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PIGMENTS OF *BOUGAINVILLEA GLABRA**†

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Abstract—The violet-red pigments (bougainvillein-v's) isolated from bracts of *Bougainvillea glabra* var. *sanderiana* have been shown to the acylated betacyanins. Four deacylated pigments were obtained by alkaline hydrolysis of the total betacyanin fraction. Two of these were proved to be respectively 6-*O*-rhamnosylsophoroside and 6-*O*-β-sophoroside of betanidin, the other two being the corresponding isobetanidin derivatives.

INTRODUCTION

WE REPORTED recently[1] the isolation and characterization of some new betacyanins from a variety of *Bougainvillea* ("Mrs. Butt") with purple bracts. Two of these red-violet pigments (bougainvillein-r's) have been shown to be 5-*O*-β-sophorosides of betanidin and isobetanidin, respectively, the remaining bougainvillein-r's being hydroxycinnamoyl derivatives of the former. We also reported earlier[2] the occurrence of a number of pigments in *B. glabra* var. *sanderiana*. Many of these compounds were subjected to acid hydrolysis and either isobetanidin or a mixture of betanidin and isobetanidin identified in the hydrolysate.

In the present study these violet-red pigments (bougainvillein-v's) have been reinvestigated in more detail and found to be acylated compounds. Alkaline hydrolysis of the total betacyanin fraction from *B. glabra* var. *sanderiana* gave four deacylated pigments. Two of these compounds were proved to be respectively 6-*O*-rhamnosylsophoroside and 6-*O*-β-sophoroside of betanidin, the other two being the corresponding isobetanidin derivatives.

RESULTS

The total betacyanin fraction was isolated from aqueous extracts of bracts of *Bougainvillea glabra* by chromatography on strongly acid ion-exchange resin. From an aliquot of this fraction individual pigments were obtained by further chromatography on polyamide powder and high-voltage electrophoresis. The properties and products of alkaline hydrolysis of the eleven bougainvillein-v's isolated in the course of the present work are shown in Table 1.

Hydrolysis of the unfractionated betacyanin mixture with alkali yielded four deacylated pigments (DP1, DP2, DP3 and DP4) which were separated by chromatography on polyamide and high-voltage electrophoresis.

Pigment DP1 (λ_{max} 541 nm; E_b's: pH 2·4 = 0·88, pH 4·5 = 0·90) gave, on complete acid hydrolysis, a mixture of betanidin and isobetanidin, and pigment DP2 (λ_{max} 541 nm; E_b's: pH 2·4 = 0·88, pH 4·5 = 0·75) only isobetanidin. The hydrolysate also contained

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* Part XIII of the series "Pigments of Centrospermae", for part XII, see *Phytochem.* 9, 2553 (1970).

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[1] M. PIATTELLI and F. IMPERATO, *Phytochem.*, 9, 455 (1970)

[2] M. PIATTELLI and L. MINALE, *Phytochem.* 3, 547 (1964).

glucose and rhamnose identified by paper chromatography (six solvents). Since either DP1 or DP2 when treated with alkali in the absence of oxygen gave an equilibrium mixture of both compounds, it was deduced that they are diastereoisomers and DP1 is a betanidin and DP2 the corresponding isobetanidin derivative.

Diazomethane methylation of the mixture of the diastereoisomeric pigments followed by alkali fusion gave 6-hydroxy-5-methoxyindole-2-carboxylic acid; hence the sugar moiety is linked to the hydroxyl group at position 6 of the aglycones.

TABLE 1. PROPERTIES OF BOUGAINVILLEIN-V'S

Bougainvillein-v	E_b* pH 2·4	pH 4·5	λ_{max} in water (nm)	Products of alkaline hydrolysis Deacylated pigments	Hydroxycinnamic acids†
A	0·70	0·87	547, 322	DP3 and DP4	PC
B	0·35	0·69	546, 321, 277	DP3 and DP4	PC
C	0·19	0·58	542, 315	DP3 and DP4	PC
D1	0·19	0·54	548, 318	DP3 and DP4	PC
D2	0·64	0·80	550, 315, 277	DP3 and DP4	PC
E	0·21	0·34	551, 322	DP3 and DP4	PC
F	0·35	0·32	551, 317	DP3 and DP4	PC
G	0·42	0·55	522, 325	DP1 and DP2	PC
H1	0·22	0·40	550, 318	DP1 and DP2	CA
H2	0·78	0·74	543, 320, 285	DP3 and DP4	CA and PC
I	0·15	0·24	551, 310	DP3 and DP4	CA and PC

* Migration in paper electrophoresis (potential gradient: 75 V/cm) relative to betanin.
† Abbreviations: PC, *p*-coumaric acid; CA, caffeic acid.

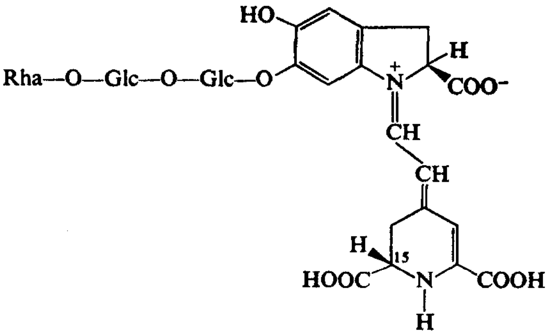
TABLE 2. R_G VALUES OF THE SUGARS PRESENT IN THE HYDROLYSATE OF A MIXTURE OF DP1 AND DP2

Designation	R_G*	Identity
S1	2·04	Rhamnose
S2	1·00	Glucose
S3	0·76	Rhamnosylglucose
S4	0·60	Sophorose
S5	0·41	Rhamnosylsophorose

* In butanol–acetic acid–water (12:3:5, v/v) on paper.

Controlled acid hydrolysis of DP1 and DP2 mixture with 10% acetic acid (3·5 hr under reflux) gave five sugars, whose chromatographic properties are reported in Table 2. Comparison with marker sugars allowed S1 to be identified as rhamnose and S2 as glucose. Compound S3, eluted with water from preparative scale chromatograms, was unaffected by β-glucosidase. On acid hydrolysis it gave glucose and rhamnose and no intermediate could be detected during the degradation. These properties are in agreement with the compound being a rhamnosylglucose. Substance S4, which was hydrolysed by β-glucosidase giving glucose, was identified as sophorose by direct comparison with authentic material by co-chromatography. The material S5 was not attacked by β-glucosidase and gave, by acid hydrolysis, glucose and rhamnose, with the intermediate formation of sophorose and

S3. These data are consistent with S5 being a linear trisaccharide (rhamnosylsophorose) and therefore DP1 can be represented as I, pigment DP2 differing only in the configuration at the C-15 carbon atom.

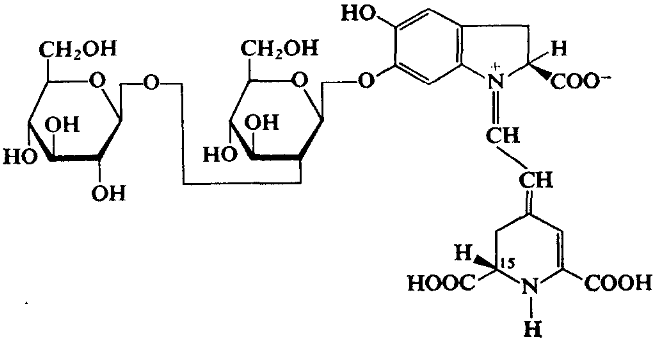


(I)

Pigments DP3 (λ_{max} 541 nm; E_b's: pH 2·4 = 0·92, pH 4·5 = 0·93) and DP4 (λ_{max} 541 nm; E_b's: pH 2·4 = 0·92, pH 4·5 = 0·78) could be equilibrated by treatment with dilute alkali at room temp. in the absence of oxygen and therefore they are diastereoisomers. Since DP3 gave on acid hydrolysis the diastereoisomeric aglycones betanidin and isobetanidin and DP4 only isobetanidin, the former is a betanidin and the latter the corresponding isobetanidin derivative. Additional work on the structure of these pigments was carried out by using a mixture of both. Glucose was the only sugar obtained by total acid hydrolysis (22% HCl; 5 min at 80°) whereas under mild conditions (10% acetic acid, 3·5 hr under reflux) a disaccharide was formed which was identified as sophorose by direct comparison with authentic material.

When a mixture of DP3 and DP4 was subjected to diazomethane methylation followed by alkali fusion, 6-hydroxy-5-methoxyindole-2-carboxylic acid was obtained. From this it was inferred that the sugar moiety is bound to the hydroxyl group at position 6 of the aglycones.

Since controlled hydrolysis (1 N HCl; 10 min at 80°) of the glucosides mixture gave, in addition to the products of total hydrolysis, small amounts of gomphrenins I and II (6-*O*-β-D-glucopyranosides of betanidin and isobetanidin, respectively) the disaccharide–aglycone linkage is β. These observations are completely consistent with structure II for DP3, pigment DP4 being its C-15 diastereoisomer.



(II)

EXPERIMENTAL

Plant Material

Bracts of *Bougainvillea glabra* var. *sanderiana* were collected in Catania, Italy.

Isolation of Pigments

Violet bracts of *B. glabra* (1 kg) were homogenized in a blendor under ice water (1 l.) and extracted for 12 hr at 5°. The aqueous extract was filtered and the solid residue re-extracted with ice water (1 l.). The combined extracts were adjusted to pH 3 (1 N HCl) and centrifuged. The supernatant was percolated through a column of Dowex 50W-X2 (H^+, 40 × 3·5 cm) at 5°. The column was washed with 0·1% HCl and eluted with water. The eluant was concentrated *in vacuo* (30°) to about 10 ml and chromatographed on a column of polyamide powder (40 × 3·5 cm) with 5% citric acid in 15% (0·5 l.), 30% (0·5 l.), 50% (1 l.) and 70% (1 l.) aq. methanol.

Seven of the nine fractions (A–I) thus obtained were homogeneous in paper electrophoresis. Two pigments (D1 and D2) were isolated from fraction D by preparative paper electrophoresis and two (H1 and H2) from fraction H.

Deacylated Pigments

The crude betacyanin fraction isolated by chromatography on strongly acid ion-exchange resin from a 1 kg batch of bracts of *B. glabra* was dissolved in deoxygenated water (25 ml) and treated with 2 N NaOH (2·5 ml). The reaction mixture was allowed to stand in N_2 for 3 hr at room temp. and then acidified with 2 N HCl. The resulting acidic solution was chromatographed on a polyamide column (40 × 3·5 cm) using 5% aq. citric acid as the eluant. Two fractions (I and II) were collected, which emerged from the column after 2·1 l. and 3·0 l. These fractions, freed from citric acid by resin treatment, gave on evaporation to dryness 9 and 32 mg of product, respectively. An aliquot of fraction I was used for the isolation of DP1 and DP2 by high-voltage electrophoresis (pyridine formate 0·05 M, pH 4·5; potential gradient: 75 V/cm). By the same procedure, samples of DP3 and DP4 were obtained from an aliquot of fraction II.

Methods of Structural Determination of Pigments

Previously described methods[3] were used throughout this work.

[3] L. MINALE, M. PIATTELLI, S. DE STEFANO and R. A. NICOLAUS, *Phytochem.* 5, 1037 (1966).