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Coumarin glucosides from *Cruciata taurica*

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Abstract

Two new coumarin glycosides (**1** and **2**) along with two known coumarin glucosides, daphnin (**3**) and daphnetin glucoside (**4**) were isolated from the aerial parts of *Cruciata taurica*. The structures of the new compounds were elucidated by spectral methods and chemical means as 7-*O*-(6′-acetoxy-β-D-glucopyranosyl)-8-hydroxycoumarin (**1**) and 7-*O*-[6′-*O*-(3″,4″-dihydroxycinnamoyl)-β-D-glucopyranosyl]-8-hydroxycoumarin (**2**). The phylogenetic significance of coumarins in *C. taurica* was discussed.

Keywords: *Cruciata taurica*; Rubiaceae; Coumarin, Phylogenetic relationships

1. Introduction

Cruciata Mill. (Rubiaceae) is a small genus distributed in the Europe and Mediterranean region. An extensive information about morphological, karyological and ecogeographic differentiation of the genus has been accumulated (Ehrendorfer and Schönbeck-Temesy, 1982; Ehrendorfer and Krendl, 1976; Ehrendorfer, 1971; Pobedimova, 1958). In continuation of the chemotaxonomic investigation on the genus *Cruciata* (De Rosa et al., 2001; Mitova et al., 1996) herein, we report the isolation of coumarin glucosides from the extremely polymorphic polyploid species *Cruciata taurica* (Pallas ex Willd.) Ehrend. Coumarin pattern of investigated *Cruciata* species showed unexpected relations between species of this genus.

To our knowledge in *C. taurica* the coumarins: umbeliferon, scopoletin (Borisov, 1974) and cruciatin (Borisov, 1967), flavonoids: hyperoside, quercetin (Borisov, 1967) and rutin (Temizer et al., 1995) and iridoid glucoside asperuloside (Borisov, 1967) were found. Recently, we reported the isolation of two new aromatic monoterpenoid glycosides (De Rosa et al., 2001).

2. Results and discussion

The methanol extract of the aerial parts of *C. taurica* was partitioned between chloroform and water. The water-soluble part, upon repeated droplet counter current chromatography (DCCC), column chromatography (CC) over silica gel and high-pressure liquid chromatography (HPLC) afforded four pure compounds. By means of spectroscopic data and published information two of them were identified as the known coumarin glycosides: daphnin (**3**) and daphnetin glucoside (**4**) (Jewers and Zirvi, 1978), and two appeared to be new compounds (**1** and **2**).

Compound **1**, isolated as amorphous solid, was assigned the molecular formula $C_{17}H_{18}O_{10}$ as determined from HRFABMS and NMR data (Table 1). Its UV spectrum with λ_{max} 266 and 322 nm was characteristic of a coumarin derivative. The IR spectrum showed the presence of an ester function and an α–β-unsaturated lactone (ν_{max} 1735 and 1720 cm^{-1}). The ^1H NMR spectrum of **1** showed two proton doublets at δ 7.86 and 6.31 ($J = 9.5$ Hz) characteristic for the H-3 and H-4 of coumarins (Joseph-Nathan et al., 1984). The presence of further two proton doublets at δ 7.15 and 7.07 ($J = 8.7$ Hz) in the ^1H NMR spectrum, and resonance of carbons bearing two oxygen moieties at δ 149.4 (*s*) and 135.7 (*s*) indicated a 7,8-disubstituted coumarin (Mikhova and Duddeck, 1996). Thus, the structure of

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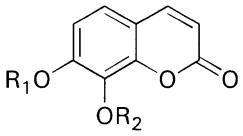

1 R_1 = 6 Ac-Glc; R_2 = H
2 R_1 = 6-O-(3,4-dihydroxycinnamoyl)Glc; R_2 = H
3 R_1 = Glc; R_2 = H
4 R_1 = H; R_2 = Glc
5 R_1 = R_2 = H

Table 1
^{13}C NMR spectral data of compound **1** (CD_3OD) and **2** (DMSO-d_6), (δ ppm)[a]

	1	2
2	162.8 *s*	160.0 *s*
3	114.6 *d*	113.4 *d*
4	146.0 *d*	144.5 *d*
5	119.4 *d*	117.7 *d*
6	114.0 *d*	112.0 *d*
7	149.4 *s*	148.0 *s*
8	135.7 *s*	134.4 *s*
9	116.4 *s*	114.4 *s*
10	144.3 *s*	142.8 *s*
1′	103.1 *d*	101.4 *d*
2′	74.7 *d*	73.2 *d*
3′	77.3 *d*	75.5 *d*
4′	71.4 *d*	70.0 *d*
5′	75.6 *d*	74.0 *d*
6′	64.6 *t*	63.2 *t*
$\underline{CO}CH_3$	172.6 *s*	
$CO\underline{CH_3}$	20.7 *q*	
1″		166.3 *s*
2″		113.6 *d*
3″		145.3 *d*
4″		125.3 *s*
5″		114.8 *d*
6″		145.7 *s*
7″		148.6 *s*
8″		115.8 *d*
9″		121.4 *d*

[a] Chemical shifts are referred to residual solvents resonance. Assignments were established by DEPT, HMQC and HMBC spectra.

the aglycone was similar to daphnetin (**5**) (Mikhova et al., 1996). Acid hydrolysis of **1** afforded glucose, which was confirmed by TLC and high performance anion exchange (HPAE–FAD) and daphnetin. The spectral data of daphnetin (**5**) were in excellent agreement with those reported in the literature (Mikhova et al., 1996). The signal of the anomeric proton at δ 4.97 (*d*, J = 7.5 Hz), in the ^{1}H NMR spectrum of **1**, showed that glucose was bound in β-configuration. The presence of an acetyl group [δ 1.95 (3H, *s*); 20.7 (*q*) and 172.6 (*s*)] in the NMR spectra of **1** and fragment peak (*m*/*z* 178), in the EIMS spectrum, due to the loss of acetate hexose unit, suggested that the acetyl group is located on the glucose moiety. HMBC correlations observed between the two protons of glucose methylene group (δ 4.46 and 4.28) and the acetyl carbonyl group (δ 172.6), and between the anomeric proton at δ 4.97 and the carbon at δ 149.4, defined the esterification at C-6′ and the glucosidation at C-7, respectively. Thus, compound **1** is the acetyl derivative of daphnin (**3**) and its structure was elucidated as 7-*O*-(6′-acetoxy-β-D-glucopyranosyl)-8-hydroxy coumarin.

Compound **2**, isolated as amorphous solid, was assigned the molecular formula $C_{24}H_{22}O_{12}$ as determined from HRFABMS and NMR data (Table 1). A preliminary analysis of its spectral data showed that **2** is related to compound **1**. In fact, acid hydrolysis of **2** afforded glucose (TLC; HPAE–FAD), daphnetin and cinnamic acid. The IR spectrum showed the presence of an α,β-unsaturated ester function and an α,β-unsaturated lactone (ν_{max} 1725 and 1720 cm^{-1}). Its FABMS exhibited fragment ion peaks at *m*/*z* 341 $[MH–162]^+$ and 179 $[MH–162–161]^+$ indicating the loss of an ester unit and a hexose. The signal of the anomeric proton at δ 4.94 (*d*, J = 7.1 Hz) showed that glucose was attached in the β-configuration. The ^{13}C NMR spectrum of **2** contained 24 carbon signals, six of which were readily assigned to a glucopyranosyl moiety, nine fitted with a daphnetin moiety, and the remaining nine carbons to an aromatic acyl unit. The NMR analysis of the ester unit of **2** showed a triple-substituted aromatic ring, a double bond and a carbonyl group. Taking into account the molecular formula, and the presence of two carbon singlets at δ 148.6 and 145.7 in the ^{13}C NMR spectrum, it was assumed that two of the ring substituents were hydroxyl groups. The signals of the aromatic protons at δ 7.06 (*d*, J = 1.8 Hz), 7.00 (*dd*, J = 8.5 and 1.8 Hz) and 6.79 (*d*, J = 8.5 Hz) indicated the presence of a 1,3,4-trisubstituted benzene ring. The coupling constant value of the olefinic protons (J = 15.9 Hz) defined the *E*-configuration for the double bond. Thus, the aromatic acyl unit was identified as a 3,4-dihydroxycinnamoyl moiety. HMBC correlations observed between the two protons of the glucose methylene group (δ 4.44 and 4.20) and the ester carbonyl group (δ 166.3), and between the anomeric proton at δ 4.94 and the carbon at δ 148.0, defined the acylation at C-6′ and the glucosidation at C-7, respectively. From the above evidence, compound **2** was characterised as 7-*O*-[6′-*O*-(3″,4″-dihydroxycinnamoyl)-β-D-glucopyranosyl]-8-hydroxycoumarin.

The coumarin pattern of *C. taurica* and *C. glabra* is very similar. These species contain two common coumarins: daphnin (**3**) and daphnetin glucoside (**4**) (Mitova et al., 1996), and additionally *C. taurica* contain two daphnin esters (**1** and **2**). According to the classical taxonomical schemes of the genus *Cruciata* (Ehrendorfer and Krendl, 1976; Ehrendorfer and Schönbeck-Temesy,

1982; Pobedimova, 1958) these species are not considered closely related. The similar coumarin pattern could be explained by convergent evolution regarding the coumarins of *C. taurica* and *C. glabra* or by relationships between these species before the inflorescens evolution of the genus *Cruciata* (cymes without bracteols, *C. glabra*; cymes with bracteols, *C. taurica*). Furthermore, the finding of aromatic monoterpenoid glycosides in *C. taurica* (De Rosa et al., 2001), does not explain this question. The chemical investigation of more *Cruciata* species could clarify the relationships in this genus.

3. Experimental

3.1. General

UV spectra were obtained on a Varian DMS 90 spectrophotometer. IR spectra were recorded on a Bio-Rad FTS-7 FT–IR spectrometer. Optical rotations were measured on a Jasco DIP 370 polarimeter, using a 10-cm microcell. FABMS were obtained on a VG-ZAB instrument, using glycerol as matrix. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker AM 500 instrument, under Aspect X32 control. The 2D NMR spectra were obtained using Bruker's microprograms. DCCC was performed on a Büchi 670 apparatus by ascending mode. Aluminum sheets silica-gel 60 F_{254} were used for TLC. Merck silica-gel 60 (0.063–0.2) were used for CC. Preparative HPLC purifications were carried out on a Waters apparatus equipped with a Kromasil C-18 column (7.8 mm i.d.×30 cm) and with UV detector.

3.2. Plant material

C. taurica was collected at florescence, in May 1999, at Hatay, Turkey and dried in shade at room temperature. The voucher specimens CO434 was deposited in the herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM).

3.3. Extraction and isolation

Dry aerial parts (27 g) were extracted twice with MeOH, and the concentrated extract (4.0 g) partitioned between CH_3Cl and H_2O. Ascending DCCC with $CHCl_3$–MeOH–H_2O–n-PrOH separated the aqueous phase (3.0 g) (9:12:8:2). The flow-rate was 25 ml/h. Fractions of 12.5 ml were collected. The MeOH soluble part, of fractions 15–19, contained daphnin (**3**, 386 mg). From frs 26–34 (112 mg), after silica-gel column, eluted with $CHCl_3$–MeOH–H_2O (30:11:2) and purification on HPLC Kromasil C-18 column, eluted with CH_3CN–H_2O (1:3; flow 2 ml/min) were obtained daphnetin glucoside (**4**, 6.2 mg; eluted after 4.8 min) and compound **2** (7.1 mg; eluted after 10.5 min). Compound **1** (58 mg; with R_f on TLC of 0.92 in $CHCl_3$–MeOH, 7:3) was obtained after additional purification of frs 46–56 (325 mg) using silica gel column, eluted with $CHCl_3$–MeOH–H_2O (30:11:2).

3.4. 7-O-(6′-Acetoxy-β-D-glucopyranosyl)-8-hydroxycoumarin (*1*)

Amorphous solid, $[\alpha]_D = -76.7^\circ$ (MeOH; *c* 0.004); UV (MeOH) λ_{max} nm (log ε): 322 (4.07), 266 (3.51); IR ν_{max} (KBr) cm^{-1} 3600–3400, 1735, 1720, 1690, 1620, 1500, 1290, 1200 and 1100; HRFABMS (positive) *m/z*: 383.1015 $[M+H]^+$ ($C_{17}H_{19}O_{10}$ requires 383.1004); EIMS *m/z* %: 382 $[M]^+$ (0.5), 322 $[M-AcH]^+$ (1), 309 $[M-CH_2OCOCH_3]^+$ (1), 178 $[M-GlcAc]^+$ (100), 150 (95); ^{1}H NMR (CD_3OD): δ 7.86 (1H, *d*, *J* = 9.5 Hz, H-4), 7.15 (1H, *d*, *J* = 8.5 Hz, H-6), 7.07 (1H, *d*, *J* = 8.5 Hz, H-5), 6.31 (1H, *d*, *J* = 9.5 Hz, H-3), 4.97 (1H, *d*, *J* = 7.5 Hz, H-1′), 4.46 (1H, *dd*, *J* = 11.9, 2.0 Hz, H-6a′), 4.28 (1H, *dd*, *J* = 11.9, 6.4 Hz, H-6b′), 3.72 (1H, *m*, H-5′), 3.60 (1H, *m*, H-2′), 3.55 (1H, *m*, H-3′), 3.44 (1H, *m*, H-4′), 1.95 (3H, *s*, CO<u>CH$_3$</u>); ^{13}C NMR: see Table 1.

3.5. 7-O-[6′-O-(3″,4″-Dihydroxycinnamoyl)-β-D-glucopyranosyl]-8-hydroxycoumarin (*2*)

Amorphous solid, $[\alpha]_D = -25.9^\circ$ (MeOH; *c* 0.009); UV (MeOH) λ_{max} nm (log ε): 320 (4.52), 264 (3.48), 227 (4.03); IR ν_{max} (KBr) cm^{-1} 3600–3400, 1725, 1720, 1690, 1620, 1500, 1490, 1290, 1200 and 1060; HRF ABMS (positive) *m/z*: 503.1210 $[M+H]^+$ ($C_{24}H_{23}O_{12}$ requires 503.1221), 341 $[MH-162]^+$, 179 $[MH-162-161]^+$; ^{1}H NMR (CD_3OD): δ 7.81 (1H, *d*, *J* = 9.7 Hz, H-4), 7.47 (1H, *d*, *J* = 15.9 Hz, H-3″), 7.09 (1H, *d*, *J* = 8.5 Hz, H-6), 7.06 (1H, *d*, *J* = 1.8 Hz, H-5″), 7.00 (1H, *dd*, *J* = 8.5, 1.8 Hz, H-9″), 6.98 (1H, *d*, *J* = 8.5 Hz, H-5), 6.79 (1H, *d*, *J* = 8.5 Hz, H-8″), 6.29 (1H, *d*, *J* = 9.7 Hz, H-3), 6.27 (1H, *d*, *J* = 15.9 Hz, H-2″), 4.94 (1H, *d*, *J* = 7.1 Hz, H-1′), 4.44 (1H, *bd*, *J* = 11.4 Hz, H-6a′), 4.20 (1H, *dd*, *J* = 11.4, 6.4 Hz, H-6b′) 3.73 (1H, *m*, H-5′) 3.42–3.30 (3H, *m*, H-2′, H-3′ and H-4′); ^{13}C NMR: see Table 1.

3.6. Acid hydrolysis

Compound **1** (5 mg) or compound **2** (2 mg) dissolved in 0.5 ml of 2 N HCl was refluxed for 1 h. The reaction mixture was neutralised and extracted with EtOAc to obtain aglycone. The water-soluble residue was analysed by TLC [mobile phase—iPrOH–toluen–EtOAc–H_2O (50:10:25:12.5)], and HPAE–PAD (Dionex) equipped with a Carbopac PA1 column eluted with 15 mM NaOH (1 ml min^{-1}) and with a Pulsed Amperometric detector, giving D-glucose, identified by comparison of its retention time with that of the authentic sample.

Compound **1** gave daphnetin (**5**) as aglycone. Compound **2** gave daphnetin (**5**) and 3,4-dihydroxycinnamic acid. The aglycones were identified by TLC [mobile phase—$CHCl_3$–MeOH–H_2O (60:15:4, lower layer)] in comparison with authentic samples.

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