

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

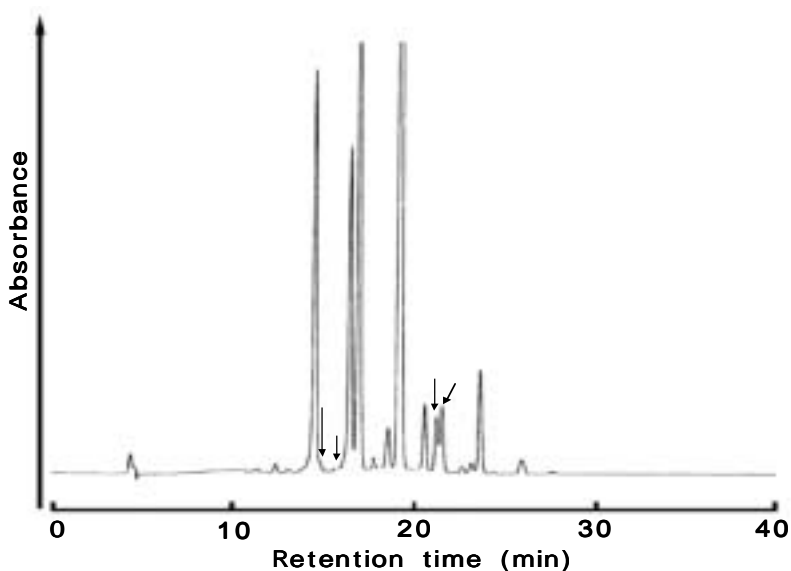
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***Abstract*-----Two novel 6-hydroxydelphinidin glycosides were isolated from the red-purple flowers of *Alstroemeria* 'Westland' along with ten known anthocyanins. These novel pigments were determined to be 6-hydroxydelphinidin 3-*O*- $\beta$ -D-glucoside and 6-hydroxydelphinidin 3-*O*-[6-*O*-(malonyl)- $\beta$ -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 anthocyanins 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<sup>1,2,5</sup> and others,<sup>3,4</sup> 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 °C and kept in the refrigerator at 10 °C. Twelve anthocyanins were observed in the MAW (MeOH/HOAc/H<sub>2</sub>O, 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’.

cyanidin 3-glucoside  
 cyanidin 3-rutinoside  
 cyanidin 3-malonylglucoside  
 delphinidin 3-glucoside  
 delphinidin 3-rutinoside  
 delphinidin 3-malonylglucoside  
 6-hydroxycyanidin 3-glucoside  
 6-hydroxycyanidin 3-rutinoside  
 6-hydroxycyanidin 3-malonylglucoside  
 6-hydroxydelphinidin 3-glucoside (pigment 2)  
 6-hydroxydelphinidin 3-rutinoside  
 6-hydroxydelphinidin 3-malonylglucoside (pigment 1)  
 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<sub>2</sub>O = 4 : 1 : 2) and HPLC.<sup>6</sup> 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<sub>2</sub>O, 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*,<sup>1-5</sup> 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 colour | Rf values (x 100) <sup>*</sup> |       |       |     | Spectral data in 0.1% HCl-MeOH |                        |                   | HPLC                                |          | FAB-MS           |
|---|----------------|--------------------------------|-------|-------|-----|--------------------------------|------------------------|-------------------|-------------------------------------|----------|------------------|
|   |                | BAW                            | BuHCl | 1%HCl | AHW | $\lambda_{\max}$ (nm)          | $E_{440}/E_{\max}$ (%) | AlCl <sub>3</sub> | $\lambda_{\max}$ (nm) <sup>**</sup> | Rt (min) | [M] <sup>+</sup> |
| 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              |

<sup>\*</sup> BAW; *n*-BuOH:HOAc:H<sub>2</sub>O=4:1:2(v/v/v), BuHCl; *n*-BuOH:2N HCl=1:1(upper phase), 1% HCl; HCl:H<sub>2</sub>O=3:97(v/v), AHW; HOAc:HCl:H<sub>2</sub>O=15:3:82(v/v/v).

<sup>\*\*</sup> Visible max of anthocyanins was measured by PDA of HPLC.

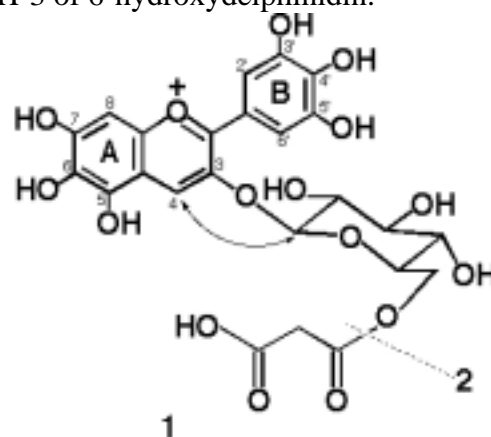
The structure determination of the new pigments (1) and (2) was carried out as follows. By acid hydrolysis with 2N HCl at 100 °C for 2 h pigments (1) and (2) gave 6-hydroxydelphinidin<sup>7</sup> as their anthocyanidin, and glucose as sugar.

The FAB-MS measurement of pigment (1) gave a molecular ion [M]<sup>+</sup> at 567 *m/z* in agreement with the mass calculated for C<sub>24</sub>H<sub>23</sub>O<sub>16</sub> (*m/z*, 567.098) indicating the presence of one molecule each of 6-

hydroxydelphinidin, glucose, and malonic acid, respectively. The exact structure of pigment (**1**) was determined by the analysis of its  $^1\text{H}$  NMR,  $^1\text{H}$ - $^1\text{H}$  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  $^1\text{H}$  NMR spectrum. The signal of an anomeric proton of glucose appeared at  $\delta$  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  $\beta$ -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 ( $\delta$  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- $\beta$ -D-glucopyranoside] (Figure 2), which is a new pigment.<sup>8</sup>

The FAB-MS measurement of pigment (**2**) gave a molecular ion  $[\text{M}]^+$  at 481 *m/z* in agreement with the mass calculated for  $\text{C}_{21}\text{H}_{21}\text{O}_{13}$  (481.098), indicating the presence of one molecule each of 6-hydroxydelphinidin and glucose. The demalonyl pigment, 6-hydroxydelphinidin 3-glucoside, of pigment (**1**) was prepared by treatment of pigment (**1**) with 1N HCl- $\text{H}_2\text{O}$  for one week.<sup>9</sup> The *R<sub>f</sub>* values of TLC, *R<sub>t</sub>* (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  $^1\text{H}$  NMR and  $^1\text{H}$ - $^1\text{H}$  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



**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.

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*- $\beta$ -D-glucopyranoside (Table 2, Figure 2), which is also a new anthocyanin.<sup>7</sup>

**Table 2.** <sup>1</sup>H NMR spectral data of pigments (1) and (2) from the flowers of *Alstroemeria* 'Westland' (500 MHz, DCI-DMSO-*d*<sub>6</sub>, 1:9 at 25 °C, standard TMS).

|                             | Pigment 1                                     | Pigment 2                              |
|-----------------------------|---|--|
| <b>6-hydroxydelphinidin</b> |   |  |
| 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)                           |
| <b>glucose</b>              |   |  |
| H-1                         | 5.46 (1H, <i>d</i> , <i>J</i> =8.0 Hz)        | 5.42 (1H, <i>d</i> , <i>J</i> =8.0 Hz) |
| H-2                         | 3.61 (1H, <i>t</i> , <i>J</i> =8.0 Hz)        | 3.56 (1H, <i>m</i> )                   |
| H-3                         | 3.44 (1H, <i>m</i> )                          | 3.41 (1H, <i>m</i> )                   |
| H-4                         | 3.22 (1H, <i>t</i> , <i>J</i> =9.3 Hz)        | 3.28 (1H, <i>m</i> )                   |
| H-5                         | 3.78 (1H, <i>m</i> )                          | 3.53 (1H, <i>m</i> )                   |
| H-6a                        | 4.15 (1H, <i>dd</i> , <i>J</i> =8.2, 12.0 Hz) | 3.51 - 3.64 ( <i>m</i> )               |
| H-6b                        | 4.41 (1H, <i>brd</i> , <i>J</i> =12.0 Hz)     | 3.64 - 3.74 ( <i>m</i> )               |
| <b>malonic acid</b>         |   |  |
| -CH <sub>2</sub>            | 3.25 - 3.41 ( <i>m</i> )                      |  |

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<sub>3</sub>PO<sub>4</sub>, 20% HOAc, 25% MeCN in H<sub>2</sub>O) in solvent A (1.5% H<sub>3</sub>PO<sub>4</sub> in H<sub>2</sub>O). 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 λ<sub>max</sub> (0.1% HCl-MeOH) 532, 283 nm, E<sub>440</sub>/E<sub>max</sub> = 0.15, AlCl<sub>3</sub> +; TLC R<sub>f</sub>-values (x 100) BAW (*n*-BuOH : HOAc : H<sub>2</sub>O = 4 : 1 : 2) 23, BuHCl (*n*-BuOH : 2N HCl = 1 : 1) 18, 1% HCl 0, AHW (HOAc : HCl : H<sub>2</sub>O = 15 : 3 : 82) 1, Forestal (HOAc : HCl : H<sub>2</sub>O = 30 : 3 : 10) 12; FAB MS [M]<sup>+</sup> *m/z* 319; HPLC R<sub>t</sub> (min) 15.74. <sup>1</sup>H NMR (500 MHz, DCl/DMSO-*d*<sub>6</sub>, 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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