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6-HYDROXYANTHOCYANIDIN GLYCOSIDES IN THE FLOWERS OF *ALSTROEMERIA*

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Key Word Index—*Alstroemeria*, Alstroemeriaceae, flower colour, anthocyanins, 6-hydroxycyanidin 3-rutinoside, 6-hydroxydelphinidin 3-rutinoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside.

Abstract—Two 6-hydroxyanthocyanidin glycosides and two common anthocyanidin glycosides are present in the coloured petals of *Alstroemeria* species and cultivars 6-Hydroxycyanidin 3-rutinoside is particularly abundant in the orange, red and pink flowers of *A aurantiaca*, *A pulchellia*, *A ligtu* var *angustifolia* × *A haemantha* and also 11 cultivars. A novel anthocyanidin glycoside, 6-hydroxydelphinidin 3-rutinoside, occurs only in the pink-purple flowers of five cultivars. Cyanidin 3-rutinoside was observed in all coloured flowers of *Alstroemeria*; delphinidin 3-rutinoside was restricted to pink and purple flowers of 10 cultivars, and also in one species, *A. violacea*

INTRODUCTION

Recently we found a new anthocyanidin, 6-hydroxycyanidin, in the red flowers of *Alstroemeria* cultivars [1]. For further study of anthocyanin occurrence in *Alstroemeria*, four species, one specific hybrid and 16 cultivars were analysed. As a result another novel anthocyanidin, 6-hydroxydelphinidin, was found to co-occur with 6-hydroxycyanidin, delphinidin and cyanidin glycosides

RESULTS AND DISCUSSION

The major anthocyanins of the plants investigated (Table 1) are the 3-rutinosides of cyanidin, delphinidin and 6-hydroxycyanidin A small amount of a further cyanidin glycoside was also observed but its structure could not be determined (Table 1) Another novel 6-hydroxyanthocyanidin glycoside was found in the pink purple flowers of five cultivars 'Jubilee', 'Patricia', 'Rosello', 'Rita', and 'Zeranon', as a minor component and was identified as 6-hydroxydelphinidin 3-rutinoside

The new pigment, after purification by TLC (solvents, BAW and Bu–HCl), gave a small shift on the addition of aluminium trichloride showing the presence of free *ortho*-dihydroxyls in the B-ring (see Table 2) On acid hydrolysis, it gave an aglycone, glucose and rhamnose Partial acid hydrolysis gave rise to one intermediate, 6-hydroxydelphinidin 3-glucoside. Rutinose was obtained by the hydrogen peroxide degradation of this pigment From these data this pigment was determined to be a 3-rutinoside of a new anthocyanidin From the R_f data of PC and TLC, this new anthocyanidin appeared to contain seven hydroxyl groups The λ_{max} of this aglycone were 280 and 530 nm in 0 1% HCl–MeOH, the latter wavelength being 17 nm shorter than that of delphinidin (547 nm). A similar hypsochromic shift of λ_{max} was also observed between 6-hydroxycyanidin and cyanidin ($\Delta\lambda = -18$ nm, as shown in Table 2) [1–3] From these data, this aglycone was presumed to be 6-hydroxydelphinidin ^{1}H NMR (Table 3) and mass spectroscopic measurements for the pigment and related anthocyanins were fully in agreement with this structural assignment. Furthermore, FAB-MS established the molecular ion peak at *m/z* 627, supporting the molecular formula as $C_{27}H_{31}O_{17}^+$ This is the first report of a 6-hydroxydelphinidin glycoside occurring in the plant world.

The other three anthocyanins, cyanidin 3-rutinoside, delphinidin 3-rutinoside and 6-hydroxycyanidin 3-rutinoside, were isolated and identified by standard procedures (Table 1) [1, 4, 5]. These structures were confirmed by analyses of ^{1}H NMR and FAB mass spectra (Table 3)

The 16 cultivars of *Alstroemeria* can be grouped into three, according to the anthocyanin types present The first group is composed of cyanidin and 6-hydroxycyanidin. Anthocyanidins of the second group are cyanidin and delphinidin, and the colour of these plants are crimson pink The last group has four anthocyanidins, cyanidin, delphinidin, 6-hydroxycyanidin and 6-hydroxydelphinidin, and the colour of this group is pink-purple

EXPERIMENTAL

Fresh petals of four species of *Alstroemeria*, *A violacea*, *A pelegrina rosea*, *A. aurantiaca*, *A. pulchellia*, and one specific hybrid (*ligtu* hybrid; *A. ligtu var angustifolia* × *A haemantha*) were collected from Miyake Nursery, Mobara, Chiba in May Also fresh petals of 16 cultivars of *Alstroemeria* (Table 1) were collected from Fukkaen Seeds Co., Mie, and Seiwa Nursery, Mobara, Chiba These petals were extracted with 0 1% HCl–MeOH at room temp for 20 hr The extract was concd to small vols The concd extract was separated by TLC (cellulose, *n*-BuOH–AcOH–H_2O, 4 1·5, AcOH–HCl–H_2O, 15 3 82), and

Table 1 Distribution of anthocyanins in flower extracts of *Alstroemeria*

Species and cultivars	Dp	6HDp	Cy	6HCy	Cyx	Flower colour
Alstroemeria violacea	+		+			bright lilac
A pelegrina rosea			+ +		+	deeper red
A aurantiaca			+ +	+ +		golden-yellow
A pulchellia			+ +	+ +		dark red
A ligut var angustifolia × *A haemantha* (ligtu hybrids)			+	+		pale pink
Jubilee	+ + +	+ +	+ + +	+	±	pink-purple
Patricia	+ +	+ +	+ +	+ +	±	
Rosello	+ +	+ +	+ + +	+ +	±	
Rita	+ +	+	+ + +	+ +	±	
Zeranon	+ + +	+ +	+ + +	+ +	±	
Luciana	+ + +		+ + +		+ +	crimson-pink
Pascal	+ + +		+ + +		+	
Tango	+ + +		+ + +		+ +	
Regina	+ + +		+ + +		±	
Mona Lisa	+ +		+ + +		±	ivory
King Cardinal			+ +	+ + +	±	orange-red
Apple Blossom			+ + +	+ + +	±	
Atlas			+ + +	+ + +	±	
Rosita			+ +	+ + +	±	
Campfire			+ + +	+ +	+	
Yellow King			+ + +	+ + +		ivory

Key Dp delphinidin 3-rutinoside, 6HDp 6-hydroxydelphinidin 3-rutinoside, Cy cyanidin 3-rutinoside, 6HCy 6-hydroxycyanidin 3-rutinoside, Cyx cyanidin glycoside Visual rating of anthocyanins in concentration on TLC + + + high, + + intermediate, + low, ± trace.

Table 2 R_f values, spectral properties and colour of 6-hydroxyanthocyanidin glycosides and related pigments

		R_f values (× 100)*						Spectral data in		
Pigment	Visible colour	Forestal	Formic	BAW	BuHCl	1% HCl	AcOHCl	MeOH–HCl	$AlCl_3$	E_{440}/E_{max}
Cyanidin	magenta	43	18	47				273 536	+	20
6-Hydroxycyanidin	red	25	11	28				282 518	+	22
Delphinidin	purple	24	10	29				277 547	+	18
6-Hydroxydelphinidin	pink-purple	11	5	10				280 530	+	17
6-Hydroxydelphinidin 3-rutinoside	pink-purple			3	4	4	19	280 525	+	16
Delphinidin 3-rutinoside	purple			20	23	11	35	277 542	+	16
6-Hydroxycyanidin 3-rutinoside	red			15	16	11	35	283 514	+	20
Cyanidin 3-rutinoside	magenta			28	37	17	45	281 530	+	20

*Solvent key Forestal, acetic acid–HCl–H_2O (30 3 10), Formic, formic acid–HCl–H_2O (5 2 3), BAW, *n*-butanol–acetic acid–H_2O (4 1 5), BuHCl, *n*-butanol–2N HCl (1 1), AcOHCl, acetic acid–HCl–H_2O (15 3 82)

10 mg of 6-hydroxydelphinidin 3-runtinoside was obtained from the extracts of a pink-purple cultivar 'Jubilee' (fresh petals 1 0 kg) 20 mg of cyanidin 3-rutinoside, 20 mg of delphinidin 3-rutinoside and small amounts of 6-hydroxycyanidin 3-rutinoside were obtained from the combined extracts of 16 cultivars (fresh petals *ca* 500 g)

For the further study of the native pigments of *Alstroemeria*, the pigments were extracted with the methanol–acetic acid–water solvent in accordance with the procedure of ref [6] However, we could not observe any acylated anthocyanins in the extracts Therefore, the native pigments are identical with the ones extracted with the methanolic HCl solvent

The absorption spectra and R_f values of these anthocyanins were compared by standard methods with authentic specimens of cyanidin 3-rutinoside, delphinidin 3-rutinoside and 6-hydroxycyanidin 3-rutinoside [2, 5]

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Table 3 ^{1}H NMR and FAB-MS analyses of *Alstroemeria* anthocyanins using DMSO-d_6 with DCl (chemical shifts in ppm from TMS)

	Delphinidin 3-rutinoside	6-Hydroxydelphinidin 3-rutinoside	Cyanidin 3-rutinoside	6-Hydroxycyanidin 3-rutinoside*
H-4	8.72 (*br s*)	8 85 (*br s*)	8 81 (*br s*)	8 94 (*br s*)
H-6	6 78 ($J=2$)	—	6 79 ($J=2$)	—
H-8	6 86 ($J=2$)	7.19 (*br s*)	6 90 ($J=2$)	7 19 (*br s*)
H-2′	7 73 † (*br s*)	7 71† (*br s*)	7 98 ($J=2$)	8 04 ($J=2$)
H-5′	—	—	7 02 ($J=8$ 5)	7 04 ($J=8.5$)
H-6′	—	—	8 22 ($J=2, 8.5$)	8 20 ($J=2, 8$ 5)
Anomeric H glucose	5 36 ($J=7$ 5)	5 39 ($J=7.5$)	5 34 ($J=7$ 5)	5 39 ($J=7$ 5)
Anomeric H rhamnose	4 46 (*br s*)	4 51 (*br s*)	4 50 (*br s*)	4 69 (*br s*)
Rhamnose Me	1 20 ($J=6.5$)	1 16 ($J=6$ 5)	1 25 ($J=6$ 5)	1 17 ($J=6$ 5)
FABMS [M^+] (*m/z*)	611	627	596	611

*N Saito *et al* (1985) *Phytochemistry* **24**, 2125
†H-2′ and 6′

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