Notes

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C-Glycosylflavones in *Lespedeza cuneata*

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C-Glycosylflavones were isolated from leaf material of *Lespedeza cuneata*. Isovitexin, isoorientin, vicenin-2 and lucenin-2 were identified.

Keywords——Leguminosae; *Lespedeza cuneata* G. Don; C-glycosylflavonoids; isovitexin: isoorientin; vicenin-2; lucenin-2

In the preceding paper[2] we reported that a C-glycosylflavonoid, 6,8-di-C-pentosylapigenin, which is a feeding stimulant for the larvae of the yellow butterfly, *Eurema hecabe mandarina de l'Orza*, was isolated from leaf material of their host plant, *Lespedeza cuneata* G. Don. The present paper describes the occurrence of four other C-glycosylflavonoids in the plant leaves.

From the biologically active methanol extract of leaf material of *L. cuneata*, two flavonoid-rich fractions, an *n*-butanol ext. (Fr. 6) and an aqueous ext. (Fr. 7), were obtained as shown in Charts 1 and 2 of the previous paper.[2] Subsequent fractionation and purification by droplet countercurrent chromatography (DCC) resulted in the isolation of compounds I and II from Fr. 6 and compounds III and IV from Fr. 7.

The appearance and behavior of the ultraviolet (UV) spectra in various media[3] indicated that I and III are both apigenin derivatives with free hydroxy groups at the 4′, 5 and 7 positions. This was supported by the proton magnetic resonance (PMR) spectra of their trimethylsilyl (TMS) ether derivatives. The same spectra showed I and III to contain seven and fourteen sugar protons and indicated the absence of protons at the 6 position and the 6, 8 positions, respectively. On treatment with acid, I gave vitexin (the Wessley-Moser interconversion product), but III was not similarly converted into an equilibrium mixture. In the mass spectra (MS) of their permethylated (PM) derivatives, I and III exhibited molecular ions at *m/e* 530 and *m/e* 748, respectively.

Compounds II and IV both gave the same luteolin-type UV spectra, and the spectral shifts in various media indicated that all their phenolic hydroxyl groups are unsubstituted. The PMR spectra of II and IV showed a pattern characteristic of luteolin derivatives, except for the absence of signals due to protons at the 6 position and 6, 8 positions, respectively. The MS of their PM derivatives indicated that the molecular weights of II and IV are 448 and 610, respectively.

We therefore, concluded that I, II, III and IV are 6-C-β-D-glucosylapigenin (isovitexin),[4] 6-C-β-D-glucosylluteolin (iso-orientin),[5] 6,8-di-C-β-D-glucosylapigenin (vicenin-2),[6] and 6,8-di-

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C-β-D-glucosylluteolin (lucenin-2),[5] respectively. The MS of the PM derivatives of I, II, III and IV closely matched, both in the presence of expected fragmentation ions and in the relative intensities of these ions, those[5] of the PM derivatives of isovitexin, isoorientin, vicenin-2 and lucenin-2, respectively. Comparison of the IR spectra of I and II with those of authentic samples confirmed their identities. Authentic samples of vicenin-2 and lucenin-2 were not available for comparison of IR spectra, but an IR spectrum of vicenin-2, kindly supplied by Prof. J. Chopin, agreed with that of III.

These flavonoids have previously been isolated from a variety of other sources, but this is the first report of their occurrence in this plant.

Experimental

PMR spectra were taken on a Hitachi R 40 spectrometer operating at 90 MHz. Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane (TMS) as an internal standard. MS were taken on a Hitachi RMU-7L spectrometer. TMS ether derivatives were prepared by a previously described procedure.[7] PM derivatives were prepared by the method of Chopin.[6] Paper partition chromatography (PPC) was conducted on Toyo Roshi No. 50 paper by the ascending method using *n*-BuOH–AcOH–H_2O (4:1:2) as a solvent. DCC was carried out with a chromatograph equipped with one hundred glass tubes (60 cm × 2 mm) packed with the upper layer (stationary phase) of $CHCl_3$–MeOH–H_2O–*n*–BuOH (10: 10: 6: 1). The flow rate of the moving phase (lower layer) was 0.21 ml/min; fractions of 15 ml were collected.

Extraction and Isolation——Fresh leaves (1.8 kg) of *L. cuneata* were extracted with several solvents and the MeOH extract (180 g) obtained was fractionated as shown in Charts 1 and 2 in our previous paper.[2] DCC of the flavonoid-rich fractions gave pure crystals of I (1.08 g) and II (198 mg) from the *n*-BuOH ext. (Fr. 6), and of III (15 mg) and IV (13 mg) from the H_2O ext. (Fr. 7).

Isovitexin (I)——Yellow needles, mp 225—230° (from MeOH), PPC; *Rf* 0.62. Hydrolysis, conducted by heating with 2 N hydrochloric acid for 5 hr, gave vitexin as yellow needles, mp 256—260° (from MeOH), identical with an authentic specimen. PMR of its TMS ether derivatives ($CDCl_3$) δ: 3.1—4.0 (5H, CH–O–), 4.27 (1H, t, J=8 Hz, CH–O–), 4.75 (1H, d, J=9 Hz, H-1″), 6.42 (1H, s, H-8), 6.46 (1H, s, H-3), 6.91 (2H, d, J=9 Hz, H-3′, 5′), 7.74 (2H, d, J=9 Hz, H-2′, 6′). MS of its PM derivative *m/e*: 530 (M^+).

Isoorientin (II)——yellow needles, mp 213—217° (from MeOH), PPC; *Rf* 0.53. PMR (CD_3OD) δ: 3.4—5.0 (CH–O–), 6.46 (1H, s, H-8), 6.50 (1H, s, H-3), 6.92 (1H, d, J=9 Hz, H-5′), 7.36 (1H, d, J=9 Hz, H-6′), 7.37 (1H, s, H-2′). MS of its PM derivative *m/e*: 560 (M^+).

Vicenin-2 (III)——Yellow needles, mp 258—261° (dec.) (from 50% EtOH–H_2O), PPC; *Rf* 0.35. PMR of its TMS ether derivative ($CDCl_3$) δ: 3.14—4.4 (CH–O–), 4.52 (2H, t, J=8 Hz, CH–O–), 4.73 (2H, d, J=10 Hz, H-1″), 6.56 (1H, s, H-3), 6.96 (2H, d, J=9 Hz, H-3′, 5′), 7.90 (2H, d, J=9 Hz, H-2′, 6′). MS of its PM derivative *m/e*: 748 (M^+).

Lucenin-2 (IV)——Yellow needles, mp 240—247° (dec.) (from 40% H_2O–MeOH), PPC; *Rf* 0.28. PMR of its TMS ether derivative ($CDCl_3$) δ: 3.1—4.9 (CH–O–), 6.52 (1H, s, H-3), 6.98 (1H, d, J=9 Hz, H-5′), 7.26 (1H, d, J=9 Hz, H-6′), 7.26 (1H, s, H-2′). MS of its PM derivative *m/e*: 778 (M^+).

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