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ISOSCUTELLAREIN 7-GLUCOSYL(1 → 2)XYLOSIDE FROM SIXTEEN SPECIES OF *SIDERITIS*

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Abstract—A new flavone diglycoside isolated from the aerial parts of *Sideritis lutoela* was characterized as isoscutellarein 7-*O*-*β*-D-glucopyranosyl(1 → 2)*β*-D-xylopyranoside by means of spectral analyses, applying 2D NMR techniques and NOE experiments. Its presence in 15 other species of *Sideritis* is described.

INTRODUCTION

The genus *Sideritis* grows in the Mediterranean zone and its species are widely used in folk medicine as antiinflammatory and antiulcer drugs. These properties are attributed to their flavonoids and terpenoids such as borjatriol [1, 2], sideritoflavone [3] and hypolaetin 8-glucoside [4–7]. We report here the structural elucidation of a new naturally occurring flavonoid and its presence in 16 species of *Sideritis* collected in Spain. The isolation of the new compound was carried out by a new reversed-phase HPLC method.

RESULTS AND DISCUSSION

The ethyl acetate extract of *Sideritis luteola* Cav. was subjected to HPLC chromatography on a Hypersil ODS column (see Experimental) affording the glycoside **1**. Its UV spectra and its changes after addition of shift reagents [8, 9] suggested the presence of a tetrahydroxyflavone with a sugar moiety attached to the hydroxyl group at C-7. The isoscutellarein (8-hydroxyapigenin) structure was assigned by the ^{1}H NMR spectrum, which showed two *ortho*-coupled doublets ($J = 8.3$ Hz) at δ 8.02 and 7.03 assigned to H-2′, H-6′ and H-3′, H-5′, and two singlets at δ 6.88 and 6.74 assigned to H-6 and H-3, respectively. After acid hydrolysis, the sugars were identified as xylose and glucose by TLC on silica gel [10]. Evidence for the localization of the sugar moiety at C-7 position was given by comparison with ^{13}C NMR data of 7-glycosylated 8-hydroxyapigenins [11]. The signal at δ 84.0, corresponding to the C-2 of the xylose moiety is indicative of a disaccharide, in which the xylose is attached to the of the xylose. The ^{13}C values for C-1 of both sugars at δ 103.7 and 102.9, respectively, suggest pyranose rings with β linkages. This was confirmed by the ^{1}H NMR spectrum, which showed two anomeric proton doublets at δ 5.15 (glucosyl) and δ 5.03 xylosyl with large coupling constants of $J = 8.3$ and $J = 6.9$ Hz, respectively. The assignment of all glycosyl proton chemical shift values was based on COSY H-H experiments. NOE effects were observed between the anomeric proton of the xylose at δ 5.03 and the proton H-6 at δ 6.88 of the aglycone. This confirms a xylose linkage at C-7 of isoscutellarein. NOE effects were also observed between the H-2 proton ($\delta = 3.8$) of the internal xylose and the anomeric proton of the glucose, indicating that the biose is a glucopyranosyl(1 → 2)xylopyranoside, which has been found in flavonoids only once before [12]. Thus the new flavone is isoscutellarein 7-*O*-[*β*-D-glucopyranosyl(1 → 2)*β*-D-xylopyranoside (**1**). It was also found in 15 other species of *Sideritis* (Table 1).

EXPERIMENTAL

Plant material. Sixteen species of *Sideritis* from different places in Spain were collected. A voucher specimen of each species is on deposit in the Botany Department Herbarium (MAF), Faculty of Pharmacy, UCM (Madrid, Spain). These data, including name, voucher specimen nos, date and collection place can be obtained from the authors. The samples were dried at room temperature and powdered.

General procedure. HPLC analysis was carried out on a Varian 2510 pump, (Varian Polychrom 9065 photodiode-array detector operating at 254 nm and a Varian DS 654 data processor). Chromatographic runs was added to prevent tailing) and acetonitrile for pump B. The gradient elution for 20 min started with 70% A and 30% B and went on decreasing: $t_{10\,\mathrm{min}} = 45\%$ A, $t_{11\,\mathrm{min}} = 20\%$ A, $t_{20\,\mathrm{min}} = 20\%$ A. The flow rate was 1 ml/min and samples of 10 μl were injected. The isolation of the new flavonoid was carried out with a semipreparative column (Hypersil ODS 250 mm × 7 mm, 5 μm, Tecknochroma, Spain) under the same conditions than the analytical one. The flow rate was 3 ml/min and the injection volume, 500 μl. To check the purity, the eluates were monitored with a photodiode-array detector (200–400 nm). The three spectra corresponding to the upslope, apex and downslope of each peak were normalized and superimposed. Peaks were considered pure when there was exact coincidence between the spectra (match factor ≥ 99.5). TLC was carried out on silica gel 60 F 254, 20 cm × 20 cm, sheets. Acid hydrolysis and recording of UV spectra with the usual shift reagents were made according to standard procedures. Sugars were identified by TLC on silica gel (EtOAc–MeOH–HOAc–H_2O, 12:3:3:2) in the presence of *p*-anisidine phtalate reagent. Glucose and xylose were identified by comparison with authentic samples. ^1H NMR spectra were recorded at 250 MHz and ^{13}C NMR at 62.5 MHz. The residual signal of the solvent was used as secondary reference for chemical shifts.

Extraction and isolation of **1**. The dried and powdered samples were extracted successively with solvents of increasing polarity [*n*-hexane (4 × 100 ml, 48 h), EtOH–H_2O (7:3; 4 × 100 ml, 48 hr)]. The aqueous alcoholic extracts were concentrated to eliminate the EtOH and the aqueous solution was extracted successively with Et_2O, EtOAc and *n*-BuOH (10 × 25 ml). The extracts were concentrated to dryness below 50°. Isoscutellarein glycoside was isolated from the EtOAc extracts. The HPLC retention time of the flavonoid was 3.05 min. The content of **1**, expressed in per cent referred to dry plant, is shown in Table 1.

Isoscutellarein 7-O-[*β-O-glucopyranosyl*(1→2) *β-*D-*xylopyranoside*]. UV λ^{MeOH} nm: 203, 273, 302; (NaOMe) 212, 273, 368; ($AlCl_3$) 205, 279, 320, 347; ($AlCl_3$/HCl) 201, 275, 318; (NaOAc) 218, 266, 379. ^1H NMR (CD_3OD): δ 3.55 (1H, *dd*, $J = 8$, 2.9 Hz, H-2 glc), 3.58 (1H, *dd*, $J = 5.5$, 2 Hz, H-4 xyl), 3.73 (1H, *m*, H-4 glc), 3.75 (1H, *m*, H-3 xyl), 3.83 (1H, *m*, H-2 xyl), 3.85 (1H, *m*, H-5 xyl), 4.05 (1H, *dd*, $J = 12$, 2 Hz, H-5 xyl), 4.13 (1H, *ddd*, $J = 10$, 4.9, 2 Hz, H-5 glc), 4.2 (1H, *dd*, $J = 2.9$, 2.9 Hz, H-3 glc), 4.35 (1H, *dd*, $J = 12$, 4.9 Hz, H-6 glc), 4.40 (1H, *dd*, $J = 12$, 2 Hz, H-6 glc), 5.03 (1H, *d*, $J = 6.9$ Hz, H-1 xyl), 5.15 (1H, *d*, $J = 8$ Hz, H-1 glc), 6.74 (1H, *s*, H-3), 6.88 (1H, *s*, H-6), 7.03 (2H, *d*, $J = 8.3$ Hz, H-3′ and H-5′), 8.02 (2H, *d*, $J = 8.3$ Hz, H-2′ and H-6′). ^{13}C NMR (CD_3OD): δ 62.2 (C-6 glc), 65 (C-5 xyl), 68.5* (C-4 glc), 70.7* (C-4 xyl), 72.6 (C-2 glc), 73.1 (C-3 xyl), 77.5 (C-3 glc), 78.5 (C-5 glc), 84 (C-2 xyl), 101.7 107.7 (C-10), 117 (C-3′ and C-5′), 123.2 (C-1′), 129.7 (C-8), 129.8 (C-2′ and C-6′), 148.1 (C-9), 152 (C-5), 154.1 (C-7), 163 (C-4′), 166.8 (C-2), 184.5 (C-4).

Table 1. Quantity of **1** (% dry wt) in *Sideritis* species

Sideritis species	%
foetens Clem. ex Lag.	0.002
luteola F.Q.	0.20
almeriensis Pau.	0.06
hirsuta L.	0.13
leucantha Cav. var. *serratifolia* Willk.	0.78
biflora Porta et Rigo.	1.50
leucantha Cav. ssr. *incana* var. meridionalis	0.31
bourgeana Boiss. & Reut.	0.60
pusilla (Lge.) Pau. *ssr. almeriensis* (Pau.) H. T. Malag.	0.02
ibanyezii Pat.	0.19
pusilla (Lge.) Pau. ssr. *pusilla* var. *granatensis*	0.64
leucantha Cav. var. *incana* (Willk.) F. Q.	0.38
cillensis	1.25
pusilla (Lge.) Pau.	0.48
glauca Cav.	0.37
incana L. ssr. *incana*	1.01

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