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# ACYLATED PELARGONIDIN 3, 7-GLYCOSIDES FROM RED FLOWERS OF *DELPHINIUM HYBRIDUM*

NORIO SAITO,† KENJIRO TOKI,‡ AKIGO SUGA‡ and TOSHIO HONDA§*

†Chemical Laboratory, Meiji-Gakuin University, Totsuka, Yokohama, Japan; ‡Laboratory of Floriculture, College of Horticulture, Minami-Kyushu University, Takanabe, Miyazaki, Japan; §Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa, Tokyo, Japan

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**Key Word Index**—*Delphinium hybridum*; Ranunculaceae; red flower colour; acylated pelargonidin 3-rutinoside-7-glucosides and 3,7-diglucosides; *p*-hydroxybenzoic acid; malonic acid.

**Abstract**—Six pelargonidin glycosides were isolated from the red flowers of *Delphinium hybridum* cv "Princess Caroline" as major anthocyanins. These pigments were classified into two groups, based on the deacylglycosides 3-rutinoside-7-glucoside and 3, 7-diglucoside. Three pigments belonging to the former group are pelargonidin 3-rutinoside-7-glucoside, pelargonidin 3-rutinoside-7-[6-(*p*-hydroxybenzoyl)glucoside] and pelargonidin 3-rutinoside-7-[6-(4-(glucosyl)-*p*-hydroxybenzoyl)-glucoside]. The other three pigments are pelargonidin 3-[6-(malonyl)-glucoside]-7-glucoside, pelargonidin 3-glucoside-7-[6-(4-(glucosyl)-*p*-hydroxybenzoyl)-glucoside] and pelargonidin 3-[6-(malonyl)-glucoside]-7-[6-(4-(glucosyl)-*p*-hydroxybenzoyl)-glucoside]. 

## INTRODUCTION

*Delphinium* is a popular ornamental plant genus and the flower colour of the cultivars ranges from white, yellow, red to blue. The common flower colour of *Delphiniums* is blue-purple. Recently, Legro has developed the University Hybrids of *Delphinium*, by introducing the red flower colours of *Delphinium cardinale* and *D. nudicaule* [1]; these produce flowers in the wide range of sharp pink, brilliant red and apricot-orange. However, to the best of our knowledge, there have been no detailed reports on the red or pink flowers of this plants. In continuing work on flower colour variation due to acylated anthocyanins in *Delphinium* and *Consolida* [2], red flowers of *Delphinium* cv "Princess Caroline" were studied and we report here the occurrence of five acylated pelargonidin 3, 7-glycosides in this plant and their structure determination.

## RESULTS AND DISCUSSION

Six anthocyanins (**1-6**) were observed by HPLC in the red flowers of *Delphinium hybridum* cv "Princess Caroline". Their relative concentrations were 24% (**1**), 4% (**2**), 22% (**3**), 6% (**4**), 5% (**5**) and 16% (**6**). The isolation of these pigments was performed according to previous procedures [2], and their chromatographic and spectral data are shown in Table 1. On acid hydrolysis, all gave pelargonidin and glucose. Moreover, similar treatment of **1–3** gave rhamnose as an additional sugar component. As acyl moieties, **2, 3** and **5** yielded only *p*-hydroxybenzoic acid, whereas, **4** and **6** gave malonic acid in addition to *p*-hydroxybenzoic acid in the case of **6**. By alkaline hydrolysis, **2** and **3** produced pelargonidin 3-rutinoside-7-glucoside which was identical with **1**, and **4–6** gave pelargonidin 3, 7-diglucoside (**7**) as their deacyl anthocyanins, respectively. The structures of these two deacyl anthocyanins (**1** and **7**) were fully determined to be 3-rutinoside-7-glucoside and 3, 7-diglucoside of pelargonidin by analyses of TLC, HPLC and UV-Vis spectra containing partial acid hydrolysis (Table 1). The structures of these six anthocyanins (**1–6**) were determined and/or confirmed by analyses of FAB mass and $^1$H NMR spectra as follows (Table 2 and Experimental). In general, the solvent system with DMSO-$d_6$-TFA-d has been employed to obtain fairly good NOE spectra in the region of 4.5–5.5 ppm of these types of pigments. However, it was observed that the acidity of this solvent system was too weak to form the flavylium cationic structures for the pigments (**1–5**), which rapidly transformed into the stable carbinol pseudobase forms [3–5]. Therefore, $^1$H NMR analyses for these pigments (**1–5**) were performed as the pseudobase forms using this solvent system, and their flavylium cationic forms were also confirmed

*Author for correspondence: Phone 081 03 5498 5791; Fax 0081 03 3787 0036; E-mail: honda@hoshi.ac.jp

Table 1. Chromatographic and spectral properties of anthocyanins from the red flowers of *Delphinium*

| Antho-cyanins* | Rf values ( × 100)** | | | | Rt† | Spectral data in 0.1% HCl-MeOH | | | FAB-MS |
|---|---|---|---|---|---|---|---|---|---|
| | BAW | BuH | 1%HCl | HOAc-HCl | (min) | λmax(nm) | $E_{250}/E_{max}$ (%) | $E_{440}/E_{max}$ (%) | $[M]^+$ |
| 1 | 53 | 14 | 66 | 79 | 7.9 | 280,504 | - | 39 | 741 |
| 2 | 73 | 67 | 53 | 76 | 20.1 | 259,506 | 152 | 37 | 861 |
| 3 | 54 | 20 | 66 | 84 | 16.6 | 252,508 | 120 | 36 | 1023 |
| 4 | 58 | 25 | 47 | 72 | 12.8 | 279,503 | - | 41 | 681 |
| 5 | 56 | 21 | 74 | 52 | 13.4 | 253,507 | 102 | 37 | 877 |
| 6 | 57 | 27 | 59 | 76 | 17.4 | 254,508 | 119 | 35 | 963 |
| 7 | 78 | 11 | 40 | 68 | 6.3 | 279,503 | - | 43 | - |

*1. pelargonidin 3-rutinoside-7-glucoside.
2. pelargonidin 3-rutinoside-7-(6-(*p*-hydroxybenzoyl)glucoside).
3. pelargonidin 3-rutinoside-7-(6-(4-(glucosyl)-*p*-hydroxybenzoyl)glucoside).
4. pelargonidin 3-(6-malonyl)glucoside)-7-glucoside.
5. pelargonidin 3-glucoside-7-(6-(4-(glucosyl)-*p*-hydroxybenzoyl)glucoside).
6. pelargonidin 3-(6-(malonyl)glucoside)-7-(6-(4-(glucosyl)-*p*-hydroxybenzoyl)glucoside).
7. pelargonidin 3,7-diglucoside.
**BAW, *n*-BuOH-HOAc-$H_2O$ (4:1:5); BuH, *n*-BuOH-2MHCl (1:1); HOAc-HCl, HOAc-HCl-$H_2O$ (15:3:82)
†Analytical conditions; See Experimental

using the solvent system with DMSO-$d_6$-DCl [5–8]. On the other hand, the conversion of flavylium cationic form into pseudobase form for the pigment (**6**) having three acyl groups was incomplete in DMSO-$d_6$-TFA-d, where the pigment (**6**) existed as a mixture of several forms in equilibrium. Finally, the structure of **6** was elucidated on the basis of NMR analyses of its flavylium cationic form in DMSO-$d_6$-DCl.

*Pigments* **1**, **2** *and* **3**

FAB mass spectrum of **1** gave its molecular ion at 741 *m/z* $[M]^+$, in good agreement with the mass calculated for $C_{33}H_{41}O_{19}$, indicating the presence of pelargonidin with two molecules of glucose and one of rhamnose. This result was confirmed by analysis of its $^1H$ NMR spectrum (Table 2). The proton signals of its sugar parts were observed in the region of $\delta$ 5.01–1.17. Signals of two anomeric protons of glucose appeared at $\delta$ 5.01($\delta$, J = 8.2 Hz, Glc A) and 4.81($\delta$, J = 7.6 Hz, Glc B), and all observed vicinal coupling constants of the two glucose moieties (Glc A and B in Fig. 1) were 7.6–10.7 Hz. Therefore, both glucose units must be $\beta$-D-glucopyranosides. Appearance of an anomeric proton of rhamnose unit at $\delta$ 4.62 as a singlet, and one doublet signals of methyl protons ($\delta$ 1.17, $\delta$, J = 6.4 Hz) at C-5 of Rhm, suggested the existence of $\alpha$-L-rhamnopyranose. By analysis of the $^1H$-$^1H$ COSY spectrum of **1**, four aromatic protons H-2′, 6′ ($\delta$ 7.32, 2H, $\delta$, J = 8.5 Hz) and H-3′, 5′ ($\delta$ 6.73, 2H, d, J = 8.5 Hz) were observed as $A_2B_2$ coupling patterns indicating the presence of a *p*-hydroxyphenyl group at the 2-position of pelargonidin moiety. Morover, three proton chemical shifts of aglycone assigned to H-6 ($\delta$ 6.11, d, J = 2 Hz), H-8 ($\delta$ 6.17, d, J = 2 Hz) and H-4 ($\delta$ 6.28, s), were identical with the data of carbinol pseudobase form of malvidin glycoside [3, 9]. These results clearly suggest the presence of the carbinol pseudobase form for the pigment **1**.

To confirm the linkages of sugars, the negative NOE difference (DIFNOE) method was performed [6]. Since irradiation of H-1 of Glc B caused strong NOEs to both signals at $\delta$ 6.11(H-6) and $\delta$ 6.17 (H-8), and also irradiations of H-6 and H-8 gave a strong NOE to H-1 of Glc B, Glc B was obviously linked with the 7-hydroxyl of pelargonidin. Irradiation of H-1 of Glc A gave a strong NOE to H-4 of pelargonidin suggesting that the OH-3 of pelargonidin was glycosylated with Glc A. Furthermore rutinose was produced by $H_2O_2$ degradation of **1**. Therefore, **1** is pelargonidin 3-*O*-[6-*O*-($\alpha$-L-rhamnopyranosyl)-$\beta$-D-glucopyranoside]-7-*O*-$\beta$-D-glucopyranoside. This structure was confirmed as the flavylium cation form of **1** by analysis of its $^1H$ NMR spectrum measured in the strong acidic solution (DMSO-$d_6$-DCl)(Experimental).

The FAB mass spectrum of **2** and **3** gave their molecular ions $[M]^+$ at 861 and 1023 *m/z*, respectively, in good agreement with the mass calculated for $C_{40}H_{45}O_{21}$ and $C_{46}H_{55}O_{26}$. Analyses of the $^1H$ NMR spectra of **2** and **3** revealed the presence of one molecule of pelargonidin, two of glucose and one of rhamnose in both pigments. Furthermore, the presence of an additional *p*-hydroxybenzoic acid in **2** and glucosyl-*p*-hydroxybenzoic acid in **3** was confirmed in their $^1H$ NMR spectra. The $^1H$ NMR spectrum of **2** was superimposable on that of **1** except for the signals of Glc B and *p*-hydroxybenzoic acid moieties, and also the $^1H$ NMR spectrum of **3** was in good agreement with that of **2** without the signals of Glc C moiety of **3** (Fig. 1 and Table 2). The detailed structures of **2** and **3** were elucidated by $^1H$-$^1H$ COSY and DIFNOE

Table 2. $^1$H NMR data of *Delphinium* anthocyanins

| H | 1* | 2* | 3* | 4* | 5* | 6† |
|---|---|---|---|---|---|---|
| Pelargonidin | | | | | | |
| 4 | 6.29 s | 6.30 s | 6.30 s | 6.28 s | 6.27 s | 8.93 s |
| 6 | 6.11 d (2) | 6.14 br s | 6.16 br s | 6.11 d (2) | 6.15 d (2) | 7.00 br s |
| 8 | 6.17 d (2) | 6.18 br s | 6.19 br s | 6.17 d (2) | 6.19 d (2) | 7.49 br s |
| 2′, 6′ | 7.32 d (8.5) | 7.30 d (8.5) | 7.32 d (8.6) | 7.31 d (8.5) | 7.32 d (8.5) | 8.65 d (8.9) |
| 3′,5′ | 6.73 d (8.5) | 6.69 d (8.5) | 6.71 d (8.6) | 6.73 d (8.5) | 6.71 d (8.5) | 7.91 d (8.9) |
| p-Hydroxybenzoic acid | | | | | | |
| 2,6 | | 7.80 m | 7.93 d (8.6) | | 7.92 d (8.6) | 7.89 d (8.9) |
| 3,5 | | 6.89 d (8.9) | 7.15 d (8.6) | | 7.15 d (8.6) | 7.13 d (8.9) |
| (A) Glucose** | | | | | | |
| 1 | 5.01 | 5.02 | 5.02 | 5.05 | 5.01 | 5.48 |
| 2 | 3.77 | 3.79 | 3.78 | 3.79 | 3.77 | 3.52 |
| 3 | 3.47 | 3.56 | 3.47 | 3.53 | 3.53 | 3.50–3.28 |
| 4 | 3.19 | 3.18 | 3.21 | 3.25 | 3.24 | 3.23 |
| 5 | 3.50 | 3.75–3.30 | 3.52 | 3.68 | | 3.89 |
| 6a | 3.67 | | 3.67 | 4.14 | 3.72–3.31 | 4.12 |
| (B) 6b | 3.88 | 3.88 | 3.86 | 4.43 | | 4.47 |
| 1 | 4.81 | 4.90 | 4.91 | 4.81 | 4.91 | 5.35 |
| 2 | 3.19 | 3.24 | 3.26 | 3.17 | 3.25 | 3.41 |
| 3 | | 3.31 | 3.35 | 3.36–3.25 | 3.38 | 3.40–3.10 |
| 4 | | 3.33 | 3.39 | | 3.37 | 3.43 |
| 5 | 3.70–3.15 | 3.66 | 3.70 | 3.44 | 3.72 | 3.97 |
| 6a | | 4.20 | 4.31 | 3.55 | 4.29 | 4.37 |
| (C) 6b | | 4.50 | 4.52 | 3.69 | 4.52 | 4.62 |
| 1 | | | 5.02 | | 5.01 | 5.01 |
| 2 | | | 3.32 | | 3.24 | 3.28 |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | 3.72–3.19 | | 3.72–3.31 | 3.69–3.00 |
| 6a | | | | | | |
| 6b | | | | | | |
| Rhamnose | | | | | | |
| 1 | 4.62 | 4.62 | 4.63 | | | |
| 2 | 3.67 | 3.69 | 3.67 | | | |
| 3 | 3.51 | 3.25 | 3.50–3.10 | | | |
| 4 | 3.17 | 3.34 | | | | |
| 5 | 3.45 | 3.45 | 3.45 | | | |
| 6-$CH_3$ | 1.17 | 1.16 | 1.17 | | | |
| Malonic acid -$CH_2$- | | | | 3.55–3.26 | | 3.48–3.35 |

**Signal assignments based on DIFNOE and $^1$H-$^1$H COSY experiments. Coupling constants (J in Hz) are given in parentheses.

*In DMSO-$d_6$-TFA-d (9:1), carbinol pseudobase form.

†In DMSO-$d_6$-DCl (9:1), flavylium cationic forms.

spectral methods as described for the structure determination of **1**. The aromatic proton signals of pelargonidin and *p*-hydroxybenzoic acid of **2** and **3** were assigned as shown in Table 2. The signals of the sugar moieties of **2** were observed in the region of $\delta$ 5.02–1.16. The signals of three anomeric protons of **2** appeared at $\delta$ 5.02 ($\delta$, J = 8.2 Hz, Glc A), $\delta$ 4.90 ($\delta$, J = 7.9 Hz, Glc B) and $\delta$ 4.62 (s, Rhm), and these assigned glucose protons had coupling constants J = 7.9–11.6 Hz indicating the glucose moieties must be of $\beta$-D-glucopyranose form. In the rhamnose moiety of **2**, one singlet signal of an anomeric proton ($\delta$ 4.62) and one doublet signals of methyl protons ($\delta$ 1.16, d, J = 6.4 Hz) at C-5 suggested the existence of $\alpha$-L-rhamnopyranose form. The two characteristic protons ($\delta$ 4.20 and 4.50) being shifted to a lower magnetic field were assigned to be a pair of methylene protons of Glc B indicating that the OH-6 of Glc B is esterified with *p*-hydroxybenzoic acid. Therefore, **2** is pelargonidin 3-*O*-[rutinoside]-7-*O*-[6-*O*-(*p*-hydroxybenzoyl)-$\beta$-D-glucopyranoside], which is a new pigment in plants [10, 11].

In the $^1$H NMR spectrum of the pigment **3**, the signals of the sugar moieties were observed in the region of $\delta$ 5.02–1.17 (Table 2). Three anomeric protons of glucose were observed at $\delta$ 5.02, 5.02 and 4.91,

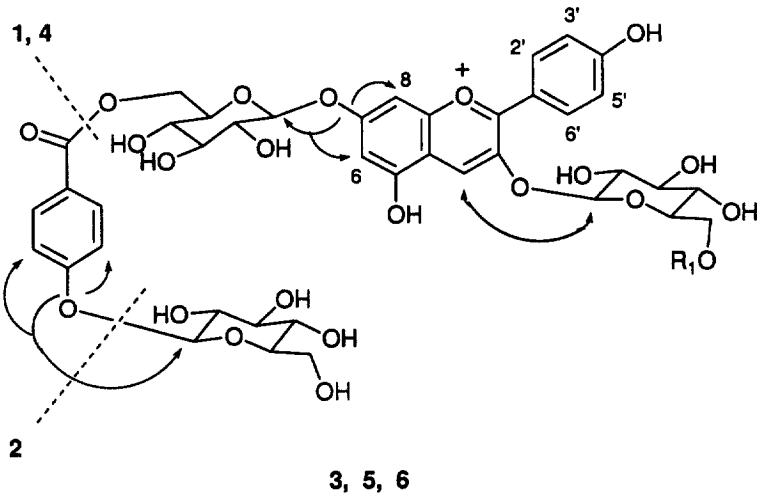


Fig. 1. Pelargonidin-based anthocyanins isolated from *D. hybridum* cv. 'Princess Caroline'. Observed NOEs are indicated by arrows. **1**. $R_1$ = rhamnose; **2**. $R_1$ = rhamnose; **3**. $R_1$ = rhamnose; **4**. $R_1$ = malonic acid; **5**. $R_1$ = H; **6**. $R_1$ = malonic acid.

and one anomeric proton of rhamnose was at $\delta$ 4.63. Their conformations were confirmed to be $\beta$-D-glucopyranose form for the glucose moieties and $\alpha$-L-rhamnopyranose form for the rhamnose moiety analogously to **1** and **2** by coupling constants. As similar to **2** characteristic protons ($\delta$ 4.31 and 4.52) being shifted to a lower magnetic field were assigned to be the methylene protons of Glc B indicating the OH-6 of Glc B is bonded with *p*-hydroxybenzoic acid. Irradiation of H-1 of Glc C ($\delta$ 5.02) and A ($\delta$ 5.02) gave strong NOEs to H-3, 5 of *p*-hydroxybenzoic acid and also rather weak NOEs to H-2, 6 of *p*-hydroxybenzoic acid as well as a strong NOE to H-4 of pelargonidin, suggesting that Glc C is attached to the OH-4 of *p*-hydroxybenzoic acid and also Glc A is bonded to the OH-3 of pelargonidin. Therefore, **3** is pelargonidin 3-*O*-[rutinoside]-7-*O*-[6-*O*-(4-*O*-($\beta$-D-glucopyranosyl)-*p*-hydroxybenzoyl)-$\beta$-D-glucopyranoside], which is a new pigment in plants [10, 11].

*Pigments* **4**, **5** *and* **6**

The FAB mass spectra of **4**, **5** and **6** gave their molecular ions $[M]^+$ at 681, 877 and 963 *m/z*, respectively, in good agreement with the mass calculated for $C_{30}H_{33}O_{15}$, $C_{40}H_{45}O_{22}$ and $C_{43}H_{47}O_{25}$. Analyses of the $^1$H NMR spectra of **4**–**6** revealed the presence of pelargonidin 3,7-diglucoside as their partial structures in these three pigments, and the presence of an additional molecule of malonic acid in **4**, one molecule of glucosyl-*p*-hydroxybenzoic acid in **5**, and also each of one molecule of malonic acid and glucosyl-*p*-hydroxybenzoic acid in **6**. The detailed structures of **4**–**6** were elucidated by $^1$H-$^1$H COSY and DIFNOE spectral methods as described for the structural determinations of **1**–**3**. In the pigment **4**, seven aromatic protons of pelargonidin were assigned as those of **1**–**3** (Table 2), and also two anomeric proton signals of glucose were assigned at $\delta$ 5.05 (d, J = 8.2 Hz, Glc A) and $\delta$ 4.81 (d, J = 7.6, Glc B). By DIFNOE spectral method it was confirmed that Glc A and B were bonded with the OH-3 and OH-7 of pelargonidin, respectively. The two methylene protons of Glc A being shifted to a lower magnetic field were assigned at $\delta$ 4.14 and 4.43 by analysis of $^1$H-$^1$H COSY and DIFNOE spectra indicating that the OH-6 of Glc A is esterified with malonic acid (Fig. 1). Therefore, **4** is pelargonidin 3-*O*-[6-*O*-(malonyl)-$\beta$-D-glucopyranoside]-7-*O*-[$\beta$-D-glucopyranoside], which is a new pigment in plants [10, 11].

The $^1$H NMR spectrum of **5** was superimposable on that of **3**, expect for the signals of rhamnose moiety (Fig. 1 and Table 2). In this spectrum, only two methylene protons of Glc B were shifted to lower magnetic field at $\delta$ 4.29 and 4.52 supporting that the OH-6 of Glc B is acylated with *p*-hydroxybenzoic acid. Since the methylene protons of Glc A were not shifted, the OH-6 of Glc A was assumed to be free from any acyl group. Irradiation of H-1 of Glc C gave strong NOEs to H-3, 5 of *p*-hydroxybenzoic acid and also rather weak NOEs to H-2, 6 of *p*-hydroxybenzoic acid indicating that Glc C was attached to the OH-4 of *p*-hydroxybenzoic acid through glycosidic bond. Therefore, **5** is pelargonidin 3-*O*-[$\beta$-D-glucopyranoside]-7-*O*-[6-*O*-(4-*O*-($\beta$-D-glucopyranosyl)-*p*-hydroxybenzoyl)-$\beta$-D-glucopyranoside], which is a new pigment in plants [10, 11].

In the $^1$H NMR spectrum of **6**, a number of proton signals arose from its flavylium cationic, quinonoidal base and carbinol pseudobase forms in the DMSO-$d_6$-TFA-d solution. Since these signals were heavily overlapping with each other, it was impossible to make reasonable assignments in this solvent. Thus, we decided to measure the $^1$H NMR spectra of its flavylium cationic form by use of the strong acidic solvent (DMSO-$d_6$-DCl, 9:1) (Table 2). Eleven aro-

matic proton signals of **6** were assigned to be one molecule of pelargonidin and one of *p*-hydroxybenzoic acid by $^1$H-$^1$H COSY spectrum, and confirmed by DIFNOE spectra as shown in Table 2. Three anomeric proton signals appeared at $\delta$ 5.48 (Glc A), $\delta$ 5.35 (Glc B) and $\delta$ 5.01 (Glc C) with large coupling constants (J = 7.0–7.6 Hz). The four methylene proton signals were also assigned at $\delta$ 4.12, 4.47 (Glc A) and $\delta$ 4.37, 4.62 (Glc B) being shifted to a lower magnetic field. Therefore, both OH-6 of Glc A and Glc B were acylated with malonic acid or *p*-hydroxybenzoic acid. The application of DIFNOE method made it possible to elucidate the linkages and/or the position of attachments of glucose, malonic acid and *p*-hydroxybenzoic acid units in **6** (Fig. 1). Hence, three glucoses (Glc A, B, C) are determined to be linked through the OH-3 and OH-7 of pelargonidin, and the OH-4 of *p*-hydroxybenzoic acid, respectively. By irradiations of H-2 and -6 of *p*-hydroxybenzoic acid and also of H-1 of Glc B, it was revealed that *p*-hydroxybenzoic acid was bonded with the OH-6 of Glc B. Therefore, **6** is pelargonidin 3-*O*-[6-*O*-(malonyl)-$\beta$-D-glucopyranoside]-7-*O*-[6-*O*-(4-*O*-($\beta$-D-glucopyranosyl)-*p*-hydroxy-benzoyl)-$\beta$-D-glucopyranoside], which is a new pigment in plants [10, 11].

Previously, Willstäter and Mieg [12] firstly isolated delphinidin from larkspur flowers (*Delphinium consolida*), and reported that this pigment consisted of 1 mol of delphinidin and 2 mol each of glucose and *p*-hydroxybenzoic acid. Thereafter, Harborne [13] isolated an anthocyanin from the same plant and suggested it to be delphinidin 3, 5-diglucoside. Shibata and Yoshitama [14] also isolated an anthocyanin from the same larkspur, and postulated it to be *p*-hydroxybenzoylcaffeoyldelphinidin 3-diglucoside. Asen *et al.* [15] isolated an anthocyanin from the flowers of *Consolida ambiqua* and reported that this pigment was delphinidin 3-di-(*p*-hydroxybenzoyl)glucosylglucoside. These outdated reports are clearly conflicting. Recently, three papers appeared on the acylated anthocyanins of *Delphinium hybridum* [16, 17] and *Consolida armeniaca* [2]. Kondo *et al.* reported the occurrence of di- and tetra-*p*-hydroxybenzoyl anthocyanins from the flowers of *Delphinium hybridum*; one is violdelphin [16] and another is cyanodelphin [17]. Both pigments consist of delphinidin 3-rutinoside-7-glycoside as their deacyl anthocyanins. Saito *et al.* [2] found four polyacylated anthocyanins with *p*-hydroxybenzoic acid and malonic acid, and these deacyl anthocyanins were determined to be delphinidin 3-glucoside-7-glycosides. Furthermore these major anthocyanins were determined to be acylated at the 7-glycosyl residue with *p*-hydroxybenzoic acid and at the 3-glucosyl residue with malonic acid. In this study, four *p*-hydroxybenzoylated anthocyanins are newly found in the flowers of *Delphinium*. These pigments are classified into two groups by their deacyl glycoside types; e.g. group 1 is based on delphinidin 3, 7-diglucoside like the pigments of *C. armeniaca*, and group 2 is based on delphinidin 3-rutinoside-7-glucoside as the pigments of *D. hybridum*. Characteristically the pigments of group 1 are acylated at the 3-glucose residue with malonic acid. Further anthocyanin studies of *Delphinium* and *Consolida* will be desirable.

## EXPERIMENTAL

### *Materials*

The plants of *D. hybridum* "Princess Caroline" were obtained from Daiichi Engei Nursery. The plants were cultivated in a greenhouse of experimental farm of Minami-Kyushu University. Fresh petals were collected and air dried at 45°.

### *Extraction and isolation*

The dried petals (200 g) were extracted with 5% HOAc at room temp. overnight. The filtered extract was adsorbed on Diaion HP-20 column, washed with 1% HOAc and then eluted with 5% HOAc in 70% MeOH. After concn, the eluate was fractionated by chromatography on a Sephadex LH-20 using HOAc-MeOH-H2O (1:6:12). The red frs were further purified with PC (*n*-BuOH-HOAc-$H_2O$, 4:1:2 and 15% HOAc) and HPLC. HPLC was performed on a Hitachi 6200 system, using an Inertsil ODS-2 (20Å × 250 mm) column and HOAc solvent system. Pigment **1** (6.2 mg), **2** (4.1 mg), **3** (13.7 mg), **4** (3.3 mg), **5** (9.2 mg) and **6** (22.1 mg) were obtained as orange-red powder.

### *Analysis*

Pigment identifications were carried out by standard procedures involving $H_2O_2$ oxidation, alkaline deacylation and acid hydrolysis [2, 7]. $^1$H NMR spectra were recorded at 500 M Hz in 10% TFA-d-90% DMSO-$d_6$ or DMSO-$d_6$-DCl with TMS as int. standard. The mass spectra were performed to obtain the positive mode with a magic bullet matrix and the negative mode with a glycerol matrix.

Analytical HPLC was performed on a Inertsil ODS-2 column (4.6Å × 250 mm) at 35° with a flow rate of 0.8 ml min$^{-1}$ monitoring at 530 nm. Solvent systems used were as follows: a linear gradient elution for 40 min from 25 to 85% B (1.5% $H_3PO_4$, 20% MeOH, 20% HOAc and 25% MeCN in $H_2O$) in solvent A (1.5% $H_3PO_4$).

## $^1$H NMR SPECTRA OF ANTHOCYANIN FLAVILIUM FORMS (DMSO-$d_6$-DCl, 9:1, 500 MHz)

### *Pigment* **1**

Pelargonidin $\delta$ 8.93 (1 H, s, H-4), 6.99 (1 H, brs, H-6), 7.44 (1 H, brs, H-8), 8.65 (2 H, d, J = 9.2 Hz, H-2′,6′), 7.15 (2 H, d, J = 9.2 Hz, H-3′, 5′), sugars 5.48 (1 H, d, J = 7.9 Hz, Glc A-H-1), 5.21 (1 H, d, J = 7.3 Hz, Glc B-H-1), 4.78 (1 H, s, Rh-H-1).

*Pigment* **2**

Pelargonidin $\delta$ 8.90 (1 H, s, H-4), 7.06 (1 H, brs, H-6), 7.42 (1 H, brs, H-8), 8.57 (2 H, d, J = 9.1 Hz, H-2′, 6′), 7.10 (2 H, d, J = 9.1 Hz, H-3′, 5′), *p*-hydroxybenzoic acid 7.78 (2 H, d, J = 8.4 Hz, H-2, 6), 6.90 (2 H, d, J = 8.4 Hz, H-3, 5), sugars 5.43 (1 H, d, J = 7.4 Hz, Glc A-H-1), 5.29 (1 H, d, J = 7.4 Hz, Glc B-H-1), 4.74 (1 H, s, Rh-H-1).

*Pigment* **3**

Pelargonidin $\delta$ 8.90 (1 H, s, H-4), 7.07 (1 H, brs, H-6), 7.46 (1 H, brs, H-8), 8.65 (2 H, d, J = 9.2 Hz, H-2′, 6′), 7.15 (2 H, d, J = 9.2 Hz, H-3′, 5′), *p*-hydroxybenzoic acid 7.90 (2 H, d, J = 8.5 Hz, H-2, 6), 7.11 (2 H, d, J = 8.5 Hz, H-3,5), sugars 5.43 (1 H, d, J = 7.6 Hz, Glc A-H-1), 5.30 (1 H, d, J = 7.0 Hz, Glc B-H-1), 4.75 (1 H, s, Rh-H-1).

*Pigment* **4**

Pelargonidin $\delta$ 8.91 (1 H, s, H-4), 7.07 (1 H, brs, H-6), 7.43 (1 H, brs, H-8), 8.66 (2 H, d, J = 9.2 Hz, H-2′, 6′), 7.15 (2 H, d, J = 9.2 Hz, H-3′, 5′), sugars 5.45 (1 H, d, J = 7.6 Hz, Glc A-H-1), 5.20 (1 H, d, J = 7.3 Hz, Glc B-H-1).

*Pigment* **5**

Pelargonidin $\delta$ 9.00 (1 H, s, H-4), 7.08 (1 H, brs, H-6), 7.48 (1 H, brs, H-8), 8.66 (2 H, d, J = 8.9 Hz, H-2′, 6′), 7.10 (2 H, d, J = 8.9 Hz, H-3′, 5′), *p*-hydroxybenzoic acid 7.80 (2 H, d, J = 8.9 Hz, H-2, 6), 7.13 (2 H, d, J = 8.9 Hz, H-3,5), sugars 5.41 (1 H, d, J = 7.9 Hz, Glc A-H-1), 5.33 (1 H, d, J = 8.2 Hz, Glc B-H-1).

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