

## ACYLATED BETACYANINS FROM *DROSANTHEMUM FLORIBUNDUM*\*

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**Key Word Index**—*Drosanthemum floribundum*; Aizoaceae; betacyanins; hydroxycinnamic acid derivatives.

**Abstract**—Four new acylated betacyanins have been isolated from flowers of *Drosanthemum floribundum* (Haw.) Schwant. These pigments were characterized as hydroxycinnamoyl derivatives of betanin and isobetanin.

### INTRODUCTION

SEVERAL acylated betacyanins have been recently isolated from plants of the order Centrospermae.<sup>1-6</sup> Although aliphatic acids (malonic, 3-hydroxy-3-methylglutaric and citric acids) were sometimes found as acyl constituents, hydroxycinnamic acids were more frequently present.

The present paper reports the isolation and characterization of four novel acylated betacyanins from flowers of *Drosanthemum floribundum* (Haw.) Schwant. (Aizoaceae).

### METHODS AND RESULTS

The total betacyanin fraction was isolated by chromatography on strongly acid exchange resin. Column chromatography on cellulose powder of this crude fraction yielded, in addition to betanin, isobetanin, betanidin and isobetanidin, two major red-violet bands (I and II). When subjected to high-voltage electrophoresis, these bands were each split into two pigments (Ia, Ib and IIa, IIb). The UV spectral characteristics of the isolated pigments indicated the presence of hydroxycinnamic acyl components, which was confirmed by degradation methods.

On alkaline hydrolysis Ia and Ib, which had virtually identical spectral properties, yielded the same products, i.e. caffeic and ferulic acids, and an equilibrium mixture of betanin and isobetanin. The molar ratio of caffeic to ferulic acid was determined by TLC and found to be 1:1. Due to the poor recovery of betanin-isobetanin, the ratio of deacylated betacyanin to caffeic acid to ferulic acid was estimated from the  $E_{UV}/E_{vis}$  ratio and found to be 1:1:1. On acid hydrolysis Ia gave a mixture of betanidin and isobetanidin, while Ib yielded isobetanidin only. From this it can be inferred<sup>7</sup> that Ia is a caffeoyl-feruloyl-betanin and Ib its epimer (caffeoyl-feruloyl-isobetanin).

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<sup>1</sup> MINALE, L., PIATTELLI, M., DE STEFANO, S. and NICOLAUS, R. A. (1966) *Phytochemistry* **5**, 1037.

<sup>2</sup> MINALE, L., PIATTELLI, M. and DE STEFANO, S. (1967) *Phytochemistry* **6**, 703.

<sup>3</sup> PIATTELLI, M. and IMPELLIZZERI, G. (1969) *Phytochemistry* **8**, 1595.

<sup>4</sup> PIATTELLI, M. and IMPERATO, F. (1970) *Phytochemistry* **9**, 455.

<sup>5</sup> PIATTELLI, M. and IMPERATO, F. (1970) *Phytochemistry* **9**, 2557.

<sup>6</sup> PIATTELLI, M. and IMPERATO, F. (1971) *Phytochemistry* **10**, 3133.

<sup>7</sup> PIATTELLI, M. and MINALE, L. (1964) *Phytochemistry* **3**, 307.

Also pigments IIa and IIb were found to be diastereoisomers since they had indistinguishable UV and visible spectra and gave rise on alkaline hydrolysis to the same products (betanin-isobetanin mixture and caffeic acid); on acid hydrolysis IIa yielded betanidin and isobetanidin, and IIb only isobetanidin. From this, and from the  $E_{UV}/E_{vis}$  ratio which suggested the presence of only one molecule of acid, they were characterized as caffeyl-betanin and caffeyl-isobetanin, respectively.

TABLE 1. PROPERTIES OF BETACYANINS FROM *Drosanthemum floribundum*

Pigment	$\lambda_{max}$ in water (nm)	$R_f (\times 100)$ in TLC*	Migration in paper electrophoresis relative to betanin	
			pH 2.4	pH 4.5
Ia	539, 318, 290	25	0.31	0.60
Ib	539, 318, 290	25	0.31	0.56
IIa	540, 321, 292	16	0.18	0.48
IIb	540, 321, 292	16	0.18	0.45

\* On cellulose using EtOH-phosphate buffer (0.2 M, pH 6.8) 1:9, as the solvent system.

Following diazomethane methylation and then alkaline fusion, all the pigments gave 5-hydroxy-6-methoxyindole-2-carboxylic acid, thus showing that the phenolic hydroxyl at position 6 of the aglycones was always free. Therefore, it is likely that in all the isolated betacyanin the acyl groups are linked to the sugar moiety.

#### EXPERIMENTAL

Flowers of *Drosanthemum floribundum* (1 kg) were extracted with aq. MeOH and the total betacyanin fraction isolated by chromatography on Dowex 50W X2. Column chromatography on cellulose powder (EtOH-0.2 M phosphate buffer pH 6.8, 1:9, v/v as the eluant) gave, besides two major red-violet bands (betanin-isobetanin and betanidin-isobetanidin), two slower migrating small bands (I and II, as a whole ca. 5% of the total betacyanin fraction) which were individually desalted by resin treatment and subjected to high-voltage paper electrophoresis (0.05 M pyridinium formate pH 4.5). Fraction I thus gave two pigments, Ia (12 mg) and Ib (5 mg), and fraction II was similarly resolved into IIa (7 mg) and IIb (4 mg).

Alkaline and acid hydrolyses, diazomethane methylation followed by alkali fusion and identification of degradation products were carried out according to the methods earlier reported.<sup>1,2</sup>