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TWO BETALAINS FROM *PORTULACA GRANDIFLORA*

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Key Word Index—*Portulaca grandiflora;* Portulacaceae; flowers betalain; betaxanthin; portulacaxanthin.

Abstract—We report here two new natural betaxanthins that occur in the flowers of *Portulaca grandiflora.* The first, portulacaxanthin II, was shown to be the tyrosine-immonium conjugate of betalamic acid and the second, portulacaxanthin III, the glycine-immonium conjugate of the same chromophore.

INTRODUCTION

The betalains [1, 2], once thought to be 'nitrogenous anthocyanins' [3], are pigments typical of the order Caryophyllales [4]. The betalains are also present in fungi of the order Agaricales, notably *Amanita muscaria* [5-8]. All betalain pigments, including the magenta betacyanins [9-11] and the yellow betaxanthins [12-16], contain the common chromophore betalamic acid. Betaxanthins result from the conjugation of betalamic acid with amino acids or amines. All protein amino acids and any of the 220 known non-protein amino acids found in plants can potentially give rise to yellow betaxanthin pigments [17]. Previously described betaxanthins include (the respective amino acid or amine moiety is given in parentheses) portulacaxanthin (hydroxyproline) [16], humilixanthin (hydroxynorvaline) [18], dopaxanthin (DOPA) [15], miraxanthin III (tyramine) and miraxanthin V (dopamine) [14]. We report here the identification of two new betaxanthin pigments from the petals of *Portulaca grandiflora,* for which we propose the trivial names portulacaxanthin II and portulacaxanthin III. These are the tyrosine- and glycine-imino conjugates of betalamic acid, respectively. This nomenclature follows that used by ref. [16] in describing the first yellow pigment observed in *P. grandiflora.*

RESULTS AND DISCUSSION

Portulaca grandiflora produces and accumulates betaxanthins and betacyanins in its stem and flower. A variety of phenotypes were observed with petal colour ranging from white to pale yellow, deep yellow, orange, red and magenta. Betalain analysis (HPLC) of *P. grandiflora* petal extracts revealed the presence of two major yellow pigments which could not be identified by comparison with any available reference material. Together, these two pigments constituted between 0 and 85% (w/w, Table 1) of the total petal pigment content depending upon the genotype. Visible wavelength absorption spectra (at pH 4.5, in PO_4^{3-} buffer) for the two compounds were typical of betaxanthin-type compounds. An alkali-induced shift in absorption maximum from λ_{max} 470-472 nm to λ_{max} 424 nm, indicated the presence of betalamic acid which is liberated upon hydrolysis of any betaxanthin. The two unknown compounds were tentatively identified as the tyrosine and glycine immonium conjugates of betalamic acid as determined by reference to HPLC R_f data for a range of semisynthetic betaxanthin standards [17]. We propose the trivial names of portulacaxanthin II and portulacaxanthin III for the tyrosine and glycine conjugants of betalamic acid, respectively.

Portulacaxanthin III was found in petals exhibiting the red, orange and yellow phenotypes, but was only poorly resolved during HPLC analysis, appearing as a shoulder on a second betaxanthin peak identified as vulgaxanthin I (R_t 8.6 min). Amino acid analysis of the co-chromatographing peaks demonstrated the presence of two amino acids, glutamine and glycine, in a ratio of 3-4:1. The collected dual peak was resolved into two broad peaks by further HPLC analysis using an acetic acid (100 mM) mobile phase applied isocratically at 1.0 ml min^{-1}. Amino acid analysis of the two resulting peaks confirmed the identity of vulgaxanthin I (glutamine) and portulacaxanthin III (glycine). The identity of portulacaxanthin III, (1), was further confirmed by comparison of its spectral properties and HPLC retention characteristics with that of a semi-synthetic standard. This pigment exhibits an absorption maximum at λ 470 nm (pH 4.5) and ε_{480} of 38 900.

The second new pigment, for which we propose the trivial name portulacaxanthin II (2), eluted with a retention time (R_t 13.8 min) corresponding to that of the tyrosine-imino conjugate of betalamic acid. This pigment

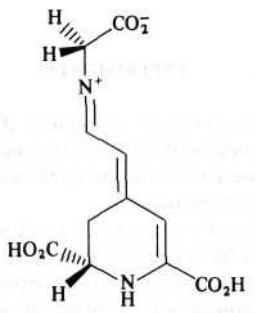

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Table 1. Betalain content of four *Portulaca grandiflora* phenotypes

Phenotype	VXI	PXIII	DX	PXII	dX	Betanin
Violet	—	—	0.15 ±0.08	0.36 ±0.20	0.08 + 0.03	17.8 ±3.00
Red	0.24 ±0.20	0.06 + 0.04	1.00 ±0.20	2.50 ±0.40	1.2 ±0.05	12.5 ±2.30
Pale yellow	0.08	0.01	0.19	1.70 ±0.40	—	—
Deep yellow	1.20 + 0.60	0.40 ±0.20	2.70 ±0.10	2.46 + 0.60	2.60 ±1.30	—

VXI; vulgaxanthin I (gin), PX III; portulacaxanthin HI (gly), DX; dopaxanthin (DOPA), PX II, portulacaxanthin II (tyr), dX; miraxanthin V (dopamine), values are expressed in mg g^{-1} petal dry weight.

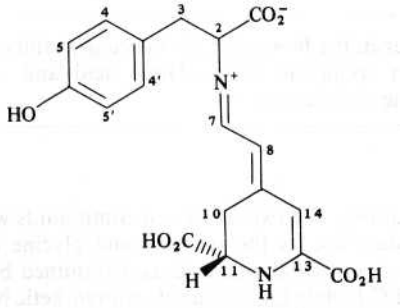

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Table 2. ^{1}HNMR spectral data for compound 2 (in CD_3OD)

H	2
2	4.45 *t*
3	3.15 *d*
4	6.75 *m*
4'	6.75 *m*
5	7.05 *m*
5'	7.05 *m*
7	7.60 *d* ($J_{7,8}$ = 12 Hz)
8	5.95 *d* ($J_{8,7}$ = 12 Hz)
10A	3.10 *m*
10B	2.90 *m*
11	4.30 *m*
14	6.10 *s*

was present in the petals of all P. *grandiflora* phenotypes analysed, except the white variety, and accounted for between 2 and 85% (w/w, Table 1) of the total pigment content depending upon the genotype examined.

Under standard HPLC conditions, portulacaxanthin II was well separated from the other betalain components of *P. grandiflora* petal extracts and fractions containing this compound were easily collected. Amino acid analysis of the hydrolysis products of portulacaxanthin II confirmed tyrosine to be the imino substituent of this betaxanthin. Incubation of the alkaline hydrolysis products of portulacaxanthin II in the presence of a 20-fold excess of proline at pH 6.0 followed by HPLC analysis revealed two peaks with R_ts analogous to those of free tyrosine (R_t 8.2 min) and the proline imino conjugate of betalamic acid, indicaxanthin (R_t 11.1 min). This experiment demonstrated without ambiguity that tyrosine is the imino substituent of portulacaxanthin II. Further evidence was provided by ^{1}HNMR spectroscopy (Table 2) which revealed signals typical of betalamic acid [19] and tyrosine, thereby confirming the molecular structure of portulacaxanthin II (2).

EXPERIMENTAL

Plant material. Portulaca grandiflora (Hook.) plants were chosen from commercially available seed stocks and maintained under greenhouse conditions. Selected phenotypes were propagated vegetatively *in vitro*.

Extraction of petal pigments. To avoid moisture loss, entire flower buds were harvested and their fr. wt determined. Petals were frozen in liq. N_2 and ground to a fine powder using a mortar and pestle. The powder was extracted, on ice, in 3 ml of soln A (MeOH-0.05 M HC1,4:1) and centrifuged at 20 000 g for 15 min at 4°. The pellet was re-extracted in a further 3 ml of soln A and centrifuged. Supernatant firs were pooled and dried under red. pres. Betalain extracts were resuspended in H_2O and stored at -20° prior to HPLC analysis.

Identification of new betalain pigments: A. Portulacaxanthin II: NMR analysis. 'HNMR was performed at room temp, with a 200 MHz spectrometer (solvent CD_3CD). The identity of portulacaxanthin II was confirmed by substitution of the amino acid moiety with proline and subsequent detection of the liberated tyrosine residue under the HPLC conditions described in ref. [17].

B. Portulacaxanthin II and III: HPLC analysis. Portulacaxanthin III and vulgaxanthin I co-chromatographed under the HPLC conditions described in ref. [17]. Complete separation was achieved by eluting pooled, concd frs corresponding to the joint portulacaxanthin III + vulgaxanthin I peak by replacing solvent A, in the HPLC protocol described in ref. [17], with solvent C (100 mM HOAc). The amino acid moieties of portulacaxanthin II, portulacaxanthin III and vulgaxanthin I were liberated by acid hydrolysis and identified by HPLC of their phenylisocyanate derivatives [20]. The natural compounds co-migrated with standards prepd by partial synthesis as in ref. [17].

Quantification of betalain pigments. Quantification of betalain pigments was achieved by reference to external standards. Known amounts of betanin (ε_{536} = 56 600), indicaxanthin (ε_{480} = 50 000) and betalamic acid (ε_{424} = 25 000), as determined spectrophotometrically were used to establish regression functions with the respective peak surface area. For unknown pigments, their concentration was determined using the extinction coefficient for indicaxanthin as follows:

$$\text{Absorption at 480 nm} = \varepsilon_{480\text{indicaxanthin}} * [\text{conc}_{\text{indicaxanthin}}]$$

$$= \varepsilon_{\text{xanthin}} * \text{conc}_{\text{xanthin}}$$

Therefore the concentration of unknown betaxanthins is:

$$conc_{xanlhin} = conc_{indicajanthin} * \varepsilon_{480indicaxanthin}/e_x$$

where

$conc_{xanthin}$: concentration of the unknown betaxanthin

$\varepsilon_{xanthin}$: molar extinction coefficient of the desired betaxanthin

The molar extinction coefficients of miraxanthin V and dopaxanthin were unknown, but assumed to be equal to that of portulacaxanthin II [17].

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