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THREE ACYLATED FLAVONE GLYCOSIDES FROM *SIDERITIS SYRIACA*

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Key Word Index—*Sideritis syriaca*; Labiatae; acylated flavone glycosides: 5,8,3'-trihydroxy-4'-methoxyflavone 7-(6'''-*O*-acetylsophoroside); apigenin 7-(6''-*p*-coumaroylglucoside); apigenin 7-(4''-*p*-coumaroylglucoside).

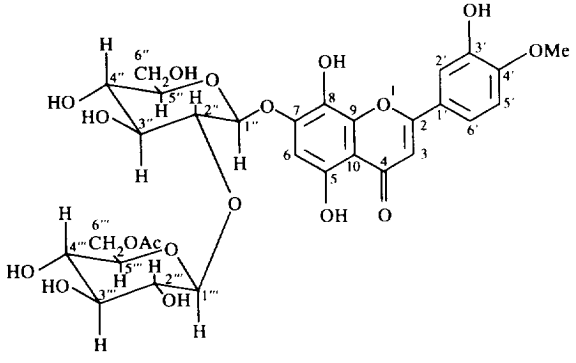
Abstract—From the aerial parts of *Sideritis syriaca* a new flavone glycoside, 5,8,3'-trihydroxy-4'-methoxyflavone 7-(6'''-*O*-acetylsophoroside) was identified together with apigenin 7-(6''-*p*-coumaroylglucoside) and apigenin 7-(4''-*p*-coumaroylglucoside) which are reported for the first time in the genus *Sideritis*. The compounds were characterized using ^{1}H NMR, ^{13}C NMR, MS and chemical methods.

INTRODUCTION

Sideritis syriaca L. (Labiatae) is a perennial herb from the mountain area of Sicily, which has been used in traditional medicine. The active principles of this plant are unknown but as many flavonoids are biologically active it is possible that the flavonoid constituents in this herb may contribute to its therapeutic properties [1]. As part of a phytochemical investigation of the genus *Sideritis* [2–4] we now report the isolation and structural elucidation of three acylated flavone glycosides (**1**–**3**) from *S. Syriaca*.

RESULTS AND DISCUSSION

The UV spectrum of **1** in methanol suggested a flavone substituted at the 7-hydroxyl (absence of a bathochromic shift in the NaOAc band II). The $AlCl_3 + HCl$ spectrum indicated a free 5-hydroxyl group and the possibility of a free 8-hydroxyl group [5].



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The substitution pattern of ring A and B was deduced also by ^{1}H NMR spectroscopy. Thus, the methoxyl group at 3.89 ppm was assigned to C-4' and the integration of the signals in a complex multiplet in the 3.20–4.40 ppm region and the number of glycosyl peaks indicated that the compound was a diglycoside. An extra singlet signal for three protons at 2.04 ppm was observed suggesting that one of the sugar hydroxyl groups was acetylated.

Acetylation of **1** gave the acetate, which showed signals in ^{1}H NMR for seven aliphatic and three aromatic acetyl groups. Acid hydrolysis of **1** yielded glucose and two aglycone isomers, the products of the well known Wessely–Moser rearrangement [6]. Enzymic hydrolysis of **1** with β-glucosidase using standard conditions yielded 5,7,8,3'-tetrahydroxy-4'-methoxyflavone. The structure of the aglycone was confirmed by methylation and by comparison (mp, IR and ^{1}H NMR) with values reported for isosinensetin [7, 8]. Methylation of **1** followed by acid hydrolysis yielded a compound whose properties were the same as those reported for 7-hydroxy-5,6,3',4'-tetramethoxyflavone. This suggested that a disaccharide moiety was attached to the aglycone at the 7 position.

The characterization of **1** was completed by ^{13}C NMR and FAB-MS. On the basis of shift assignments for flavonoid glucosides [9–11] we assigned the resonances to carbon atoms as described in Table 1. The ^{13}C NMR spectrum clearly proved that a glucosylpyranosyl group was attached to the flavone moiety at C-7 [9–11] and showed the presence of an acetyl group [signals at 20.89 ppm (Me) and 169.54 ppm (C=O)]. The absence of an aromatic methine carbon signal in the range 90.0–96.0 ppm indicated that C-8 was substituted. The clear differentiation between a 5,6,7 and a 5,7,8-trihydroxy-pattern on the basis of the chemical shift of the A ring methine resonance has been previously described

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Table 1. ^{13}C NMR data* for glycoside **1** (80 MHz, DMSO-d_6)

C		C	
2	164.0	G′ 1″	99.1
3	102.8	2″	82.6
4	182.3	3″	77.1
5	161.4	4″	69.4
6	99.9	5″	76.3
7	152.5	6″	60.7
8	127.4	G″ 1‴	103.4
9	144.4	2‴	74.7
10	105.5	3‴	77.1
1′	123.2	4‴	69.4
2′	113.3	5‴	73.2
3′	146.8	6‴	62.2
4′	150.9	OMe	55.9
5′	112.3	Acetyl	20.9
			169.5
6′	118.9		

*TMS as int. standard.

[11]. An upfield shift for C-5′ showed an *ortho* carbon resonance in accordance with the assignment of a methoxyl group at the 4′ position. The signal at 82.59 ppm corresponding to the C-2″ of the glucosyl moiety is indicative of a disaccharide in which the glucosyl group is attached to the aglycone and has a second glucosyl unit attached at C-2″ while the chemical shift values of the second glucose unit (downfield shift of 1.49 ppm in the C-6‴ and a upfield shift of 3.18 ppm in the C-5‴ signal) indicate that the site of acetylation must be at C-6‴ [12–14]. The ^{13}C NMR values for C-1 of the glucosyl units suggested a pyranose ring with β-linkages. This was confirmed by the ^1H NMR, which exhibited two doublets for two anomeric protons at 5.10 ppm and 5.15 ppm with large coupling constants [15].

Compound **1** (Table 2) showed a quasi-molecular ion $(M + H)^+$ at *m/z* 683 in its positive ion FAB-MS spectrum and an ion $(M - H)^-$ at *m/z* 681 in the negative-ion FAB-MS spectrum, which confirmed **1** as a diglycoside.

Table 2. FAB mass spectral data of glycoside **1**

Ions	PI	NI
Quasi-molecular (M)	(M+H)	(M−H)
m/z (rel. int. %)	683 (4.73)	681 (1.20)
M-Me	668 (6.20)	666 (4.70)
M-COMe	640 (3.30)	638 (1.50)
M-Me-COMe	625 (2.66)	623 (1.05)
M-acetylglucose	462 (4.20)	460 (4.70)
Aglycone	317 (93.40)	
Aglycone-Me	302 (100.00)	
Aglycone-Me	273 (44.23)	
Aglycone-Me	167 (2.02)	
Aglycone-Me	151 (1.58)	
Aglycone-Me	133 (2.33)	

PI: Positive ion mode; NI: negative ion mode.

Two significant fragments at *m/z* 640 and at *m/z* 462 were observed, which indicated that the acetyl group must be located on the second hexose moiety. Furthermore, the fragments at *m/z* 317 and 302 indicated that the flavone contained four hydroxyls and one methoxyl group while the RDA fragments confirmed the presence of one hydroxyl and one methoxyl group in the B-ring. Considering these data, the structure of **1** is proposed to be 5,8,3′-trihydroxyflavone 4′-methoxy-7-*O*-β-(6‴-*O*-acetylsophoroside).

It is noteworthy that this is the first report of the occurrence of an 8-hydroxyflavone 7-diglucoside in the genus *Sideritis*. Previously only allosylglucosides of 8-hydroxyflavones have been described in *Sideritis* species [16–19] and related genera [20–22].

Glycosides **2** and **3** both gave apigenin, D-glucose and *trans-p*-coumaric acid as acid hydrolysis products and apigenin 7-*O*-β-glucoside and *trans-p*-coumaric acid on alkaline hydrolysis. The ^1H NMR and ^{13}C NMR spectra of both **2** and **3** showed the presence of only one sugar unit and indicated that the acyl group must be attached to the sugar. In contrast to other cited structures from *S. raeseri* [23] where the acid is esterified to the flavonoid nucleus, the data UV, IR and NMR clearly confirmed that the glucopyranosyl was attached to the 7-hydroxyl of the flavone [11]. The ^{13}C values for C-1 of the glucosyl units suggested a pyranose ring with β-linkage, which was confirmed by two doublets ($J = 7.0$ Hz) for one anomeric proton at 5.18 and 5.25 ppm, respectively in the ^1H NMR spectra of **2** and **3** (Table 3).

In addition to the expected signals for apigenin [24, 25], a pair of doublets with large coupling constants ($J = 16$ Hz) for the *trans* olefinic hydrogens support the presence of *trans-p*-coumaric acid. The position of the *p*-coumaroyl residue on the sugar was unequivocally determined by NMR spectroscopy, which showed that the spectra of the two glycosides (**2** and **3**) differed in the resonances of the glucose unit. A careful examination of

Table 3. ^{13}C NMR data* for glycosides **2** and **3** (80 MHz, DMSO-d_6)

C	2	3	C	2	3
2	164.2	164.2	1‴	124.9	125.0
3	102.5	103.3	2‴, 6‴	130.0	130.3
4	181.9	181.9	3‴, 5‴	115.6	115.8
5	161.3	161.1	4‴	159.7	159.8
6	99.5	99.5	7‴	144.8	145.0
7	162.7	162.8	8‴	113.7	114.1
8	94.3	95.0	9‴	166.3	165.8
9	156.8	156.9	Glucose		
10	105.6	105.4	1″	99.5	99.7
1′	120.9	121.0	2″	73.0	73.2
2′, 6′	128.5	128.5	3″	76.2	74.3
3′, 5′	115.9	115.9	4″	70.1	71.0
4′	161.1	161.3	5″	73.8	74.8
			6″	62.1	60.4

*TMS as int standard.

this portion of the spectrum indicated that the *p*-coumaroyl group in **2** was located at C-6″. Thus a downfield shift of the 6″-CH_2OH signals in the ^1H NMR spectrum (Table 4) proved the attachment of the acyl-group at C-6″ [26]. This was confirmed by the ^{13}C NMR which showed the expected downfield of the signal for C-6″ at 62.1 ppm and the signal due to C-5″ moved upfield by 3 ppm [12–14].

A close comparison of the sugar carbon signals in the ^{13}C NMR spectrum of **3** with that of apigenin 7-*O*-*β*-glucoside showed that the signals due to C-3″ and C-5″ had moved upfield by 2.42 and 1.46 ppm, respectively, whereas the C-4″ signal was deshielded. These data confirmed that the acyl group in **3** was present at C-4″ [27]. Also in the ^1H NMR spectrum, the chemical shift for the H-4″ (a triplet at 4.82 ppm, $J = 10$ Hz) was indicative of acylation at C-4″ of the glucose moiety [26].

Therefore, these two isomers differ in the point of attachment of the *p*-coumaroyl moieties to the glucose unit and are, respectively, characterized as apigenin 7-*O*-*β*-D-(6″-*O*-*p*-coumaroylglucopyranoside) **2** [28, 29] and apigenin 7-*O*-*β*-D-(4″-*O*-*p*-coumaroylglycopyranoside) **3** [30].

Flavonoid *p*-coumaroylglucosides have been reported from several other *Sideritis* species, which were identified by UV spectra analysis and chromatographic comparison (HPLC) [31, 32].

EXPERIMENTAL

Mps: uncorr. IR: nujol mull. MS: PM-136-A1-FAB in a Gly-Thio matrix. TLC: silica gel F_{254} (Merk). HPLC (Varian 5000) on MicroPak NH_2-10, 10 *μ*m alkylamine (25 × 0.2 cm). All the products here reported gave satisfactory elemental analyses.

Plant material. Plants of *Sideritis syriaca* were collected on high summits of Madonie Mounts (Sicily) and voucher specimens have been deposited in the Herbarium of the Department of Scienze Botaniche of the Faculty of Scienze Palermo, University.

Extraction and isolation. Aerial parts of *S. syriaca* were ground and extracted (Soxhlet) successively with petrol and MeOH. The concd MeOH extract was run on a silica gel column, eluted successively with EtOAc/MeOH mixts of increasing polarity and the fr. further sepd by prep. TLC using EtOAc:MeOH (2:1) followed by repeated recrystallization from MeOH to give **1**–**3**.

Table 4. ^1H NMR data* for sugars of **2** and **3** (200 MHz, DMSO-d_6)

H	2	3
1″	5.18 (*d*, $J = 7.0$ Hz)	5.25 (*d*, $J = 7.0$ Hz)
4″		4.82 (*t*, $J = 10.0$ Hz)
5″	3.84 (*t*, $J = 8.0$ Hz)	3.85 (1H, *m*)
6″	4.17 (*dd*, $J = 7.0$, 12.0 Hz)	
6″	4.48 (*d*, $J = 12.0$ Hz)	
2″, 3″, 4″	3.15–3.50 (*m*)	
2″, 3″, 6″		3.30–3.65 (4H, *m*)
OH	5.29, 5.38, 5.50 (3*d*, $J = 5.5$ Hz)	4.90, 5.45, 5.70 (3*d*, $J = 5.5$ Hz)

*TMS as internal standard.

Compound **1** was obtained from MeOH, mp 270–272°; UV λ_{max} (MeOH) 227, 285, 296 (sh), 330 nm; (MeOH + $AlCl_3$) 297 (sh), 321, 342 (sh), 378 nm; no bathochromic shift in the presence of NaOAc; IR ν cm^{-1}: 3400 (OH), 1720 and 1250 (-OCOMe), 1660 (C=O), 1480, 1090, 800, 740.

^1H NMR (200 MHz, DMSO-d_6): δ 2.04 (3H, *s*, OCOMe); 3.89 (3H, *s*, OMe); 3.20–4.40 (12H, *m*); 5.10 (1H, *d*, $J = 7.0$ Hz, H-1‴); 5.15 (1H, *d*, $J = 7.0$ Hz, H-1″); 6.68 (2H, *s*, H-3 and H-6); 7.10 (1H, *d*, $J = 8.5$ Hz, H-5′); 7.98 (1H, *d*, $J = 2.5$ Hz, H-2′); 8.06 (1H, *dd*, $J = 8.5$ and $J = 2.5$ Hz, H-6′); 8.50 and 9.55 (*br*, 2-OH); 12.40 (*s*, 5-OH).

^{13}C NMR: Table 1. FAB-MS: Table 2.

Acetylation of **1**. (Py, Ac_2O, 0.5 hr) Gave a compound with mp 190–192° (MeOH). ^1H NMR (80 MHz, $CDCl_3$): δ 1.99, 2.01, 2.03, 2.08 (21H, 4*s*, 7-OCOMe); 2.14, 2.42, 2.50, (9H, 3*s*, 3-OCOMe); 3.89 (3H, *s*, -OMe); 4.0–4.30 (6H, *m*); 4.70–5.45 (7H, *m*); 5.56 (1H, *d*, $J = 7.0$ Hz, H-1″); 6.58 and 6.60 (2H, 2*s*, H-3 and H-6); 7.10 (1H, *d* $J = 8.5$ Hz, H-5′); 7.65 (1H, *d*, $J = 2.5$ Hz, H-2′); 7.80 (1H, *dd*, $J = 8.5$ and 2.5 Hz, H-6′).

Acid hydrolysis of **1**. Compound **1** was refluxed for 3 hr with 7% alcoholic H_2SO_4 and the aglycone extracted with Et_2O. Repeated crystallization from MeOH gave an inseparable mixt. of 2 aglycones. The aq. soln was neutralized with Amberlite MB-3 and evapd to yield the sugar fr.

Identification of tne sugar. Sugar analysis was carried out by HPLC at 80°; solvent: MeOH–H_2O (5:1) flow rate 0.2 ml min^{-1} for 0–10 min, after 0.5 ml min^{-1} for 30 min; detection at 192 nm. The sample (20 *μ*l in H_2O) was injected in a concn of 2 mg ml^{-1}, the ref. compounds in a concn of 1 mg ml^{-1} each.

One peak was obtained corresponding to glucose (R_t 15.1 min). Allose (R_t 18.8 min) could not be detected [16–19]. The osazone was prepd by heating the sugar soln with phenylhydrazine in HOAc for 1 hr at 95°. It crystallized from aq. EtOH, mp 206–207° and gave identical IR and mmp with a sample prepd from authentic D-glucose.

Enzymic hydrolysis was carried out at 5° in an acetate buffer sol. (pH 5.0). Compound **1** was completely hydrolysed after 2 hr. The aglycone, 5,7,8,3′-tetrahydroxy-4′-methoxyflavone, crystallized from MeOH, mp 250–252°. UV λ_{max} (MeOH) 230, 285, 302 (sh), 340 nm. ^1H NMR (80 MHz, DMSO-d_6): δ 3.88 (3H, *s*, OMe); 6.68 (2H, *s*, H-3 and H-6); 7.12 (1H, *d*, $J = 8.5$ Hz, H-5′); 8.02 (2H, *m*, H-2′ and H-6′); 8.70, 9.50 and 10.30 (3*s*, *br*, 3-OH); 12.50 (*s*, OH-5).

Methylation of **1**. Methylation of **1** gave 5,7,8,3′,4′-pentamethoxyflavone, mp 196–197° (mmp undepressed and ^1H NMR identical with that of an authentic sample) [7, 8]. Methylation of **1** followed by acid hydrolysis [33] gave 7-hydroxy-5,8,3,4′-tetramethoxyflacone, mp 205–206° (MeOH). UV λ_{max} (MeOH): 230, 283, 302 (sh), 313 (sh) nm; ^1H NMR (80 MHz, DMSO-d_6): δ 3.85 and 3.97 (2*s*, 12H, 4-OMe); 6.43 (*s*, 1H, H-6); 6.66 (*s*, 1H, H-3); 6.87

(1H, *d*, $J = 8.5$ Hz, H-5'); 7.50 (*d*, 1H, $J = 2.5$ Hz, H-2'); 8.0 (1H, *dd*, $J = 8.5$ and $J = 2.5$ Hz, H-6'); 10.40 (*br s*, OH-7).

Compound **2**. Compound **2** crystallized from MeOH as a yellow powder, mp 268–270°; UV λ_{max} (MeOH) 270, 320; (MeOH + NaOAc): 267, 317, 389 nm; IR ν cm^{-1}: 3305–3650 (OH), 1700 (C=O ester), 1657 (C=O), 986 (CH=CH). ^{1}H NMR: Table 4; ^{13}C NMR: Table 3.

Compound **3**. Compound **3** crystallized from MeOH as a yellow powder, mp 248–250°; [27]; UV λ_{max} (MeOH) 270, 320; (MeOH + NaOAc): 267, 317, 389 nm; IR ν cm^{-1}: 3305–3600 (OH), 1700 (C=O ester), 1658 (C=O), 987 (CH=CH). ^{1}H NMR: Table 4; ^{13}C NMR: Table 3.

Alkaline hydrolysis of **2** *and* **3**. Gave *trans-p*-hydroxycinnamic acid, mp 210–211° and apigenin 7-glucoside, as pale yellow needles, mp 233–234° [14] (UV, IR, ^{1}H NMR comparison with an authentic sample).

Acid hydrolysis of **2** *and* **3**. With 2N H_2SO_4–MeOH (1:1) for 6 hr on b. m. gave apigenin, mp 335–340° (mmp and UV identical with authentic specimen), *trans-p*-coumaric acid and D-glucose.

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