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Technical Note

Anthocyanins from *Tibouchina grandiflora*

ABSTRACT

From the flowers of Tibouchina grandiflora, *four pigments were extracted and two of them were identified as peonidin-3-sophoroside and malvidin-3,5-diglucoside. Another two pigments were tentatively identified as malvidin-3-*(p-*coumaroyl)-sambubioside-5-glucoside and peonidin-3-sambubioside. Three pigments were also present in* T. grandiflora *but in amounts insufficient to permit identification.*

INTRODUCTION

Tibouchina is a large family of ornamental plants that grows easily in tropical and sub-tropical climates. Harborne (1964) identified malvidin-3,5-diglucoside as the major pigment in the flower of *Tibouchina semidecandra*, and Francis *et al.* (1982) found, in the flower of *Tibouchina granulosa*, the following anthocyanins: malvidin-3-(di-*p*-coumaroyl xyloside)-5-glucoside and malvidin-3-(*p*-coumaroyl xyloside)-5-glucoside.

Continuing our studies on natural pigments and possible sources for natural food colours, we identified the anthocyanins extracted from the flowers of *Tibouchina grandiflora*, a branching shrub of approximately 5 ft with very showy and quite abundant dark blue flowers, easily grown as a green fence for gardens or lining roadsides.

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MATERIALS AND METHODS

Extraction and purification of pigments

Flowers of *T. grandiflora* were extracted with 0·1 % HCl in methanol at 5 °C under N_2 (Francis & Harborne, 1966). The methanolic solution was concentrated under reduced pressure at 30 °C and the extract purified by paper chromatography on Whatman 3MM with two different solvent systems: BAW (*n*-butanol–glacial acetic acid–water, 6:1:2) and 1 % HCl (concentrated HCl–water, 3:97). The pigments were separated by TLC on cellulose plates developed with BAW.

Spectral analysis

The spectral data were obtained with a Unicam SP ultraviolet recording spectrophotometer.

Identification of individual pigments

The individual anthocyanins were identified through acid, alkaline, and controlled hydrolysis (Du & Francis, 1973; Chen & Luh, 1967; Francis & Harborne, 1966; Francis *et al.*, 1982). Sugars in 3C position were obtained through peroxide hydrolysis (Chandler & Harper, 1961) and identified by paper chromatography and GLC (Sweeley *et al.*, 1963), under conditions already described (Bobbio *et al.*, 1983). The amounts of total pigments were determined according to Francis (1982).

RESULTS AND DISCUSSION

The total amount of red pigments in the flowers of *T. grandiflora* was determined to be 0·62 % (w/w).

TLC of the pigments on cellulose plates with BAW yielded four bands designated A, B, C and D. Three other pigments were present in trace amounts.

Spectral analysis

The spectral data (Table 1) showed that all four pigments had no vicinal-free hydroxyl groups; pigments A and D had sugars attached only in the

TABLE 1
Characteristic Data of *Tibouchina grandiflora* Anthocyanins

		Pigments			
		A	*B*	*C*	*D*
$R_f \times 100$	BAW[b]	30	30	40	34
	1% HCl	38	15	30	38
	AWH[a]	60	47	52	50
Ultraviolet fluorescence		negative	dark red	negative	dark red
$\lambda_{vis\ max}$ (nm)		520	530	528	524
$\lambda_{UV\ max}$ (nm)		277	280	275, 325[c]	256
$\Delta\lambda_{vis\ max\ with\ AlCl_3}$ (nm)		0	0	0	0
$E440/E_{\lambda_{vis\ max} \times 100}$		45	17	14	28

[a] AWH = acetic acid–water–12N HCl (15:82:3).
[b] BAW = *n*-Butanol–acetic acid–water (6:1:2).
[c] Indicates the presence of organic acid (Harborne, 1967; Chen & Luh, 1967).

TABLE 2
Characteristic Data for Hydrolysis Intermediates of *Tibouchina grandiflora*

		Intermediates				
		A_1	B_1	C_1	C_2	D_1
$R_f \times 100$	BAW[d]	43(41)[a]	41(38)[b](43)[c]	32(31)[a]	40(38)[b](43)[c]	43(41)[a]
	1% HCl	09(09)[a]	06(06)[b](04)[c]	15(13)[a]	05(06)[b](04)[c]	07(09)[a]
	AWH[e]	36(33)[a]	27(29)[b](22)[c]	45(42)[a]	26(29)[b](22)[c]	33(33)[a]
	Forestal[f]	—	—	—	—	—
Ultraviolet fluorescence		negative	negative	dark red	negative	negative
$\lambda_{vis\ max}$ (nm)		—	—	—	—	—
$\lambda_{UV\ max}$ (nm)		—	—	—	—	—
$\Delta\lambda_{vis\ with\ AlCl_3}$ (nm)		—	—	—	—	—

[a] Numbers in parentheses are R_f values obtained by Harborne (1967).
[b,c] Values obtained by Harborne (1967) for malvidin-3-glucoside and malvidin-5-glucoside, respectively.
[d] BAW = *n*-Butanol–acetic acid–water (6:1:2).
[e] AWH = acetic acid–water–12N HCl (15:82:3).
[f] Forestal = acetic acid–conc. HCl–water (30:3:10).

TABLE 3
Chromatographic Characteristics of Hydrolysis Products

Samples	*$R_f \times 100$*					*Retention time (s)*
	BAW	*Forestal*	*EtAc-Py-W*	*Bu-Py-W*	*PrN*[b]	
Aglycones						
A	75(71)[a]	64(63)[a]				
B	55(58)[a]	65(60)[a]				
C	58(58)[a]	65(60)[a]				
D	72(71)[a]	63(62)[a]				
Acid						
C	97				85	
Sugars (total hydrolysis)						
A			21	18		
B			20	16		
C			22–41	16–33		
D			20–40	17–34		
Sugars (H_2O_2 hydrolysis)						
A						10·5
B			20	17		
C						
D						
Standards						
p-Coumaric acid	97				85	
Glucose			20	17		
Xylose			41	35		
Sophorose						10·5

[a] Numbers in parentheses are R_f values obtained by Harborne (1967).
[b] *n*-Propanol–NH_4OH, 7:3.

3C position while pigments C and D had both positions 3C and 5C substituted; only pigment C showed absorbance in the region characteristic of acylated anthocyanins (Harborne, 1967; Chen & Luh, 1967; Vaccari *et al.*, 1981).

Total hydrolysis

Hydrolysis data are shown in Tables 2 and 3. Total hydrolysis (Table 3) yielded peonidin as the aglycone for pigments A and D, and malvidin for

pigments B and C; glucose was the only sugar identified in pigments A and B; pigments C and D produced glucose and xylose.

Controlled hydrolysis

Controlled hydrolysis (Table 2) yielded only one intermediate for pigments A, B and D designated A_1, B_1 and D_1, and two intermediates for pigment C, indicated by C_1 and C_2. Pigments A_1 and D_1 were identified by paper chromatography as peonidin-3-glucoside and C_1 had the same mobility as malvidin-3,5-diglucoside. Both intermediates B_1 and C_2 could be either malvidin-3-glucoside or malvidin-5-glucoside. An unquestionable identification could not be made for these compounds due to the similarity of the mobility of malvidin-3-glucoside and malvidin-5-glucoside.

Peroxide hydrolysis

Peroxide hydrolysis (Table 3) demonstrated the presence of sophorose in pigment A (identified by GLC), glucose for pigment B (identified by paper chromatography) and a glucose and xylose containing disaccharide for pigments C and D.

Alkaline hydrolysis

Pigment C was the only pigment with spectral characteristics of an acylated anthocyanin. Alkaline hydrolysis of this pigment produced *p*-coumaric acid, identified by paper chromatography (Table 3). The same yellow colour was obtained when a solution of the acid and of a pure sample of *p*-coumaric acid were treated with an aqueous 2% $FeCl_3$ solution (Pifferi, 1965).

On the basis of the results, pigments A and B could be identified as peonidin-3-sophoroside and malvidin-3,5-diglucoside, respectively. Unfortunately the lack of reference made impossible an identification of the sugar in the 3C position of pigments C and D. However, since controlled hydrolysis of pigment C produced malvidin-3,5-diglucoside while from pigment D peonidin-3-glucoside was obtained, pigments C and D could be tentatively identified as malvidin-3-(*p*-coumaroyl)-sambubioside-5-glucoside and peonidin-3-sambubioside.

CONCLUSION

The major pigment extracted from the flowers of *Tibouchina grandiflora* was identified as peonidin-3-sophoroside. A second pigment was identified as malvidin-3,5-diglucoside. Two other pigments were tentatively identified as malvidin-3-(*p*-coumaroyl)-sambubioside-5-glucoside and peonidin-3-sambubioside, the latter occurring in very small amounts. There were trace amounts of three other pigments.

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