A New Isoflavone from *Genista corsica*

Luisa Pistelli,*,† Isa Giachi,† Donatella Potenza,‡ and Ivano Morelli†

Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy, and Dipartimento di Chimica Organica e Industriale, Università di Milano, Via Venezian 21, 20133 Milano, Italy

Received June 9, 1999

A new isoflavone (**1**), dihydroisoderrondiol [(3″*S*,4″*R*)-5,7,3″,4″-tetrahydroxy-2″,2″-dimethyl-3″,4″-dihydropyrano(5″,6″;3″,4″)isoflavone], was isolated from aerial parts of *Genista corsica*, together with 11 previously known compounds [daidzein, isoprunetin, isoderrone (**2**), ficuisoflavone (**3**), luteolin, luteolin 4′-*O*-*β*-glucoside, luteolin 7-*O*-*β*-glucoside, taxifolin, 5-methoxytaxifolin, sucrose, and D-pinitol]. The structure of **1** was elucidated by spectroscopic methods.

Genista corsica (Loisel.) D.C. (Leguminosae) is a perennial endemic shrub widely distributed in Corsica and Sardinia, flowering from April to June.[1] Previous phytochemical studies of this species have led to the isolation of flavonoids (daidzein and luteolin)[2] and quinolizidine alkaloids (anagyrine, cytisine, *N*-methylcytisine, lupanine, retamine, and sparteine),[3] which are characteristic compounds of the genus *Genista*.[4]

In a continuation of our study of plants of the genus *Genista*,[5] we report herein the isolation and characterization of a novel compound, namely, dihydroisoderrondiol (**1**). Nine known flavonoids, sucrose, and D-pinitol were also detected.

The dried aerial parts of *G. corsica* were extracted in a Soxhlet apparatus with *n*-hexane, $CHCl_3$, and MeOH used, in turn, to obtain residues R_H, R_C, and R_M, respectively. Fractionation of R_C led to the isolation of four pure compounds: isoprunetin;[6] isoderrone (**2**);[7,8] ficuisoflavone (**3**), recently isolated and structurally characterized from *Ficus microcarpa* (Moraceae);[9] and a new natural flavonoid, dihydroisoderrondiol (**1**). From residue R_M, nine known pure compounds were isolated: sucrose;[10] D-pinitol;[11] the dihydroflavonol, 5-methoxytaxifolin[12,13] and taxifolin;[12,14] the isoflavones isoprunetin[6] and daidzein;[15] the flavones luteolin,[14,16] luteolin 4′-*O*-*β*-glucopyranoside,[16,17] and luteolin 7-*O*-*β*-glucopyranoside.[16] The known compounds were identified by comparison with published spectral data.

Compound **1** appeared as a pale yellow solid, whose formula was established as $C_{20}H_{18}O_7$ by HREIMS ($[M]^+$ *m*/*z* 370.1049). The presence of a singlet at δ 8.06 in the ^1H NMR spectrum and UV absorption bands at 263 and 299 (sh) nm, suggested that it was an isoflavone. The UV spectrum of **1** showed a bathochromic shift upon addition of NaOAc, suggesting a free hydroxyl group at C-7; no shift was apparent in the presence of $AlCl_3$.

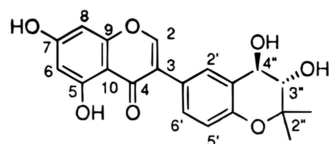
Two *meta*-coupled doublets at δ 6.32 (1H, d, $J = 1.8$ Hz) and 6.22 (1H, d, $J = 1.8$ Hz), which correlated to carbons at 100.1 and 94.8 ppm in the HETCOR spectrum, were characteristic of H-6 and H-8 protons of the A ring of **1**. The other signals in the aromatic region of the ^1H NMR spectrum (δ 7.61, 1H, d, $J = 1.8$ Hz; δ 7.33, 1H, dd, $J = 8.3$, 1.9 Hz; δ 6.79, 1H, d, $J = 8.4$ Hz) were in agreement with an ABX spin system in the B ring. These data are closely related to those of isoderrone (**2**) and ficuisoflavone (**3**), also isolated from *G. corsica*, having a 2,2-dimethylpyrano and a 3-hydroxy-3,4-dihydro-2,2-dimethylpyrano group in the B ring, respectively.

The mass spectra of compounds **2** and **3** showed molecular ions at *m*/*z* 336 and 354, showing differences of 34 and 16 amu, respectively, in comparison with compound **1**; these data could be explained by the presence of two hydroxyl groups on the pyran ring. This hypothesis was confirmed by signals occurring at high field in the ^1H NMR spectrum, in which two singlets at δ 1.45 (3H) and 1.21 (3H), attributable to two methyl groups, and two doublets at δ 4.53 (1H, d, $J = 8.3$ Hz, H-4″) and 3.54 (1H, d, $J = 8.3$ Hz, H-3″), corresponding to protons of a 2,2-dimethylchromane moiety,[18] were observed. The ^{13}C NMR spectrum of **1** showed 20 resonances, which were determined by a DEPT experiment as two methyls, eight methines, and 10 quaternary carbons. The presence of signals for two methyl carbons (27.1 and 19.4 ppm), two carbinol carbons (76.9 and 70.1 ppm; C-3″ and C-4″), and one quaternary carbon at 79.4 ppm (C-2″) was evident from these spectra. The complete assignments, and the relative trans configuration of 3″ and 4″ protons were established by HETCOR, $^1H-^1H$ NOESY, and ROESY experiments. H-4″ showed NOESY correlations with H-2′ and CH_3 at 1.21 ppm, which is possible only supposing an axial configuration of H-4″. The coupling constant ($J = 8.3$ Hz) between the carbinol hydrogens and the absence of any NOE enhancement between the H-3″ and H-4″ protons suggested that they have a diaxial-like relationship. Based on the biosynthetic relationship with ficuisoflavone (**3**),[9] we propose the absolute stereochemistry (3″*S*, 4″*R*) for compound **1**, in which the OH in 4″ has a β configuration, as shown in Figure 1. Thus, compound **1** was identified as (3″*S*,4″*R*)-3″,4″,5,7-tetrahydroxy-2″-dimethyl-3″,4″-dihydropyrano(5″,6″;3′,4′)-isoflavone, to which the trivial name dihydroisoderrondiol has been given.

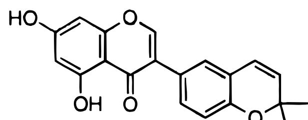
During the present phytochemical study on *G. corsica*, no quinolizidine alkaloids were detected by extraction with 0.5 M H_2SO_4, followed by alkalinization with 25% NH_4OH. Quinolizidine alkaloids occur in the 10 most primitive tribes of the Papilionoideae,[19] but their distribution appears to depend on the time of harvesting, on the stage of plant growth, and the geographical source of the plant material.[20] All the identified flavonoids, except daidzein and luteolin, have been isolated for the first time in this species and reflect the distinctive characteristics of tribe Genisteae, namely, high concentrations of isoflavones, the absence of leucoanthocyanidins, and the common occurrence of glycoflavones, flavonols, and the flavone luteolin, which, in phylogenetic terms, is of a more advanced character than

* To whom correspondence should be addressed. Tel.: +39 50 44074. Fax: +39 50 43321. E-mail: luipi@farm.unipi.it.
† Universita di Pisa.
‡ Univesita di Milano.

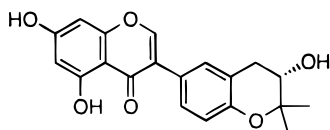
10.1021/np990282k CCC: $19.00 © 2000 American Chemical Society and American Society of Pharmacognosy
Published on Web 03/24/2000



Dihydroisoderrondiol (1)



Isoderrone (2)



Ficuisoflavone (3)

Figure 1.

the flavonols.[2] The presence of isoprunetin is another taxonomic characteristic of the tribe Genisteae.[21]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter using a 1-mL cell; UV spectra were recorded on a Perkin-Elmer Lambda 11 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AC-200 spectrometer and a Bruker Avance 400 spectrometer, using TMS as internal standard; all the 1D and 2D NMR experiments were performed using the standard Bruker library of microprograms. EIMS were obtained on a Hewlett-Packard 5988A spectrometer (70 eV); 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 mm 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. corsica* were collected in May 1996, at Gennargentu, Sardinia, Italy, and were identified by Prof. Antonio Manunta (Istituto di Botanica ed Orto Botanico, Università di Urbino, Italy). A voucher specimen (URB-1742/96) 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. corsica* (650 g) were extracted in a Soxhlet apparatus with *n*-hexane, $CHCl_3$, and MeOH, in turn. After removal of solvent in vacuo at temperatures up to 40 °C, the following residues were obtained: R_H (15.51 g), R_C (13.65 g), and R_M (51.62 g).

R_C was chromatographed on Sephadex LH-20 eluted with MeOH−$CHCl_3$ (9:1) to obtain eight crude fractions (C_1−C_8). An aliquot (418 mg) of fraction C_6 was applied to Si gel column chromatography eluted with $CHCl_3$−Et_2O (8:2) and $CHCl_3$−MeOH (9:1), yelding eight fractions ($C_{6.1}$−$C_{6.8}$). From fractions $C_{6.2}$ and $C_{6.3}$, chromatographed over Lobar RP_8 eluted with MeOH−H_2O (7:3), pure isoderrone (**2**) 15.5 mg, and ficuisoflavone (**3**), 4.1 mg, were obtained, respectively. Fraction $C_{6.5}$ (123.3 mg), after chromatography on a Si gel column eluting with EtOAc−toluene−MeOH (100:30:1), furnished **1** (27.7 mg) and a mixture of flavonoids. This mixture was further purified by preparative TLC using EtOAc−toluene−MeOH (100:30:1) as eluent to give isoprunetin (4.1 mg).

Two portions of residue R_M (13.93 and 12.15 g) were chromatographed separately over a Sephadex LH-20 column with MeOH as eluent, to obtain 10 (M_1−M_{10}) and 11 (M'_1−M'_{11}) fractions, respectively. D-Pinitol (81 mg) and isoprunetin (8.2 mg) were afforded after crystallization from fractions M_3 and M_7, while both M_8 and M_9 gave luteolin 7-*O*-*β*-glucopyranoside (55 mg and 158 mg). The mother liquors of these two fractions were combined and then subjected to column chromatography over Si gel eluted with toluene−EtOAc−MeOH (6:3:1) to obtain eight subfractions (I−VIII). Subfraction IV was further purified on a Lobar RP_8 column and gradient eluted with MeOH−H_2O (1:1) to MeOH, to afford daidzein (8.8 mg). Subfraction VI, chromatographed over Lobar RP_8, by gradient elution with MeOH−H_2O (45:55) to MeOH, yielded 5-methoxytaxifolin (6.2 mg). Fraction M_{10} was subjected to Si gel column chromatography using $CHCl_3$−MeOH (85:15 and 8:2) as eluents to give 10 fractions ($M_{10.1}$−$M_{10.10}$). Pure luteolin (6.1 mg) was obtained from fraction $M_{10.7}$, after column chromatography on a Si gel column with $CHCl_3$−EtOAc (6:4), and final purification by preparative TLC ($CHCl_3$−EtOAc 7:3). Fraction $M_{10.8}$ was chromatographed over a Lobar RP_8 column with MeOH−H_2O (6:4) and further purified by preparative TLC (toluene−EtOAc−HCOOH, 5:4:1), to afford taxifolin (5 mg). Fractions M'_3 and M'_{11}, after crystallization, furnished sucrose (77 mg) and luteolin (265.7 mg), respectively. The mother liquor of M'_{11}, chromatographed over a Sephadex LH-20 column with MeOH−H_2O (8:2) as eluent, gave 17 subfractions (I−XVII). Luteolin 4′-*O*-*β*-glucopyranoside (3.5 mg) was obtained after crystallization as a pure compound from subfraction VIII.

Dihydroisoderrondiol (1): amorphous pale yellow solid, mp 168−174 °C; $[\alpha]^{25}_D$ −0.020° (*c* 2.69, MeOH); UV (MeOH) λ_{max} (log ϵ) 263 (4.30), 299 sh (3.93); (MeOH + MeONa) 274 (4.30), 330 sh (3.87); (MeOH + $AlCl_3$) 273 (4.32), 310 (3.87), 367 sh (3.63); (MeOH + $AlCl_3$ + HCl) 272 (4.34), 310 sh (3.87), 360 sh (3.73); (MeOH + NaOAc) 327 (4.40); (NaOAc + H_3BO_3) 263 nm (4.34); ^{1}H NMR (CD_3OD) δ 8.06 (1H, s, H-2), 7.61 (1H, d, J = 1.8 Hz, H-2′), 7.33 (1H, dd, J = 8.3, 1.9 Hz, H-6′), 6.79 (1H, d, J = 8.4 Hz, H-5′), 6.32 (1H, d, J = 1.8 Hz, H-8), 6.22 (1H, d, J = 1.8 Hz, H-6), 4.53 (1H, d, J = 8.3 Hz, H-4″), 3.54 (1H, d, J = 8.3 Hz, H-3″), 1.45 and 1.21 (2 × CH_3); ^{13}C NMR (CD_3OD) δ 182.1 (C-4), 166.0 (C-7), 163.8 (C-5), 159.7 (C-9), 154.9 (CH-2), 153.9 (C-4′), 130.1 (CH-2′), 129.1 (CH-6′), 125.1 (C-1′), 124.6 (C-3), 124.4 (C-3′), 117.7 (CH-5′), 106.3 (C-10), 100.1 (CH-6), 94.8 (CH-8), 79.4 (C-2″), 76.9 (CH-3″), 70.1 (CH-4″), 27.1 and 19.4 (2 × CH_3); EIMS *m*/*z* 370 $[M]^+$ (10), 352 $[M - H_2O]^+$ (2), 185 (2), 153 (21), 149 (4), 124 (25), 91 (15), 69 (52), 55 (37), 43 (100); HREIMS *m*/*z* 370.1049 (calcd for $C_{20}H_{18}O_7$ 370.1052); TLC R_f 0.51 [EtOAc−toluene−MeOH (100:30:1)].

Acknowledgment. This work was supported by a grant from M.U.R.S.T.−Italy (Ministero dell'Università e della Ricerca Scientifica e Tecnologica).

References and Notes

(1) Pignatti, S. *Flora d'Italia*; Edagricole: Bologna, 1982; Vol. 1, pp 636−644.
(2) Harborne, J. B. *Phytochemistry* **1969**, *8*, 1449−1456.
(3) Bernasconi, R.; Gill, S.; Steinegger, E. *Pharm. Acta Helv.* **1965**, *40*, 246−256.
(4) Southon, I. W. In *Phytochemical Dictionary of the Leguminosae*; Bisby, F. A., Buckingham, J., Harborne, J. B., Eds.; Chapman & Hall: London, 1994; Vol. 1, pp 313−330.
(5) Pistelli, L.; Bertoli, A.; Giachi, I.; Manunta, A. *J. Nat. Prod.* **1998**, *61*, 1404−1406.
(6) Adesanya, S. A.; O'Neill, M. J.; Roberts, M. F. *Phytochemistry* **1985**, *24*, 2699−2702.
(7) Tahara, S.; Orihara, S.; Ingham, J. L.; Mizutani, J. *Phytochemistry* **1989**, *28*, 901−911.
(8) Maximo, P.; Lourenço, A. *Phytochemistry* **1998**, *48*, 359−362.
(9) Li, Y. C.; Kuo, Y. H. *J. Nat. Prod.* **1997**, *60*, 292−293.

(10) Pfeffer, P. E.; Valentine, K. M.; Parrish, W. F. *J. Am. Chem. Soc.* **1979**, *107*, 1265−1274.
(11) Collins, P. M. In *Carbohydrates*; Collins, P. M., Ed.; Chapman & Hall: London, 1987; p 289.
(12) Agrawal, P. K.; Agrawal, S. K.; Rastogi, R. P.; Osterdahal, B. G. *Planta Med.* **1981**, *43*, 82−85.
(13) Foo, L. Y. *Phytochemistry* **1987**, *26*, 813−817.
(14) Markham, K. R.; Geiger, H. In *The Flavonoids: Advances in Research*, Harborne J. B., Mabry T. J., Eds.; Chapman & Hall: London, 1994; pp 441−498.
(15) Sree Rama Murthy, M.; Venkata Rao, E. *Magn. Reson. Chem.* **1986**, *24*, 225−230.
(16) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389−1397.
(17) Yoshizaki, M.; Fujino, H.; Masuyama M.; Arisawa, M.; Morita, N. *Phytochemistry* **1987**, *26*, 2557−2558.
(18) Halpin, R. A.; El-Naggar, S. F.; McCombe, K. M.; Vyas, K. P.; Boyd, D. R.; Jerina, D. M. *Tetrahedron Lett.*, **1982**, *23*, 1655−1658.
(19) Kinghorn, A. D.; Balandrin, M. F. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Wiley Interscience: New York, 1984; Vol. 2, pp 105−239.
(20) Howard, A.; Michael, J. P. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1986; Vol. 28, pp 183−308.
(21) Harborne, J. B.; Turner, B. L. *Plant Chemosystematics*; Academic Press: London, 1984; p 156.

NP990282K