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BETACYANINS FROM *BOUGAINVILLEA**†

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Abstract—The isolation of seven new betacyanins from purple bracts of *Bougainvillea* "Mrs. Butt" is described. Two of these pigments, bougainvillein-r-I and isobougainvillein-r-I, have been shown to be 5-*O*-β-sophorosides of betanidin and isobetanidin, respectively, the remaining five being hydroxycinnamoyl derivatives of bougainvillein-r-I and isobougainvillein-r-I.

INTRODUCTION

THE GENUS *Bougainvillea* (Nyctaginaceae) contains about fourteen shrubby species indigenous to South America with inconspicuous flowers enclosed by showy bracts whose colour ranges from white, yellow, orange, various shades of red to purple and violet.

Previous work relating to the pigments of members of the genus *Bougainvillea* is scanty. During a survey for anthocyanins, Robinson and Robinson[1, 2] recorded the presence in the bracts of *B. glabra* of a pigment (bougainvillein) resembling betanin. No conclusive results were obtained in a chemical study of this pigment.[3] By the use of paper electrophoresis and chromatography, Wyler and Dreiding[4] later found two betacyanins in *B. spectabilis* and four in *B. glabra*. Acid hydrolysis of one of these pigments gave a crystalline mixture of the diastereoisomeric aglycones, betanidin and isobetanidin. More recently, Piattelli and Minale[5] using chromatography on polyamide have demonstrated the occurrence of five betacyanins in *B. fastuosa* and up to sixteen pigments in *B. glabra* var. *sanderiana*. Many of these compounds were subjected to acid hydrolysis and betanidin and/or isobetanidin identified in the hydrolysates. Further data on the chemistry of the betacyanins of the genus *Bougainvillea* have not been reported.

In the present investigation, purple bracts of a horticultural variety of *Bougainvillea* ("Mrs. Butt") were examined. Repeated chromatography and electrophoresis yielded, besides betanin and isobetanin, seven previously unreported pigments. These pigments (bougainvillein-r's) are red-violet in colour, and thus differ from the bougainvilleins present in *B. glabra* which are violet-red. Two of these compounds, bougainvillein-r-I and isobougainvillein-r-I, have been shown to be the 5-*O*-β-sophorosides of betanidin and isobetanidin, respectively. The other five pigments proved to be hydroxycinnamoyl derivatives of the former.

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* Part XI of the series "Pigments of Centrospermae", for Part X, see *Phytochem.* **8**, 1595 (1969).

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[1] G. M. ROBINSON and R. ROBINSON, *Biochem. J.* **26**, 1647 (1932).

[2] G. M. ROBINSON and R. ROBINSON, *Biochem. J.* **28**, 1712 (1934).

[3] J. R. PRICE and R. ROBINSON, *J. Chem. Soc.* 449 (1937).

[4] H. WYLER and A. S. DREIDING, *Experientia* **17**, 23 (1961).

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

METHODS AND RESULTS

Isolation

The total betacyanin fraction was isolated from purple bracts of *Bougainvillea* "Mrs. Butt" by chromatography on strongly acid ion-exchange resin. Further chromatography on polyamide powder and high-voltage electrophoresis were used to purify the individual pigments.

Bougainvillein-r-I and Isobougainvillein-r-I

Complete acid hydrolysis of bougainvillein-r-I ($E^{1\,\mathrm{cm}}_{1\%} = 630$ at 538 nm in water; E_b[*]'s: pH $2{\cdot}4 = 0{\cdot}98$, pH $4{\cdot}5 = 0{\cdot}97$) gave a mixture of the diastereoisomeric aglycones betanidin and isobetanidin, and isobougainvillein-r-I ($E^{1\,\mathrm{cm}}_{1\%} = 625$ at 538 nm in water; E_b's: pH $2{\cdot}4 = 0{\cdot}98$, pH $4{\cdot}5 = 0{\cdot}91$) only isobetanidin. The hydrolysates also contained a single sugar identified as glucose by paper chromatography (six solvents) and by the use of glucose oxidase.

Since either bougainvillein-r-I or isobougainvillein-r-I on treatment with dilute alkali at room temperature and in the absence of oxygen gave an equilibrium mixture of both pigments, it can be deduced that they are diastereoisomers. Furthermore, taking into account the result of the acid hydrolysis, bougainvillein-r-I is evidently a betanidin and isobougainvillein-r-I the corresponding isobetanidin derivative. Elucidation of the structure of these pigments was therefore carried out using a crystalline mixture of both since this can be more easily obtained than the individual compounds. The pigment mixture was neither hydrolysed by emulsin nor attacked by maltase.

Partial acid hydrolysis with 10 per cent acetic acid (3·5 hr under reflux) gave, along with a small amount of glucose, a sugar which on paper chromatography behaved as a disaccharide and was hydrolysed by β-glucosidase giving glucose. The disaccharide was isolated by chromatography on paper and treated with methyl iodide in dimethylformamide in the presence of silver oxide. The permethylated product subjected to acid hydrolysis gave 3,4,6-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose, identified by paper and thin-layer chromatography. From these results it can be inferred that the type of sugar–sugar linkage in the disaccharide is β-1 $\rightarrow$ 2. The disaccharide was identified as sophorose by direct comparison with authentic material by paper co-chromatography (five solvents). Furthermore, it gave the expected colours with appropriate sugar reagents[6] and could be separated by chromatography from all the other β-glucosylglucose.

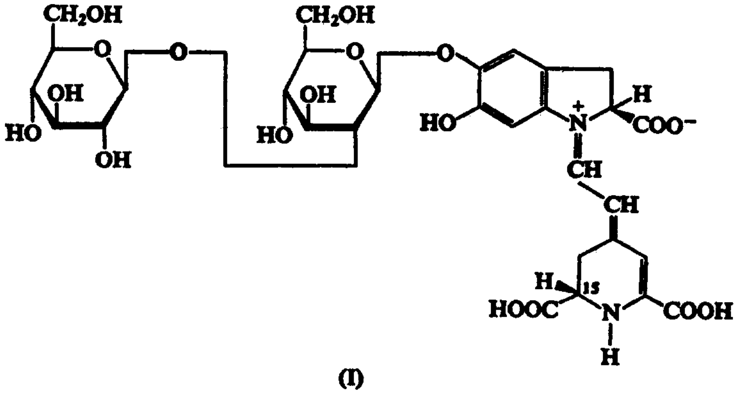
Diazomethane methylation of the mixture of the two diastereoisomeric pigments followed by alkali fusion gave 5-hydroxy-6-methoxyindole-2-carboxylic acid, thus showing that the sugar moiety is linked to the hydroxyl group at position 5 of the aglycones.

Since mild acid hydrolysis (1 N HCl; 10 min at 80°) of the diglucosides mixture gave, in addition to the products of total hydrolysis, small amounts of betanin and isobetanin the disaccharide–aglycone linkage is β.

All the above results are in complete agreement with structure I for bougainvillein-r-I, isobougainvillein-r-I differing only in the configuration at the C-15 carbon atom.

[*] E_b equals migration in paper electrophoresis relative to betanin.

[6] R. W. Bailey, *J. Chromatog.* **8**, 57 (1962).



(I)

Bougainvillein-r-II and Isobougainvillein-r-II

Bougainvillein-r-II (λ_{max} 280, 320 and 541 nm; E_b's: pH 2·4 = 0·40, pH 4·5 = 0·60) on total acid hydrolysis gave a mixture of betanidin and isobetanidin and isobougainvillein-r-II (λ_{max} 280, 320 and 541 nm; E_b's: pH 2·4 = 0·40, pH 4·5 = 0·41) only isobetanidin.

The two pigments could be equilibrated by treatment with 5% citric acid (24 hr at room temp.) and therefore they are C-15 diastereoisomers. Upon alkaline hydrolysis both gave bougainvillein-r-I, isobougainvillein-r-I, *p*-coumaric acid and caffeic acid. Identification of deacilated pigments was based on analytical column chromatography and paper electrophoresis. The molar ratio of *p*-coumaric to caffeic acid was found to be 2:1 by quantitative TLC.

Lack of material prevented a more detailed investigation.

Bougainvillein-r-III, Bougainvillein-r-IV and Bougainvillein-r-V

Bougainvillein-r-III (λ_{max} 312 and 541 nm; E_b's: pH 2·4 = 0·40, pH 4·5 = 0·44), bougainvillein-r-IV (λ_{max} 319 and 542 nm; E_b's: pH 2·4 = 0·17, pH 4·5 = 0·16) and bougainvillein-r-V (λ_{max} 314 and 539 nm; E_b's: pH 2·4 = 0·18, pH 4·5 = 0·20) were obtained in very minute amounts. All three gave on alkaline hydrolysis *p*-coumaric acid and a mixture of bougainvillein-r-I and isobougainvillein-r-I, and on acid hydrolysis a mixture of betanidin and isobetanidin.

The amounts isolated were not sufficient for further studies.

EXPERIMENTAL

Plant Material

Several collections of bracts of *Bougainvillea* "Mrs. Butt" were made in 1968 and 1969 at different places in the Catania area. No qualitative difference in their betacyanin composition was observed.

Authentic Samples

Samples of betanin, isobetanin, betanidin and isobetanidin were available from an earlier study.[7] A sample of sophorose was kindly supplied by Dr. J. B. Harborne (University of Reading). Laminaribiose was synthesized according to Bächli and Percival.[8]

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

[8] P. BÄCHLI and E. G. V. PERCIVAL, *J. Chem. Soc.* 1243 (1952).

Isolation of Pigments

Plant material (2 kg) was homogenized in a blendor in ice water (4 l.) and extracted for 12 hr at 5°. The aqueous extract was filtered through cheese-cloth and the residue re-extracted with ice water (2 l.). The combined extracts at 5° were adjusted to pH 3 (1 N HCl) and centrifuged. The supernatant was percolated through a column of Dowex 50W-X2 (H^+ form) at 5°. The column was washed with 0·1% HCl and eluted with H_2O. The eluant was concentrated *in vacuo* to about 20 ml and chromatographed on a polyamide column (5°) with 10% Na acetate in 50% aq. MeOH. Five fractions (A–E) were obtained, which emerged from the column after 230, 450, 710, 2300 and 3000 ml, respectively. The resolved bands were desalted with resin.

Fraction A on chromatography on polyamide with 5% citric acid as the eluant gave two bands (*A*1 and *A*2), which were collected after 800 and 1600 ml, respectively. Band *A*1, freed from citric acid by resin treatment, on concentration and standing overnight at 4° gave a crystalline mixture of bougainvillein-r-I and isobougainvillein-r-I (42 mg).

An aliquot (5 mg) of this mixture was used for the isolation of the individual pigments by high-voltage electrophoresis (pyridine–formate, 0·05 M, pH 4·5; potential gradient: 75 V/cm). Band *A*2 was shown to be a mixture of betanin and isobetanin, identified by co-electrophoresis with authentic samples, analytical column chromatography on polyamide and hydrolysis with β-glucosidase. *Fraction B*, by preparative high-voltage electrophoresis gave two pigments (bougainvillein-r-II and isobougainvillein-r-II) which were purified by Et_2O precipitation from MeOH. *Fractions C, D* and *E*, homogeneous in high-voltage electrophoresis, were dissolved in MeOH and precipitated with Et_2O giving bougainvillein-r-III, bougainvillein-r-IV and bougainvillein-r-V, respectively.

Methods of Structural Determination of Pigments

Methods described in an earlier publication[7] were used. Identification of sugars, methylated sugars, hydroxycinnamic acids have also been described.[7]