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ANTHOCYANINS IN CHILEAN SPECIES OF *ALSTROEMERIA*

RIKKE NØRBAEK,* LARS P. CHRISTENSEN, GUSTAV BOJESEN† and KIRSTEN BRANDT

Research Group for Plant Breeding and Propagation, Department of Ornamentals, Research Center Årslev, Danish Institute of Plant and Soil Science, Kirstinebjergvej 10, DK-5792 Årslev, Denmark; †Department of Chemistry, Odense University, DK-5230 Odense M, Denmark

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Key Word Index—*Alstroemeria*; Alstroemeriaceae; flower pigments; acylated anthocyanins; monomalonates; HPLC; FAB-MS; chemotaxonomy.

Abstract—The tepals of 28 Chilean *Alstroemeria* species were investigated by HPLC for their content of anthocyanins. The investigation revealed the presence of at least seven anthocyanins of which six were identified. The anthocyanins were isolated from the cultivars 'Regina' and 'Cana' and identified as 6-hydroxydelphinidin 3-rutinoside, 6-hydroxycyanidin 3-rutinoside, delphinidin 3-rutinoside, cyanidin 3-rutinoside, delphinidin 3-malonylglucoside and cyanidin 3-malonylglucoside. The position of the malonyl group in the acylated anthocyanins could not be determined due to their instability and low amounts. Most of the *Alstroemeria* species investigated can be placed into three groups regarding their contents of major pigments. The use of anthocyanins as taxonomic markers in *Alstroemeria* is discussed.

INTRODUCTION

The flowers of *Alstroemeria* (Alstroemeriaceae) vary in colour from yellow, orange, pink through scarlet to purple and violet. Only a few reports on the flower pigments from *Alstroemeria* have occurred, which include the investigation of 16 cultivars and a few species [1, 2]. The anthocyanins so far isolated from *Alstroemeria* are the 3-rutinosides of 6-hydroxydelphinidin, 6-hydroxycyanidin, delphinidin and cyanidin and the 3-glucoside of 6-hydroxycyanidin [1, 2]. The presence of a further cyanidin glycoside has also been described but its structure could not be determined [2]. Acylated anthocyanins have not been detected earlier in *Alstroemeria*, although acylated anthocyanins seem to occur regularly in Liliaceae [3–5], a family closely related to Alstroemeriaceae. We have now investigated 28 Chilean *Alstroemeria* species for flower colour pigments in order to determine their distribution and use as taxonomic markers.

RESULTS AND DISCUSSION

The tepals of 28 *Alstroemeria* species were extracted with a mixture of MeOH–HCO_2H–H_2O (10:3:3) and the extracts investigated by analytical HPLC. Seven anthocyanins (**1–7**) were detected and their distribution in the *Alstroemeria* species are shown in Table 1. The pigments were isolated from the cultivars 'Regina' and 'Cana' by semi-preparative HPLC (Experimental). The major anthocyanins with retention times at 14.8, 15.1 and 16.4 min on HPLC (Table 2) were identified as 6-hydroxycyanidin 3-rutinoside (**2**), delphinidin 3-rutinoside (**3**) and cyanidin 3-rutinoside (**4**), respectively, by UV (Table 2), FAB-MS and partial acid hydrolysis (Experimental). The structures were also confirmed by analyses of their ^1H NMR spectra, which were found to be in accordance with literature values [1, 2]. The pigment with a retention time at 14.5 min on HPLC showed λ_{max} at 280 and 525 nm indicating that it was 6-hydroxydelphinidin 3-rutinoside (**1**) [2]. The structure was confirmed by its FAB-mass spectrum which gave a molecular ion peak at *m/z*: 627 ($C_{27}H_{31}O_{17}^{+}$) with fragments corresponding to 6-hydroxydelphinidin 3-glucoside (*m/z*: 481) and 6-hydroxydelphinidin (*m/z*: 319). Pigments **6** and **7** had retention times on HPLC that were longer than found for simple anthocyanidin glycosides and rutinosides, indicating that these anthocyanins were acylated [6–8]. The characterization of malonic acid as the only acylating group in **6** and **7** was based on FAB-MS, whereby **6** and **7** were established as monomalonates. Partial acid hydrolysis of pigment **7** gave rise to one intermediate, cyanidin 3-glucoside, indicating that **7** is cyanidin 3-malonylglucoside. The structure of **7** was confirmed by HPLC co-chromatography with authentic material, UV (Table 2) and FAB-MS, which showed a molecular ion at *m/z*: 535 ($C H O^{+}$) and fragment

Table 1. Distribution of anthocyanins in Chilean species of *Alstroemeria*

Species* and cultivars	**1**	**2**	**3**	**4**	**5**	**6**	**7**	C_T†
Alstroemeria angustifolia Herbert‡		++		+++		±	±	20
A. aurea Graham‡		++		+++				30
A. exserens Meyen‡		+		+++	±		±	50
A. garaventae Bayer		++		+++				30
A. ligtu L.‡		+++		++				40
A. ligtu L. ssp. *simsii* Bayer‡		++		+++				30
A. pallida Graham‡		++		+++	±		±	20
A. presliana Herbert‡		+++	±	++	±	±	±	50
A. pseudospathulata Bayer		++		+++				10
A. versicolor Ruiz et Pavon		+	±	+++		±		150
A. diluta Bayer		±		–			+++	10
A. diluta Bayer ssp. *chrysantha* Bayer		±		+++			+	10
A. hookeri Loddiges		±	±	+++			++	110
A. hookeri Loddiges ssp. *recumbens* Bayer		±	±	++			+++	50
A. leporina Bayer et Grau‡		±		++	±		+++	50
A. pelegrina L.		±	±	+			+++	110
A. magenta Bayer	±		+++	+		++	++	200
A. philippii Baker‡	±		+++	+		++	+	50
A. pulchra Sims‡	±		+	+++		+	++	110
A. pulchra Sims ssp. *maxima* Philippi	±		+	+++		++	+	140
A. werdermannii Bayer‡	+		+++	+++		++	++	60
A. paupercula Philippi			+++	+++		++	++	10
A. polyphylla Philippi‡			+	++		+++	+	20
A. revoluta Ruiz et Pavon‡	±		++	+++		+	+	60
A. presliana Herbert ssp. *australis* Bayer‡		+++		++	+		+	80
A. hookeri Loddiges ssp. *cummingiana* Bayer‡		±		+++	+		+	30
A. magnifica Herbert ssp. *maxima* Bayer			±	+++	+		++	240
A. schizanthoids Grau			±	±		+++	±	10
Regina	+	±	+++	++	±	+	+	300
Cana		+++	±	+++	+	±	+	350

Key: **1**, 6-hydroxydelphinidin 3-rutinoside; **2**, 6-hydroxycyanidin 3-rutinoside; **3**, delphinidin 3-rutinoside; **4**, cyanidin 3-rutinoside; **5**, acylated 6-hydroxycyanidin 3-rutinoside; **6**, delphinidin 3-malonylglucoside; **7**, cyanidin 3-malonylglucoside. Rating of anthocyanins in relative concentration on HPLC. +++: high, ++: intermediate, +: low, ±: trace. *Author names in accordance with Bayer [15]. †C_T: Approximate total anthocyanin concentration in μM. C_T was calculated from the HPLC chromatograms by using the extinction coefficient of cyanidin 3-glucoside (log $\varepsilon = 4.47$) [17]. ‡A large genotypic variation in relative amounts of the major anthocyanins was observed. The relative concentration given are those most frequently found.

Table 2. Analytical HPLC retention times and spectral data of anthocyanins found in *Alstroemeria*

		In 0.1% HCl–MeOH		
Pigment	R_t^* (min)	λ_{max}	(nm)	$AlCl_3$ shift
6-Hydroxydelphinidin 3-rutinoside (**1**)	14.5	280	525	+
6-Hydroxycyanidin 3-rutinoside (**2**)	14.8	282	515	+
Delphinidin 3-rutinoside (**3**)	15.1	277	542	+
Cyanidin 3-rutinoside (**4**)	16.4	281	531	+
Acylated 6-hydroxycyanidin 3-Rutinoside (**5**)†	17.6	282	515	+

delphinidin [2]. This was supported by the results of partial acid hydrolysis, which gave delphinidin and the intermediate, delphinidin 3-glucoside. The FAB-MS of **6** gave a molecular ion peak at *m/z*: 551 ($C_{24}H_{23}O_{15}^{+}$) and fragments corresponding to delphinidin 3-glucoside (*m/z*: 465) and delphinidin (*m/z*: 303), which clearly established **6** as delphinidin 3-malonylglucoside. Furthermore, small amounts of a very unstable pigment (**5**), which appeared at 17.6 min on HPLC was also isolated. The compound decomposed easily to 6-hydroxycyanidin 3-rutinoside, indicating that the pigment was acylated. However, it was not possible to determine the acylating group.

The position of malonic acid in pigment **6** and **7** remain to be determined. However, almost all anthocyanins isolated with a monomalonated 3-glucoside have been 6″-malonates [4, 8–14], which could indicate that the malonoyl group in **6** and **7** are in the 6″-position. A more detailed structure determination was, however, not possible due to the small amounts available for analysis and the instability of these pigments, even though HCl was omitted from all solvents. Malonated anthocyanins, when extracted by standard procedures are demalonated within a short time [4, 7], which may explain why this is the first report of malonated anthocyanins in *Alstroemeria*. Cyanidin 3-malonylglucoside (**7**) occurs regularly in the Compositae [4, 7, 9, 10, 13] and delphinidin 3-malonylglucoside (**6**) has previously been isolated from *Hibiscus syriacus* [11], *Verbena hybrida* [12] and from *Lupinus* hybrids [14].

The *Alstroemeria* species given in Table 1, except for a few species, can be placed into three groups regarding their contents of major anthocyanins, even though a large genotypic variation in the relative amounts was observed within several species. The first group contains 6-hydroxycyanidin 3-rutinoside and cyanidin 3-rutinoside. The second group is characterized by having cyanidin 3-rutinoside and cyanidin 3-malonylglucoside as major anthocyanins. Anthocyanins in the last group are the 3-rutinosides and 3-malonylglucosides of delphinidin and cyanidin. Within a group, species are distinguished by large differences in the total anthocyanin concentration (Table 1).

Anthocyanins may be used for taxonomic purpose in *Alstroemeria*. Species belonging to the first group seem to be related as they contain 6-hydroxycyanidin 3-rutinoside as a major anthocyanin. The presence of cyanidin 3-rutinoside in this group seem to be of less importance from a chemotaxonomic point of view as this pigment is widely distributed in *Alstroemeria* (Table 1 and refs [1, 2]). The presence of cyanidin 3-malonylglucoside as one of the major anthocyanins in the second group indicates a relationship between species of this group, because the ability to produce acylated anthocyanins is limited to 18 of the 28 species investigated (Table 1). The *Alstroemeria* species in the due to the presence of 6-hydroxycyanidin 3-rutinoside as one of the major anthocyanins. The anthocyanin composition of *A. magnifica* ssp. *maxima* and *A. hookeri* ssp. *cummingiana* are to some extent similar to that found in species of the second group, whereas *A. schizanthoides* is different from all the other species investigated, because it only contains delphinidin 3-malonylglucoside as a major anthocyanin (Table 1). The cultivars are characterized by a combination of many compounds, and differ from most species; this is probably because they are of hybrid origin.

EXPERIMENTAL

Plant material. *Alstroemeria* species were produced from seeds in a greenhouse and identified according to Bayer [15]. Voucher specimens are retained at the Department of Ornamentals, Research Center Årslev, Danish Institute of Plant and Soil Science. Tepals from *Alstroemeria* species were collected from January to December 1994 and tepals from ‘Regina’ and ‘Cana’ were harvested in April 1995. The fresh tepals were frozen (−20°) and analysed within one week.

Isolation and quantification by HPLC. Anthocyanins were isolated from the cultivars ‘Regina’ and ‘Cana’ because of the large supplies of these plant tissues. Frozen tepals of ‘Regina’ (ca 500 g) and ‘Cana’ (ca 600 g) were extracted with a mixt. of MeOH–HOAc–H_2O (7 : 1 : 6) at room temp for 24 hr. The extracts were filtered and centrifuged at room temp and concd to small vols. Pigments were isolated by semi-prep. HPLC [LiChrospher 100 RP-18 (10 μm; 10 mm × 25 cm)] at 35° using a gradient solvent system consisting of solvent A (H_2O–HOAc, 49 : 1) and solvent B (MeOH–H_2O, 1 : 1). The elution profile was as follows: 0 min 0% B, 3 min 15% B, 8 min 37% B, 22 min 97% B, 25–45 min 100% B, flow rate: 3 ml min^{-1}; injection volume: 500 μl; detection: UV 535 and 360 nm. The purified pigments were stored at 0° as solids, after evaporation to dryness.

The pigment concentrations in *Alstroemeria* species (Table 1) were determined by analytical HPLC. Frozen tepals were ground in 4 ml MeOH–HCO_2H–H_2O (10 : 3 : 3) per g fr. wt with an Ultra-Turrax T25. After centrifugation the extracts were separated by HPLC [LiChrospher 100 RP-18 (5 μm, 4.0 mm × 11.9 cm)] at 35° using a gradient solvent system consisting of solvent A (H_2O–HCO_2H, 19 : 1) and solvent B (MeOH–H_2O–HOAc, 10 : 7 : 3). The elution profile was as follows: 0 min 0% B, 3 min 10% B, 8 min 25% B, 22 min 60% B, 25–45 min 100% B; flow rate: 1 ml min^{-1}; detection: UV 535 nm; injection volume: 10 μl.

FAB-MS. FAB spectra were recorded on a Kratos MS 50 RF double focusing mass spectrometer at 8 kV accelerating voltage and a resolution of 1200. The samples were dissolved in mixtures of MeOH and HCO_2H with subsequent addition of 1 : 1 mixt. of sion of air into the first field free region at a pressure where a 50% attenuation of the main beam was observed. Data collection and treatment was done through a Kratos Mach3 data system and data reported represented the major ions attributed to the sample, ions from the matrix have been omitted. Calibrations were done with CsI. FAB-MS (**2**) m/z: 611 $[M]^+$, 465 $[M - \text{rhamnose}]^+$, 303 $[\text{6-hydroxyCy}]^+$; FAB-MS (**3**): m/z: 611 $[M]^+$, 465 $[M - \text{rhamnose}]^+$, 303 $[\text{Dp}]^+$; FAB-MS (**4**) m/z: 595 $[M]^+$, 449 $[M - \text{rhamnose}]^+$, 287 $[\text{Cy}]^+$. For FAB-mass spectra of pigment **1**, **6** and **7** see text.

Partial acid hydrolysis. Hydrolysis of anthocyanins were made in 1 M HCl at 100°. At suitable time intervals (2, 5, 10, 20, 40 and 60 min) samples were taken out, cooled in an icebath and the hydrolysates analysed by HPLC. Pigments **3**, **4**, **7** and their hydrolysates were identified by HPLC co-chromatography with authentic material isolated from *Ribes nigrum* [16] and *Dendranthema* cultivars [10]. The hydrolysates of **5** and **6** were identified by comparison with the hydrolysates obtained from **2** and **3**, respectively.

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