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SHORT COMMUNICATION

COMPLEX ANTHOCYANINS FROM *IPOMOEA CONGESTA**

A. B. Pomilio and J. F. Sproviero†

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, Buenos Aires, Argentina

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Key Word Index—*Ipomoea congesta*; Convolvulaceae; peonidin-3-sophoroside-5-glucoside cinnamic esters.

Abstract—Six acylated anthocyanins have been isolated from the flowers of *Ipomoea congesta* R. Brown. One has been previously described as an acylated peonidin derivative. Three others are isomers, derived from peonidin-3-(caffeylsophoroside)-5-glucoside. The fifth was characterised as peonidin-3-(*p*-coumarylcaffeylsophoroside)-5-glucoside and the last as peonidin-3-(coumarylsophoroside)-5-glucoside. It is noteworthy that the anthocyanins found in this species have the same glycosidic pattern, 3-sophoroside-5-glucoside, as those reported for the cyanidin derivatives in *Ipomoea cairica* flowers.[1] Acylated anthocyanin occurrence in Tubiflorae order is of chemotaxonomical value.

INTRODUCTION

In preliminary work[1] we reported the isolation of several anthocyanins from the flowers of *Ipomoea cairica* (L.) Sweet. These were all derived from cyanidin-3-sophoroside-5-glucoside esterified with caffeic and *p*-coumaric acid. This paper is concerned with the identification of six acylated anthocyanins present in *Ipomoea congesta* R. Br. flowers. Five of them are new and only one pigment (I) has been previously described.[2] The anthocyanins are all peonidin derivatives acylated with *p*-coumaric and caffeic acid. Acylated peonidin pigments only a few are known such as peonanin, isolated from *Petunia hybrida*[3] and *Solanum tuberosum*,[4] which contains *p*-coumaric acid whereas caffeic acid is the acyl constituent of the *Vitis vinifera*[5] and *Ipomoea batatas*[2] pigments.

RESULTS AND DISCUSSION

Paper chromatographic methods were used to isolate and purify the pigments.[4] Four magenta bands (I, II, III, IV) were obtained when the original flower extract was chromatographed for 48 hr in BAW.

As we recently reported[1] in the separation of *I. cairica* flower pigments, isolation is laborious because of the presence of secondary bands accompanying the main ones. These minor pigments were not present in sufficient quantity to allow their identification. Attempts

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* Part II in the series "Acylated Anthocyanins". For Part I see Ref. 1.

† Research member of the Consejo Nacional de Investigaciones Científicas y Técnicas.

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to characterize them in order to establish whether they are hydrolysis products of the original pigments or other artifacts produced during the isolation process were unsuccessful.

TABLE 1. ANTHOCYANINS FROM *Ipomoea congesta*

Pigments	Acid produced on alkaline hydrolysis*	BAW	Bu–HCl	1% HCl	HOAc–HCl
I	Caffeic acid	30	32	33	58
IIa	Caffeic acid	26	14	5	24
IIb	Caffeic acid	25	4	2	17
IIc	Caffeic acid	24	2	4	11
III	*p*-Coumaric- and caffeic acid	14	8	5	15
IV	*p*-Coumaric acid	10	5	6	24

* Peonidin-3-sophoroside-5-glucoside was present in each case.
On Whatman No. 1 paper.
Abbreviations: BAW (*n*-BuOH–HOAc–H_2O; 4:1:5, v/v); Bu–HCl (*n*-BuOH–2N HCl; 1:1, v/v); 1% HCl (conc. HCl–H_2O; 3:97 v/v); HOAc–HCl (HOAc–conc. HCl–H_2O; 15:3:82, v/v).

Rechromatography of band II in 15% HOAc yielded three magenta bands, (IIa, IIb, IIc) that exhibited different colours under UV light: IIa dull magenta, IIb dull pink and IIc bright pink (main band).

The pigments, in decreasing order of relative concentration were: IV, III, II, and I the latter being present only in trace amounts. Several steps in purification were necessary to free pigments from other plant constituents.

TABLE 2. SPECTRAL DATA OF ANTHOCYANINS FROM *Ipomoea congesta*

Pigment	λ_{max} (nm)*†	$\frac{E_{acyl}\text{ peak}\%}{E_{vis.}\text{ max.}}$	$\frac{E_{440}\%}{E_{vis.}\text{ max.}}$
I	281·2; 321·6; 529	88	13·6
IIa	279; 325; 526·5	55·2	16·8
IIb	280; 321; 528	66·4	19
IIc	282·5; 321; 529	47·4	20·5
III	284·6; 316·5; 532·5	85·2	17
IV	280; 313; 534	61·1	20·2

* In methanol containing 0·01% conc. HCl.
† The addition of 3 drops of $AlCl_3$ in EtOH (5% w/v) gave no shift.

The anthocyanins were identified by their R_f values,[4] degradative oxidation,[6] acid[7] and alkaline[8] hydrolysis, and also by spectral characteristics in the visible and ultraviolet regions[9] (Tables 1 and 2). On acid hydrolysis all the pigments gave peonidin, λ_{max} 277; 532 nm; $\Delta\lambda(AlCl_3) = 0$; R_f 0·60 (Forestal) and 0·72 (BAW). Glucose was the only sugar detected. On alkaline hydrolysis, the pigments yielded the same deacylated anthocyanin, identified as peonidin-3-sophoroside-5-glucoside by means of R_f values: 0·20 (BAW); 0·10 (Bu–HCl); 0·57 (1% HCl); 0·71 (HOAc–HCl), and spectral data: λ_{max} 272; 524 nm; $\Delta\lambda(AlCl_3) = 0$; $E_{uv}/E_{vis} = 42\%$. Acyl moieties were characterized as caffeic and *p*-coumaric acids (Table 1). Besides the R_f values,[10], the characteristic colours under UV light,

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with diazotised *p*-nitroaniline[11] and on exposure to ammonia vapour, contributed to their identification. Hydrogen peroxide oxidation of deacylated anthocyanins yielded only sophorose, which must be thus attached to position 3.[6] No maxima shift was observed in the spectra on addition of aluminium chloride to the cell[12] (Table 2). The E_{acyl}/E_{max} (%) ratio[4] suggested that pigments I and III contained two molecules of acid whereas IIa, IIb, IIc, and IV only one. Two maxima were observed in the UV region (280 and 320 nm) and one in the visible. No absorption at 290 nm could be detected. Tanchev and Timberlake[13] previously reported absorption at 296 nm in mono- and di-sinapyl derivatives of cyanidin-3-sophoroside-5-glucoside isolated from *Brassica oleracea*. Similar results were obtained by us with caffeyl and coumaryl derivatives of the same deacylated anthocyanin isolated from *Ipomoea cairica* flowers.[1] This maximum (290 nm) was not detected in spectra of *Ipomoea congesta* pigments. The UV spectra of caffeic and *p*-coumaric acid showed maxima at 218·6; 242; 294; 325·5 nm and 225; 298·5; 310 nm respectively, when methanol containing 0·01 % HCl was used as solvent. Consequently, absorptions at 290 and 325 nm correspond to the acyl constituents. So far we have been unable to explain the lack of absorption at 290 nm in peonidin derivatives acylated with caffeic acid.

The IR spectra determined on KBr pellets containing small amounts of the pigments and using beam reference attenuator, were also of value in detecting the presence of hydroxycinnamic acid acylation. Characteristic absorptions were at 1700 and 1620 cm^{-1}, arising from the double bond conjugated with ester carbonyl group.[14]

Thus the six acylated anthocyanins were characterised as (I), peonidin-3-(dicaffeyl-sophoroside)-5-glucoside; (IIa), peonidin-3-(caffeyl-sophoroside)-5-glucoside; (IIb), peonidin-3-(caffeylsophoroside)-5-glucoside; (IIc), peonidin-3-(caffeyl-sophoroside)-5-glucoside; (III), peonidin-3-(*p*-coumaryl-caffeyl-sophoroside)-5-glucoside; and (IV), peonidin-3-(*p*-coumaryl-sophoroside)-5-glucoside. So far as we know these pigments have not been previously reported as occurring in the plant world, except for I which was originally found in *Ipomoea batatas* (L.) Lam.[2]

Pigments IIa, IIb, and IIc are isomers since they gave rise to the same hydrolytic and oxidative products, and could only be distinguished by their chromatographic behaviour (Table 1). It must considered that the possibilities of structural isomerism in these acylated anthocyanins is clearly considerable, as well as the probable existence of *cis-trans* isomerism in the caffeic and *p*-coumaric acid moiety. The occurrence of $O \rightarrow O$ acyl migration during the isolation and purification steps is unlikely, since the medium was always acid.[15]

It is noteworthy that in *I. congesta*, the isolated anthocyanins are all based on peonidin, whereas in *I. cairica* only cyanidin containing pigments were found. Nevertheless, the most remarkable feature of all these *Ipomoea* anthocyanins is that their glycosidic pattern -3-sophoroside-5-glucoside, is the same. Although, in *I. cairica* pigments, caffeic acid has the same hydroxylation pattern as ring B of cyanidin, there is no parallel in the *I. congesta* pigments. Thus, *O*-methylation does not occur in the cinnamic acids. Cinnamic acid derivatives with the same hydroxylation pattern as the co-occurring flavonoids have been found in several plants.[16] However, this is not an invariable rule and it is clear that the hydroxylating methylating genes for the anthocyanins do not necessary affect the cinnamic acids.

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Work is in progress towards establishing to which sugar position and glucose unit each cinnamic acid moiety is attached in the anthocyanins isolated.

EXPERIMENTAL

Flowers were collected from plants growing in Buenos Aires and La Plata from November to April. Extraction was carried out with 0·1% HCl–MeOH, at 0°, in the darkness under N_2. Oxidation, acid and alkaline hydrolyses were carried out according to the conventional methods earlier reported.[1]

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