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Iridoid and Phenylethanoid Glycosides from *Euphrasia pectinata*

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Two specimens of *Euphrasia pectinata* collected from different regions of northern Anatolia were studied to determine their iridoid and phenylethanoid glycosides. Four iridoid glucosides, aucubin (**1**), euphroside (**2**), ixorodoside (**3**), and boschnaloside (**4**), and two phenylethanoid glycosides, acteoside (**5**) and leucosceptoside A (**6**), were isolated, and characterized from the aerial parts of *E. pectinata* collected from Zigana pass (Trabzon). From the aerial parts of *E. pectinata* collected from Kartalkaya (Bolu), a phenethyl alcohol glycoside, decaffeoylacteoside (**7**), was isolated in addition to previously isolated compounds. The structures of the isolated compounds were established by spectroscopic evidence.

Key Words: *Euphrasia pectinata*, Scrophulariaceae, iridoid glucosides, aucubin, euphroside, ixoroside, boschnaloside, phenylethanoid glycosides, acteoside, leucosceptoside A, decaffeoylacteoside.

Introduction

The genus *Euphrasia* is represented by ten species in the flora of Turkey[1]. *Euphrasia pectinata* Ten. is a widely distributed plant in the flora and its flowering herb is used for wound healing in Anatolian folk medicine[2]. Iridoid glucosides, boschnaloside and 7-hydroxyboschnaloside, were previously reported from a Russian sample of *E. pectinata*[3]. Our previous research on *E. pectinata*, collected from Kartalkaya-Bolu, led to the isolation of six iridoid glucosides, 5β,6β-dihydroxyboschnaloside, 6β-hydroxyboschnaloside, aucubin, euphroside, plantarenaloside and geniposidic acid as well as two phenylethanoid glycosides, acteoside and leucosceptoside A[4]. In the continuation of our research on *E. pectinata*, we now report the isolation and structure elucidation of the iridoid glucosides aucubin (**1**), euphroside (**2**), ixoroside (**3**) and boschnaloside (**4**) along with the phenylethanoid glycosides acteoside (**5**) and leucosceptoside A (**6**) from the samples collected from Zigana pass (Trabzon). Further investigation of the previously studied *E. pectinata* specimen[4] has now resulted in the isolation of a phenethyl alcohol glycoside, decaffeoylacteoside (**7**).

Experimental

General Experimental Procedures: UV ($\lambda_{max.}$) specta were recorded on a Hitachi HP 8452 A spectrophotometer. IR (cm^{-1}) spectra were recorded on a Perkin-Elmer 2000 FTIR spectrophotometer, using KBr pellets. NMR measurements in CD_3OD were recorded on a Varian Unity 500 spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C. ^1H-^{13}C HSQC, and HMBC experiments were recorded by employing conventional pulse sequences. ESIMS were recorded on a Finnigan LCQDECA ion trap mass spectrometer. Silica gel 60 (0.063-0.200 mm, Merck) was used for vacuum-liquid chromatography (VLC) (column 5.2x20 cm, i.d.) and open CC. MPLC separations were performed on a Labomatic glass column (1.8x35.2 cm, i.d.), packed with LiChroprep RP-18, using a Lewa M5 peristaltic pump. TLC analyses were carried out on pre-coated silica gel 60 F_{254} aluminium sheets (Merck). Compounds were detected by UV fluorescence and spraying 1% vanillin/H_2SO_4, followed by heating at 100° C for 1-2 min.

Plant Materials. *Euphrasia pectinata* Ten. (Scrophulariaceae) were collected from Kartalkaya-Bolu in August 1996, as reported previously[4], and from Zigana pass (Trabzon) in July 1998. Voucher specimens have been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 96-006 and HUEF 98-084, resp.).

Extraction and Isolation. The air-dried and powdered aerial parts of *E. pectinata* (36 g) collected from Zigana pass were extracted twice with MeOH (2x250 ml) at 40° C. The combined extracts were evaporated under reduced pressure and the crude extract (1.57 g) was fractioned over Si gel vacuum liquid chromatography. Elution with $CHCl_3$-MeOH-H_2O (90:10:0.5→70:30:3) yielded 3 main fractions (A-C) [Fr. A (78.3 mg), Fr. B (608.5 mg), Fr. C (410 mg)]. Fraction B was subjected to C_{18} medium pressure liquid chromatography (MPLC) using gradient MeOH-H_2O (5-45%) mixtures to yield **1** (3.45 mg), **2** (2.85 mg), **3** (6.05 mg), **4** (3.60 mg), **5** (29.1 mg) and **6** (19.8 mg). The extraction and isolation procedure for *E. pectinata* samples collected from Kartalkaya-Bolu region was reported in our previous study[4]. An aliquot of fraction C_2 (396 mg) was subjected to RP-18 medium-pressure liquid chromatography (MPLC) and elution with MeOH-H_2O mixtures (5-65%), affording **7** (2.5 mg).

Results

Aucubin (1): UV $\lambda_{max.}$(MeOH) nm: 210; IR $\upsilon_{max.}$ (KBr) cm^{-1}: 3369, 2918, 1655, 1230, 1045; .^1H NMR (500 MHz, CD_3OD): Table 1; ^{13}C NMR (125 MHz, CD_3OD): Table 2.

Euphroside (2): UV $\lambda_{max.}$(MeOH) nm: 237; IR $\upsilon_{max.}$ (KBr) cm^{-1}: 3400, 1700, 1640; ^1H NMR (500 MHz, CD_3OD): Table 1; ^{13}C NMR (125 MHz, CD_3OD): Table 2.

Ixoroside (3): UV $\lambda_{max.}$(MeOH) nm: 249; IR $\upsilon_{max.}$ (KBr) cm^{-1}: 3400, 1700, 1640; positive-ion ESIMS m/z 383 $[M+Na]^+$ (20); negative-ion ESIMS m/z 359 $[M-H]^-$ (100), 719 $[2M-H]^-$ (16); ^1H NMR (500 MHz, CD_3OD): Table 1; ^{13}C NMR (125 MHz, CD_3OD): Table 2.

Boschnaloside (4): UV $\lambda_{max.}$(MeOH) nm: 249; IR $\upsilon_{max.}$ (KBr) cm^{-1}: 3350, 1665, 1630; positive-ion ESIMS m/z 367 $[M+Na]^+$ (20); ^1H NMR (500 MHz, CD_3OD): Table 1; ^{13}C NMR (125 MHz, CD_3OD): Table 2.

Acteoside (5): UV $\lambda_{max.}$(MeOH) nm: 232, 218, 203; IR $\upsilon_{max.}$ (KBr) cm^{-1}: 3500, 1695, 1635, 1610, 1520; ^1H NMR (500 MHz, CD_3OD): Table 3; ^{13}C NMR (125 MHz, CD_3OD): Table 4.

Leucosceptoside A (6): UV $\lambda_{max.}$(MeOH) nm: 322, 288, 201; IR $\upsilon_{max.}$ (KBr) cm^{-1}: 3400, 1700, 1630, 1605, 1515; ^{1}H NMR (500 MHz, CD_3OD): Table 3; ^{13}C NMR (125 MHz, CD_3OD): Table 4.

Decaffeoylacteoside (7) UV $\lambda_{max.}$(MeOH) nm: 225 (sh), 283.5; IR $\upsilon_{max.}$(KBr) cm^{-1}: 3450, 1610, 1530; positive-ion ESIMS: m/z 485 [M+Na]$^{+}$ (100), 463 [M+H]$^{+}$ (30); negative-ion ESIMS: m/z 461 [M-H]$^{-}$ (100); ^{1}H NMR (500 MHz, CD_3OD): Table 3; ^{13}C NMR (125 MHz, CD_3OD): Table 4.

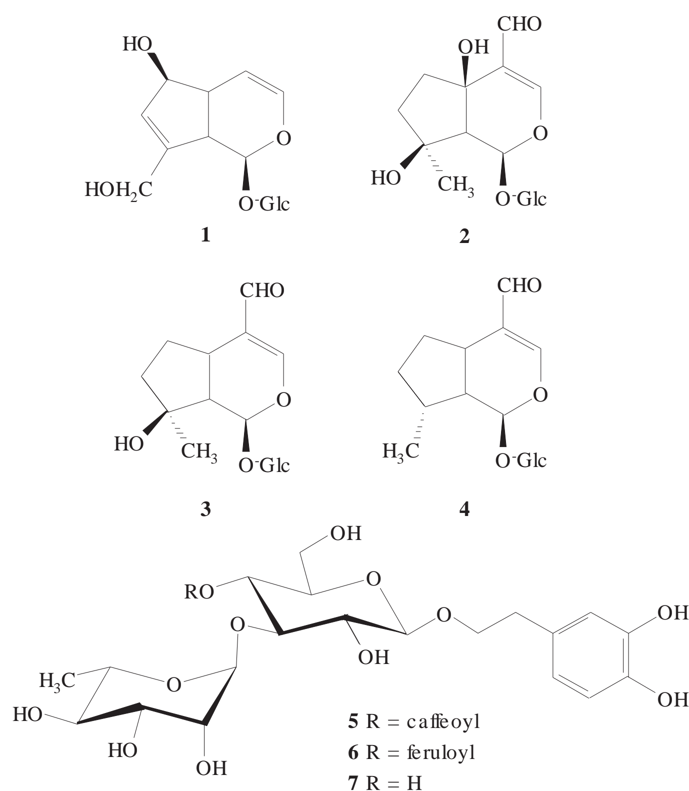

Figure 1. Iridoid and phenylethanoid glycosides isolated from *E. pectinata*.

Discussion

Compound **1** was obtained as a colourless amorphous compound. The UV ($\lambda_{max.}$ 210 nm), and the IR ($\upsilon_{max.}$ 3369, 2918, 1655 cm^{-1}) spectra of **1** were characteristic of a non-conjugated iridoid enol-ether system for C-4 non-substituted iridoids, which was supported by the NMR data. In the ^{1}H NMR spectrum (Table 1), the signals at δ_H 6.34 (dd, J = 6.1/1.9 Hz) and 5.12 (dd, J = 6.1/3.9 Hz) were attributed to H-3 and H-4, respectively, whose splitting pattern suggested C-5 to be unsubstituted. Therefore, the multiplet signal at δ_H 2.68 was assigned to H-5 (δ_C 46.3). H-7 resonance (δ_H 5.79 br.s) was highly deshielded due to the presence of a second double bond. The chemical shift values of both C-7 (δ_C 130.3) and C-8 (δ_C 148.1) and a doublet-like triplet signal at δ_H 2.92 (J = 7.3 Hz, H-9) clearly showed the presence of a second double bond between C-7 and C-8. Two doublets (AB system), at δ_H 4.37 (J = 15.4 Hz, H-10$_b$) and 4.19 (J = 15.4 Hz, H-10$_a$) were assigned to the methylene protons of a primary alcohol unit located at C-8. On the other hand, the proton resonance at δ_H 4.43 (dd, J = 3.4/1.7 Hz) was assigned to a secondary hydroxyl-bearing carbon atom, C-6 (δ_C 82.8). Furthermore, a characteristic anomeric proton resonance at δ_H 4.70 (d, J =

7.8 Hz) suggested that compound **1** contains a β-glucopyranoside moiety. On the basis of the spectroscopic data and a comparison with the published data in the literature, compound **1** was identified as aucubin[5,6].

Table 1. ^{1}H NMR data of aucubin (**1**), euphroside (**2**), ixoroside (**3**)*, and boschnaloside (**4**)* (500 MHz, CD_3OD)

Proton	**1** δ (ppm) J (Hz)	**2** δ (ppm) J (Hz)	**3** δ (ppm) J (Hz)	**4** δ (ppm) J (Hz)
Aglycone				
1	4.98 d (7.1)	5.85 br. s	5.51 br. s	5.60 d (3.8)
3	6.34 dd (6.1/1.9)	7.35 s	7.24 s	7.30 s
4	5.12 dd (6.1/3.9)	-	-	-
5	2.68 m	-	3.07 m	2.89 m
6	4.43 dd (3.4/1.7)	2.25 m 1.90 m	2.19 m 1.35 m	1.98 m 1.57 m
7	5.79 br. s	2.10 m 1.50 m	1.60 m	1.75 m 1.26 m
8	-	-	-	2.26 m
9	2.92 dd (t) (7.3)	2.45 br. s	2.15 m	2.25 m
10	4.37 d (15.4) 4.19 d (15.4)	1.20 s	1.20 s	1.03 s
11	-	9.25 s	9.10 s	9.10 s
Glucose				
1′	4.70 d (7.8)	4.62 d (7.8)	4.58 d (7.8)	4.70 d (7.8)
2′	3.18 dd (7.8/9.0)	3.18 dd (7.8/9.0)	3.15 dd (7.8/9.0)	3.18 dd (7.8/9.0)
3′	3.37 t (9.0)	3.38 t (8.5)	3.38 t (9.0)	3.37 t (9.0)
4′	3.30 t (9.0)	3.34 t (8.5)	3.34 t (9.0)	3.32 t (9.0)
5′	3.32 m	3.32 m	3.32 m	3.34 m
$6'_B$	3.87 dd (11.7/1.6)	3.85 dd (11.7/2.0)	3.80 dd (11.0/2.0)	3.85 dd (11.7/2.0)
$6'_A$	3.67 dd (11.7/5.3)	3.65 dd (11.7/6.1)	3.55 dd (11.0/6.0)	3.60 dd (11.7/6.1)

*All proton assignments are based on 2D NMR (DQF-COSY and HSQC)

The UV and IR spectra of compounds **2-4** showed the presence of a conjugated enol-ether system. The ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectra exhibited characteristic signals for a conjugated iridoid structure and indicated a close structural relationship between **2-4**. The following ^{1}H NMR signals were readily assignable: a broad singlet (doublet for **4**) [δ_H 5.85, 5.51, 5.60 (J = 3.8 Hz), resp.] due to H-1; a singlet (δ_H 9.25, 9.10, 9.15, resp.) due to an α,β-unsaturated aldehyde function (H-11); a singlet (δ_H 7.35, 7.24, 7.30, resp.) due to H-3 and a doublet [4.62 (J = 7.8 Hz); 4.58 (J = 7.8 Hz); 4.70 (J = 7.8 Hz), resp.] typical of the anomeric proton of a β-D-glucopyranose unit. These assumptions were also supported by the ^{13}C NMR spectra (*see* Table 2), in which 16 carbon resonances were observed.

In the ^{1}H NMR spectrum of **2**, the chemical shift value and the multiplicity of H-3 (δ_H 7.35, s) were indicative of an oxygen substitution at C-5. Therefore, the quaternary carbon resonance at δ_C 71.3 was readily assigned to C-5. Furthermore, the ^{1}H NMR spectrum of compound **2** exhibited signals arising from two methylene groups, assignable to H_2-6 (δ_H 2.25, m and 1.90, m) and H_2-7 (δ_H 2.10, m and 1.50, m). This proposal was confirmed by the ^{13}C NMR signals assigned for C-6 (δ_C 37.6, t) and C-7 (δ_C 40.3, t). The singlet signal at δ_H 1.20 was attributed to a tertiary methyl group (H_3-10). The multiplicity of H-9 (δ_H 2.45, br.s) and the chemical shift values of both C-8 (δ_C 78.9) and H_3-10 were indicative of the presence of a tertiary hydroxyl function and the methyl group at C-8. Consequently, according to its NMR data and a comparison with those given in the literature, the structure of **2** was established as euphroside[7].

Table 2. ^{13}C NMR data of aucubin (**1**), euphroside (**2**), ixoroside (**3**)*, and boschnaloside (**4**)* (125 MHz, CD_3OD)

	1		**2**		**3**		**4**	
C	Mult	δ (ppm)	Mult	δ (ppm)	Mult	δ (ppm)	Mult	δ (ppm)
Aglycone								
1	CH	97.7	CH	95.3	CH	96.6	CH	97.5
3	CH	141.6	CH	163.1	CH	163.2	CH	164.2
4	CH	105.7	C	126.4	C	126.1	C	126.3
5	CH	46.3	C	71.3	CH	30.1	CH	32.4
6	CH	82.8	CH_2	37.6	CH_2	29.7	CH_2	31.3
7	CH	130.3	CH_2	40.3	CH_2	41.0	CH_2	33.6
8	C	148.1	C	78.9	C	80.2	CH	37.1
9	CH	47.9	CH	61.3	CH	52.1	CH	44.1
10	CH_2	62.7	CH_3	23.6	CH_3	24.6	CH_3	16.6
11		-	C	192.6	C	193.1	C	193.1
Glucose								
1′	CH	100.0	CH	99.8	CH	100.0	CH	99.9
2′	CH	74.9	CH	74.2	CH	74.7	CH	74.8
3′	CH	77.9	CH	78.3	CH	78.0	CH	78.5
4′	CH	71.7	CH	71.6	CH	71.7	CH	71.7
5′	CH	78.3	CH	77.3	CH	78.5	CH	78.1
6′	CH_2	61.4	CH_2	62.7	CH_2	62.9	CH_2	63.0

*All carbon assignments are based on 2D NMR (DQF-COSY, gHSQC and gHMBC)

The molecular formula of **3** was established as $C_{16}H_{24}O_9$ by means of positive ESIMS (m/z 383 $[M+Na]^+$) and negative ESIMS (m/z 359 $[M-H]^-$ and 719 $[2M-H]^-$) together with ^{13}C NMR data (Table 2). The ^{1}H and ^{13}C NMR spectra of **3** were almost identical to that of **2** and exhibited characteristic signals for an iridoid structure with a 10-carbon skeleton. The complete assignments of all proton and carbon resonances were based on the results of DQF-COSY (Figure 2), HSQC (Figure 3) and HMBC (Figure 4) experiments. A DQF-COSY experiment allowed the establishment of the spin system sequence from H-1 to H-7. In the ^{1}H-^{1}H COSY spectrum, the H-1 signal (δ_H 5.51, br.s) was correlated to H-9 (δ_H 2.15, m), which in turn coupled to H-5 (δ_H 3.07, m), indicating C-5 to be non-substituted. Further proof of this assignment was provided by gHMBC correlations observed between C-3/H-5, C-4/H-5, C-6/H-5 and C-9/H-5. Since H-9 did not show any other homonuclear interaction, the C-8 position was supposed to be totally substituted. A ^{1}H-^{13}C HMBC correlation from C-8 (δ_C 80.2) to H_3-10 (δ_H 1.20, s), and heteronuclear long-range couplings observed between C-10/H_2-7, C-10/H-9 and C-8/H-9, showed the attachment of the methyl group at C-8. On the other hand, the chemical shift values of C-8 and H_3-10 indicated the presence of tertiary hydroxyl group at the C-8 position as in the case of **2**. H-5 exhibited an additional homonuclear coupling with the geminally coupled methylene protons (δ_H 2.19 m, and 1.35 m, H_2-6), and the latter proton resonances correlated to C-7 methylene protons (δ_H 1.60 m, H_2-7). Geminal couplings for C-7 methylene protons were not well established in the DQF-COSY spectrum, but a ^{1}H-^{13}C HSQC experiment revealed its unambigious assignment. Final analysis of the ^{1}H and ^{13}C NMR data indicated that the structure of **3** was almost identical to that of euphroside (**2**), except for the absence of the hydroxyl group at C-5. Based on the NMR data and a comparison with those given in the literature, compound **3** was identified as ixoroside[7,8].

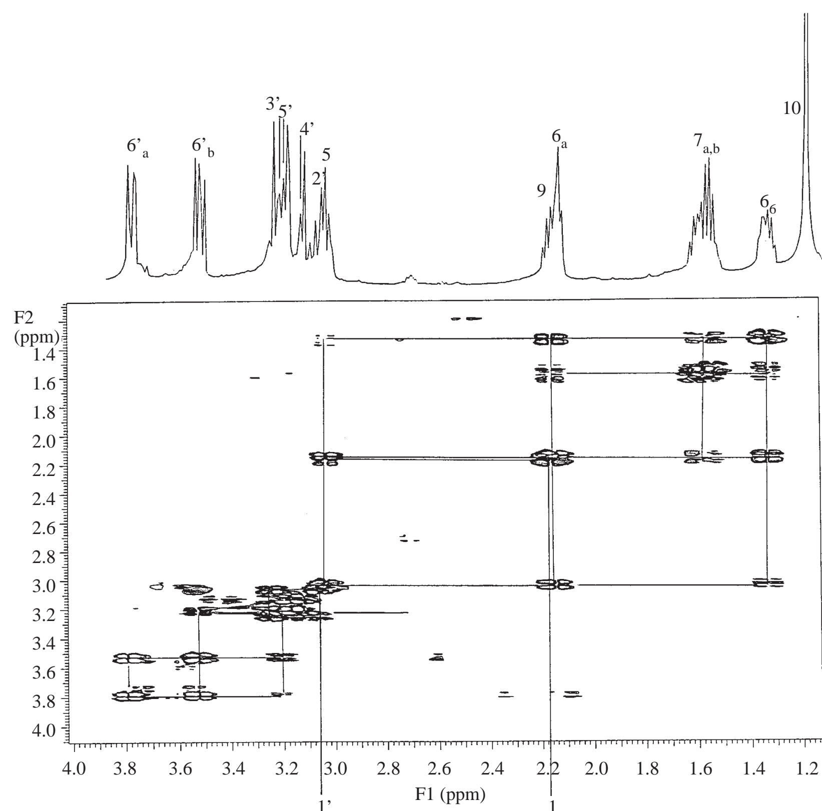

Figure 2. ^{1}H-^{1}H COSY spectrum of **3**.

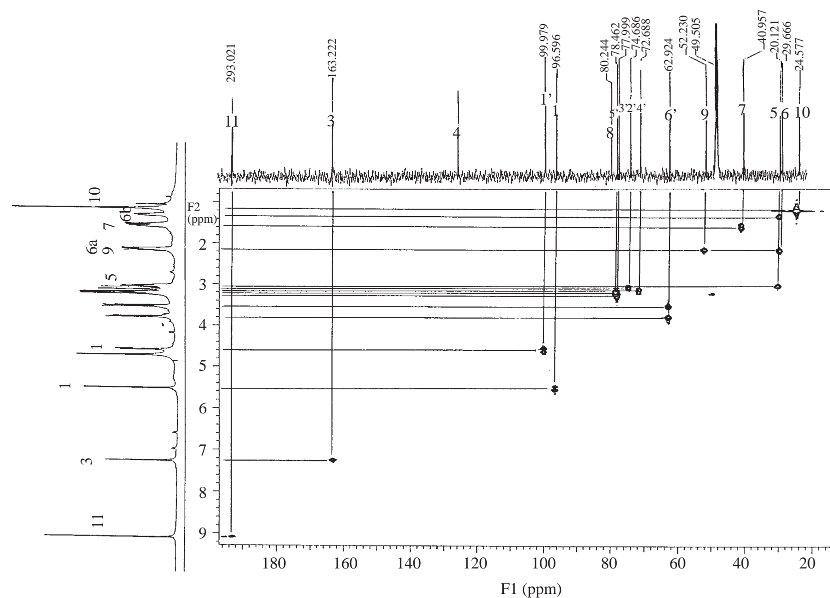

Figure 3. ^{1}H-^{13}C gHSQC spectrum of **3**.

Compound **4** was isolated as an amorphous powder. The positive ESIMS exhibited a pseudomolecular ion $[M+Na]^{+}$ at m/z 367, and the negative ESIMS showed the ions $[2M-H]^{-}$ at m/z 687, compatible with the molecular formula $C_{16}H_{24}O_{8}$, and in good agreement with the observation of 16 resonances in the ^{13}C NMR spectrum. All structural assignments were substantiated by the 2D shift-correlated DQF-COSY, HSQC, and

HMBC spectra. The ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra of **4** exhibited resonances almost identical to those in ixoroside (**3**). However, the pseudomolecular ion $[M+Na]^{+}$at m/z 367 in the positive ESIMS of **4** was 16 mass units less than that of **3** (m/z 383 $[M+Na]^{+}$), suggesting a loss of an oxygen function in **4**. In the ^{1}H-^{1}H COSY spectrum, the H-5 signal were coupled to geminally coupled C-6 methylene protons. H_2-6 signals showed a homonuclear coupling to geminally coupled C-7 methylene protons, which in turn were coupled to H-8 (δ_H 2.26, m). The chemical shift values of C-8 (δ_C 37.1) and the methyl protons (δ_H 1.03, s) and ^{1}H-^{1}H COSY interaction from H-8 to H_3-10 suggested the presence of a secondary methyl function at C-8. The gHMBC couplings between C-8/H_3-10, C-7/H_3-10 and C-9/H_3-10 confirmed the attachment of the methyl group at the C-8 position. Consequently, the structure of **4** was identified as boschnaloside[8,10].

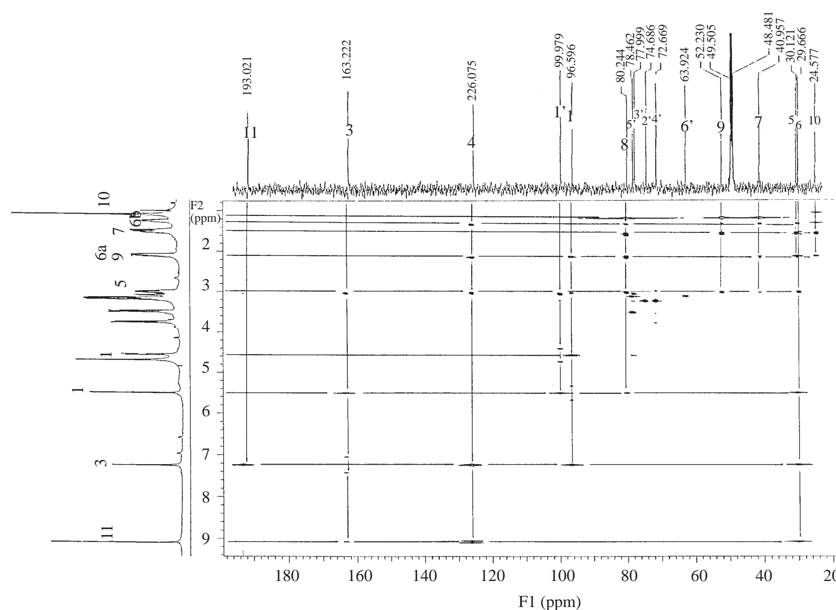

Figure 4. ^{1}H-^{13}C gHMBC spectrum of **3**.

Compounds **5-7** were obtained as colourless, amorphous compounds. UV spectra indicated their phenolic structures. IR bands for hydroxyl groups and aromatic rings were observed for **5-7**; however, the IR spectra of **5** and **6** exhibited additional absorption bands for an α,β-unsaturated ester function.

The ^{1}H NMR spectrum of compound **5** (Table 3) exhibited typical resonances arising from six aromatic protons (2 ABX systems; δ_H 7.05-6.56 region), two *trans*-olefinic protons (AB system, J_{AB} = 15.9 Hz), a benzylic methylene at δ_H 2.79 (2H, t, J = 7.2 Hz) and two non-equivalent proton signals at δ_H 3.72 m and 4.05 (each 1H, m). These data were consistent with the presence of a (*E*)-caffeic acid unit and 3,4-dihydroxyphenethyl alcohol moiety. In addition, two anomeric proton signals at δ_H 4.37 (d, J= 7.9 Hz) and 5.18 (d, J= 1.8 Hz) were attributed to the β-glucose and α-rhamnose units, respectively, indicating the disaccharide structure of **5**. The acyl group was positioned at the C-4′ position of the glucose unit, on the basis of the strong deshielding of the H-4′ signal (δ_H 4.95 t, J= 9.4 Hz) of the glucose unit. In the ^{13}C NMR spectrum (Table 4), the C-3′ (δ_C 81.7) resonance of the glucose unit showed a remarkable downfield shift (±4 ppm), indicating that the rhamnose moiety was attached to the C-3′ position of the glucose. Therefore, based on the NMR data, the structure of **5** was identified as acteoside[10].

The proton and carbon resonances of **6** due to the aglycone and sugar moieties were in good agreement with those of **5**, indicating the similar substructures. However, the methoxyl signal at δ_H 3.89 (3H, s) and the corresponding carbon resonance at δ_C 56.7 as well as the deshielded C-3‴ resonance (δ_C 150.7) suggested that the acyl group in **6** was (*E*)-ferulic acid. Therefore, the structure of **6** was established as leucosceptoside A[11].

Table 3. ^{1}H NMR data of acteoside (**5**) leucosceptoside A (**6**) and decaffeoylacteoside (**7**) (500 MHz, CD_3OD)

Proton	**5** δ (ppm) *J* (Hz)	**6** δ (ppm) *J* (Hz)	**7** δ (ppm) *J* (Hz)
Aglycone			
2	6.69 d (1.8)	6.69 d (2.0)	6.64 d (2.0)
5	6.67 d (8.2)	6.68 d (8.2)	6.70 d (8.0)
6	6.56 dd (8.2/1.8)	6.58 dd (8.2/2.0)	6.55 dd (8.2/2.0)
α	3.72 n	3.72 m	3.72-2.60†
	4.05 m	4.05 m	3.90 m
β	2.79 t (7.2)	2.80 t (7.3)	2.77 t (7.3)
Glucose			
1′	4.37 d (7.9)	4.38 d (7.9)	4.28 d (7.8)
2′	3.39 dd (9.1/7.9)	3.39 dd (9.1/7.9)	3.27 dd (9.5/7.9)
3′	3.81 t (9.1)	3.81 t (9.1)	3.72 -3.60†
4′	4.95 t (9.4)	4.95 (9.4)	3.33 t (9.5)
5′	3.55 m	3.55 m	3.39 m
$6'_B$	3.61 dd (12.2/2.0)	3.83 dd (11.6/2.0)	3.85 dd (12.0/2.0)
$6'_A$	5.53 dd (12.2/6.4)	3.61 br. d (11.6/6.4)	3.72-3.60†
Rhamnose			
1″	5.18 d (1.8)	5.20 d (2.1)	5.14 d (1.7)
2″	3.91 dd (3.4/1.8)	3.91 dd (3.0/2.1)	3.93 dd (1.7/3.2)
3″	3.57 dd (9.7/3.4)	3.56 dd (9.3/3.6)	3.72-3.60†
4″	3.28 t (9.7)	3.29 t (8.2)	3.47 t (10.0)
5″	3.54 m	3.54 m	1.24 d (6.2)
6″	1.09 d (6.1)	1.10 d (6.2)	
Acyl moiety			
2‴	7.05 d (1.4)	7.20 d (1.7)	
5‴	6.77 d (8.2)	6.81 d (8.2)	
6‴	6.96 dd (8.2/1.4)	7.09 dd (8.2/1.7)	
α′	6.28 d (15.9)	6.38 d (15.9)	
β′	7.59 d (15.9)	7.66 d (15.9)	
OCH_3	-	3.89 s	

†Signal patterns are unclear due to overlapping

Compound **7** was obtained as an amorphous powder with the molecular formula $C_{20}H_{30}O_{12}$, confirmed by the observation of 20 resonances in the ^{13}C NMR spectrum (Table 4) andthe pseudomolecular ions in the positive ESIMS (*m/z* 485 $[M+Na]^+$ and 463 $[M+H]^+$), and the negative ESIMS (*m/z* 461 $[M-H]^-$). The ^{1}H and ^{13}C NMR spectra of compound **7** (Tables 3 and 4) revealed a strong resemblance to those of acteoside (**5**), lacking, however, the (*E*)-caffeoyl moiety. Consequently, the structure of **7** was determined to be decaffeoylacteoside[12,13].

Table 4. ^{13}C NMR data of acteoside (**5**), leucosceptoside A (**6**) and decaffeoylacteoside (**7**) (125 MHz, CD_3OD)

C	Mult.	**5** δ (ppm)	**6** δ (ppm)	**7** δ (ppm)
Aglycone				
1	C	131.5	131.7	131.5
2	CH	117.2	117.1	117.1
3	C	146.7	146.1	146.1
4	C	144.3	144.6	144.7
5	CH	116.4	116.4	116.3
6	CH	121.3	121.2	121.2
α	CH_2	72.4	72.3	72.4
β	CH_2	36.6	36.6	36.6
Glucose				
1′	CH	104.3	104.3	104.3
2′	CH	76.3	76.1	77.9
3′	CH	81.7	81.3	84.6
4′	CH	70.7	70.3	70.2
5′	CH	76.1	76.0	75.7
6′	CH_2	62.4	62.4	62.7
Rhamnose				
1″	CH	103.1	102.7	102.8
2″	CH	72.3	72.0	72.1
3″	CH	72.1	72.3	72.3
4″	CH	73.9	73.9	74.0
5″	CH	70.5	70.7	70.1
6″	CH_3	18.5	18.2	17.9
Acyl moiety				
1‴	C	127.7	127.8	
2‴	CH	115.3	112.3	
3‴	C	146.9	150.7	
4‴	C	149.9	149.4	
5‴	CH	116.6	116.6	
6‴	CH	123.2	124.2	
α ′	CH	114.8	115.3	
β ′	CH	148.1	147.7	
C=O	C	168.3	168.3	
OCH_3	CH_3	-	56.7	

Conclusion

Our present study on two *Euphrasia pectinata* samples collected from two different regions of northern Anatolia partly confirmed our previous results[4]. The iridoid glucosides, 5β,6β-dihydroxyboschnaloside, 5β-hydroxyboschnaloside, plantarenaloside and geniposidic acid, previously isolated from samples from the Kartalkaya (Bolu) region were not detected in the Ziganapass (Trabzon) samples. As stated above, from the *E. pectinata* samples of the Zigana region, iridoid glucosides, ixoroside and boschnaloside were isolated, in addition to aucubin and euphroside. The latter two iridoids can be considered the common iridoid glucosides, since they were isolated from the two samples of *E. pectinata*. The difference in the iridoid content of the same plant species collected from two different regions may be of chemotaxonomical significance in future. On the other hand, the phenylethanoid glycosides, acteoside and leucosceptoside A

were isolated from both plant samples. A phenylethyl alcohol glycoside, decaffeoylacteoside, was isolated from the Kartalkaya-Bolu samples, in addition to the formerly isolated compounds[4]. Decaffeoylacteoside (**7**) has been previously reported as a constituent of *Osmanthus fortunei* (Oleaceae)[12], *Cistanche salsa* (Orobanchaeae)[13], *Rehmannia glutinosa* var. *purpurea* (Scrophulariaceae)[14], *Harpagophytum procumbens* (Pedaliaceae)[15] and *Stachys sieboldii* (Labiatae)[16]. To our knowledge, this is the first report of the isolation of decaffeoylacteoside (**7**) from a member of the genus *Euphrasia*.

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