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Anthocyanins of the Brazilian Fruit *Cyphomandra betaceae*

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ABSTRACT

The anthocyanin pigments present in the dark red seeds and reddish-brown peel of the fruit of the tree tomato (Cyphomandra betaceae) *were identified. Pelargonidin-3-glucosyl-glucose was detected in both peel and seeds. Two other pigments were present in the seeds, identified as peonidin-3-glucosyl-glucose and malvinidin-3-glucosyl-glucose. All pigments had an identical sugar moiety tentatively identified as maltose. Besides the fact that seeds are not a very common source of anthocyanins, maltose has never been reported in the literature as the disaccharide of these compounds.*

INTRODUCTION

The fruit of the Brazilian tree tomato *Cyphomandra betaceae* has reddish-brown peel, orange pulp and dark red seeds. The orange colour of the pulp is due to carotenoids, the identification and quantification of which has been reported by Rodriquez-Amaya *et al.* (1983). Although the peel also contains carotenoids, the reddish tinge could not be accounted for by these pigments. The dark red colour of the seeds is also not derived from carotenoids.

Wrolstad & Heatherbell (1974) found in a fruit named tamarillo and classified as *Cyphomandra betaceae*, the following anthocyanins:

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pelargonidin, cyanidin and delphinidin-3-rutinosides and 3-glucosides. The fruit originates from Perú but is available in New Zealand.

In the present paper we report the identification of the anthocyanin pigments of the peel and seeds of *C. betaceae* fruit which grows wild in Brazil.

Our research on natural pigments has a twofold objective (i) to characterize the pigments responsible for the colour of Brazilian fruits and (ii) to identify possible plants sources of natural pigments for use as food colourants.

MATERIAL AND METHODS

Extraction and purification of pigments

Seeds and peel from fruits of *C. betaceae* were separately macerated overnight with 1 % HCl in methanol under N_2 at a temperature of 5 °C. The extract was decanted and the process was repeated twice. The methanolic extracts were combined and concentrated in a rotatory evaporator under reduced pressure at 30 °C and were purified by TLC on cellulose plates with the following solvent systems: BAW (*n*-butanol–glacial acetic acid–water, 6:1:2); 1 % HCl (concentrated HCl–water, 3:97); Forestal (glacial acetic acid–concentrated HCl–water, 30:3:10); AWH (glacial acetic acid–water–concentrated HCl, 15:82:3).

Spectral analysis

The spectral analyses were obtained with a Unicam SP ultraviolet recording spectrophotometer with the pigments dissolved in 0·01 % HCl methanolic solution.

Acid hydrolysis

Acid hydrolysis was accomplished by adding 2 ml of 2N HCl to a methanolic solution of the pigment and boiling the solution for 30 min as described by Du & Francis (1973). After cooling, the aglycones were removed with amyl alcohol. The aqueous solution containing the sugar was dried at room temperature and subjected to paper chromatography with two different solvent systems: EtAc–Py–W (ethyl acetate–pyridine–water, 8:2:1) and Bu–Py–W (*n*-butanol–pyridine–water,

6:3:1). The chromatograms were visualized with a solution of $AgNO_3$ in acetone followed by a solution of NaOH in methanol.

Authentic samples of glucose, mannose, xylose, galactose, arabinose and rhamnose were used as standards.

The aglycones were identified by paper chromatography, using Forestal and BAW as developing solutions, and by the spectral data.

Peroxide analysis

A methanolic solution of the pigment was treated with 30% hydrogen peroxide according to Chandler & Harper (1961). After concentration of the solution at room temperature the sugar was identified by GLC of TMS derivatives prepared by the method of Sweeley *et al.* (1963). GLC was performed with a Pye Series 104 Chromatograph, with a hydrogen flame ionization detector, and a glass column (1·30 m × 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^{-1} to 275 °C and maintained at 275 °C for 4 min.

The linkage position of the disaccharide was detected by spotting the sample on paper and spraying with a solution prepared by dissolving 4 g of diphenylamine and 4 ml aniline in 200 ml acetone and mixing the solution with 20 ml orthophosphoric acid (specific gravity 1·70 at 20 °C) following the work of Toba & Adachi (1978).

Controlled hydrolysis

A methanolic solution of the pigment was hydrolysed by the addition of 2 ml of 2 N HCl and heating to boiling following the method outlined by Francis and Harborne (1966). Samples were withdrawn at 0, 2, 4, 6, 8, 12, 16, 20 and 32 min, spotted on Whatman No. 1 paper and developed with BAW, 1% HCl, AWH and Forestal.

RESULTS AND DISCUSSION

Anthocyanin pigments of the seeds

TLC of the crude pigment in cellulose plates indicated the presence of more than one pigment. Satisfactory separation was obtained by

successive development with different solvents. Chromatography with BAW yielded three bands designated A, B and C, with band A closest to the line of application and C the fastest moving band.

None of the pigments was fluorescent under ultraviolet light, indicating a free hydroxyl group in the 5C position. The absence of absorption in the region between 300 and 330 nm and the existence of only one peak in the region below 300 nm indicated that neither cinnamic acid (Chen & Luh, 1967; Harborne, 1967) nor vanillic acid residues (Vacari *et al.*, 1982) are present. The absorption ratio $\lambda_{440}/\lambda_{max}$ for the three pigments confirmed the absence of substitution in the 5C position. No shifts in the absorption values of the pigments were observed after addition of a methanolic solution of $AlCl_3$, showing the absence of vicinal free hydroxyl groups in the B ring.

The properties of the pigments are summarized in Table 1.

Acid hydrolysis of A, B and C yielded malvidin, peonidin and pelargonidin, identified by comparing the R_F values obtained with those of the literature and by the properties discussed above (Table 1).

Acid hydrolysis yielded from all three pigments a single sugar identified as glucose by comparing the R_F value of the samples with that of an authentic sample of glucose or paper chromatography. On peroxide hydrolysis of pigments A, B and C only a glucosyl-glucose moiety was obtained. Since the spot corresponding to the sugar partially overlapped with that of ammonium salts on paper chromatography, identification was accomplished by GLC and the colour reaction of Toba & Adachi (1978). The retention time of the derivative of the sample coincided with

TABLE 1
Characteristic Data of *Cyphomandra betaceae* Anthocyanins

		Pigments		
		A	*B*	*C*
$R_F \times 100$	BAW	19	26	41
	1% HCl	09	15	45
	AWH	41	21	61
Ultraviolet fluorescence		negative	negative	negative
$\lambda_{vis\,max}$ (nm)		530	520	504
$\lambda_{UV\,max}$ (nm)		272	270	273
$\Delta\lambda_{vis\,max}$ with $AlCl_3$ (nm)		0	0	0
$E_{440}/E_{\lambda vis\,max}$ ($\times 100$)		38	41	45

that of the maltose derivative. Spiking of the sample with the TMS derivative of maltose produced an increase in the area of the peak corresponding to the TMS disaccharide derivative.

The retention time of the sample was distinctly different from cellobiose, indicating that a difference in the type of linkage is sufficient to alter the retention time. Unfortunately sophorose was not available, thus preventing us from excluding definitely sophorose as the possible sugar moiety on the basis of the GLC data. A further confirmation was thus undertaken. The disaccharide obtained on peroxide hydrolysis of all three pigments of *C. betaceae* was treated with an acetone solution of diphenylamine–aniline–orthophosphoric acid, giving a blue colour consistent with a 1,4 linkage and thus excluding a 1,2 linkage which gives an orange colour according to the work of Toba & Adachi (1978).

Controlled hydrolysis of A, B and C showed in each case the presence of only one intermediate pigment A_1, B_1 and C_1, respectively, identified by paper chromatography as malvidin-3-glucoside, peonidin-3-glucoside and pelargonidin-3-glucoside, respectively (Table 2).

Anthocyanin pigments of the peel

Extraction, purification and identification of the pigment from the peel, as described for the seeds, yielded a major pigment identified as being the same as pigment C.

TABLE 2

Characteristic Data of Controlled Hydrolysis Products of *Cyphomandra betaceae*

		Pigments					
		Intermediates			*Aglycones*		
		A_1	B_1	C_1	A_2	B_2	C_2
$R_F \times 100$	BAW	46 (38)[a]	42 (41)	45 (44)	60 (60)	73 (71)	80 (80)
	1% HCl	05 (06)	09 (09)	15 (14)	—	—	—
	AWH	30 (29)	33 (33)	33 (35)	—	—	—
	Forestal	—	—	—	57 (58)	63 (63)	68 (68)
Ultraviolet fluorescence		negative	negative	negative	negative	negative	negative
$\lambda_{vis\,max}$ (nm)		—	—	—	542 (542)	530 (532)	520 (520)
$\lambda_{UV\,max}$ (nm)		—	—	—	274 (275)	276 (277)	270 (270)
$\Delta\lambda_{vis\,max}$ with $AlCl_3$ (nm)		—	—	—	0	0	0

[a] Numbers in parentheses are R_F values obtained by Harborne (1967).

Another pigment was also observed on the thin layer plates but the amount was too small to permit its identification.

Besides the fact that seeds are not a common source of anthocyanins (Timberlake & Bridle, 1975), to the best of our knowledge maltose has not been reported in the literature as the sugar moiety of anthocyanins. The main biosides present in these compounds, according to Timberlake (1980), are rutinose, sambubiose, lathyrose and sophorose.

CONCLUSION

The three pigments extracted from the seeds of *Cyphomandra betaceae* were identified as malvidin-3-glucosyl-glucose, peonidin-3-glucosyl-glucose and pelargonidin-3-glucosyl-glucose. The major pigment extracted from the peel was identified as pelargonidin-3-glucosyl-glucose. The glucosyl-glucose in all three pigments, was tentatively identified as maltose. Another pigment was detected in the peel but it was present in amounts too small to permit identification.

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