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TWO NOVEL 6-HYDROXYANTHOCYANINS IN THE FLOWERS OF *ALSTROEMERIA* 'WESTLAND'

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***Abstract*-----Two novel 6-hydroxydelphinidin glycosides were isolated from the red-purple flowers of *Alstroemeria* 'Westland' along with ten known anthocyanins. These novel pigments were determined to be 6-hydroxydelphinidin 3-*O*-β-D-glucoside and 6-hydroxydelphinidin 3-*O*-[6-*O*-(malonyl)-β-D-glucoside] by spectral and chemical methods. The ten known pigments were also identified as cyanidin 3-glucoside, delphinidin 3-glucoside, 6-hydroxycyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside, 6-hydroxycyanidin 3-rutinoside, 6-hydroxydelphinidin 3-rutinoside, cyanidin 3-malonylglucoside, delphinidin 3-malonylglucoside, and 6-hydroxycyanidin 3-malonylglucoside, respectively.**

In the investigation of floral anthocyanins in the flowers of *Alstroemeria* species and cultivars, five reports on the occurrence of ten anthcocyanins such as cyanidin 3-glucoside, delphinidin 3-glucoside, 6-hydroxycyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside, 6-hydroxycyanidin 3-rutinoside, 6-hydroxydelphinidin 3-rutinoside, cyanidin 3-malonylglucoside, delphinidin 3-malonylglucoside, and 6-hydroxycyanidin 3-malonylglucoside have been published by us[1,2,5] and others,[3,4] independently. As an extension of our work on the flower color variation of *Alstroemeria* cultivars, we have isolated two new anthocyanins from the red-purple flowers of *Alstroemeria* 'Westland' together with the above ten known anthocyanins. In the present paper, we wish to report the results of a structural study of these new anthocyanins.

The red-purple flowers of *Alstroemeria* 'Westland' were obtained at the flower market in Sapporo, and their color was measured to be Red-Purple 67B by R. H. S. color chart, and 0.01 (b*/a*) as their chromaticity value. The fresh flowers of this plant were dried overnight at 37℃ and kept in the refrigerator at 10 ℃. Twelve anthocyanins were observed in the MAW (MeOH/HOAc/H_2O, 4:1:5) extract by the analysis of HPLC, (Figure 1).

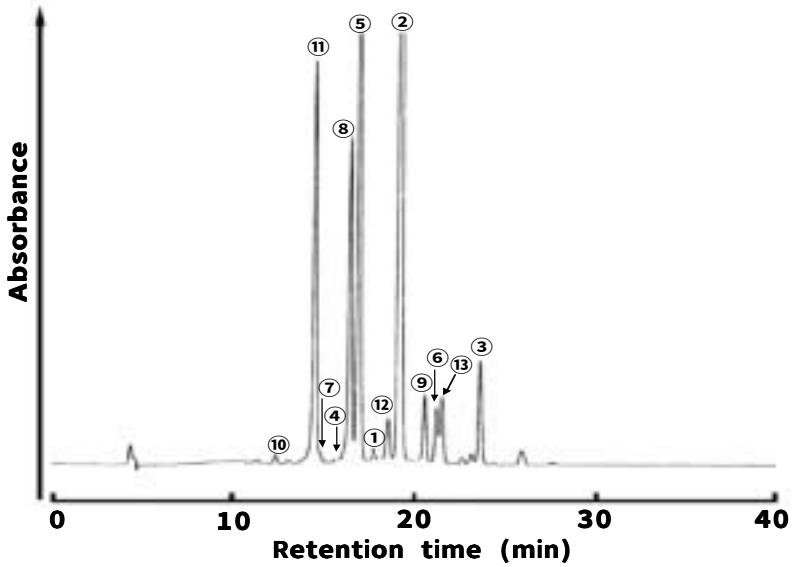

Figure 1. HPLC anthocyanin profile of the extract with MAW solvent from the flowers of *Alstroemeria* 'Westland'.

① cyanidin 3-glucoside
② cyanidin 3-rutinoside
③ cyanidin 3-malonylglucoside
④ delphinidin 3-glucoside
⑤ delphinidin 3-rutinoside
⑥ delphinidin 3-malonylglucoside
⑦ 6-hydroxycyanidin 3-glucoside
⑧ 6-hydroxycyanidin 3-rutinoside
⑨ 6-hydroxycyanidin 3-malonylglucoside
⑩ 6-hydroxydelphinidin 3-glucoside (pigment 2)
⑪ 6-hydroxydelphinidin 3-rutinoside
⑫ 6-hydroxydelphinidin 3-malonylglucoside (pigment 1)
⑬ unknown

The dried perianthes (*ca*. 150 g) were immersed in 10% HOAc-MeOH (1.5 L, HOAc-MeOH, 1:9) at room temperature for overnight. The 10% HOAc-MeOH extract was concentrated to *ca.* 50 mL, and purified

by Diaion HP-20 gel column chromatography, preparative TLC (BAW; *n*-BuOH : HOAc : H_2O = 4 : 1 : 2) and HPLC.[6] The pigment fractions were evaporated *in vacuo* to dryness. The residues were dissolved in a small amount of 10% HOAc-MeOH followed by addition of an excess Et_2O, and then dried to obtain twelve pigment powders. Finally, cyanidin 3-glucoside (*ca*. 1 mg), cyanidin 3-rutinoside (*ca*. 20 mg), cyanidin 3-malonylglucoside (*ca*. 2 mg), delphinidin 3-glucoside (*ca*. 1 mg), delphinidin 3-rutinoside (*ca*. 5 mg), delphinidin 3-malonylglucoside (*ca*. 2 mg), 6-hydroxycyanidin 3-glucoside (*ca.* 1 mg), 6-hydroxycyanidin 3-rutinoside (*ca.* 5 mg), 6-hydroxycyanidin 3-malonylglucoside (*ca.* 2 mg), 6-hydroxydelphinidin 3-rutinoside (*ca.* 5 mg), pigment (**1**) (*ca.* 2 mg) and pigment (**2**) (*ca.* 1 mg), were obtained, respectively (Table 1). Among these twelve isolated anthocyanins, ten pigments were known to be present in the flowers of *Alstroemeria*,[1-5] and determined based on their TLC, HPLC and spectroscopic properties, and also by direct comparison with the authentic samples (Table 1).

Table 1. Chromatographic and spectral properties of anthocyanins from flowers of *Alstroemeria* 'Westland'.

Anthocyanin	Visible	Rf values (x 100)*				Spectral data in 0.1% HCl-MeOH			HPLC		FAB-MS
	colour	BAW	BuHCl	1%HCl	AHW	λ_{max} (nm)	E_{440}/E_{max} (%)	$AlCl_3$	λ_{max} (nm)**	Rt (min)	$[M]^+$
1: cyanidin 3-glucoside	magenta	37	23	3	13	282,529	27	+	516	16.26	-
2: cyanidin 3-rutinoside	magenta	35	23	8	33	280,529	25	+	519	17.68	-
3: cyanidin 3-malonylglucoside	magenta	41	31	3	17	280,529	25	+	519	22.07	-
4: delphinidin 3-glucoside	purple	23	9	1	6	280,541	18	+	524	14.19	-
5: delphinidin 3-rutinoside	purple	26	14	5	12	277,543	18	+	526	15.18	-
6: delphinidin 3-malonylglucoside	purple	30	18	3	11	276,544	18	+	525	19.50	-
7: 6-hydroxycyanidin 3-glucoside	red	15	5	3	8	283,513	24	+	499	13.04	465
8: 6-hydroxycyanidin 3-rutinoside	red	20	8	5	19	284,514	23	+	500	14.73	611
9: 6-hydroxycyanidin 3-malonylglucoside	red	21	16	3	13	282,514	24	+	500	18.82	551
10: 6-hydroxydelphinidin 3-glucoside (pigment 2)	pink-purple	7	2	1	4	280,525	21	+	507	11.08	481
11: 6-hydroxydelphinidin 3-rutinoside	pink-purple	12	5	3	12	279,525	17	+	509	12.54	627
12: 6-hydroxydelphinidin 3-malonylglucoside (pigment 1)	pink-purple	15	9	2	9	275,526	21	+	509	16.78	567

* BAW; n-BuOH:HOAc:H_2O=4:1:2(v/v/v), BuHCl; *n*- BuOH:2N HCl=1:1(upper phase), 1% HCl; HCl:H_2O=3:97(v/v), AHW; HOAc:HCl:H2O=15:3:82(v/v/v).

** Visible max of anthocyanins was measured by PDA of HPLC.

The structure determination of the new pigments (**1**) and (**2**) was carried out as follows. By acid hydrolysis with 2N HCl at 100℃ for 2 h pigments (**1**) and (**2**) gave 6-hydroxydelphinidin[7] as their anthocyanidin, and glucose as sugar.

The FAB-MS measurement of pigment (**1**) gave a molecular ion $[M]^+$ at 567 *m/z* in agreement with the mass calculated for $C_{24}H_{23}O_{16}$ (*m/z* 567.098) indicating the presence of one molecule each of 6-

hydroxydelphidin, glucose, and malonic acid, respectively. The exact structure of pigment (**1**) was determined by the analysis of its ^{1}H NMR, ^{1}H-^{1}H COSY and negative NOE difference (DIFNOE) spectra (Table 2). The chemical shifts of four characteristic aromatic protons (H-4, 8, 2' and 6') of anthocyanidin were assigned as shown in Table 2. However, the signal of H-6 was not observed in the ^{1}H NMR spectrum. The signal of an anomeric proton of glucose appeared at δ 5.46 (*d*, *J*=8.0 Hz), and all the observed vicinal coupling constants of glucose were *J*=7-12 Hz, indicating the glucose unit must be β-D-glucopyranose form. By irradiation at H-4 of 6-hydroxydelphinidin, the observation of strong DIFNOE at H-1 of glucose indicated that glucose was bonded at OH-3 of 6-hydroxydelphinidin.

The proton chemical shifts of methylene in glucose moiety were shifted to a low magnetic field (δ 4.15 and 4.41), supporting that 6-OH of glucose is bonded with malonic acid. Therefore, the structure of pigment (**1**) was determined to be 6-hydroxydelphinidin 3-*O*-[6-*O*-malonyl-β-D-glucopyranoside] (Figure 2), which is a new pigment.[8]

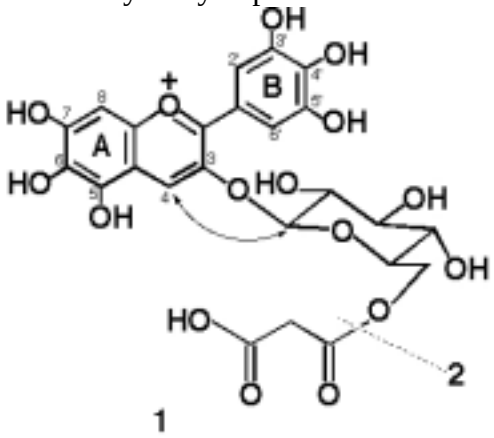

Figure 2. 6-hydroxydelphinidin 3-malonylglucoside (1) and 6-hydroxydelphinidin 3-glucoside (2) from the red-purple flowers of *Alstroemeria* 'Westland'. NOEs are indicated by arrows.

The FAB-MS measurement of pigment (**2**) gave a molecular ion $[M]^+$ at 481 *m/z* in agreement with the mass calculated for $C_{21}H_{21}O_{13}$ (481.098), indicating the presence of one molecule each of 6-hydroxydelphidin and glucose. The demalonyl pigment, 6-hydroxydelphinidin 3-glucoside, of pigment (**1**) was prepared by treatment of pigment (**1**) with 1N HCl-H_2O for one week.[9] The Rf values of TLC, Rt (min) of HPLC and spectral data of the demalonyl pigment (**1**) were identical with those of pigment (**2**) (Table 1). Furthermore, the exact structure of pigment (**2**) was elucidated based on measurements of its ^{1}H NMR and ^{1}H-^{1}H COSY spectra. The proton chemical shifts of pigment (**2**) were in agreement with those of pigment (**1**) except the proton signals of malonic acid and, H-5 and methylene proton signals of glucose moiety (Table 2). In particular, the methylene proton signals (3.51 – 3.74 ppm) of glucose in

pigment (**2**) were observed in the upper magnetic field than those (4.15 and 4.41 ppm) of pigment (**1**), indicating that the OH-6 of glucose was free from malonic acid. Therefore, the structure of pigment (**2**) was determined to be demalonyl pigment (**1**), 6-hydroxydelphinidin 3-*O*-β-D-glucopyranoside (Table 2, Figure 2), which is also a new anthocyanin.[7]

Table 2. **^{1}H NMR spectral data of pigments (1) and (2) from the flowers of Alstroemeria 'Westland' (500 MHz, DCl-DMSO-*d*$_6$, 1:9 at 25 ℃, standard TMS.**

	Pigment 1	Pigment 2
6-hydroxydelphinidin		
H-4	**8.88 (1H, *s*)**	**8.94 (1H, *s*)**
H-8	**7.24 (1H, *s*)**	**7.25 (1H, *s*)**
H-2',6'	**7.72 (2H, *s*)**	**7.73 (2H, *s*)**
glucose		
H-1	**5.46 (1H, *d* , *J* =8.0 Hz)**	**5.42 (1H, *d* , *J* =8.0 Hz)**
H-2	**3.61 (1H, *t* , *J* =8.0 Hz)**	**3.56 (1H, *m*)**
H-3	**3.44 (1H, *m*)**	**3.41 (1H, *m*)**
H-4	**3.22 (1H, *t* , *J* =9.3 Hz)**	**3.28 (1H, *m*)**
H-5	**3.78 (1H, *m*)**	**3.53 (1H, *m*)**
H-6a	**4.15 (1H, *dd* , *J* =8.2, 12.0 Hz)**	**3.51 - 3.64 (*m*)**
H-6b	**4.41 (1H, *brd* , *J* =12.0 Hz)**	**3.64 - 3.74 (*m*)**
malonic acid		
-CH $_2$	**3.25 - 3.41 (*m*)**	

In this study, it revealed that four different type of anthocyanidins were found in the same flower of *Alstroemeria* 'Westland' at the same time, and also the three analogous glycoside sets were detected in the same flower such as 3-glucosides, 3-rutinosides and 3-malonylglucosides of cyanidin, delphinidin, 6-hydroxycyanidin and 6-hydroxydelphinidin, respectively.

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6. Preparative HPLC was run on a Waters C18 (19ϕ x 150 mm) column at 40 °C with a flow rate of 4 mL/min and monitored at 530 nm for anthocyanins. A solvent system used was linear gradient elution for 15 min from 40 to 60% solvent B (1.5% H_3PO_4, 20% HOAc, 25% MeCN in H_2O) in solvent A (1.5% H_3PO_4 in H_2O). Analytical HPLC was performed using a Waters C18 (4.6ϕ x 250 mm) at 40 °C with a flow rate of 1 mL/min and monitoring at 530 nm. The solvent was applied as a linear gradient from 20 to 85 % solvent B in solvent A.

7. 6-Hydroxydelphinidin; UV λmax (0.1% HCl-MeOH) 532, 283 nm, E_{440}/E_{max} = 0.15, $AlCl_3$ +; TLC Rf-values (x 100) BAW (*n*-BuOH : HOAc : H_2O= 4 : 1 : 2) 23, BuHCl (*n*-BuOH : 2N HCl = 1 : 1) 18, 1%HCl 0, AHW (HOAc : HCl : H_2O = 15 : 3 : 82) 1, Forestal (HOAc : HCl : H_2O = 30 : 3 : 10) 12; FAB MS $[M]^+$ *m/z* 319; HPLC Rt (min) 15.74. ^1H NMR (500 MHz, DCl/DMSO-d_6, 1:9): δ 8.80 (1H, *s*, H-4), 7.73 (2H, *s*, H-*2*',6'), 7,20 (1H, *s*, H-8).

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