Flavonoids from *Genista ephedroides*

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Three new flavonoids (**1**−**3**) and 11 known compounds (genistein, isoprunetin, wighteone, laburnetin, alpinumisoflavone, genistin, genistein 8-*C*-glucoside, apigenin, isokaempferide, licoflavone C, and D-pinitol) were isolated from the aerial parts of *Genista ephedroides*. The structures of the new compounds were established as hydroxyalpinumisoflavone (**1**) [4′,5-dihydroxy-2″-methyl-2″-hydroxymethylpyrano(5″,6″:6,7)isoflavone], ephedroidin (**2**) [4′,5,7-trihydroxy-8-(2-hydroxy-3-methyl-3-butenyl)flavone], and genisteone (**3**) (7-*O*-glucosylwighteone) by means of spectroscopic methods.

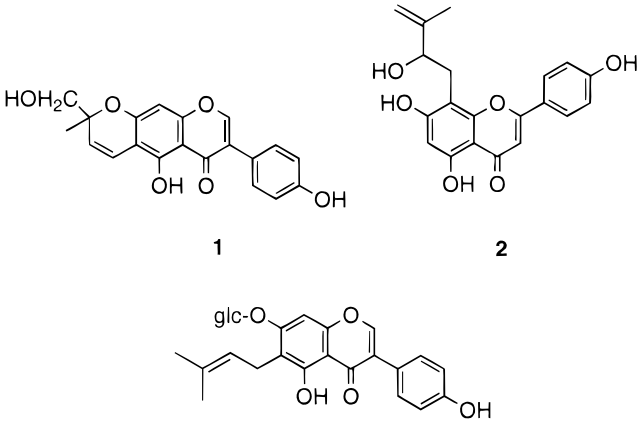
Genista ephedroides D.C. (family Leguminosae, subfamily Papilionoideae, tribe Genisteae) is a perennial endemic shrub in Sardinia. The plant is commonly known in Italy as "Ginestra del Gasparrini", and grows on rocky ground near the sea, where it flowers from April to May.[1]

The genus *Genista* is known to contain numerous flavonoids as well as lupine-type quinolizidine alkaloids.[2] Earlier chemical investigations on this species have led to the isolation of a number of interesting quinolizidine alkaloids, among them retamine as the most abundant example.[3] However, phytochemical studies on phenolic compounds in *Genista ephedroides* have not been reported as yet.

The present paper deals with the isolation and structure elucidation of three novel compounds, hydroxyalpinumisoflavone (**1**), ephedroidin (**2**), and genisteone (**3**). Ten known flavonoids and D-pinitol were also detected.

The dried aerial parts of *Genista ephedroides* were extracted in a Soxhlet apparatus with *n*-hexane, $CHCl_3$, and MeOH in turn, to obtain residues R_H, R_C, and R_M, respectively. Fractionation of R_C led to the isolation of several pure compounds. The known compounds, wighteone,[4] laburnetin,[5,6] alpinumisoflavone,[7] licoflavone C,[8] and isokaempferide[9] were identified by comparison with published spectral data. Compounds **1** (hydroxyalpinumisoflavone) and **2** (ephedroidin), were obtained as new natural flavonoids. From the residue R_M, seven pure compounds were isolated—a new isoflavone, genisteone (**3**), and the known compounds genistein,[10] isoprunetin,[11] genistin,[10] genistein 8-*C*-glucopyranoside,[10] apigenin,[12] and D-pinitol.[13] Again, the known isolates were identified by comparison with literature data.

The spectral data of **1** suggested that it was an isoflavone with a *para*-substituted B ring, a chelated hydroxyl, and a dimethylpyran group in the A ring (see Experimental Section). The ^{13}C NMR spectrum showed 20 resonances, which were sorted by the DEPT experiment as one CH_3, one CH_2, eight CH, and 10 quaternary carbons. A molecular formula of $C_{20}H_{16}O_6$ was determined by HREIMS (*m*/*z* 352.0942). The mass spectrum (EIMS) of **1** revealed intense mass fragments at *m*/*z* 337 $[M - CH_3]^+$ and 321 $[M - CH_2OH]^+$ (base peak); *retro*-Diels−Alder fragments occurred in high abundance at *m*/*z* 203 (ring A) and 118 (ring B). All these data were in close agreement with those previously reported for alpinumisoflavone.[7] Compound **1** differed only by a hydroxyl substituted on one of the geminal methyl groups of the dimethylpyranyl ring fused to ring A (δ 3.46, d, J = 5.5 Hz, CH_2OH and δ 5.10, t, J = 5.5 Hz, CH_2OH). Confirmatory evidence for structure **1** was provided by the ^{13}C NMR data (see Experimental Section), where the resonances attributable to the pyranyl moiety were consistent with a hydroxyl group on C-6″ (δ 67.03 for C-6″). Thus, the structure of **1** was concluded to be 4′,5-dihydroxy-2″-methyl-2″-hydroxymethylpyrano(5″,6″:6,7)-isoflavone, to which the trivial name hydroxyalpinumisoflavone has been given.



The new compound **2** showed the presence of a molecular peak at *m*/*z* 354 in the EIMS, corresponding to the molecular formula $C_{20}H_{18}O_6$, as confirmed by the elemental analysis. The UV spectrum suggested that it was a typical flavone, showing absorption maxima at 272.5, 310, and 327.5 nm. The small bathochromic UV shifts observed by adding the $AlCl_3$ and NaOAc reagents suggested that **2** had free hydroxyl groups at the C-5 and C-7 positions. The ^{13}C NMR spectrum showed 20 resonances, sorted by DEPT experiment as one CH_3, two CH_2, seven CH, and 10 quaternary carbons. Its ^1H NMR spectrum (CD_3OD) showed signals [δ 6.17 (1H, s, H-3), 6.87 and 7.95 (each 2H, d, J = 9.8 Hz, H-3′/5′ and H-2′/6′, respectively)] corresponding to a flavone structure. Ring A was found to be trisubstituted as revealed from the singlet at δ 6.52 (H-6). The ^1H NMR spectrum was very closely related to that of licoflavone C,[8] except for the presence of signals attributable to a 2-hydroxy-3-methyl-3-butenyl moiety [δ 1.80 (3H, s, H-5″), 3.11

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(2H, m, H-1″), 4.39 (1H, m, H-2″), 4.74 and 4.84 (each 1H, br s, H-4″)] instead of a 3,3-dimethylallyl chain. The location of the hydroxy prenyl substituent at C-8 of **2** was unambiguously determined by comparison of the ^{13}C NMR chemical shifts of C-6 and C-8 (δ 100.9 and 106.3, respectively) with those for apigenin and 6- or 8- substituted 5,7-dihydroxyflavones.[14] Thus, compound **2** was identified as 8-(2-hydroxy-3-methyl-3-butenyl)apigenin, to which the trivial name ephedroidin has been given.

Compound **3**, named genisteone, was tentatively assigned as an isoflavone derivative (characteristic ^1H NMR signal at δ 8.13 ascribable to H-2; δ_C 155.1 for C-2). The ES−MS of **3** gave an [M + H] peak at *m/z* 501.5, consistent with the molecular formula $C_{26}H_{28}O_{10}$, supported also by elemental analysis. The ^{13}C and ^1H NMR spectra of **3** displayed signals for one sugar residue, which, on the basis of the chemical shifts of the sugar carbons as well as the coupling constant (7.1 Hz) of the anomeric proton, clearly indicated a β-glucopyranoside.[14] The sugar was confirmed to be glucose, as deduced by TLC analysis after acid hydrolysis. The oxygenation pattern of the aglycon could be derived unequivocally from the number and multiplicity of the aromatic protons in the ^1H NMR spectrum. The NMR data were very close to those of wighteone. The site of glycosylation at C-7 was suggested by the upfield shift of the C-7 resonance (−1.2) and the downfield shift resonances of the C-6 (+2) and C-8 (+0.9). Therefore, genisteone (**3**) is 7-*O*-glucosylwighteone.

In agreement with a chemotaxonomic study by Harborne,[15] *G. ephedroides* exhibited high concentration levels of isoflavones and the absence of any leucoanthocyanidins, and luteolin was not detected (among the flavonoids, apigenin and of its two derivatives were present). Isoflavonoids have been identified in many species in the tribe Genisteae. Daidzein and genistein are the most representative for the genus. All the other such compounds, isolated herein, have been identified for the first time in this genus. The presence of isoprunetin is a taxonomic character of the tribe.[16]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin−Elmer 241 polarimeter using a 1-mL cell; IR spectra were acquired with a Unicam Mattson 3000 FTIR spectrophotometer, and UV spectra were recorded on a Perkin−Elmer Lambda 11 spectrophotometer. ^1H and ^{13}C NMR spectra were measured on a Bruker AC-200 spectrometer, using TMS as internal standard; all the 1D NMR experiments were performed using the standard Bruker library of microprograms. EIMS were obtained on a Hewlett−Packard 5988A spectrometer (70 eV); electrospray mass spectra (ES−MS) were determined on a HP-1100 MSD instrument; and HREIMS was recorded with a VG70−250S mass spectrometer. The following adsorbents were used for purification: flash chromatography, Merck Kieselgel 60 (230−410 mesh); low-pressure chromatography, Merck Lobar Lichroprep RP-8 (31 × 2.5 i.d.); gel filtration chromatography, Pharmacia Fine Chemicals Sephadex LH-20; analytical TLC, Merck Kieselgel 60 F_{254}. TLC chromatograms were visualized under UV light at 254 and 366 nm and/or sprayed with ceric sulfate or Naturstoffreagents-PEG. Known compounds were isolated and identified by comparison of their spectral and physical data with those of the literature and, when available, by comparison with authentic samples.

Plant Material. The aerial parts of *G. ephedroides* D. C. were collected in May 1995, at Capo Testa, Sardinia, Italy, and identified by one of us (A. M.). A voucher specimen (voucher URB-167/94) is held in the Herbarium of the Istituto di Botanica ed Orto Botanico (Università di Urbino-URB).

Extraction and Isolation. Powdered air-dried aerial parts of *G. ephedroides* (680 g) were defatted with *n*-hexane and then extracted in a Soxhlet apparatus with $CHCl_3$ followed by MeOH. After removal of solvents in vacuo at up to 40 °C, the following residues were obtained: R_H (7.35 g), R_C (11.45 g), and R_M (58.74 g).

R_C was chromatographed on Sephadex LH-20 eluted with MeOH−$CHCl_3$ (9:1) to obtain nine crude fractions (C_1−C_9). From fraction C_6 (974 mg), chromatographed on Si gel column with $CHCl_3$−MeOH (4:1 and 3:2), then over Lobar RP-8 eluted with MeOH−H_2O (4:1), followed by flash chromatography with $CHCl_3$−Et_2O (4:1), pure laburnetin (4 mg) was obtained. Fraction C_7 (1.75 g), after crystallization, furnished licoflavone C (151 mg). The mother liquor was chromatographed on Si gel using $CHCl_3$−Et_2O (4:1 and 1:1) as eluent to obtain 10 subfractions (I−X): subfractions II, IV, and VI, after crystallization, gave alpinumisoflavone (11 mg), wighteone (33 mg), and laburnetin (31 mg), respectively. Compound **1** (10.3 mg) was obtained, after crystallization, from subfraction IX; the mother liquor was further chromatographed by preparative TLC using $CHCl_3$−MeOH−NH_3 (85:15:1) as eluent to give 6.3 mg of isokaempferide and 7.7 mg of **2**.

Residue R_M, chromatographed over a Sephadex LH-20 column with MeOH as eluent, furnished 10 fractions, M_1−M_{10}. Fraction M_3, after flash chromatography with $CHCl_3$−MeOH−H_2O (6:4:1) followed by Si gel column chromatography with the same solvent, gave pure D-pinitol (314 mg). Fraction M_6 was further chromatographed on a Lobar RP-8 column, with subfractions I−XX obtained. Elution occurred with MeOH−H_2O (1:1) to MeOH, to afford genistein 8-*C*-glucoside (9.6 mg), genistin (11 mg), isoprunetin (16 mg), and **3** (6.0 mg) from subfractions IV, VI, XIV, and XVIII, respectively. Fraction M_9, chromatographed over Lobar RP-8 eluted with MeOH−H_2O (8:2), yielded genistein (20 mg) and apigenin (4.3 mg).

Hydroxyalpinumisoflavone (1): amorphous yellow solid; $[\alpha]^{25}_D$ +39.9° (*c* 0.158, DMSO); TLC R_f 0.65 [$CHCl_3$−MeOH−NH_3 (85:15:1)]; UV (MeOH) λ_{max} 283.3; (MeOH + MeONa) 289, 340.5 sh; (MeOH + $AlCl_3$) 295.7, 350 sh; (MeOH + $AlCl_3$ + HCl) 296.4, 360 sh; (MeOH + NaOAc) 282.7, 342 sh nm; IR (Nujol) ν_{max} 3410 (OH) and 1655 (intramolecularly hydrogen-bonded C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.6 (1H, s, OH), 8.35 (1H, s, H-2), 7.37 (2H, d, J = 8.7 Hz, H-2′ and H-6′), 6.81 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 6.67 (1H, d, J = 9.3 Hz, H-1″), 6.45 (1H, s, H-8), 5.71 (1H, d, J = 10.2 Hz, H-2″), 5.11 (1H, d, J = 5.8 Hz, CH_2−O*H*), 3.46 (2H, d, J = 5.8 Hz, H-5″), 1.33 (3H, s, H-4″), ^{13}C NMR (DMSO-d_6) δ 180.4 (C-4), 159.7 (C-7), 157.4 (C-9), 156.7 (C-5), 155.8 (C-4′), 154.2 (C-2), 130.1 (C-2′ and C-6′), 126.2 (C-2″), 122.3 (C-3), 121.01 (C-1′), 116 (C-1″), 115.07 (C-3′ and C-5′), 105.3 (C-10), 104.8 (C-6), 94.8 (C-8), 81.2 (C-3″), 67.03 (C-5″), 23.2 (C-4″); EIMS *m/z* 352 (2), 337 (1), 321 (100), 203 (26), 160 (3), 118 (12); HREIMS *m/z* 352.0942 ([M]$^+$ calcd for $C_{20}H_{16}O_6$, 352.0947).

Ephedroidin 2: $[\alpha]^{25}_D$ +16.23° (*c* 0.043, MeOH); TLC R_f 0.42 [$CHCl_3$−MeOH−NH_3 (85:15:1)]; UV (MeOH) λ_{max} 272.5, 310, 327.4; (MeOH + MeONa) 280.7, 329.8sh, 396.4; (MeOH + $AlCl_3$) 279.6, 307.0, 342.3, 383 sh; (MeOH + $AlCl_3$ + HCl) 280.0, 306.3, 342.0; (MeOH + NaOAc) 280.3, 310.5 sh, 393.2 nm; ^1H NMR (CD_3OD) δ 7.85 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 6.90 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 6.52 (1H, s, H-6), 6.18 (1H, s, H-3), 4.84 (1H, br s, H-4″), 4.74 (1H, br s, H-4″), 4.39 (1H, m, H-2″), 3.11 (2H, m, H-1″), 1.80 (3H, s, H-5″). ^{13}C NMR (CD_3OD) δ: 183.8 (C-4), 165.7 (C-2), 163.04 (C-5 and C-7), 161.2 (C-4′), 156.9 (C-9), 149 (C-3″), 129.3 (C-2′ and C-6′), 123.4 (C-1′), 117.1 (C-3′ and C-5′), 111.1 (C-4″), 106.3 (C-8), 103.1 (C-3), 100.9 (C-6), 77.1 (C-2″), 30.7 (C-1″), 18.01 (C-5″); EIMS *m/z* 354 [M]$^+$ (0.2), 336 [M − H_2O]$^+$ (0.3), 321 [M − H_2O − Me]$^+$ (1.3), 283 [M − C_4H_7O]$^+$ (42),149 (33), 109 (24), 91 (47), 69 (100), 83 (46), 55 (85); *anal*. C 67.51%, H 5.23%, calcd for $C_{20}H_{18}O_6$, C 67.79%, H 5.08%.

Genisteone 3: $[\alpha]^{25}_D$ −31.62° (*c* 0.043, MeOH); TLC R_f 0.19 [MeOH − H_2O (6:4)]; UV (MeOH) λ_{max} 267, 330 sh; (MeOH + MeONa) 277.0; (MeOH + $AlCl_3$) 267.0; (MeOH + $AlCl_3$ + HCl) 267.8; (MeOH + NaOAc) 266.7 nm; ^1H NMR (CD_3OD) δ: 8.13 (1H, s, H-2), 7.38 (2H, d, J = 8.3 Hz, H-2′ and H-6′), 6.84 (2H, d J = 8.3 Hz, H-3′ and H-5′), 6.78 (1H, s, H-8), 5.26 (1H, m,

H-2″), 5.08 (1H, d, J = 7.1 Hz, H-1‴), 1.80 (3H, s, H-4″), 1.65 (3H, s, H-5″); ^{13}C NMR (CD_3OD) δ 182.5 (C-4), 162.4 (C-7 and C-5), 158.8 (C-4′), 157.4 (C-9), 155.1 (C-2), 132.3 (C-3″), 131.4 (C-2′ and C-6′), 124.9 (C-3), 123.3 (C-1′ and C-2″), 116.2 (C-3′ and C-5′), 115.0 (C-6), 101.7 (C-1‴), 94.3 (C-8), 78.4 (C-3‴), 78.3 (C-5‴), 74.9 (C-2‴), 71.3 (C-4‴), 62.5 (C-6‴), 25.9 (C-4″), 18.5 (C-5″); ESMS m/z 501.1 $[M + H]^+$, m/z 499.5 $[M - H]^-$; *anal*. C 62.67%, H 5.36%, calcd for $C_{26}H_{28}O_{10}$, C 62.37%, H 5.58%.

Acid Hydrolysis. Compound **3** (2 mg) was treated with 2 N HCl in a sealed tube at 100° for 1 h. The aglycon was extracted with Et_2O and identified by spectroscopic methods as wighteone. The identification of glucose was performed by TLC on Si gel (solvent system: toluene−$CHCl_3$−Me_2CO 8:5:7) with authentic sample as reference.

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