Identification of a Peonidin-3-cinnamylsophoroside in the Flowers of Tabebuia impetiginosa

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Tabebuia impetiginosa, a tall, fast-growing tree of the family Bignoniaceae, completely loses its leaves in May or June and becomes covered by large clusters of bluish pink flowers from May to July or August. Pomilio and Sproviero (8) found in the flowers of Argentine Tabebuia spp. three anthocyanins: cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-rutinoside. This paper reports the identification of a major anthocyanin present in the flowers of T. impetiginosa.

Fresh flowers of T. impetiginosa were collected in Campinas, in the months of May, June, and July, and extracted overnight at 5°C with methanol containing 0.1% HCl, under nitrogen. The methanolic solution of anthocyanins was concentrated at 30°C under reduced pressure and chromatographed on paper and on cellulose plates (1). Developing solvents were BAW; 1% HC1; (3 concentrated HCl : 97 water) and AWH (15 glacial acetic acid : 82 water ; 3 concentrated HCl). Sugars and organic acids used as markers in chromatographic methods were all analytical grade. Authentic pigments used as standards in chromatography and spectral analysis were prepared from Myrciaria jaboticaba (10).

Spectral data were obtained with the pigment dissolved in methanol containing 0.01% HCl. Nuclear magnetic resonance (NMR) spectrum was obtained with a Varian A-100 Spectrometer with CDCl$3$ as solvent and tetramethylicative (TMS) as internal standard. Total and controlled hydrolyses of the pigment were carried out with 2 n HCl according to Harborne (5) and Pomilio and Sproviero (8). The resulting products were identified by spectral data and paper chromatography. Developing solvents were BAW; 1% HCl; AWH; Forestal (30 glacial acetic acid : 3 concentrated HCl: 10 water). The sugar at position 3 was obtained by oxidation of the pigment with 30% hydrogen peroxide followed by the method of Chandler and Harper (2) and identified by GLC of the TMS derivative with a Pye Series 105 Chromatograph with a hydrogen flame ionization detector, and a glass column (1.30 m x 4 mm i.d.) packed with SE 52 on Carbowax WHP. Nitrogen at a flow rate of 45 ml·min$^{-1}$ served as carrier gas. The operating temperatures were: detector 320°C; injector, 150°C, column oven initially at 100°C for 1 min, programmed at 20°C/min to 275°C and maintained at 275°C for 4 min.

The acyl group was identified after treating a solution of the pigment with NaOH at room temperature according to Francis and Harborne (3) and the acid produced identified by TLC on silica gel developed with BAW; PrN (7 n-propanol: 3 concentrated ammonium hydroxide); TAE (5 toluene: glacial acetic acid: 1 ethanol), and by its NMR spectrum.

The amount of total pigments in the flowers of T. impetiginosa was determined according to Francis (4). It varied from 180 to 300 mg per 100 g of fresh flowers, depending on the harvesting time.

The purified pigment extract yielded one major spot when chromatographed on paper and on cellulose plates developed with BAW, 1% HCl, and AWH. The major pigment $Rf = 12$ (BAW); 28 (AWH); 07 (1% HCl); $\lambda_{max}$ 520, 275 nm showed no fluorescence under UV light precluding glycosidation in position 5. This was confirmed by the ratio E440/E vis. max. = 27. No shift in the visible region was observed by the addition of a methanolic solution of AlCl$_3$ ruling out the presence of vicinal free hydroxyl groups in the B ring.

Total acid hydrolysis produced peonidin as the aglycon identified by spectral data: $\lambda_{max}$ 276, 530 nm; $\Delta \lambda$ (AlCl$_3$) = 0, and by paper co-chromatography with authentic peonidin. Glucose was identified by paper chromatography as the only sugar produced by acid hydrolysis. A chromatogram of the acid hydrolyzate sprayed with bromocresol green showed one yellow spot indicating the presence of an acid.

Alkaline hydrolysis of the pigment confirmed the presence of an acid, identified as cinnamic acid by: a) TLC on silica gel using a pure sample of cinnamic acid as marker followed by observation of the spot under UV light before and after exposure to NH$_3$ vapors and by spraying the chromatogram with an aqueous solution of FeCl$_3$ according to Pifferi (7); b) its NMR spectrum; the NMR spectrum of the acid exhibited two strong doublets, at $\sigma$ 6.45 ($J$ = 16) and 7.8 ($J$ = 16) that were assigned to the ethylene protons of cinnamic acid; a signal at $\sigma$ 13.21 and a narrow peak at $\sigma$ 7.45 (m) were assigned to the carbonyl and phenyl protons, respectively (6, 9). Also the wide single band at 275 nm showed in the UV spectrum of T. impetiginosa also can be attributed to cinnamic acid, which has a $\lambda_{max}$ 273 nm, a value close to the one for the UV band of peonidin-3-glycosides and peonidin-3,5-diglycosides.

Controlled hydrolysis of the pigment after removing the acyl group, yielded only one intermediate identified as peonidin-3-glucoside by means of $Rf$ values: 41 (BAW); 36 (AWH); 08 (1% HCl), and spectral data: $\lambda_{max}$ 273, 521 nm; $\Delta \lambda$ (AlCl$_3$) = 0, E440/Evis. = 28%, EUV/Evis. = 58%. Peroxide oxidation produced sophorose, which was identified by GLC by comparison with the retention time of an authentic sample of sophorose.

On the basis of the results obtained, the pigment was identified as a peonidin-3-cinnamylsophoroside. So far as we know, cinnamic acid has not been reported as the acyl moiety of acylated anthocyanins. A second pigment was present in the extract from T. impetiginosa, but in amounts not sufficient to permit identification.

Literature Cited


