1″	5.47 d (1.4)	110.4	3″, 4″
2″	3.95 d (1.4)	77.8	5″
3″	–	80.6	
4″a	3.79 d (9.5)	75.2	1″, 3″
4″b	4.05 d (9.5)		5″
5″	3.54 s	65.8	2″, 3″, 4″

[a] ^{1}H and ^{13}C assignments aided by COSY, TOCSY, HSQC and HMBC experiments.
[b] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

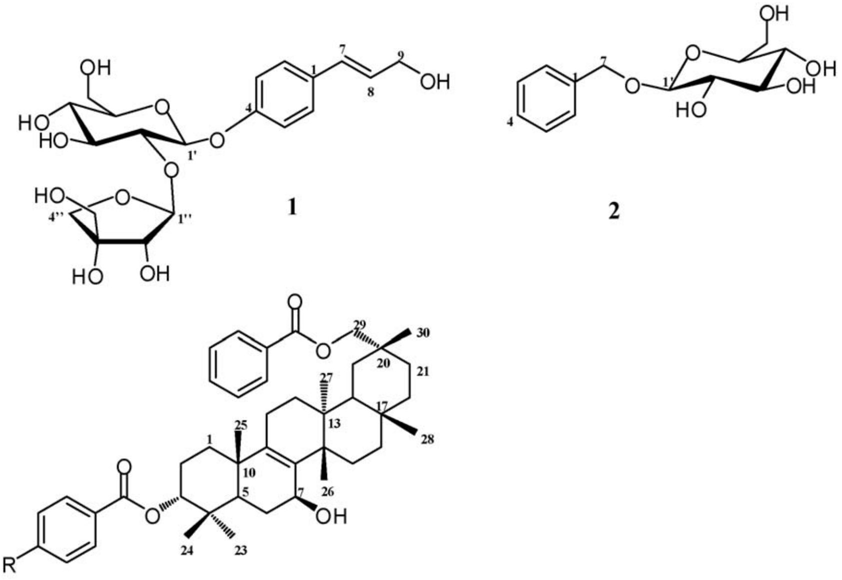
quasi-axial positions. The 2D-NOESY spectrum of **1** contains cross-peaks between H-2″ and the protons of the hydroxymethyl group (δ_H 3.54). Similar NOE cross-peaks was observed between H_2-5″ (δ_H 3.54) and H-4″b (δ_H 4.05) indicating that H-2″, H_2-5″ and H-4″b are found on the same side.

By HSQC and HMBC experiments (Table 1) a total of 20 carbon signals were detected, 11 signals assignable to glucopyranosyl and apiofuranosyl moieties. The low-field chemical shift of glucose C-2′ (δ_C 78.2) suggests that the hydroxyl group at C-2 of glucose bears the apiose unit. The interglycosidic linkage was determined by the HMBC correlation evidenced between δ_H 3.64 (H-2′ Glc) and δ_C 110.4 (C-1″ Api). H-1″ (δ_H 5.47) also displayed cross-peaks with C-3″ (δ_C 80.6) and C-4″ (δ_C 75.2) by ^3J correlations to reveal an apiofuranoside. The attachment of the sugar chain at C-4 of the aromatic ring was supported by the HMBC correlation observed between δ_H 4.96 (H-1′ Glc) and δ_C 157.4 (C-4) proving the C-4 glycosylation. *E*-geometry of C-7/C-8 double bond was inferred by the *J*(H-7/H-8) value (16.3 Hz). Inspection of ^{1}H NMR spectrum indicated that all signals relative to aglycone moiety were superimposable to that of (*E*)-4-hydroxycinnamyl alcohol 4-*O*-β-D-glucopyranoside isolated from the leaves of *Lilium cordatum* (Nakano et al., 1989).

On the basis of the foregoing the structure of **1** was determined to be (*E*)-4-hydroxycinnamyl 4-*O*-(2′-*O*-β-D-apiofuranosyl)(1″ → 2′)-β-D-glucopyranoside.

The molecular formula of compound **2** was deduced from its positive ion FAB-MS as $C_{13}H_{18}O_6$ which showed a $[M+Na]^+$ ion at *m*/*z* 293. The ^{1}H NMR spectrum of compound **2,** in addition to the signals due to the β-glucopyranosyl moiety (anomeric signals: δ_C 102.1 and δ_H 4.36, $J = 7.7$ Hz), showed a set of monosubstituted aromatic proton signals at δ 7.42, 7.33, 7.28 and a hydroxymethyl signal at δ 4.93 suggesting the presence of a benzyl alcohol moiety. The significant downfield shift of the H_2-7 (δ_H 4.93 and 4.67, each d, $J = 11.9$ Hz) and C-7 (δ_C 70.5) signals of **2** supported the location of glucosyl linkage also confirmed by the HMBC data. HMBC correlations were observed for H-7 (δ_H 4.93)/C-1′ (δ_C 102.1 Glc), H-7/C-1 (δ_C 138.0), H-7/C-2(6) (δ_C 128.0), proving that the glucose unit was linked to the benzyl carbon. Thus the structure of **2** was defined as benzyl *O*-β-D-glucopyranoside. This compound was previously identified in *Vitis vinifera* grapes and has been described as the corresponding peracetylated derivative (Williams et al., 1983).

Compounds **3** and **4** were found to be known multiflorane triterpene esters. By spectroscopic analysis the structures of the two compounds were determined to be 3,29-*O*-dibenzoylmultiflor-8-en-3α,7β,29-triol (**3**) previously isolated from the seeds of *Trichosanthes kirilowii* (Wu et al., 2005) and 3-*O*-*p*-amino-benzoyl-29-*O*-benzoylmultiflor-8-en-3α,7β,29-triol (**4**) identified only from the seeds of pumpkin (Appendino et al., 1999) and seeds of zucchini (Appendino et al., 2000).



1

2

3 R=H (OBz)
4 R=NH_2 (OPABA)

Fig. 1. Compounds from *Cucumis melo* seeds.