C-GLYCOSYLFLAVONOIDS OF *PASSIFLORA SERRATIFOLIA*

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Passiflora and related genera in the Passifloraceae serve as larval food plants for about 65 species of neotropical butterflies (*Heliconius*) (1). In *Passiflora serratifolia*, feeding on young leaves kills the larvae while older leaves are a suitable diet. We report here the first of a series of chemical investigations designed to determine the chemical basis of coevolution in the *Passiflora-Heliconius* system. Although it is known that *Passiflora* species contain C-glycosylflavonoids (2–5), this is the first chemical investigation of *P. serratifolia* L. The leaves of the plant yielded five C-glycosylflavonoids, namely, vitexin, isovitexin, orientin, 2"-xylosylvitexin and 2"-xylosylisovitexin. All the compounds were identified by spectral methods as well as by direct comparison with standard samples.

EXPERIMENTAL[2]

PLANT MATERIAL.—The plant material was collected from plants grown in Dr. L. E. Gilbert's greenhouse collection, Department of Zoology, University of Texas at Austin. The rootstocks were collected 20 miles south of Naranjo, San Luis Potosi, Mexico in 1970, by Gilbert, Voucher No. 70179, Univ. of Texas at Austin Live Collection.

EXTRACTION AND FRACTIONATION.—Air-dried and powdered leaves (95 g) of *P. serratifolia* L. were extracted with 85% aqueous methanol followed by 50% methanol and water. The combined extracts were concentrated *in vacuo*. The aqueous concentrate was extracted with n-hexane, chloroform, and ethyl acetate, successively; upon evaporation *in vacuo*, 0.9g, 2.0 g and 1.0 g of syrup was obtained from the extracts, respectively. The remaining water layer yielded 10 g of a brown syrup. Since the n-hexane and chloroform fractions appeared similar on two-dimensional paper chromatography, they were combined. The resulting material was chromatographed on a polyclar column (5 x 50 cm, 200 g). Elution with methanol afforded vitexin and isovitexin. When the material from the ethyl acetate extract was chromatographed on a polyclar column using a gradient of methanol-water, starting with methanol and then increasing the amount of water, vitexin, isovitexin, orientin, 2"-xylosylvitexin, and 2"-xylosylisovitexin were obtained. The water extract yielded a mixture of the latter two compounds. Each compound was cleaned over a Sephadex LH-20 column.

IDENTIFICATION OF FLAVONOIDS.—Uv spectra (NaOMe; $AlCl_3$; $AlCl_3$/HCl; NaOAc; NaOAc/H_3BO_3) indicated apigenin-type structures for all compounds except orientin. Pmr spectra of the TMS derivatives of vitexin and isovitexin showed the typical signals for C-8 and C-6 glucosides.

Ms of vitexin and isovitexin were similar to those of standards (M-x·H_2O peaks as well as A_1 and B_1 peaks); tlc comparison with standard samples using various solvents (40% HOAc; 15% HOAc; BAW; TBA) gave the same Rf values. Hydrolysis of xylosylvitexin and xylosylisovitexin with 0.1 N TFA (trifloroacetic acid) yielded xylose (tlc comparison with a standard) and vitexin and isovitexin (uv, pmr, ms and tlc comparison with standards). Acetyl derivatives of xylosylvitexin and xylosylisovitexin were prepared and pmr were recorded in $CDCl_3$. Since signals were not observed for 2"-acetyl groups (at 1.74 for the former and 1.80 for the latter) the xylosyl must be attached to the 2" position of the C-glucosyl moiety (6). Other acetyl peaks as well as signals for the aromatic protons were the same as previously reported (6, 7).

ELECTROPHORESIS OF C-GLYCOSYLFLAVONOIDS.—All the C-glycosylflavonoids were further identified by high voltage (1.5 KV) electrophoresis on Whatman 3MM paper using a Gilson Medical Electronics Model D (tank type) electrophorator for 1½ hr at pH 1.9 (formic acid-acetic acid-water; 33:147:1820). Under these conditions, 6-C-glycosides (as well as 6,8-di-C-glycosides) migrate towards the cathode approximately twice as fast as 8-C-glycosides (4.8 cm versus 2.6 cm).

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[2]Uv spectra were recorded in a Beckman DU model recording instrument; pmr spectra in a Varian HA 100. Ms data were recorded in a DuPont 21-491 instrument. Polyclar powder (GAF), precoated cellulose plates (E. Merck), and precoated polyamide plates (Macharey-Nagel).

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