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ANTHOCYANIDIN GLYCOSIDES FROM THE FLOWERS OF *ALSTROEMERIA*

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Key Word Index—*Alstroemeria*; Alstroemeriaceae; 6-hydroxycyanidin; 6-hydroxycyanidin 3-rutinoside; 6-hydroxycyanidin 3-glucoside; cyanidin 3-rutinoside.

Abstract—Three anthocyanins were isolated from the red flowers of four cultivars of *Alstroemeria*. Two compounds were novel anthocyanidin glycosides; the 3-rutinoside and 3-monoglucoside of 6-hydroxycyanidin. Cyanidin 3-rutinoside was also present in the flower petals.

INTRODUCTION

The flowers of *Alstroemeria* species vary in colour from yellow, orange, red to purple. All the species are native to Chile, Peru and North Brazil, South America, and have recently been introduced into Europe as ornamental plants and hybridized in Europe.

Since no report on the flower pigments in the *Alstroemeria* species and cultivars has been found [1–3] and they have unusual orange or red flower colour, analyses of the flower colour pigments in the several species and cultivars of *Alstroemeria* flowers were undertaken to determine anthocyanin pigments in their flowers.

RESULTS AND DISCUSSION

6-Hydroxycyanidin 3-rutinoside (**1**) was isolated from red flowers of cultivars, 'Red Sunset', 'Red Sunrise' and 'King Cardinal' as a major pigment with 0.1% HCl–MeOH, and purified by TLC (solvents, BAW and Bu-HCl).

This pigment gave a small shift on the addition of $AlCl_3$ showing the presence of free *ortho*-dihydroxyls in the B-ring [1]. Compound 1 showed the λ_{max} at 515 and 282 nm in 0.1% HCl–MeOH, and the value of $E_{440}/E_{vis\ max}$ was 27%. R_f values and spectral data of this anthocyanin and the related pigments are shown in Table 1. On acid hydrolysis, this pigment gave 6-hydroxycyanidin, glucose and rhamnose. Partial acid hydrolysis gave rise to one intermediate, 6-hydroxycyanidin 3-glucoside. Moreover, rutinose was obtained by the H_2O_2 degradation of this pigment. The 100 MHz ^{1}H NMR spectrum [solvent: CD_3OD + DCl (one drop)] of **1** revealed signals at 8.94 at δ (*br s*, H-4), 8.20 (*d*, J = 2, 8.5 Hz, H-6′), 8.04 (*d*, J = 2 Hz, H-2′), 7.19 (*br s*, H-6 or 8), 7.04 (*d*, J = 8.5 Hz, H-5′), 5.39 (*br s*, Glc anomeric H), 4.69 (*br s*, Rha anomeric H), 1.17 (3H, *d*, J = 6 Hz, Rha Me), and 10 sugar protons. FAB mass spectrometry established the M_r as 611, m/z: $C_{27}H_{31}O_{16}{}^+$. These data indicate that this pigment is 6-hydroxycyanidin 3-rutinoside.

Also, 6-hydroxycyanidin 3-glucoside (**2**) was found in the red flowers of 'Red Sunset' and three other cultivars as a minor component. Compound **2** showed λ_{max} at 512 and 282 nm in 0.1% HCl–MeOH, and the value of $E_{440}/E_{vis\ max}$ was 26%. The 6-hydroxycyanidin skeleton was confirmed by the ^{1}H NMR spectrum.

The aglycone, 6-hydroxycyanidin, obtained from **1** by the complete hydrolysis with 2 N HCl for 1 hr exhibited a bathochromic shift on the addition of $AlCl_3$ (10% in EtOH). This indicates the presence of a dihydroxylic B-ring. It showed λ_{max} at 518 and 282 nm in 0.1% HCl–MeOH, and the value of $E_{440}/E_{vis\ max}$ was 22%. This aglycone showed a low R_f in comparison with those of cyanidin in the Forestal and Formic solvents, and was almost identical with delphinidin (Table 1). These data are identical to those of 6-hydroxycyanidin obtained synthetically by Harborne [1]. The 100 MHz ^{1}H NMR spectrum

Table 1. R_f values, spectral properties and colour of 6-hydroxycyanidin glycosides and related pigments

Visible pigment	Colour	R_f values (× 100)*						Spectral data in		
		Forestal	Formic	BAW	BuHCl	1%HCl	AcOHCl	MeOH–HCl	$AlCl_3$	E_{440}/E_{max}
6-Hydroxycyanidin	red	30	12	39				282, 518	+	22
	(red	30	12	39				283, 518	+	25)†
Cyanidin	magenta	49	22	68				273, 536	+	20
Delphinidin	purple	32	13	42				277, 547	+	18
1	red			16	19	7	24	282, 515	+	27
2	red			22	7	3	7	282, 512	+	26
Cyanidin 3-Rutinoside	magenta			42	43	15	35	281, 528	+	30

*Solvent key: Forestal, HOAc–HCl–H_2O (30:3:10); Formic, HCO_2H–HCl–H_2O (5:2:3); BAW, *n*-BuOH–HOAc–H_2O (4:1:5); Bu-HCl, *n*-BuOH–HCl–H_2O (7:2:5); AcOHCl, HOAc–HCl–H_2O (82:3:15).

†Data according to Harborne [1].

[solvent; $CD_3OD + DCl$ (one drop)] of this aglycone revealed signals at δ 8.61 (*br s*, H-4), 8.22 (*d*, $J = 2$, 8.5 Hz, H-6′), 8.04 (*d*, $J = 2$ Hz, H-2′), 7.08 (*br s*, H-6 or H-8), 7.00 (*d*, $J = 8.5$ Hz, H-5′). From these data it is not possible to distinguish between 6- and 8-hydroxylation. However, the visible spectral data for **1** are decisive in indicating 6-substitution [4]. Because of the extra hydroxyl substituent in the A-ring, the pigment is more unstable than cyanidin and this instability was also apparent *in vivo* in *Alstroemeria* flowers.

The only other natural occurrence of a 6-hydroxyanthocyanidin is of 6-hydroxypelargonidin in *Impatiens* flowers [1, 4, 5]. As in this case, the effect of 6-hydroxylation is to cause a hypsochromic shift in visible colour. A similar effect in the cyanidin series has been recently observed as a result of glycosylation of the B-ring hydroxyl groups [6].

A third pigment was isolated from *Alstroemeria* flowers and identified as cyanidin 3-rutinoside by standard procedures.

EXPERIMENTAL

Fresh petals of four cultivars of *Alstroemeria*, 'Red Sunset', 'Red Star' 'Red Sunrise' and 'King Cardinal' were collected from Fukkaen Seeds Co., Mie, and Seiwa Nursery, Mobara, Chiba in May, 1984. Fresh petals (30 g) were extracted with 0.1% HCl–MeOH at room temp for 20 hr. The extract was concd to 30 ml. The concd extract was separated by TLC (cellulose: *n*-BuOH–AcOH–H_2O, 4:1:5; AcOH–HCl–H_2O, 15:3:82); 20 mg of **1**, 5 mg of **2** and 5 mg of cyanidin 3-rutinoside were obtained from the extracts. The absorption spectra and R_f values of **1** and **2** were measured by standard methods (Table 1), and the letter pigment was identical with those of 6-hydroxycyanidin 3-glucoside which was obtained from the partial hydrolysis of **1**. Another minor pigment was identified as cyanidin 3-rutinoside by co-chromatography in several solvents with an authentic specimen [1], and its proton spectrum was examined by ^1H NMR measurements.

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