0.47 (C_6H_6–$CHCl_3$–MeOH, 10:5:3), sparingly soluble in $CHCl_3$ and MeOH, insol. in petrol, C_6H_6; gave a light yellow colour with methanolic NaOH; with Mg and alcoholic HCl, it gave a light pink colour which changed to blue on addition of more Mg and finally turned to deep red. It exhibited UV λ_{max}^{MeOH} nm 270, 335 (log ε 4.14, 4.15); $\lambda_{max}^{MeOH+NaOH}$ 282, 392 nm (log ε 4.08, 4.16); IR ν_{max}^{KBr} cm^{-1}, 3100–3400, 2870, 1665, 1615, 1560, 1510, 1370, 1250, 1200, 848; 90 MHz ^{1}H NMR (DMSO-d_6) δ3.85 (3H, *s*), 6.17 (1H, *d*, J = 2 Hz), 6.44 (1H, *d*, J = 2 Hz), 6.66 (1H, *s*), 6.96 (2H, *d*, J = 8.7 Hz), 7.85 (2H, *d*, J = 8.7 Hz); MS:*m/z* 284 ([M]$^+$, 100%), 270 (20), 269 (16), 256 (18), 153 (20), 152 (15), 149 (25), 121 (12), 48 (25).

Nivegin (**2**). Fractions from C_6H_6–EtOAc (4:1) were combined according to TLC which on cryst: from MeOH furnished pale yellow crystals of **2** (14 mg), mp 262–264°, $C_{15}H_{10}O_5$ ([M]$^+$, *m/z* 270.0528), R_f 0.42 (C_6H_6–$CHCl_3$–MeOH, 10:5:3), sol. in MeOH, sparingly sol. in $CHCl_3$, insoluble in petrol and C_6H_6. It gave a pale yellow colour with methanolic NaOH and with Mg and alcoholic HCl, it gave a light pink colour which changed to blue on addition of more Mg and finally turned to deep red. It exhibited UV λ_{max}^{EtOH} nm 267, 338 (log ε 4.14, 4.20); $\lambda_{max}^{EtOH+NaOH}$ nm 278, 400 (log ε 4.09, 4.24); IR ν_{max}^{KBr} cm^{-1} 3300, 1660, 1610, 1550, 1500, 1445, 1355, 1249, 1175, 825; 90 MHz ^{1}H NMR (DMSO-d_6) δ6.16, (1H, *d*, J = 2.12 Hz), 6.44 (1H, *d*, J = 2.12 Hz), 6.65 (1H, *s*), 6.89 (2H, *d*, J = 8.76 Hz), 7.86 (2H, *d*, J = 8.76 Hz); HRMS:*m/z* 270.0528 ([M]$^+$, 100%), 242.0576 (18), 153 (22), 152 (15), 149 (24), 121 (15), 97 (10), 71 (15), 57 (28).

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A GLUCOSYLATED ACYLFLAVONE FROM *SIDERITIS RAESERI*

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Key Word Index—*Sideritis raeseri*; Lamiaceae; apigenin 7-glucoside; apigenin 7-(4-*O*-β-glucosyl-*trans*-*p*-coumarate).

Abstract—Besides apigenin 7-glucoside, a new glucosylated acylflavone has been isolated from *Sideritis raeseri* and its structure elucidated as apigenin 7-(4-*O*-β-glucosyl-*trans*-*p*-coumarate), on the basis of spectral and chemical analysis.

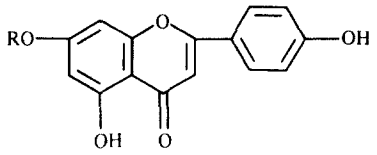
INTRODUCTION

Sideritis species (Lamiaceae) are used in Mediterranean traditional medicine for their anti-inflammatory [1] and antimicrobial activity [2]. The genus *Sideritis* is reported to contain terpenoids, sterols, flavonoids, coumarins, lignans and iridoids [3, 4]. There is however no report on the phenolic constituents of *S. raeseri* subsp. *raeseri* Boiss. et Heldr., endemic to northern Greece apart from one preliminary screening [5].

RESULTS AND DISCUSSION

We now report the isolation and structural elucidation of two apigenin derivatives from *S. raeseri* subsp. *raeseri*. Compound **1** was identified as apigenin 7-glucoside [6] and **2** as apigenin 7-(4-*O*-β-glucosyl-*trans*-*p*-coumarate) a new natural product. In **2** the flavonoid nucleus is esterified directly with a phenylacid glucoside, in contrast to other cited structures [7–10], where the acid is esterified in the sugar moiety.

UV spectroscopy of **2** using standard shift reagents defined the 5- and 4′-hydroxyl groups as free [11] and the A and B-ring *ortho*-dihydroxyl system as absent. Band I (317 nm) was much higher than band II (268 nm) in the UV spectrum suggesting the presence of an additional chromophore [12]. β-D-Glucosidase hydrolysis produced **3** and glucose. In the absorption spectrum of **3** the band I in methanol was unaffected but the intensity of band II was increased.



1 R = glc

2 R = CO—CH=CH— —O—glc

3 R = CO—CH=CH— —OH

4 R = H

On mild alkaline hydrolysis **3** afforded **4** (UV, ^{1}H NMR and MS data) and *p*-coumaric acid, indicating acylation at the 7 position of the flavonoid aglucone; *p*-coumaric acid was confirmed by co-chromatography. Acid hydrolysis of **2** with 10% HCl yielded apigenin **4**, *p*-coumaric acid and glucose and hydrolysis with 3% HCl yielded apigenin 7-*p*-coumarate **3** (UV and MS data) and glucose.

In all chemical degradation procedures the aglucone was identified as apigenin **4** by its mp, spectral analysis and R_f values on co-chromatography with an authentic sample; *p*-coumaric acid was identified by standard chromatographic methods [13] with an authentic sample and glucose on PC with BAW and phenol saturated with water [14].

The ^{1}H NMR spectrum of **2** showed most of the characteristics of a 7-*O*-substituted apigenin but the resonances of protons 6 and 8 gave a very small downfield shifts of 0.05 and 0.025 ppm, respectively in relation to the same protons of apigenin 7-*O*-glucoside. This confirmed the presence of an acyl moiety at the 7-position in accordance with the lower wavelength absorption in the UV spectrum (band I-MeOH) [10]. The appearance of the olefinic protons (H-α and H-β) at 6.41 and 7.61 ppm (J = 16 Hz), respectively suggested the presence of a *trans*-*p*-coumaroyl moiety [7] (see Experimental). Also the anomeric proton at 5.24 ppm (J = 7.5 Hz) indicated the presence of a β-glucosyl moiety.

The IR spectrum showed a strong band of a *p*-bisubstituted phenyl ring at 830 cm^{-1} and a carbonyl band characteristic of 5-hydroxyflavones at 1653 cm^{-1} flanked by a weak band around 1684 cm^{-1} of the cinnamoyl ester group.

DCIMS (NH_3) gave a molecular ion at m/z 578 corresponding to the presence of an ion $[M+1]^+$ at 579. The fragments at m/z 417 $[M-glc+1]^+$ and 433 $[M-glc+17]^+$ confirmed the presence of a hydroxycinnamoyl moiety attached to the apigenin molecule. The fragment at m/z 271 $[M-glc-coumaroyl+1]^+$ is formed from the $[M]^+$ by the loss of a glucose and a coumaroyl moiety, corresponding to the $[M+1]^+$ of the apigenin. In addition the presence of the fragments at m/z 121 $[120+H]^+$ and 138 $[120+NH_4]^+$ are due to the apigenin after the RDA scission. The DIEMS confirmed the proposed structure for the apigenin-*p*-coumarate **3** with a $[M]^+$ at 416.

EXPERIMENTAL

Plant material was collected in July 1985 from Agioneri-Prespes (1600 m altitude) northern Greece. A voucher specimen has been deposited at the herbarium of our Laboratory (E.K.) and plant material verified by the Laboratory of Systematic Botany.

Extraction. Air-dried aerial parts of the plant (400 g) were exhaustively extracted (Soxhlet) with petrol (bp 50–70°), C_6H_6, $CHCl_3$ and MeOH. The latter extract was concd and the residue redissolved in boiling H_2O. The H_2O-soluble fraction was filtered and extracted successively with Et_2O, EtOAc and *n*-BuOH.

Isolation. The Et_2O extract was concd under red. pres., to yield a ppt. (5 g, 1.25%), 2.2 g of which was chromatographed on a Polyamide column using gradient elution with C_6H_6–MeOH. Compound **2** was eluted with 20% MeOH and recryst. in EtOH–H_2O to give 25 mg of pure substance. The EtOAc extract was concd under red. pres. to yield a ppt. (8 g, 2%), 6 g of which was chromatographed in Polyamide column with a H_2O–MeOH gradient elution system to give compound **1** from 20% MeOH and **2** from 60% MeOH. After TLC on cellulose with EtOAc-HOAc–H_2O (4:1:2) and final purification on Sephadex LH-20 column (MeOH) gave 7 mg of **2** and 8 mg of **1**.

Apigenin 7-glucoside **1** was identified by mp, UV, ^{1}H NMR and EIMS spectral data [6].

Apigenin 7-(4-O-β-glucosyl-trans p-coumarate) **2**. Mp 201–203° uncorr.; TLC (cellulose): R_f 0.83 (BAW, 4:1:5), 0.06 (HOAc 15%), 0.69 ($CHCl_3$–HOAc–H_2O, 10:9:1), spot appearance dark (UV), dull green F1 (UV/NH_3); UV λ_{max}^{MeOH} nm: 225, 269, 318; +MeONa 243, 268, 308, 370; +$AlCl_3$ 225, 275, 298, 325, 382; +$AlCl_3$ + HCl 225, 276, 298, 326, 382; +NaOAc 266, 295, 315, 380; +NaOAc + H_3BO_3 266, 317; IR ν_{max}^{Nujol} cm^{-1}: 3700–3050 (OH), 1684 (ester CO), 1653 (CO), 1510, 1490 (aromatic), 1450, 1372, 1243, 1180, 1075, 830; ^{1}H NMR (200 MHz, DMSO-d_6, TMS): δ7.97 (2H, *d*, J = 9 Hz, H-2′ and H-6′), 7.61 (3H, *dd*, $J_{2'',6''}$ = 9 Hz, $J_{\beta,\alpha}$ = 16 Hz, H-2″, H-6″, H-β), 6.95 (2H, *d*, J = 9 Hz, H-3′ and H-5′), 6.90 (1H, *s*, H-3), 6.88 (1H, *d*, J = 2 Hz, H-8), 6.81 (2H, *d*, J = 9 Hz, H-3″, H-5″), 6.51 (1H, *d*, J = 2 Hz, H-6), 6.41 (1H, *d*, J = 16 Hz, H-α), 5.70 (1H, *d*, J = 5 Hz, OH-sugar), 5.43 (1H, *d*, J = 5.5 Hz, OH-sugar), 5.24 (1H, *d*, J = 7.5 Hz, H-1‴), 4.85 (2H, *m*, 2 OH-sugar), offset 11.72 (*s*, OH-5), 10.30 (*br*, OH-4′); (200 MHz, DMSO-d_6, TFA, TMS): δ8.00 (2H, *d*, J = 9 Hz, H-2′ and H-6′), 7.63 (3H, *t*, $J_{2'',6''}$ = 9 Hz, $J_{\alpha,\beta}$ = 15 Hz, H-2″, H-6″ and H-β), 7.10 (2H, *d*, J = 9 Hz, H-3′ and H-5′), 6.92 (4H, *m*, H-3, H-8, H-3″ and H-5″), 6.58 (1H, *d*, J = 1.8 Hz, H-6), 6.50 (1H, *d*, J = 15 Hz, H-α), 5.3 (1H, *d*, J = 8 Hz, H-1‴); DIEMS 70 eV, m/z (rel. int.): 416 $[M]^+$ (2), 388 $[M-CO]^+$ (1), 323 $[M-PhOH]^+$ (2), 309 $[HOC-CH=CH-C_6H_4-O-glc]^+$ (1), 270 $[M-HOC_6H_4C_2H_2CO+H]^+$ (80), 269 $[M-146]^+$ (20), 248$^+$ (20), 164 $[HOC_6H_4C_2H_2COOH]^+$ (22), 153 $[A_1+H, RDA]^+$ (25), 147 $[M-269]^+$ (50), 121 $[B_2]^+$ (50), 120 $[164-CO_2]^+$ (100), 119 $[147-CO]^+$ (75), 118 $[B_1, RDA]^+$ (24); DCIMS (NH_3) 90 eV, m/z (rel. int.): 579 $[M+1]^+$ (2), 433 $[M-glc+17]^+$ (10), 417 $[M-glc+H]^+$ (7), 271 $[M-OCC_2H_2C_6H_4-O-glc+H]^+$ (75), 180 $[glc-OH+NH_3]^+$ (25), 162 $[glc-H_2O]^+$ (12), 138 $[B_2+NH_3]^+$ (12), 121 $[164-CO_2+H]^+$ (100), 119 $[B_1+H]^+$ (30).

Enzymatic cleavage. Incubation of **2** with β-D-glucosidase for 24 h at 37° yielded glucose and **3** which on TLC (cellulose) gave R_f values: 0.02 (15% HOAc), 0.82 (EtOAc–HOAc–H_2O, 4:1:2), 0.85 ($CHCl_3$–HOAc–H_2O, 10:9:1), spot appearance dark (UV), dull yellow F1 (UV/NH_3); UV λ_{max}^{MeOH} nm: 269, 318; +MeONa 300, 370; DIEMS $[M]^+$ at m/z 416.

Alkaline hydrolysis. Product **3** (10 mg) was treated with 2 M NaOH at room temp. for 30 min. After acidification with 2 M

HCl and evapn the residue was taken in to boiling H_2O and extracted with EtOAc. TLC (cellulose) of the EtOAc extract gave *p*-coumaric acid (co-chromatography with an authentic sample) and apigenin by prep. TLC (cellulose, $CHCl_3$–HOAc–H_2O, 10:9:1, R_f 0.70), by UV, ^1H NMR, EIMS.

Acid hydrolysis. A small amount of **2** was refluxed with 3% and 10% HCl and the hydrolysis product was identified as described above.

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A STEROIDAL ALKALOID FROM *BUXUS PAPILLOSA*

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Key Word Index—*Buxus papillosa*; Buxaceae; steroidal alkaloids; buxoxybenzamine; 2D-NMR

Abstract—A new steroidal alkaloid, (−)-buxoxybenzamine, has been isolated form the leaves of *Buxus papillosa* and its structure elucidated.

INTRODUCTION

Buxus papillosa, C. K. Schneider (Buxaceae) is a commonly grown shrub of northern Pakistan. A number of steroidal alkaloids have been reported from this plant [1–5]. Continuing our investigations on the alkaloids of various *Buxus* species, we report here the isolation and structure of a new steroidal alkaloid (−)-buxoxybenzamine (**1**) from the leaves of *B. papillosa*.

RESULTS AND DISCUSSION

The ethanolic extract of air-dried leaves of *B. papillosa* was evaporated and partial separation of the alkaloids was carried out by extraction into chloroform at different pH values. The fraction obtained at pH 8.5 was loaded on a silica gel column. Elution was carried out with chloroform–methanol. Further purification of the fraction eluted in 96% chloroform–4% methanol by prep. TLC resulted in the isolation of **1**.

(−)-Buxoxybenzamine (**1**), $C_{35}H_{50}N_2O_5$, partially soluble in chloroform, showed UV absorption at 224 nm, characteristic of a secondary benzamidic chromophore [6]. The IR spectrum displayed absorptions at 3400 (OH and NH), 1730 (ester carbonyl), 1640 (amide carbonyl) and 1540 (C=C) cm^{-1} [3].