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SHORT COMMUNICATION

# ACYLATED ANTHOCYANINS FROM *IPOMOEA CAIRICA*

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**Abstract**—Four new acylated anthocyanins have been isolated from flowers of *Ipomoea cairica* (L.) Sweet. Pigments I and II were characterized as isomers of cyanidin-3-(*p*-coumaryl-caffeyl-sophoroside)-5-glucoside, IIIb as cyanidin-3-(dicaffeyl-sophoroside)-5-glucoside and IV as cyanidin-3-(caffeyl-sophoroside)-5-glucoside. Pigment IIIa was identified as cyanidin-3-(dicaffeyl-sophoroside)-5-glucoside, which has been described by Imbert *et al.*[10] in *Ipomoea batatas* (L.) Lam. Anthocyanin IIIa is isomeric with IIIb. The presence of acylated pigments in *Tubiflorae* order is of systematic interest.

## INTRODUCTION

IN RECENT years, several series of acylated anthocyanins containing cyanidin have been isolated from a range of plants. The acyl group of these pigments is frequently *p*-coumaric acid, as in perillanin from leaves of *Perilla ocimoides*,[1,2] cyananin from petals of *Solanum tuberosum*[3] and of *Viola* × *wittrockiana*,[4] raphanusin C from roots of *Raphanus sativus*[5] and hyacinthin from bulbs of *Hyacinthus orientalis*.[5] These pigments all have a ratio of glycoside to *p*-coumaric acid of 1:1.

Harborne[5] characterized ferulic acid in acylated anthocyanins from *Brassica oleracea* whereas Stroh and Seidel[6] found sinapic acid as an acyl constituent in the same species. Tanchev and Timberlake[7] later reported that the pigments were acylated with one and two moles of sinapic acid. Caffeic acid was also found acylating cyanidin derivatives as in *Orobanche minor*,[8] *Urginea maritima*,[9] and *Salvia splendens*.[8]

The object of this paper is to report the results obtained from a study on anthocyanins present in *Ipomoea cairica* flowers. This species is indigenous to Argentina, and tropical and subtropical America. Five acylated anthocyanins have been now isolated from its lilac flowers, the acyl groups of these pigments being caffeyl and *p*-coumaryl. So far as we know, the compounds have not been previously described, except for pigment (IIIa), the experimental data for which are similar to those reported for the acylated cyanidin derivative present in *Ipomoea batatas* (L.) Lam.[10]

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The genus *Ipomoea*, which belongs to the Convolvulaceae (Tubiflorae), has not been fully investigated. However, Kataoka[11] isolated pelargonidin-3,5-diglucoside from petals of *Ipomoea purpurea* without mentioning the occurrence of acylated anthocyanins in this species. Later, Imbert *et al.*,[10] described two acylated anthocyanins in *Ipomoea batatas* (L.) Lam., which were characterized as peonidin- and cyanidin-3-dicaffeylsophoroside-5-glucoside. It must be stressed that acylation of the anthocyanins is a character which distinguishes plants in Tubiflorae from those in every other sympetalous order.[8]

## RESULTS AND DISCUSSION

We have observed, in agreement with Chen and Luh,[12] that methanol containing 0·1% HCl is the most suitable solvent for acylated pigment extraction, to avoid any hydrolysis of acyl groups at this stage. It was necessary to carry out all operations carefully, since solutions of acylated anthocyanins tended to fade on exposure to light and were less stable than the simple pigments to changes in pH and temperature.

Identification depended largely on effective separation and purification of the complex anthocyanins which were more difficult to characterize than the related simple ones by conventional methods. Isolation of acylated pigments of closely related structure was

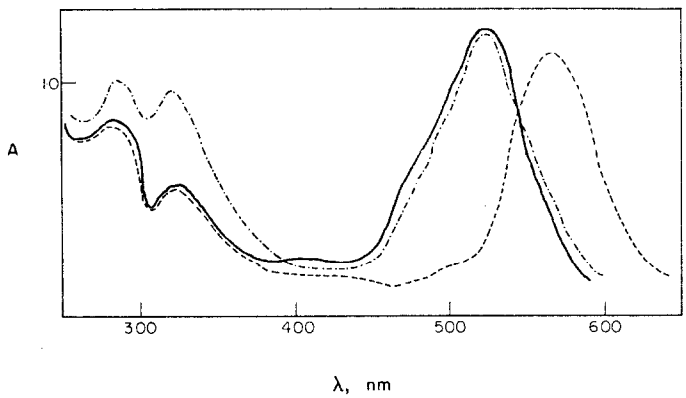


FIG. 1. ABSORPTION SPECTRA OF ACYLATED ANTHOCYANINS.
— 1 mol of caffeic acid (IV); — · — · 2 mol of caffeic acid (IIIa and IIIb); ..... $AlCl_3$–EtOH 5% (w/v).

cumbersome since they did not separate as satisfactorily as simple glycosides when present together in plant extracts. Furthermore, successive chromatographic separations gave rise to degradation of original pigments. Purification was conducted successfully by using no purely alcoholic solvents to prevent transesterification of the anthocyanin esters.

Chromatography of the original extract yielded four magenta bands designated: I, II, III, and IV. Band III when rechromatographed in 15% HOAc split into two pigments, IIIa and IIIb. $R_f$ values, as well as alkaline hydrolysis products are shown in Table 1. During examination of each hydroxycinnamic acid derivative two spots were always obtained on chromatograms presumably due to the presence of both *cis*- and *trans*-isomers.[13] The behaviour of both spots under UV light and chromogenic reagents was nearly the same.

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[13] A. H. WILLIAMS, *Chem. & Ind.* 120 (1955).

TABLE 1. ANTHOCYANINS FROM *Ipomoea cairica*

| Pigments | Alkaline hydrolysis* | $R_f$ values (× 100) in† | | | |
|---|---|---|---|---|---|
| | Cinnamic acid | BAW | Bu–HCl | 1% HCl | HOAc–HCl |
| I | *p*-coum. caff. | 37 | 18 | 19 | 52 |
| II | *p*-coum. caff. | 32 | 10 | 14 | 38 |
| IIIa | caff. | 25 | 21 | 30 | 58 |
| IIIb | caff. | 26 | 8 | 10 | 36 |
| IV | caff. | 19 | 10 | 20 | 41 |

* Cyanidin-3-sophoroside-5-glucoside was produced in each case.

† On Whatman No. 1 paper. Abbreviations: BAW (*n*-BuOH–HOAc–$H_2O$; 4:1:5, v/v); Bu–HCl (*n*-BuOH, 2 N 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); *p*-coum: *p*-coumaric acid; caff: caffeic acid.

On acid hydrolysis the isolated pigments yielded the same aglycone, cyanidin, and glucose as the only sugar.[14] Oxidation of the deacylated anthocyanins with hydrogen peroxide provided in each case the same disaccharide, sophorose.[15]

The presence of acyl components was determined by spectral means and confirmed by chromatographycal methods.[5] Visible and UV spectra of acylated anthocyanins showed two or three peaks in the UV region and one in the visible, the relative intensities of which varied depending on the kind of compound. The position of the peak between 310 and 330 nm indicated the presence of a hydroxycinnamic acid derivative and its relative intensity gave a measure of the acyl molecules present in the pigment.[8] The UV spectra of I, II, IIIa, and IIIb all exhibited a double peak at *ca.* 280 and 291 nm, perhaps due to an ester bond. This result was confirmed by the IR spectra of the pigments which showed typical absorptions at 1705 and 1620 $cm^{-1}$ indicating an ester carbonyl group conjugated with an ethylenic bond.[16] Spectral properties are described in Table 2. It is noteworthy that only the visible maximum was shifted (~40 nm) when aluminium chloride was added, whereas the maxima at 280–290 nm and 315–325 nm remained unchanged.

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

| Pigment | $\lambda_{max}$* (nm) | $\Delta\lambda_{A1C13}$† | E acyl peak‡ / E vis. max. (%) | $E_{440}$ / E vis. max. (%) |
|---|---|---|---|---|
| I | 282, 290, 319, 528 | 40·4 | 67 | 21 |
| II | 284, 291, 319, 528 | 40 | 67·6 | 19·5 |
| IIIa | 280, 291, 327, 529 | 36 | 94·1 | 19·2 |
| IIIb | 287, 291, 323, 528 | 37 | 83 | 19·3 |
| IV | 285, 320, 525 | 40·5 | 32·2 | 16 |

* In MeOH containing 0·01% conc. HCl.

† Three drops of a solution of $AlCl_3$ in EtOH (5% w/v) added to 2·5 ml solution. Only the 525–529 nm band shifts.

‡ 319–327 nm.

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[15] B. V. CHANDLER and K. A. HARPER, *Austral. J. Chem.* **14**, 586 (1961).

[16] K. NAKANISHI, *Infrared Absorption Spectroscopy; Practical.*, pp. 61, Holden-Day, San Francisco (1962).

According to these experimental data the pigments were characterized as follows: (I) Cyanidin-3-(*p*-coumaryl-caffeyl-sophoroside)-5-glucoside; (II), Cyanidin-3-(*p*-coumaryl-caffeyl-sophoroside)-5-glucoside; (IIIa), Cyanidin-3-(dicaffeyl-sophoroside)-5-glucoside; (IIIb), Cyanidin-3-(dicaffeyl-sophoroside)-5-glucoside; and (IV), Cyanidin-3-(caffeyl-sophoroside)-5-glucoside.

The most striking feature of the present study is that all pigments contain Cyanidin-3-sophoroside-5-glucoside. The pigments I and II, and IIIa and IIIb are isomers; this isomerism was previously pointed out by Birkofer *et al.*[17] The pigments only differ in their $R_f$ values, but co-chromatography in several solvents (Table 1) shows they behave as different compounds.

We have begun a systematic research on further *Ipomoea* species and a more detailed structural study on the pigments described above.

## EXPERIMENTAL

*Plant material.* Flowers were collected in Buenos Aires and its surroundings from November to April. No variation in anthocyanin pattern was observed during flowering period.

*Anthocyanin isolation.* Anthocyanins were extracted with MeOH containing 0·1% HCl, and the individual pigments were separated by paper chromatography (Whatman 3MM) using BAW as solvent. Purification was carried out with BAW, HOAc–HCl, and 15% HOAc. Hydrolysis products were identified by standard techniques. Ratio of glycoside to each hydroxycinnamic acid derivative was determined by the E(acyl peak)/E(vis. max) (%) values.

*Identification of acyl groups.* Acylated anthocyanins were hydrolysed with 2 N NaOH under $N_2$ for 20–60 min at room temp. in darkness, followed by acidification.[18] The mixture was extracted several times with $Et_2O$. Caffeic and *p*-coumaric acids were identified by comparison with authentic samples by paper chromatography using 3% HOAc, followed by observation of the colours of the spots in the UV $\pm$ $NH_3$ vapours, and after spraying with diazotized *p*-nitroaniline.[19] IR, spectra were determined in KBr discs. Other techniques were carried out as described.[14,15]

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*Key Word Index—Ipomoea cairica*; Convolvulaceae; acylated anthocyanins; cyanidin derivatives.