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C-GLYCOSYLFLAVONES FROM *PARKINSONIA ACULEATA*

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Key Word Index—*Parkinsonia aculeata*; Leguminosae; *C*-glycosylflavones; orientin; isoorientin; vitexin; isovitexin.

Abstract—The only *C*-glycosylflavones isolated from *Parkinsonia aculeata* leaves were identified as orientin (instead of epiorientin), isoorientin (instead of parkinsonin-A), vitexin and isovitexin (instead of parkinsonin-B). Orientin was definitely present in the laevorotatory form.

INTRODUCTION

In a study of the possible sugar isomerization in *C*-glycosylflavones, we sought a sample of epiorientin, isolated from *Parkinsonia aculeata* leaves by Bhatia *et al.* [1] and considered by them to be an epimer of orientin. No sample of epiorientin being available, we undertook to re-isolate the compound from its natural source in order to compare its spectral properties with those of orientin (8-*C*-β-D-glucopyranosylluteolin). Besides epiorientin, two other *C*-glycosylflavones (parkinsonins-A and B) had been isolated by the same authors who proposed for parkinsonin-A a 5-*O*-methylluteolin-8-*C*-*gluco*-pyranoside structure, and for parkinsonin-B a 5,7-*di*-*O*-methylepiorientin structure. None of these compounds has been reported since from the same or other sources.

RESULTS AND DISCUSSION

When the flavonoid mixture isolated from the leaves of *Parkinsonia aculeata* was submitted to PC in water saturated with phenol, four dark spots could be observed in UV with R_f values 0.33, 0.43, 0.54 and 0.61. In 30% HOAc, the R_f values were 0.44, 0.53, 0.60, 0.70 and this solvent was used for preparative PC of the mixture. The eluate from the band 1 (0.44) did not crystallize directly and was chromatographed on a polyamide column eluted by H_2O–MeOH. From the first fractions, crystals were isolated which showed the same chromatographic properties and the same UV and IR spectra as authentic orientin, as expected from the data given by Bhatia *et al.* [1]. However, permethylation of the compound led to one permethyl derivative which could not be distinguished from authentic permethylorientin on TLC in conditions where PM 8-*C*-β-D-glucopyranosides and PM 8-*C*-β-D-galactopyranosides are separated [2] as well as PM 8-*C*-α and β-L-arabinopyranosides and furanosides (E. Besson, unpublished results). The MS of the permethylated compound was that of a PM 8-*C*-hexosylluteolin [2]. When the ^{1}H NMR spectrum of the perdeuteriomethylated compound was compared with that of PDM vitexin, the sugar protons gave the same signals, the anomeric proton being found at δ 4.94 as a doublet (J = 10 Hz) characteristic of the β configuration in *C*-D-glucopyranosides [3]. Therefore the compound is 8-*C*-β-D-glucopyranosylluteolin, i.e. orientin and not an epimer of orientin. In agreement with this conclusion and in contradiction with the data reported by Bhatia *et al.* [1], the mps and rotations of orientin and the compound were found to be the same, but surprisingly the sign of rotation was the reverse of that described by Koeppen *et al.* [4]. This has since been confirmed by Prof. Koeppen (personal communication) who found $[\alpha]_D^{24}$ − 17.24° (*c* 1.79 in pyridine) instead of $[\alpha]_D^{20}$ + 18.4° (*c* 1.14 in pyridine). Bhatia *et al.* [1] found epiorientin to have a negligible rotation and a mp > 300°, to give an acetate very similar (mp and rotation) to orientin acetate, but a methyl ether (CH_2N_2), considered to be a tetramethyl ether, very different (mp and rotation) from orientin tetramethyl ether. This led them to write "inter-conversion during acetylation may take place". Unfortunately, they did not compare 'epiorientin tetramethyl ether' (mp 259°, $[\alpha]_D^{22}$ −39.7° in pyridine) with orientin 7,3′,4′-trimethyl ether (mp 271–273°, $[\alpha]_D$ −38° in pyridine [5]) and it now seems most likely, considering our own results, that their methylation of 'epiorientin' with diazomethane was incomplete. After elution of orientin from the polyamide column, the following fractions gave a chromatographically homogeneous compound, showing the UV spectrum and diagnostic shifts [5] of a 4′-substituted luteolin and the mobility of a flavone glycoside. Acid hydrolysis showed it was not a *C*-glycoside, luteolin and glucose being the only products obtained. Thus no *C*-glycoside other than orientin was present in band 1.

The eluate from the band 2 (0.53) was rechromatographed on paper in 15% HOAc and BAW to give two PC- and TLC-homogeneous compounds. The main one could by crystallized and showed the same UV spectrum and diagnostic shifts as apigenin and was identified (PC, TLC and IR) with vitexin (8-*C*-β-D-glucopyranosylapigenin). The other compound showed the UV spectrum and diagnostic shifts of a 3′,4′-disubstituted luteolin and the mobility of a flavone glycoside. Acid hydrolysis showed it was not a *C*-glycoside, chrysoeriol and glucose being the only products obtained. Thus no *C*-glycoside other than vitexin was present in band 2.

The eluate from band 3 (0.60) directly gave a precipitate which showed the same UV spectrum and diagnostic shifts as luteolin and could not be distinguished (PC and TLC) from isoorientin (6-*C*-*β*-D-glucopyranosylluteolin). It is worth mentioning here that the mps and rotations given by Bhatia *et al.* [1] to parkinsonin-A acetate (mp 144–145°, $[\alpha]_D^{22}$ + 25.7° in pyridine) and parkinsonin-A trimethyl ether (mp 273–274°, $[\alpha]_D^{22}$ + 51.1° in pyridine) are similar to those [4] of isoorientin acetate (mp 140–144°, $[\alpha]_D^{22}$ + 27° in acetone) and isoorientin tetramethyl ether (mp 267–268°, $[\alpha]_D^{22}$ + 37.8° in aq. 50% acetone). The mp 224° [1] of parkinsonin-A is similar to the mp 235° [5] of isoorientin but the rotations are exactly opposite: $[\alpha]_D^{22}$ − 32° (parkinsonin-A), +30.8° (isoorientin) in the same solvent pyridine.

The eluate from band 4 (0.70) was rechromatographed on paper in BAW to give one dark band under UV light which was purified on a polyamide column. The chromatographically homogeneous product showed the same UV spectrum and diagnostic shifts as apigenin and could not be distinguished (PC and TLC) from isovitexin (6-*C*-*β*-D-glucopyranosylapigenin).

No other *C*-glycosylflavone could be found in the extracts, so that, in our hands, *Parkinsonia aculeata* did not give any of the very unusual *C*-glycosylflavones epiorientin and parkinsonins-A and B, but only the quite common ones orientin, isoorientin, vitexin and isovitexin.

EXPERIMENTAL

Plant material. *Parkinsonia aculeata* (voucher specimen No. 1/79, deposited at JIPMER, Pondicherry-6) was collected in Pondicherry, south India.

Extraction. Fresh leaves (3.0 kg) were cut into small pieces and extracted with boiling 90% EtOH (2 × 12 l., 24 hr).

The combined extract was concd *in vacuo* (1.2 l.) and filtered hot through cotton. The clear brown filtrate, after cooling was extracted with C_6H_6 (5 l.), Et_2O (6 l.) and EtOAc (12 l.) in succession. The EtOAc concentrate (150 ml) on admixture with $CHCl_3$ (150 ml) yielded a yellow solid which was removed. More of the solid was obtained by concentrating the mother liquor (60 ml) and mixing with petrol (bp 40–60°) (60 ml) (total yield, 1.8 g). PC indicated the presence of at least six components showing colour reactions and UV fluorescence characteristic of flavonoids and phenyl propanoids. The mixture was subjected to chromatography over SiO_2 using $CHCl_3$–MeOH (5:1, 4:1, 3:1, 2:1, 1:1 and 1:2) when the flavonoids could be separated to less complex mixtures (2–3 components showing overlapping). Further purification was done by prep. PC using 30% HOAc as developing solvent to yield four distinct flavonoid bands (R_f: 0.44, 0.53, 0.60 and 0.70). The pigment from each band was individually purified by chromatography over polyamide (aq. MeOH) and/or PC (15% HOAc, BAW) (see Discussion) to yield pure components.

Orientin from band 1. Co-chromatography with authentic orientin: PC R_f 0.23 (HOAc 15%), 0.44 (BAW). TLC (Si gel) R_f 0.83 (EtOAc–MeCOEt–HCO_2H–H_2O, 5:3:1:1).

UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 254, 267, 347; +NaOAc 272, 276, 325i, 384; +NaOAc + H_3BO_3 260, 372; +$AlCl_3$ 274, 300i, 334i, 424; +$AlCl_3$ + HCl 263i, 275, 296, 356, 386; +NaOH 268, 275i, 334i, 403. IR (KBr) superimposable with the spectrum of authentic orientin. Mp and mmp 260° (dec.): $[\alpha]_D^{20}$ − 24° (pyridine, *c* 0.56); authentic orientin: $[\alpha]_D^{20}$ − 20° (pyridine, *c* 0.41). Permethylation and TLC (2). MS of the PM ether: *m/e* (rel. int.) 560 (M^+, 100), 399 (M − 161, 8), 385 (M − 175, 80), 371 (M − 189, 20), 355 (M − 205, 7). ^1H NMR of the PDM ether (250 MHz, $CDCl_3$): δ_{TMS} 3.2–3.9 (H-2″, 3″, 4″, 5″, 6″), 4.94 (*d*, *J* = 10 Hz, H-1″), 6.40 (*s*, H-6), 6.58 (*s*, H-3), 6.93 (*d*, J_o = 9 Hz, H-5′), 7.95 (*dd*, J_o = 9, J_m = 2, H-6′).

Luteolin O-*glucoside from band* 1. PC R_f 0.22 (15% HOAc), 0.62 (BAW). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 247i 268, 334; +NaOAc 272, 319, 353; +NaOAc + H_3BO_3 268, 338; +$AlCl_3$ 258, 278, 290i, 346, 382; +$AlCl_3$ + HCl 257i, 280, 290i, 344, 380; +NaOH 271, 298i 376. Acid hydrolysis (4 N HCl–MeOH 1:1, 100°, 4 hr) led to luteolin (UV, TLC) and glucose (TLC).

Vitexin from band 2. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 271, 302i, 333; +NaOAc 280, 301i, 370; +NaOAc + H_3BO_3 271, 302i, 336; +$AlCl_3$ 278, 304, 347, 385; +$AlCl_3$ + HCl 278, 303, 343, 383; +NaOH 280, 332, 392. IR (KBr) superimposable with the spectrum of authentic vitexin. Co-chromatography with authentic vitexin: PC R_f 0.30 (HOAc 15%), 0.48 (BAW). TLC (Si gel) R_f 0.62 (EPWM), 0.89 (EBFW).

Chrysoeriol O-*glucoside from band* 2. PC R_f 0.30 (15% HOAc), 0.60 (BAW). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 272, 290i, 335; +NaOAc 279, 311, 364; +NaOAc + H_3BO_3 272, 336; +$AlCl_3$ 263, 280, 294i, 351, 384; +$AlCl_3$ + HCl 260, 282, 294i, 346, 383; +NaOH 280, 313, 378. Acid hydrolysis led to chrysoeriol (UV, TLC) and glucose (TLC).

Isoorientin from band 3. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 255, 269, 348; +NaOAc 271, 276i, 330i, 377; +NaOAc + H_3BO_3 261, 372; +$AlCl_3$ 275, 300i, 340i, 425; +$AlCl_3$ + HCl 263i, 277, 293i, 359, 384; +NaOH 268, 274i, 346i, 403. Co-chromatography with authentic isoorientin: PC R_f 0.39 (HOAc 15%), 0.46 (BAW). TLC (Si gel) R_f 0.74 (EBFW).

Isovitexin from band 4. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 270, 302i, 333; +NaOAc 277, 296i, 374; +NaOAc + H_3BO_3 270, 300i, 336; +$AlCl_3$ 277, 301, 347, 380; +$AlCl_3$ + HCl 278, 301, 343, 379; +NaOH 277, 330, 397. Co-chromatography with authentic isovitexin: PC R_f 0.52 (HOAc 15%), 0.68 (BAW). TLC (Si gel) 0.82 (EPWM), 0.80 (EBFW).

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