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2-DESCARBOXYBETANIDIN, A MINOR BETACYANIN FROM *CARPOBROTUS ACINACIFORMIS**†

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Abstract—The flowers of *Carpobrotus acinaciformis* have been shown to contain betanin, isobetanin, betanidin, isobetanidin, lampranthin-II and isolampranthin-II. A minor pigment was also isolated and identified as 2-descarboxybetanidin. This is the first report of the natural occurrence of this compound.

INTRODUCTION

Carpobrotus acinaciformis (L.) L. Bol. syn. *Mesembryanthemum acinaciforme* L. (Aizoaceae) is a xerophilous plant native of South Africa and widely naturalized on the coast of southern Italy.

In the present investigation the purple flowers of *C. acinaciformis* have been found to contain a mixture of betacyanins of which betanidin (I), betanin (5-*O*-β-D-glucopyranoside of betanidin) and their epimers isobetanidin and isobetanin are the main components. Two acylated pigments were isolated in lesser amounts and found to be identical with two betacyanins (lampranthin-II and isolampranthin-II) recently isolated from a *Lampranthus* species.[1]

In addition to these compounds, a violet pigment was isolated in very small amount and characterized as 2-descarboxybetanidin (II) on the basis of its chemical properties and comparison with a synthetic specimen. The present isolation represent the first report of the natural occurrence of 2-descarboxybetanidin, which had been previously synthesized from indicaxanthin (III) by exchange of the proline moiety with 5,6-dihydroxy-2,3-dihydroindole.[2]

RESULTS

A crude betacyanin fraction was obtained from aqueous extracts of *Carpobrotus acinaciformis* by chromatography on strongly acid ion-exchange resin. Individual pigments were isolated from this fraction by cellulose powder column chromatography and by high-voltage electrophoresis.

Betanin, isobetanin, betanidin and isobetanidin were identified by direct comparison of their spectral, chromatographic and electrophoretic properties with those of reference samples. The identity of the isolated lampranthin-II and isolampranthin-II was established by the identification of the products of alkaline hydrolysis and by means of co-chromatography and co-electrophoresis with known compounds. The violet pigment obtained from

* Part XII of the series "Pigments of Centrospermae", for part XI, see *Phytochem.*, **9**, 455 (1970).

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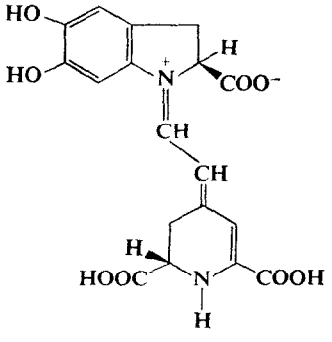
[1] M. PIATTELLI and G. IMPELLIZZERI, *Phytochem.* **8**, 1595 (1969).

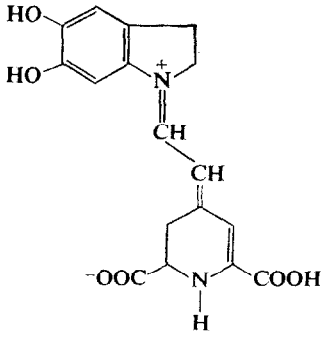
[2] L. MINALE and M. PIATTELLI, *Rend. Accad. Sci. Fis. Mat.* **32**, 165 (1965).

the last fraction of the cellulose column chromatography had u.v. spectrum (λ_{max} 542 nm in water) and bathochromic shift (10 nm) in the presence of borate very similar to those of betanidin (λ_{max} 541 nm, bathochromic shift in borate 10 nm). These spectral properties suggested the possible presence of a 1,7-diazaheptamethine system conjugated to an aromatic nucleus with *ortho*-dihydroxyl groups.

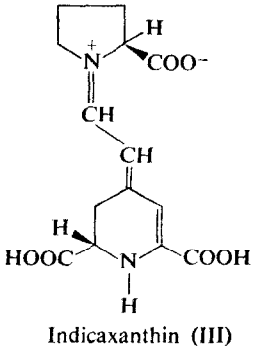
When an aqueous solution of the compound was saturated with sulphur dioxide the violet colour completely bleached in about 48 hr. The presence of betalamic acid (IV) in the colourless solution was indicated by the formation of indicaxanthin on treatment with proline. Furthermore, the pigment was shown to be a dicarboxylic acid, since methylation



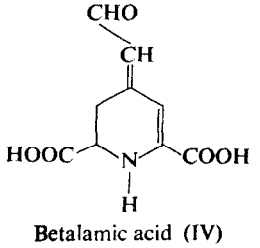
Betanidin (I)



2-Descarboxybetanidin (II)



Indicaxanthin (III)



Betalamic acid (IV)

with methanol in the presence of boron fluoride yielded a monomethyl and finally a dimethyl ester.

These data are consistent with the pigment being 2-descarboxybetanidin, a compound previously obtained synthetically and until now not reported in nature. The identification was confirmed by comparison of its spectral, chromatographic and electrophoretic properties with those of a reference sample of 2-descarboxybetanidin. Due to paucity of material, efforts directed to the assignment of the configuration of the asymmetric carbon atom were not undertaken.

The possibility that this compound was an artifact resulting by decarboxylation of betanidin during the isolation procedure appeared unlikely since betanidin by heating in aqueous solution is apt to decarboxylate preferentially at position 17.[2] Furthermore, 2-descarboxybetanidin was not detectable when a solution of betanidin was treated under

comparable conditions to those used during the isolation of betacyanins from the aqueous extract of the flowers of *C. acinaciformis*.

EXPERIMENTAL

Plant Material

Flowers of *Carpobrotus acinaciformis* were collected in Catania, Italy in spring 1969.

Authentic Samples

Samples of betanin, isobetanin, betanidin, isobetanidin, lampranthin-II and isolampranthin-II were available from earlier studies.[1,3] Indicaxanthin was isolated from fruits of *Opuntia ficus-indica* as described elsewhere.[4] 2-Descarboxybetanidin was synthesized from indicaxanthin by exchange of the proline moiety with 5,6-dihydroxy-2,3-dihydroindole,* according to a previously described procedure.[2]

Isolation of Pigments

Fresh petals of *C. acinaciformis* were macerated in 500-g batches in a blender with 2 l. ice-water. The macerate was filtered and the residue re-extracted with water. The combined extracts, adjusted to pH 3 (1 N HCl) and clarified by centrifuging, were passed through a column of Dowex 50W–X2 (H^+). After washing with 0·1 % HCl the column was eluted with water. The eluate was concentrated *in vacuo* in a cyclone evaporator (35°) and the concentrated solution was freeze-dried. From 3·5 kg (total wt.) of petals, 160 mg of crude betacyanin was obtained, which was chromatographed on a column of cellulose powder (3 × 45 cm) using water as the eluant. The course of the chromatography, monitored by paper electrophoresis, is represented in Table 1.

TABLE 1. COLUMN CHROMATOGRAPHY OF THE TOTAL BETACYANIN FROM *Carpobrotus acinaciformis*

Fraction No.	Quantity (ml)	Compounds
1	245	—
2	35	Betanin and isobetanin
3	50	Betanin, isobetanin, betanidin and isobetanidin
4	20	Betanidin and isobetanidin
5	50	Betanidin, isobetanidin, lampranthin-II and isolampranthin-II
6	70	Lampranthin-II and isolampranthin-II
7	370	—
8	350	2-Descarboxybetanidin

An attempt was made to determine if 2-descarboxybetanidin was artifact formed during the isolation procedure. When a solution of betanidin (50 mg) in water (15 l.) was worked up (absorption on ion-exchange resin, elution, concentration, chromatography on cellulose powder) under the conditions applied above to the aqueous extract of the flowers, 2-descarboxybetanidin was not detectable.

Betanin, Isobetanin, Betanidin and Isobetanidin

These pigments were identified by spectral, electrophoretic and chromatographic (polyamide column) comparison with known specimens. Moreover, betanin and isobetanin were hydrolysed by β-glucosidase giving betanidin and isobetanidin, respectively and glucose.

Lampranthin-II and Isolampranthin-II

Fraction 6 was found to consist of two components which were separated by high-voltage electrophoresis (pyridine formate 0·05 M, pH 4·5). Both pigments gave by alkaline hydrolysis a mixture of betanin and isobetanin, and ferulic and *p*-coumaric acid. The molar ratio of ferulic to *p*-coumaric acid was found to be 2:1 (quantitative TLC). In paper electrophoresis the two acylated pigments ran coincidental with authentic lampranthin-II and isolampranthin-II.

* 5,6-Dihydroxy-2,3-dihydroindole was prepared by oxidative cyclization of 3-hydroxytyramine (unpublished results).

[3] L. MINALE, M. PIATTELLI, S. DE STEFANO and R. A. NICOLAUS, *Phytochem.* **5**, 1037 (1966).
[4] M. PIATTELLI, L. MINALE and G. PROTA, *Tetrahedron* **20**, 2325 (1964).

2-Descarboxybetanidin

Fraction 8 was taken to dryness giving 0·7 mg of almost pure 2-descarboxybetanidin. Due to the low yield, no attempt was made to crystallize the product. It had the following properties: λ_{max} 542 nm in water, 552 nm in borate buffer, pH 8·7; E_b's* (75 V/cm) on paper unless otherwise stated): borate buffer 0·2 M, pH 7·7 = 0·74; pyridine formate 0·05 M, pH 4·5 = 0·10 (on paper) and 0·7 (on glass-fiber paper); formic acid 0·1 M, pH 2·4 = 0·03.

Methylation of 2-Descarboxybetanidin

2-Descarboxybetanidin (0·1 mg) was dissolved in methanol (0·1 ml) and BF_3 etherate (2 μl) was added. Samples were examined by paper electrophoresis at 2-hr intervals. Esterification proceeded with the initial formation of a violet compound (E_b's: pH 4·5 = 0·08, pH 2·4 = −0·02), followed by the formation of a second violet pigment E_b's: pH 4·5 = 0·00, pH 2·4 = −0·12).

Indicaxanthin from 2-Descarboxybetanidin

The conversion of 2-descarboxybetanidin into indicaxanthin was readily achieved using a modified version of the previously described procedure for the interconversion of betalains. A solution of 2-descarboxybetanidin (0·5 mg) in water (5 ml) was saturated with SO_2. After 48 hr the colourless solution was concentrated *in vacuo* to 0·1 ml and applied as a streak to the starting line of an electrophoretogram (phosphate buffer 0·05 M, pH 6·8). A 1% solution of proline (0·1 ml) was applied to a second starting line which was 1·5 cm from the first one and from the side of the anode. After about 0·5 hr betalamic acid had reached the slower migrating band of proline and a yellow band began to appear. The electrophoresis was allowed to run for 1·5 hr (potential gradient 20 V/cm). The yellow band was excised and identified as indicaxanthin by spectral and electrophoretic comparison with an authentic specimen.

* E_b equals migration in paper electrophoresis relative to betanin.