

**6-HYDROXYCYANIDIN 3-MALONYLGLUCOSIDE FROM THE
FLOWERS OF *ALSTROEMERIA* 'TIARA'**

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Abstract----- A novel malonylanthocyanin was isolated from the flowers of
Alstroemeria 'Tiara' and determined to be 6-hydroxycyanidin 3-*O*-[6-*O*-
malonyl- β -D-glucopyranoside].

From the flowers of *Alstroemeria* cultivars, at least, seven anthocyanins have been isolated and determined to be 3-glucoside of 6-hydroxycyanidin,¹ 3-rutinosides of delphinidin, cyanidin, 6-hydroxydelphinidin and 6-hydroxycyanidin,¹⁻⁴ and 3-malonylglucosides of delphinidin and cyanidin^{3,4} by Nørbaek and by us, independently. During the course of our further studies directed forward the structure determination of flower colors of *Alstroemeria* species, we could isolate a novel anthocyanin,

and here we wish to report the structure elucidation of a novel malonylated anthocyanin along with three known anthocyanins in the flowers of *Alstroemeria* 'Tiara'.

The red cultivars of *Alstroemeria* 'Tiara' were obtained at the flower market in Sapporo. The flowers exhibited red colors (Red 43A by R.H.S. color chart, chromaticity value (b/a=0.65). The fresh perianthes of these plants were dried overnight at 37°C and kept in the refrigerator at 10°C. Dried perianthes (ca. 50 g) of *Alstroemeria* 'Tiara' were immersed in 10% HOAc-MeOH (1 L, HOAc-MeOH, 1:9) at room temperature overnight. The pigments were then extracted with MAW (MeOH/HOAc/H₂O, 4:1:5), and the MAW extract was concentrated to ca. 10 mL, and purified by preparative HPLC. 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% H₃PO₄, 20% HOAc, 25% MeCN in H₂O) in solvent A (1.5% H₃PO₄ in H₂O). 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 Et₂O, and then dried to give four pigment powders. Finally, pigment (1)⁵ (ca. 2 mg), pigment (2)⁶ (ca. 5 mg), pigment (3)⁷ (ca. 1 mg) and pigment (4)⁸ (ca. 3 mg) were obtained, respectively.

By acid hydrolysis, pigment (1) gave 6-hydroxycyanidin as the anthocyanidin, and glucose as the sugar residue. Similar treatment of pigment (2) afforded the same anthocyanidin, 6-hydroxycyanidin together with glucose and rhamnose as the sugar residues. On the other hand, cyanidin was isolated as the anthocyanidin by acid hydrolysis of pigments (3) and (4), where glucose was obtained from the former pigment (3), and glucose and rhamnose were found in the later pigment (4). Among these four pigments, three (2, 3 and 4) of them were already known, and identified based on their spectroscopic data and also by direct comparison with the authentic samples, as 6-hydroxycyanidin 3-rutinoside, cyanidin 3-malonylglucoside, and cyanidin 3-rutinoside, respectively. Pigment (1), however, seemed

to be a new anthocyanin, and its structure was determined as follows.

The FAB-MS measurement of **1** gave a molecular ion $[M]^+$ at 551 m/z in good agreement with the mass calculated for $C_{24}H_{23}O_{15}$ (m/z 551.103) indicating the presence of each one molecule of 6-hydroxycyanidin, glucose, and malonic acid, respectively. The detailed structure of **1** was determined by the analysis of 1H NMR, 1H - 1H COSY and DIFNOE spectra of **1** (Table 1). The chemical shifts of five characteristic aromatic protons were assigned as shown

in Table 1. However, the signal of H-6 disappeared in its 1H NMR spectrum. The signal of an anomeric proton of glucose appeared at δ 5.43 (d , $J=7.9$ Hz). As all the assigned glucose protons have the vicinal coupling constants of $J=7$ -12 Hz, indicating the glucose unit must be β -D-glucopyranose form. By irradiation at H-4 of 6-hydroxycyanidin, the observation of a negative NOE signal at H-1 of glucose indicated that glucose was bonded at OH-3 of 6-hydroxycyanidin. The protons of methylene in glucose moiety were shifted to a low magnetic field (δ 4.14 and 4.50), supporting that 6-OH of glucose is bonded with malonic acid. Therefore, the structure of **1** was determined to be 6-hydroxycyanidin 3-*O*-[6-*O*-(malonyl)- β -D-glucopyranoside] (Table 1, Figure 1), which is a new pigment.⁹

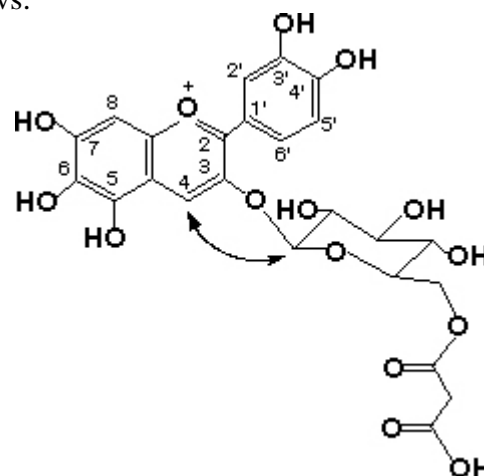


Figure 1. 6-hydroxycyanidin 3-malonyl-glucoside from *Alstroemeria* 'Tiara'. NOEs are indicated by arrows.

Table 1. 1H NMR spectral data of 6-hydroxycyanidin 3-malonylglucoside (pigment **1**) from the flowers of *Alstroemeria* 'Tiara' (500 MHz, TFA-DMSO- d_6 , 1:9 at 25°C, standard TMS)

| 6-hydroxycyanidin | | glucose | | malonic acid | |
|-------------------|--|---------|---|--------------------|-----------|
| H-4 | 8.87 (1H, <i>s</i>) | H-1 | 5.43 (1H, <i>d</i> , $J=7.9$ Hz) | -CH ₂ - | 3.20-3.50 |
| H-8 | 7.10 (1H, <i>s</i>) | H-2 |] 3.20-3.60 | | |
| H-2' | 7.99 (1H, <i>d</i> , $J=2.5$ Hz) | H-3 | | | |
| H-5' | 7.05 (1H, <i>d</i> , $J=8.6$ Hz) | H-4 | | | |
| H-6' | 8.21 (1H, <i>dd</i> , $J=2.5, 8.6$ Hz) | H-5 | 3.87 (1H, <i>m</i>) | | |
| | | H-6a | 4.14 (1H, <i>dd</i> , $J=8.0, 12.0$ Hz) | | |
| | | H-6b | 4.50 (1H, <i>brd</i> , $J=12.0$ Hz) | | |

Thus, we could determine the structure of a novel anthocyanin from the flowers of *Alstroemeria* 'Tiara'. Although the structure of a novel anthocyanin is relatively simple, the isolation of an acylated anthocyanin of 6-hydroxylated cyanidin would be of interest from the chemotaxonomical point of view.

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3. R. Nørbaek, L. P. Christensen, G. Bojesen, and K. Brandt, *Phytochemistry*, 1996, **42**, 97.
4. R. Nørbaek, L. P. Christensen, and K. Brandt, *Plant Breeding*, 1998, **117**, 63.
5. Pigment (**1**); UV λ_{\max} (0.1% HCl-MeOH) 513, 282 nm, $E_{440}/E_{\max} = 0.24$; TLC Rf-values (x100) BAW (BuOH/AcOH/H₂O, 4:1:2) 17, BuHCl (BuOH/2N-HCl, 1:1) 19, 1% HCl 7, AHW (AcOH/HCl/H₂O, 15:3:82) 19; FAB MS [M]⁺ m/z 551; HPLC Rt (min) 20.61.
6. Pigment (**2**) (6-hydroxycyanidin 3-rutinoside); UV λ_{\max} (0.1% HCl-MeOH) 515, 284 nm, $E_{440}/E_{\max} = 0.23$; TLC Rf-values (x100) BAW 14, BuHCl 16, 1% HCl 10, AHW 34; FAB MS [M]⁺ m/z 611; HPLC Rt (min) 16.40. ¹H NMR (500 MHz, TFA/DMSO-*d*₆, 1:9): 6-hydroxycyanidin δ 8.81(1H, *s*, H-4), 7.06(1H, *s*, H-8), 7.94(1H, *d*, $J=2.1$ Hz, H-2'), 7.00(1H, *d*, $J=8.9$ Hz, H-5'), 8.16(1H, *dd*, $J=2.1$, 8.9 Hz, H-6'); Glucose, δ 5.34(1H, *d*, $J=7.6$ Hz, H-1), 3.49(1H, *t*, $J=8.5$ Hz, H-2), 3.37(1H, *m*, H-3), 3.20(1H, *t*, $J=9.2$ Hz, H-4), 3.66(1H, *m*, H-5), 3.42(1H, *m*, H-6a), 3.88(1H, *d*, $J=10.1$ Hz, H-6b); Rhamnose, δ 4.50(1H, *s*, H-1), 3.59(1H, *m*, H-2), 3.45(1H, *dd*, $J=3.4$, 9.5 Hz, H-3), 3.13(1H, *t*, $J=9.5$ Hz, H-4), 3.35(1H, *m*, H-5), 1.06(3H, *m*, -CH₃).
7. Pigment (**3**) (cyanidin 3-malonylglucoside); UV λ_{\max} (0.1% HCl-MeOH) 528, 280 nm, $E_{440}/E_{\max} = 0.25$; TLC Rf-values (x100) BAW 29, BuHCl 56, 1% HCl 7, AHW 30; FAB MS [M]⁺ m/z 535; HPLC Rt (min) 23.46.

8. Pigment (4) (cyanidin 3-rutinoside); UV λ_{max} (0.1% HCl-MeOH) 529, 280 nm, $E_{440}/E_{\text{max}} = 0.25$; TLC Rf-values (x100) BAW 27, BuHCl 37, 1%HCl 17, AHW 47; FAB MS $[M]^+$ m/z 595; HPLC Rt (min) 19.16. ^1H NMR (500 MHz, TFA/DMSO- d_6 , 1:9): Cyanidin δ 8.79(1H, *s*, H-4), 8.20(1H, *br d*, H-6'), 7.97(1H, *br s*, H-2'), 7.01(1H, *d*, $J=8.5$ Hz, H-5'), 6.88(1H, *br s*, H-8), 6.69(1H, *br s*, H-6); Glucose, δ 5.35(1H, *d*, $J=7.7$ Hz, H-1), 3.88(1H, *m*, H-6b), 3.63(1H, *m*, H-5); Rhamnose, δ 4.50(1H, *s*, H-1), 3.57(1H, *br s*, H-2), 3.37(1H, *m*, H-5), 1.03(3H, *m*, -CH₃).
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