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NOTE

Two new maltol glycosides and cyanogenic glycosides from *Elsholtzia rugulosa* Hemsl.

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Abstract Two new maltol glycosides, maltol 6′-*O*-*β*-D-apiofuranosyl-*β*-D-glucopyranoside and maltol 6′-*O*-(5-*O*-*p*-coumaroyl)-*β*-D-apiofuranosyl-*β*-D-glucopyranoside, were isolated from *Elsholtzia rugulosa* Hemsl. along with 11 known compounds including prunasin and amygdalin. The structures were determined on the basis of spectroscopic and chemical evidence. This is the second example of isolation of cyanogenic glycosides from Lamiaceous plants.

Keywords *Elsholtzia rugulosa* · Lamiaceae · Maltol glycoside · Cyanogenic glycoside

Introduction

Elsholtzia rugulosa Hemsl., which belongs to the Labiatae family, is distributed in the Yunnan, Sichuan and Guizhou provinces of China. It is one of the honey plants in this region and has also been used as herbal tea or folk medicine for the treatment of colds, coughs, headaches, pharyngitis and fever [1]; however, the chemical constituents of this plant have not been clarified. This paper describes the isolation and structural determination of two new maltol glycosides together with 11 known compounds including two cyanogenic glycosides.

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Results and discussion

An aqueous acetone extract of the air-dried plant was subjected to a combination of Diaion HP-20, Sephadex LH-20, silica gel and Chromatorex ODS column chromatography with various solvent systems to furnish 13 compounds. Known compounds maltol 3-*O*-*β*-D-glucoside [2], luteolin [3], luteolin 7-*O*-*β*-D-glucoside [4], luteolin 3′-*O*-*β*-D-glucuronide [5], quercetin 3-*O*-*β*-D-glucoside [6], tuberonic acid *β*-D-glucoside [7], benzyl alcohol *β*-D-glucoside [8], prunasin [9], amygdalin [10], caffeic acid [11] and rosmarinic acid [12] were identified based on comparison of their spectral data with those of authentic samples cited in the literature. This is only the second example of isolation of cyanogenic glycosides, prunasin and amygdalin, from Lamiaceous plants: the first case was from an Australian plant *Clerodendrum grayi* [13].

Compound **1** was obtained as a white amorphous powder, and its molecular formula $C_{17}H_{24}O_{12}$ was determined by ^{13}C NMR spectral analysis, MALDI-TOF–MS (443 *m/z*, $[M+Na]^+$), and elemental analysis. The UV and IR spectral data of **1** showed the presence of an *α*, *β*-unsaturated carbonyl group (255 nm and 1,650 cm^{-1}). The ^1H and ^{13}C NMR spectra of **1** resembled those of maltol 3-*O*-*β*-D-glucoside and showed signals due to a maltol group [δ_H 8.00 (1H, d, J = 5.6 Hz, H-6), 6.45 (1H, d, J = 5.6 Hz, H-5), and 2.45 (3H, s, H-7); δ_C 164.6 (C-2), 143.3 (C-3), 177.0 (C-4), 117.2 (C-5), 157.1 (C-6), and 15.9 (C-7)]. However, two sugar anomeric proton signals appeared at δ_H 4.78 (d, J = 7.6 Hz, H-1′) and 4.95 (d, J = 2.2 Hz, H-1″). The corresponding anomeric carbon resonances

were observed at δ_C 105.0 and 110.7, respectively, in the ^{13}C NMR spectrum. Chemical shift comparison of the remaining carbon signals (Table 1) with those of known 6-*O*-apiosyl glucoside suggested the presence of glucose and apiose in **1** [14]. Appearance of a set of doublets with geminal coupling attributable to an isolated methylene (apiose H-4″) supported the presence of apiose. The relatively large *J* values (7.6 Hz) of the anomeric proton at δ_H 4.78 indicated a *β*-configuration for the glucopyranosyl moiety. The ^{13}C NMR shift of the anomeric carbon of the apiofuranosyl unit at δ_C 110.7 also indicated a *β*-configuration [14]. The location of the *β*-apiose moiety at the C-6′ of *β*-glucose was apparent from the large low field shift of the C-6′ (δ 68.5, $\Delta\delta$ 6.0) compared with that of maltol 3-*O*-*β*-D-glucoside (δ 62.5) [2, 14]. The absolute configurations of the glucose and apiose were determined to be D-glucose and D-apiose by reversed-phase HPLC after conversion of the sugars to thiocarbamoyl–thiazolidine derivatives [15].

Table 1 ^1H- and ^{13}C-NMR data for compounds **1** and **2** (^1H; 500 MHz, ^{13}C; 125 MHz, CD_3OD)

	1		2	
	^{13}C	^1H	^{13}C	^1H
Maltol				
2	164.6		164.4	
3	143.3		143.3	
4	177.0		176.9	
5	117.2	6.45 (d, 5.6)	117.2	6.38 (d, 5.6)
6	157.1	8.00 (d, 5.6)	157.0	7.89 (d, 5.6)
7	15.9	2.45 (s)	15.9	2.39 (s)
D-Glucose				
1′	105.0	4.78 (d, 7.6)	105.2	4.72 (d, 7.6)
2′	74.9	3.27–3.45 (m)	74.9	3.25–3.40 (m)
3′	77.9	3.27–3.45 (m)	78.4	3.25–3.40 (m)
4′	71.3	3.27–3.45 (m)	71.3	3.25–3.40 (m)
5′	77.4	3.27–3.45 (m)	77.4	3.25–3.40 (m)
6′	68.5	3.94 (dd, 2.0, 11.2)	68.5	3.93 (d, 11.2)
		3.60 (dd, 6.1, 11.2)		3.57 (dd, 6.0, 11.2)
D-apiose				
1″	110.7	4.95 (d, 2.2)	110.4	4.94 (d, 2.2)
2″	77.8	3.84 (d, 2.2)	77.9	3.92 (d, 2.2)
3″	80.4		78.9	
4″	75.3	3.89 (d, 9.5)	75.3	3.90 (d, 9.8)
		3.73 (d, 9.5)		3.78 (d, 9.8)
5″	65.5	3.54 (2H, s)	67.4	4.21 (d, 11.4)
				4.17 (d, 11.4)
p-coumaroyl group				
1‴			127.0	
2‴, 6‴			131.1	7.41 (d, 8.5)
3‴, 5‴			116.8	6.76 (d, 8.5)
4‴			161.2	
7‴			146.8	7.59 (d, 16.0)
8‴			114.7	6.30 (d, 16.0)
9‴			168.7	

Coupling constants (*J* in Hz) are given in parentheses

Compound **2** was obtained as a white amorphous powder, and the ^1H and ^{13}C NMR spectra were closely related to those of **1**, except for the appearance of additional 9 sp^2 carbon signals arising from a *p*-coumaroyl ester group [δ_H 7.59 (d, *J* = 16.0 Hz, H-7‴), 6.30 (d, *J* = 16.0 Hz, H-8‴), 7.41 (2H, d, *J* = 8.5 Hz, H-2‴, H-6‴), 6.76 (2H, d, *J* = 8.5 Hz, H-3‴, H-5‴); δ_C 127.0 (C-1‴), 131.1 (C-2‴, C-6‴), 116.8 (C-3‴, C-5‴), 161.2 (C-4‴), 146.8 (C-7‴), 114.7 (C-8‴), 168.7 (C-9‴)] [11]. The formula $C_{26}H_{30}O_{14}$, which was determined by MALDI-TOF–MS (589 *m/z*, $[M+Na]^+$) and elemental analysis, is consistent with **2** being a *p*-coumaroyl ester of **1**. The ^{13}C NMR spectrum showed sugar carbon signals similar to those of **1** except for the low field shift of apiose C-5″ (δ_C 67.4, $\Delta\delta$ 1.9). In addition, a large low field shift of the apiose H-5″ signal at δ 4.17 (d, *J* = 11.4 Hz) and 4.21 (d, *J* = 11.4 Hz) compared to those of **1** [δ 3.54 (2H, s)] unequivocally indicated the acylation of this position. Acid hydrolysis of **2** yielded *p*-coumaric acid and maltol along with D-apiose and D-glucose. Thus the structure of **2** was concluded to be maltol 6′-*O*-(5-*O*-*p*-coumaroyl)-*β*-D-apiofuranosyl -*β*-D-glucopyranoside.

This study revealed the presence of two cyanogenic glycosides, prunasin and amygdalin, and the total isolation yield was 0.08% from dry weight. Since Armeniacae semen (apricot kernel), which contains these compounds, has been used for cough remedies in Europe and China, this result chemically supports the use of *E. rugulosa* for the treatment of colds and coughs in China. In addition, luteolin 3′-*O*-*β*-D-glucuronide [16–19] and rosmarinic acid [20–22] were found to be major phenolic constituents, and these compounds are known to have anti-inflammatory activity.

Experimental

General

UV spectra were obtained with a Jasco V-560 UV/Vis spectrophotometer, and optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^1H and ^{13}C NMR spectra were measured in CD_3OD at 27°C with a Varian Unity plus 500 spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C. Coupling constants are expressed in Hz and chemical shifts are given on a δ (ppm) scale. MS were recorded on a Voyager DE-PRO (Applied Biosystems) and JEOL JMS-700N spectrometer, 2,5-dihydroxybenzoic acid and glycerol were used as the matrix

for MALDI-TOF–MS and FAB–MS measurements, respectively.

Plant material

The aerial parts of *E. rugulosa* Hemsl. were collected in Yunnan Province, China, and identified by Prof. Yang Chongren. A voucher specimen was deposited in the Kunming Institute of Botany, CAS, Kunming, China.

Extraction and isolation

The air-dried aerial parts of *Elsholtzia rugulosa* (3.0 kg) were extracted with 70% aqueous acetone (10 l) at room temperature three times. The acetone was removed by evaporation under reduced pressure (ca. 40°C), and the resulting dark green precipitates consisting mainly of chlorophylls and waxes were removed by filtration. The filtrate was concentrated to give an extract (170 g), which was directly subjected to Diaion HP-20 column chromatography (8 cm i.d. × 45 cm) with water containing increasing proportions of MeOH (20% step-wise elution from 0–100%) and finally with 50% aqueous acetone, and fractionated into four fractions. The first fraction (67.6 g) contained highly water soluble compounds and was mainly composed of sugars, and the last fraction (19.6 g) contained non-polar substances including chlorophylls. Fraction 3 (21.3 g) was separated into two fractions by Diaion HP-20 (0–100% MeOH in H_2O, 10% step-wise elution) and the first fraction was combined with fraction 2 because these fractions contained similar compounds. Fraction 3–2 was subjected to Sephadex LH-20 column chromatography with 60% MeOH to yield luteolin (76.3 mg). Fraction 2, after mixing with fraction 3–1, (35.0 g) was chromatographed over Diaion HP-20 (0–100% MeOH in H_2O, 10% step-wise elution) to yield four fractions: 2–1 (2.0 g), 2–2 (17 g), 2–3 (3.7 g) and 2–4 (10.0 g). Fraction 2–1 was crystallized from water to give luteolin 3′-*O*-*β*-D-glucuronide (1.72 g). Fraction 2–2 was separated by a combination of column chromatography using Sephadex LH-20 (40–80% MeOH), Chromatorex ODS (0–80% MeOH) and silica gel 60 ($CHCl_3$—MeOH–H_2O, 80:20:2—70:30:5 v/v/v) to furnish **1** (56.4 mg), **2** (44.2 mg), maltol 3-*O*-*β*-D-glucoside (52.2 mg), tuberonic acid *β*-D-glucoside (35.2 mg), benzyl alcohol *β*-D-glucoside (512.1 mg), prunasin (2.18 g), amygdalin (178.0 mg) and caffeic acid (60.4 mg). Fraction 2–3 was successively separated by Sephadex LH-20 (40–60% MeOH), Chromatorex ODS (0–80% MeOH) and then silica gel 60 ($CHCl_3$–MeOH–H_2O, 70:30:5) to yield luteolin 7-*O*-*β*-D-glucoside (50.0 mg) and quercetin 3-*O*-*β*-D-glucoside (84.0 mg). Chromatography of fraction 2–4 over Sephadex LH-20 (60–80% MeOH), Chromatorex ODS (20–60% MeOH) and Sephadex LH-20 (EtOH) afforded rosmarinic acid (874.5 mg) and caffeic acid (67.5 mg).

Maltol 6′-*O*-*β*-D-apiofuranosyl-*β*-D-glucopyranoside (1)

White amorphous powder. $[\alpha]_D^{25}$ −6.2° (*c* 0.7, MeOH). UV λ_{max}^{EtOH} (log *ε*): 255 (3.97). IR ν_{max} cm^{-1}: 3,376, 1,650. MALDI-TOF–MS *m/z*: 443 $[M+Na]^+$. *Anal.Calcd* for $C_{17}H_{24}O_{12}\cdot 3H_2O$: C, 43.04; H,6.37. Found: C, 43.01; H, 6.41. ^1H and ^{13}C NMR see Table 1.

Maltol 6′-*O*-(5-*O*-*p*-coumaroyl)-*β*-D-apiofuranosyl-*β*-D-glucopyranoside (2)

White amorphous powder. $[\alpha]_D^{25}$ −81.5° (*c* 0.6, MeOH). UV λ_{max}^{EtOH} (log *ε*): 258 (4.09), 313 (4.35). IR ν_{max} cm^{-1}: 3,402, 1,703, 1,647, 1,613. MALDI-TOF-MS *m/z*: 589 $[M+Na]^+$. *Anal.Calcd* for $C_{26}H_{30}O_{14}\cdot 4H_2O$: C, 48.90; H, 6.00. Found: C, 48.58; H, 6.12. ^1H and ^{13}C NMR see Table 1.

Acid hydrolysis of 2

A solution of **2** (3 mg) in 1 M HCl (0.5 ml) was heated at 90–100°C in a screw-capped vial for 5 h. The mixture was partitioned with EtOAc (0.5 ml), and the EtOAc layer was analyzed by HPLC [Cosmosil $5C_{18}$ AR II (250 × 4.6 mm i.d.); gradient elutions of CH_3CN in 50 mM H_3PO_4 from 4–30% in 39 min and 30–75% in15 min; flow rate, 0.8 ml/min; detection with a Jasco MD-910 photodiode array detector]. The t_R and UV absorptions of the two peaks at 14.61 and 29.55 min were coincided with those of maltol (UV λ_{max}: 223 nm) and *p*-coumaric acid (UV λ_{max}: 310 nm), respectively.

Determination of aldose configuration

A solution of **1** (0.8 mg) in 1 M HCl (0.2 ml) was heated at 90–100°C in a screw-capped vial for 5 h. The mixture was neutralized by addition of Amberlite IRA400 (OH^- form) and filtered. The filtrate was dried in vacuo, dissolved in 0.2 ml of pyridine containing L-cysteine methyl ester (10 mg/ml) and reacted at 60°C for 1 h. To this mixture a solution (0.2 ml) of *o*-torylisothiocyanate in pyridine (10 mg/ml) was added, and it was heated at 60°C for 1 h. The final mixture was directly analyzed by HPLC [Cosmosil $5C_{18}$ AR II (250 × 4.6 mm i.d., Nacalai Tesque Inc.);

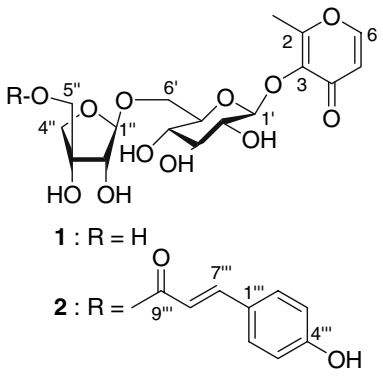

Fig. 1 Structures of compounds **1** and **2**

25% CH_3CN in 50 mM H_3PO_4; flow rate, 0.8 ml/min; detection, 250 nm]. The t_R of the two peaks at 17.48 and 28.57 min coincided with those of D-glucose and D-apiose, respectively. The standard samples of D- and L-apiose were synthesized from L- and D-ribose, respectively [23]. The aqueous layer remaining after partition with EtOAc on the acid hydrolysis of **2** was treated in the manner similar to that described for **1**, and the HPLC analysis showed the presence of D-glucose and D-apiose (Fig. 1).

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