

A NEW ISOFLAVONE FROM *LUPINUS HIRSUTUS*

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ABSTRACT.—A new isoflavone from *Lupinus hirsutus* has been characterized as 2',4'-dihydroxy-5,7-dimethoxyisoflavone. Two known flavones, 5-hydroxy-3,6,7-trimethoxyflavone and 5,7,8-trimethoxyflavone, were also isolated. Structures were confirmed by uv, ¹H-nmr, and ms spectroscopic methods.

Lupinus hirsutus L. (Leguminosae) is a plant growing in Greece known by the common name of "agriolupino" and probably is the "Thermos" of Dioscorides (1). It has been used in Greek folk medicine as an antidiabetic and emollient. This work concerns the isolation and characterization of a new isoflavone and two known methylated flavones.

The three compounds were isolated from the EtOAc fraction and were identified as 2',4'-dihydroxy-5,7-dimethoxyisoflavone, 5-hydroxy-3,6,7-trimethoxyflavone, and 5,7,8-trimethoxyflavone. The uv spectra of the new isoflavone with NaOAc relative to those in MeOH indicated a substitution of the 7-hydroxyl group. Furthermore, the shift observed in the AlCl₃ spectrum suggested a free hydroxyl either at C-5 and C-2' (2).

Confirmation of the isoflavone skeleton was provided by the ¹H-nmr spectrum, which contained the characteristic singlet at δ 8.15 ppm due to the C-2 proton. The singlets (3H) at δ 3.90 and 3.85 ppm were attributed to the methoxyl groups at C-7 and C-5. The signals between δ 6.40 and 7.02 ppm integrated for five aromatic protons. Two of these protons resonated at 6.48 and 6.50 ppm and showed meta coupling, while the three remaining aromatic signals at δ 6.40, 6.42, and 7.02 ppm represented a typical ABX system. The mass spectrum showed the base peak *m/z* = 314. Also, fragment ions at *m/z* 180 and *m/z* 134 are attributable, respectively, to an A-ring fragment bearing two methoxyl substituents and a B-

ring fragment possessing two hydroxyl groups. Thus, in the ¹H-nmr spectrum the signals at 6.48 ppm and 6.50 ppm, which display meta coupling, must be due to protons at C-6 and C-8, whereas the ABX system of signals represent B-ring protons. The location of the two methoxyl groups at C-5 and C-7 was confirmed by the uv, ¹H-nmr, and ms spectra. The two hydroxyl groups on the B ring could be located either at C-2' and C-4' or at C-2' and C-5' to account for the ABX pattern of signals. C-3' and C-4' must be excluded because spectra recorded in the presence of AlCl₃ + HCl and NaOAc + H₃BO₃ indicated the absence of a free 3',4'-ortho-hydroxyl system on the B ring. Because all known legume isoflavonoids are substituted at C-4' (3), the location of the hydroxyl groups at C-2' and C-4' was presumed. This was confirmed by the fact that the compound gave a positive reaction with Gibbs reagent and by the above ¹H-nmr data. Also, the chemical shifts of the ring-B protons of the new isoflavone are nearly identical to those of 2'-hydroxygenistein (5,6). Thus, from the uv, ¹H-nmr and ms spectral data, the structure of the new compound was identified as 2',4'-dihydroxy-5,7-dimethoxyisoflavone.

The structures of the known compounds were determined by comparing chemical and spectroscopic (uv, ¹H nmr, ms) data with data found in the literature (4).

EXPERIMENTAL

PLANT MATERIAL.—Leaves and flowers of *L.*

birsutus were collected in Dimitsana, Greece, in June 1987. A voucher specimen is deposited at the Herbarium of the Botanical Museum of the University of Thessaloniki. The samples were dried in a cool, dark place and coarsely powdered.

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: uv-vis, Perkin-Elmer 554; ^1H -nmr, Brücker AW 80 MHz; ms, Hitachi-Perkin-Elmer-61 (70 eV); tlc on silica gel GF₂₅₄ with hexane-EtOAc-MeOH (6:4:1) (I) and CHCl_3 -isoPrOH (9:1) (II); cc on polyamide-SC-6 (MN). The spots were visualized with uv light (254 nm and 366 nm) or by NH_3 vapor.

ISOLATION AND IDENTIFICATION OF COMPOUNDS.—Dried plant material (2.5 kg) was successively extracted with 95% EtOH in a Soxhlet. Evaporation of the solvent yielded a gum that was partitioned between H_2O and organic solvents: petroleum ether, EtOAc, and *n*-BuOH. Extracts were evaporated to dryness. The EtOAc extract (10.5 g) was chromatographed over a polyamide column eluted with a mixture of MeOH and H_2O . Fractions were further fractionated and purified by tlc in solvents I and II. This yielded in a pure state the following compounds: 2',4'-dihydroxy-5,7-dimethoxyisoflavone (58 mg), 5-hydroxy-3,6,7-trimethoxyflavone (48 mg), and 5,7,8-trimethoxyflavone (62 mg).

2',4'-DIHYDROXY-5,7-DIMETHOXYISOFLAVONE.—Yellow, color on tlc under uv (366 nm) deep purple, uv/ NH_3 purple; uv max (MeOH) 208, 256, 292, (NaOMe) 212, 272, 320, (AlCl_3) 207, 265 sh, 290, ($\text{AlCl}_3 + \text{HCl}$) 208, 267 sh,

290, (NaOAc) 208, 289 sh, 317, (NaOAc + H_3BO_3) 208, 288 sh, 315; ^1H nmr ($\text{DMSO}-d_6$) δ 8.15 (s, H-2), 7.03 (d, $J = 8.2$ Hz, H-6'), 6.50 (d, $J = 2.2$ Hz, H-8), 6.48 (d, $J = 2.2$ Hz, H-6), 6.42 (d, $J = 2.4$ Hz, H-3'), 6.40 (dd, $J = 8.2$, 2.4 Hz, H-5'), 3.90 and 3.85 (s, 2 OMe); ms m/z [M]⁺ 314, [$\text{M} - 2\text{OH}$]⁺ 280, 180, 134. *Anal.* found C 64.2, H 4.1; calcd for $\text{C}_{17}\text{H}_{14}\text{O}_6$, C 64.9, H 4.4%.

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