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ACYLATED DELPHINIDIN GLYCOSIDES IN THE BLUE-VIOLET FLOWERS OF *CONSOLIDA ARMENIACA*

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Key Word Index—*Consolida armeniaca*; Ranunculaceae; flower colour; acylated anthocyanins; delphinidin 3,7-di- and triglycoside; malonic acid; *p*-hydroxybenzoic acid.

Abstract—Four new acylated delphinidin 3,7-glycosides were isolated from the blue-violet flowers of *Consolida armeniaca* as major anthocyanin pigments. The first pigment was based on delphinidin 3,7-diglucoside and was identified as delphinidin 3-*O*-[6-*O*-(malonyl)-β-D-glucopyranoside]-7-*O*-[6-*O*-(4-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-*p*-hydroxybenzoyl)-β-D-glucopyranoside] by spectral methods. The other three acylated anthocyanins were based on delphinidin 3-glucoside-7-sophoroside. The second pigment was determined to be delphinidin 3-*O*-[6-*O*-(malonyl)-β-D-glucopyranoside]-7-*O*-[2-*O*-(β-D-glucopyranosyl)-6-*O*-(4-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-*p*-hydroxybenzoyl)-β-D-glucopyranoside]. The third pigment was elucidated to be delphinidin 3-*O*-[6-*O*-(malonyl)-β-D-glucopyranoside]-7-*O*-[2-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-6-*O*-(4-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-*p*-hydroxybenzoyl)-β-D-glucopyranoside], and the fourth as delphinidin 3-*O*-[6-*O*-(malonyl)-β-D-glucopyranoside]-7-*O*-glucopyranosyl[(2-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-6-*O*-(4-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-*p*-hydroxybenzoyl)-β-D-glucopyranoside].

INTRODUCTION

In continuing work on flower colour variation due to acylated anthocyanins [1–10], purple-violet flowers of *Consolida armeniaca* were used for the isolation of purple-violet pigments. Four novel acylated delphinidin glycosides with *p*-hydroxybenzoic acid and malonic acid were isolated from this plant as major pigments.

To date, six anthocyanins acylated with *p*-hydroxybenzoic acid have been reported as follows, four acylated anthocyanins from *Campanula* flowers [4, 11], two acylated anthocyanins from *Delphinium* flowers [12, 13] and one pigment each from *Dendrobium* flowers [14], *Ipomoea batatas* cell cultures [16], and *Aconitum chinense* flowers [17].

In this paper we report the isolation and the structure determination of four novel acylated delphinidin 3,7-glycosides with *p*-hydroxybenzoic acid and malonic acid from the blue-violet flowers of *C. armeniaca*.

RESULTS AND DISCUSSION

Four anthocyanins (**1**–**4**) were observed in the flower extract as major anthocyanins (Figure 1). Their relative concentrations were 8% (**1**), 30% (**2**), 41% (**3**) and 14% (**4**). The isolation of these anthocyanins was performed according to previous procedures [4, 5, 10, 14].

R_f values and spectral data of **1**–**4** and their two deacylanthocyanins (**5** and **6**) are shown in Table 1. Acid hydrolysis of **1**–**4** gave delphinidin, glucose, *p*-hydroxybenzoic acid and malonic acid. Alkaline hydrolysis of **2**–**4** under N_2 yielded the same delphinidin triglycoside, delphinidin 3-glucoside-7-sophoroside (**5**), and **1** gave delphinidin 3,7-diglucoside (**6**). As acid components these pigments produced malonic acid, *p*-hydroxybenzoic acid and 4-glucosyl-*p*-hydroxybenzoic acid by alkaline hydrolysis; these were identified by TLC and spectral data (see Experimental and Table 1). The structure of **5** was confirmed by the analysis of FAB mass and ^{1}H NMR spectra (Tables 1 and 2). However the structure confirmation of **6** could not be achieved because of the small amount obtained.

Armeniaca anthocyanin (**1**). The FAB-mass spectrum of **1** showed a molecular ion peak $[M]^+$ at 1115 *m/z*, in good agreement with the mass calculated for $C_{50}H_{51}O_{29}$ (1115.223). The ^{1}H NMR spectrum of **1** was obtained at 400 MHz using CD_3OD or DMSO-d_6 solvent containing 10% DCl or TFA-d. The ^{1}H NMR spectrum of **1** showed the presence of one molecule of delphinidin, three molecules of glucose, two molecules of *p*-hydroxybenzoic acid and one molecule of malonic acid (Table 2). The structure determination of **1** was mainly performed by the analysis of ^{1}H-^{1}H COSY spectrum. The proton chemical shifts of **1** are shown in Table 2.

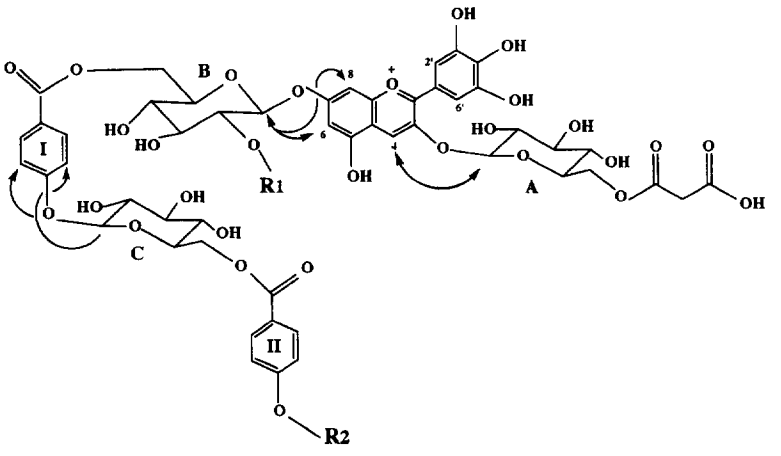

Fig. 1. *Armeniaca anthocyanins.* **1**: R_1 = H, R_2 = H; **2**: R_1 = glucose, R_2 = H; **3**: R_1 = 6-(*p*-hydroxybenzoyl)-glucose, R_2 = H; **4**: R_1 = 6-(*p*-hydroxybenzoyl)glucose, R_2 = glucose or R_1 = glucosyloxybenzoylglucose, R_2 = H. Observed NOEs are indicated by arrows.

Three anomeric protons of **1** appeared at δ 5.41 (*d*, J = 7.0 Hz, Glc A), δ 5.40 (*d*, J = 7.0 Hz, Glc B) and δ 4.15 (*m*, Glc C). As all the observed vicinal J values of Glc A, B and C were ca. 7.0–9.0 Hz, these glucose units must be β-D-glucopyranose. All methylene protons of these glucose moieties were shifted to a low magnetic field (δ 4.44, 4.72 Glc A; δ 4.21, 5.11 Glc B; δ 3.93, 4.59 Glc C), indicating three acids to be attached to all the OH-6 of glucose units (A–C). By analysis of ^{1}H-^{1}H COSY spectrum, H-1 of Glc A (δ 5.41) was correlated to H-6b of Glc A (δ 4.72), H-1 of Glc B (δ 5.40) was correlated to H-6b of Glc B (δ 5.11) and H-1 of Glc C (δ 4.15) to H-6b of Glc C (δ 4.59), respectively. The demalonyl pigment of **1** was prepared by the treatment of **1** with 2N HCl–H_2O according to the previous procedure [14, 9]. Analysis of ^{1}H NMR and ^{1}H-^{1}H COSY spectra of demalonyl **1** revealed that the proton chemical shifts were in good agreement with those of **1** without the proton signals of Glc A and malonic acid moieties (Table 2). In particular, the up-field shifts of methylene proton signals of Glc A in demalonyl **1** were observed from δ 4.72, 4.44 of **1** to δ 3.7–3.5, indicating that the OH-6 of Glc A in demalonyl **1** was free from malonyl groups. Therefore, the malonic acid was bonded with Glc A at OH-6 in **1**, and both *p*-hydroxybenzoic acids (I and II, Fig. 1) were acylated with the OH-6 groups of Glc B and C, and Glc C was attached at the OH-4 of *p*-hydroxybenzoic acid (I) through a glycosidic bond because 4-glucosyl-*p*-hydroxybenzoic acid was produced from **1** by alkaline hydrolysis. Thus, **1** is delphinidin 3-*O*-[6-*O*-(malonyl)-β-D-glucopyranoside]-7-*O*-[6-*O*-(4-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-*p*-hydroxybenzoyl)-β-D-glucopyranoside], which is a new pigment [15, 16].

Armeniaca anthocyanin (**2**). The FAB-mass spectrometry measurement of **2** gave a molecular ion $[M]^+$ at 1277 *m/z*, corresponding to the mass calculated for $C_{56}H_{61}O_{34}$ (1277.271). The ^{1}H NMR spectrum of **2** showed one molecule of delphinidin, four molecules of glucose and two molecules of *p*-hydroxybenzoic acid. The proton signals of malonic acid were not assigned in these spectra because of the heavy overlap with the proton signals of glucose (δ 5.46–3.38). The proton signals of delphinidin and two *p*-hydroxybenzoic acids (I and II) were assigned and correlated each other by 2D COSY spectral analysis. This result was confirmed by negative NOE difference (DIFNOE) spectral method [4, 5] as shown in Table 2.

The proton signals of sugar parts were observed in the region of δ 5.46–3.38, and all the observed vicinal coupling constants of four glucose moieties were J = 7.0–9.0 Hz. Signals of the four anomeric protons appeared at δ 5.46 (*d*, J = 7.7 Hz, Glc B), δ 5.42 (*d*, J = 7.7 Hz, Glc A), δ 4.70 (*d*, J = 7.7 Hz, Glc D) and δ 4.23 (*d*, J = 7.3 Hz, Glc C). Therefore, all the glucose units (A–D) must be β-D-glucopyranose. In comparison with the chemical shifts of **1** and **2**, the H-2 signal of Glc B of **2** was largely shifted down to δ 3.95 from δ 3.70 for **1**, indicating the attachment of a new glucose unit (Glc D) is at the 2-OH of Glc B in **2**.

The six characteristic protons at δ 5.10, 4.23 (Glc B), δ 4.71, 4.43 (Glc A) and δ 4.60, 3.95 (Glc C) shifted to a low magnetic field were assigned to be three methylene protons (H-6a, b) of Glc A–C, indicating all three acid units to be attached to the three 6-OH of glucose units through three ester-bonds. By analysis of the ^{1}H-^{1}H COSY spectrum, the H-1 protons of Glc A (δ 5.42), Glc B (δ 5.46) and Glc C (δ 4.23) were correlated to these methylene protons of Glc A, Glc B and of Glc C, respectively, as shown in Table 2. Therefore, the 6-OH of Glc D is free from the acid groups.

In order to determine the linkages of sugar and acid units in **2**, DIFNOE spectra of **2** were measured and confirmed these linkages as follows. Glc A is attached to the 3-OH of delphinidin, and Glc B is bonded at the OH-7 of delphinidin through glycosidic bonds. Moreover the irradiation of H-1 of Glc B gave NOE effects to H-2, 6 and H-3, 5 of *p*-hydroxybenzoic acid (I). Therefore, *p*-hydroxybenzoic acid (I) was attached to the OH-6 of

Table 1. Chromatographic and spectral properties of anthocyanins from violet-blue flowers of *C. armeniaca*

Anthocyanin	TLC R_f values (×100)*				HPLC	Spectral data (0.1% HCl–MeOH)			FAB-MS
	BAW	BuHCl	1% HCl	AHW	R_t (min)	λ_{max} (nm)	E_{acyl}/E_{max} (%)	E_{440}/E_{max} (%)	$[M]^+$
1	26	10	7	31	24.4	250,548	145	14	1115
2	18	4	8	37	25.4	250,548	133	13	1277
3	35	13	0	14	38.1	249,546	253	13	1397
4	20	3	8	39	27.7	250,550	174	12	1559
Deacyl **2–4** (**5**)	8	0	10	35	4.4	276,537	—	—	789
Deacyl **1** (**6**)	9	1	6	28	5.1	—	—	—	—

For key to abbreviations, see Experimental: *TLC, BAW = *n*-BuOH–HOAc–H_2O (4:1:5), BuHCl = *n*-BuOH–2M HCl (1:1), AHW = HOAc–HCl–H_2O (15:3:82). HPLC, column a Inertsil ODS-2 (4.6ϕ × 250 mm); solvent A = 1.5% H_3PO_4, B = 1.5% H_3PO_4, 20% HOAc, 25% MeCN in H_2O, linear gradient elution B = 25–85% in 40 min^{-1}, 1 ml min^{-1}, 35°, detected at 530 nm.

Glc B through an ester bond. To determine the attachment of malonic acid, demalonyl pigment **2** was prepared by the process described above. Analysis of the ^1H-^1H COSY spectrum of demalonyl **2** revealed that the methylene proton signals (H-6a, b) of Glc A of demalonyl **2** were shifted to a high magnetic field (δ 3.93, 4.01) from δ 4.43, 4.71 of **2** (Table 2), indicating that malonic acid was free from Glc A in demalonyl **2**. The malonic acid unit of **2** was esterified with the OH-6 of Glc A, and also Glc C was acylated with *p*-hydroxybenzoic acid (II) (Fig. 1). Therefore, Glc C was attached at the OH of *p*-hydroxybenzoic acid (I) through a glycosidic bond. The pigment **2** is delphinidin 3-*O*-[6-*O*-(malonyl)-β-D-glucopyranoside]-7-*O*-[2-*O*-(β-D-glucopyranosyl)-6-*O*-(4-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-*p*-hydroxybenzoyl)-β-D-glucopyranoside], which is a new pigment. The structure of demalonyl **2** was delphinidin 3-glucoside-7-*p*-hydroxybenzoylglucosyl-*p*-hydroxybenzoylsophoroside.

Armeniaca anthocyanin (**3**). The FAB-mass spectrum gave its molecular ion $[M]^+$ at 1397 *m/z* in good agreement with the mass calculated for $C_{63}H_{65}O_{36}$ (1397.323). Analysis of the ^1H NMR spectra including ^1H-^1H COSY spectrum indicated the presence of the same molecule components of **2** with one additional *p*-hydroxybenzoic acid (III) as shown in Table 2. These proton chemical shifts were assigned as shown in Table 2 except some heavy overlapping protons of sugar and malonic acid moieties. The eight methylene protons of all four glucose units (A–D) were assigned and correlated to each anomeric proton of Glc A–D by analysis of COSY spectrum. These proton signals (H-6a, b of Glc A–D) were shifted to a low magnetic field (δ 4.31, 4.80 Glc A; δ 4.27, 5.04 Glc B; δ 3.86, 4.71 Glc C and δ 3.99, 4.58 Glc D), indicating that all four hydroxyl groups (6-OH) of these glucose units were acylated by four acid units (three molecules of *p*-hydroxybenzoic acid (I–III) and one molecule of malonic acid).

The H-2 of Glc B in **3** was shifted to a lower magnetic field at δ 3.91 as similar to **2**, indicating that the 2-OH of Glc B is bonded with Glc D by glycosidic bond similar to that of **2**. In order to determine the attachment position of malonic acid unit in **3**, demalonyl **3** was prepared by the similar procedures mentioned for **1** and **2**. The proton chemical shifts of demalonyl **3** were investigated by analysis of ^1H-^1H COSY spectrum (Table 2). The proton chemical shifts of demalonyl **3** are identical with those of **3**, but the methylene protons (δ 4.31, 4.80) of Glc A were clearly shifted further upfield at δ 3.80–3.30 in demalonyl **3**, indicating that the 6-OH of Glc A was linked with malonic acid in pigment **3**. Therefore, **3** is delphinidin 3-*O*-[6-*O*-(malonyl)-β-D-glucoside]-7-*O*-[2-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-6-*O*-(4-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-*p*-hydroxybenzoyl)-β-D-glucopyranoside], which is a new pigment.

Armeniaca anthocyanin (**4**). The FAB-mass spectrum of **4** gave its molecular ion $[M]^+$ at 1559 *m/z* in good agreement with the mass calculated for $C_{69}H_{75}O_{41}$ (1559.376), which is composed of delphinidin with five molecules of glucose, three molecules of *p*-hydroxybenzoic

Table 2. ^{1}H NMR data of *Armeniaca* anthocyanins (DCl-DMSO-*d* 6, and DCl-CD_3OD, 1:9 at 25°)

H	1	demalonyl 1	2	demalonyl 2	3	demalonyl 3	4	demalonyl 4	Deacyl 2–4
Delphinidin									
4	8.57 *s*	8.62 *s*	8.59 *s*	8.62 *s*	8.59 *s*	8.62 *s*	8.39 *s*	8.46 *s*	8.93 *s*
6	6.69 *br s*	6.69 *d*,(2.0)	6.70 *br s*	6.69 *d*,(1.9)	6.63 *d*,(1.9)	6.65 *d*,(1.9)	6.70 *d*,(2.0)	6.67 *d*,(2.2)	6.88 *br s*
8	7.20 *br s*	7.35 *d*, (2.0)	7.14 *br s*†	7.35 *d*,(1.9)	7.30 *d*,(1.9)	7.30 *d*,(1.9)	7.38 *d*,(2.0)	7.41 *d*,(2.2)	7.01 *br s*
2′, 6′	7.77 *s*	7.95 *s*	7.76 *s*	7.95 *s*	7.89 *s*	7.92 *s*	7.89 *s*	8.00 *s*	7.92 *s*
p-Hydroxybenzoic acid									
(I)									
2,6	7.29 *d*, (8.1)	7.33 *d*,(8.7)	7.33 *d*, (8.6)†	7.34 *d*,(8.8)	7.31 *d*,(8.8)	7.33 *d*,(8.8)	7.27 *d*,(8.6)	7.35 *d*,(9.1)	
3,5	6.39 *d*, (8.1)	6.45 *d*,(8.7)	6.45 *d*, (8.6)†	6.45 *d*,(8.8)	6.42 *d*,(8.8)	6.44 *d*,(8.8)	6.81 *d*,(8.6)	6.57 *d*,(9.1)	
(II)									
2,6	7.89 *d*, (8.8)	7.96 *d*,(8.7)	7.86 *d*, (8.6)†	7.94 *d*,(8.8)	7.95 *d*,(8.8)	7.95 *d*,(8.8)	7.93 *d*,(9.0)	7.96 *d*,(9.1)	
3,5	6.77 *d*, (8.8)	6.85 *d*,(8.7)	6.77 *d*, (8.6)†	6.84 *d*,(8.8)	6.82 *d*,(8.8)	6.84 *d*,(8.8)	6.86 *d*,(9.0)	6.86 *d*,(9.1)	
(III)									
2,6					7.96 *d*,(8.5)	7.97 *d*,(8.8)	8.01 *d*,(8.6)	8.07 *d*,(9.1)	
3,5					6.86 *d*,(8.5)	6.86 *d*,(8.8)	6.81 *d*,(8.6)	6.91 *d*,(9.1)	
Glucose*									
(A)									
1	5.41	5.39	5.42†	5.37	5.51	5.51	5.46	5.38	5.49
2	3.95	3.90	3.99	3.92	3.87	3.82	3.94	3.86	
3	3.67	3.68	3.68	3.70	3.55		3.67		
4	3.54	3.49	3.40	3.39	3.44		3.48		
5	3.94		3.90	3.65	3.75	3.80~3.30	3.65	3.80~3.10	3.90~3.20
6a	4.44	3.70~3.50	4.43	3.93	4.31		4.31		
6b	4.72		4.71	4.01	4.80		4.77		
(B)									
1	5.40	5.47	5.46†	5.51	5.55	5.64	5.63	5.58	5.40
2	3.70	3.65	3.95	3.90	3.91	4.20	4.03	4.01	
3	3.69	3.63	3.86	3.86	3.86		3.87		
4	3.46	3.46	3.61	3.59	3.55	3.80~3.30	3.47	3.90~3.10	
5	4.14	4.20	4.13	4.24	4.21		3.74		3.90~3.20
6a	4.21	4.23	4.23	4.26	4.27	4.20	4.23	4.31	
6b	5.11	5.03	5.10	5.05	5.04	5.01	4.95	4.93	

(C)									
1	4.15	4.23	4.23†	4.24	4.27	4.32	4.30	4.13	4.98
2	3.49	3.49	3.42	3.61	3.42	3.48	3.65	3.42	
3	] 3.75 ~ 3.50	3.44	3.38	3.57	3.34	] 3.80 ~ 3.30	3.53	] 3.80 ~ 3.10	] 3.90 ~ 3.20
4	]	3.32	3.52	3.30	3.51	]	3.43	]	]
5	3.59	3.53	3.74	3.49	3.55	3.64	3.36	]	]
6a	3.93	3.86	3.95	3.89	3.86	3.83	3.87	3.82	]
6b	4.59	4.72	4.60	4.72	4.71	4.73	4.56	4.73	]
(D)									
1			4.70†	4.33	4.68	4.69	4.85	4.90	
2			3.38	3.44	3.44	3.44	3.51	3.52	
3			]	]	3.34	3.34	3.49	]	
4			]	]	3.70 ~ 3.30	3.80 ~ 3.30	3.44	] 3.90 ~ 3.10	
5			] 3.70 ~ 3.40	] 3.90 ~ 3.25	3.64	3.75	3.75	]	
6a			]	]	3.99	4.32	4.23	4.30	
6b			]	]	4.58	4.81	4.77	4.80	
(E)									
1							4.67	4.69	
2							3.44	3.44	
3							]	]	
4							]	]	
5							] 3.10 ~ 3.90	] 4.00 ~ 3.10	
6a							]	]	
6b							]	]	
Malonic acid									
$-CH_2-$	3.75 ~ 3.50		3.55 ~ 3.30		3.60 ~ 3.40		3.60 ~ 3.50		

* Assigned by ^1H-^1H COSY.
† Assigned by DIFNOE.
Coupling constants (J in Hz) in parentheses.

acid and one molecule of malonic acid. The structure was determined by analysis of ^{1}H NMR spectra including ^{1}H-^{1}H COSY spectra. Five proton signals of delphinidin moiety and 12 proton signals of *p*-hydroxybenzoic acid moieties (I–III) were observed as shown in Table 2. Signals of five anomeric protons appeared at δ 5.46 (Glc A), δ 5.63 (Glc B), δ 4.30 (Glc C), δ 4.85 (Glc D) and δ 4.67 (Glc E), and all the observed vicinal coupling constants of five glucose moieties were 7.7–9.0 Hz. Therefore, all the glucose units must be β-D-glucopyranoside. The eight characteristic methylene proton signals were shifted to the lower magnetic field at δ 4.31, 4.77 (Glc A), δ 4.23, 4.95 (Glc B), δ 3.87, 4.56 (Glc C) and δ 4.23, 4.77 (Glc D), and correlated to each anomeric proton signal of glucose units (A–D), respectively, by analysis of the ^{1}H-^{1}H COSY spectrum of **4**. Then the four OH-6 groups of glucose units (A–D) are acylated with three *p*-hydroxybenzoic acids (I–III) and one malonic acid, respectively. Moreover, to confirm the attachment of malonic acid in this pigment the demalonyl pigment of **4** was obtained, and its structure was analysed by 2D NMR spectrum (Table 2). This result was identical with that of **3**. Therefore, the linkages of sugars and acids are essentially same to those of **3** without the bonding of Glc E. From the above result it revealed that Glc E was free from acid units and a terminal of side chains. Nevertheless, we could not determine either the OH-4 of *p*-hydroxybenzoic acid (II) or (III) was glycosylated with Glc E in **4**, because of small amounts of **4**. Therefore, **4** was tentatively assigned to be delphinidin 3-*O*-[6-*O*-(malonyl)-β-D-glucopyranoside]-7-*O*-glucopyranosyl [2-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-6-*O*-(4-*O*-(6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-*p*-hydroxybenzoyl)-β-D-glucopyranoside], which is a new pigment.

To date, there are six reports on the occurrence of *p*-hydroxybenzoyl anthocyanins in the flowers of *Delphinium hybridum* [12, 13], *Campanula medium* [4, 11], *Dendrobium* cv. Pramot [14] and *Aconitum chinense* [17]. From the chemotaxonomic point of view, the occurrence of acylated anthocyanins with *p*-hydroxybenzoic acid in the plants of Ranunculaceae is still restricted [15, 16]. Only two acylated pigments were identified to be violdelphin in *D. hybridum* [12] and *A. chinense* [17], and cyanodelphin in *D. hybridium* [13]. The former is di-*p*-hydroxybenzoyl delphinidin 3-rutinoside-7-glucoside and the latter is tetra-*p*-hydroxybenzoyl delphinidin 3-rutinoside-7-laminaritrioside. In addition to these two pigments, four new *p*-hydroxybenzoyl anthocyanins have now been isolated from the flowers of *Consolida armeniaca*, and identified to be a triacyl delphinidin 3,7-diglucoside, two triacyl delphinidin 3-glucoside-7-sophorosides, and a tetraacyl delphinidin 3-glucoside-7-sophoroside. Furthermore, *Armeniaca* anthocyanins have a different acylation pattern such as 3-malonylglucoside, and also different glycosylation patterns such as the 3,7-diglucoside or 3-glucoside-7-sophoroside instead of the 3-rutinoside-7-glucoside or 3-rutinoside-7-laminaritrioside in *D. hybridium* and *A. chinense*.

EXPERIMENTAL

Plant material. The blue-violet flowers of *C. armeniaca* were collected in July 1990–1992 at Bayburt-Gumushame, Turkey, and identified. Then these were dried at 40° under shadow, and sent to Japan. These dried petals were kept in silica-gel desiccators at 7° until experiments.

Isolation of anthocyanins. Dried blue-purple flowers (200 g) were immersed with 3% HCO_2H at room temp. for 24 hr and its red-violet pigment extract was filtered. The extract was absorbed on a Diaion HP-20 resin column, and successively washed with 1% HOAc. Then the pigments were eluted with 3% HCO_2H–70% EtOH from the column. After concn, the eluted was fractionated through Sephadex LH-20 CC using HCO_2H–HOAc–MeOH–H_2O (1:2:12:24). The frs containing major anthocyanins were further purified by prep. PC with a solvent (*n*-BuOH–HOAc–H_2O, 4:1:2) and HPLC. Prep. HPLC was performed on Hitachi 6200 system, using a Inertsil ODS-2 (20ϕ × 250 mm) column and a HCO_2H solvent system. The pigment frs were evapd *in vacuo* to dryness. After these processes pigment **1–4** were dissolved in a small vol. of 1% TFA–MeOH, respectively, and pptd by addition of excess Et_2O. Then the pigment **1** was collected and dried to powder (*ca* 8 mg), also, **2** (*ca* 30 mg), **3** (*ca* 15 mg) and **4** (*ca* 20 mg).

Analysis of anthocyanins. Pigment identification was carried out by standard procedures involving H_2O_2 oxidation, alkaline deacylation, demalonylation by acid and acid hydrolysis [9, 19]. Solvents used for TLC were BAW (*n*-BuOH–AcOH–H_2O, 4:1:5), BuH (*n*-BuOH–2M HCl, 1:1), HOAc–HCl (HOAc–HCl–H_2O, 15:3:82) and 1% HCl for anthocyanins, BEW (*n*-BuOH–EtOH–H_2O, 4:2:2.2), PFW (Phenol–HCOOH–H_2O, 75:1:25), EAA (EtOAc–AcOH–H_2O, 3:1:1) for sugars and organic acids. The sample spots on chromatograms were detected with a UV-lamp and bromocresol green spray reagent for acids, and aniline hydrogen phthalate spray regent for sugars. HPLC was performed on a Hitachi 6200 pump system. Analytical HPLC was run on a Inertsil ODS-2 (4.6ϕ × 250 mm) column at 35° with a flow rate of 0.8 ml min^{-1} monitoring at 530 nm. Solvent systems employed were as follows; a linear gradient elution for 40 min from 25 to 85% 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).

Mass and NMR spectrometry. Positive and negative ion FAB-MS of pigments were measured by the JEOL JMS SX-102 mass spectrometer; positive FAB-MS recorded in HCl–MeOH + glycerol/α-thioglycerol and negative FAB-MS in HCL–MeOH + glycerol. ^{1}H NMR spectra of pigments were measured by the JEOL FX-400 spectrometer in 10% DCl–90% DMSO-d_6, 10% DCl–90% CD_3–OD or 10% TFA-*d*-90% DMSO-d_6.

Preparation of deacylanthocyanins, p-hydroxybenzoic acid and glucosyl-p-hydroxybenzoic acid. Pigments **1–4** were dissolved in 2M NaOH–50% MeOH and kept for 30 min under N_2, respectively. The reaction mixtures were acidified with 2M HCl–MeOH and the pigments

were pptd with Et_2O. The Et_2O layer was evapd to dryness and used for preparation of *p*-hydroxybenzoic acid and its glycoside. The residues were diluted with 1% TFA, and the deacyl anthocyanins were absorbed to Amberlite XAD-200 columns, respectively, and washed with 1% TFA. The XAD-2000 column of pigment **1** was eluted with 1% TFA in 70% EtOH, and obtained a deacyl anthocyanin of **1**. The XAD-2000 columns of deacylpigments **2–4** were also eluted with 1% TFA in 70% EtOH, and obtained another deacylanthocyanin of **2–4**. From these eluted solutions, consequently, *p*-hydroxybenzoic acid, 4-glucosyloxybenzoic acid and two kinds of deacylanthocyanin were sepd and purified by HPLC on an ODS-2 column with a HCO_2H solvent system. Each fr. of *p*-hydroxybenzoic acid, 4-glucosyl-*p*-hydroxybenzoic acid and two deacylanthocyanins was evaporated to dryness under reduced pressure [4, 14].

p-Hydroxybenzoic acid and 4-glucosyloxybenzoic acid. *p*-Hydroxybenzoic acid MS (EI, JEOL-D 300), 138 (M^+); ^1H NMR (in CD_3OD), 7.86 ppm (2H, *d*, $J = 8.6$), 6.80 ppm (2H, *d*, $J = 8.6$).; R_f values of TLC, 0.93 (BAW), 0.97 (BEW). 4-glucosyloxybenzoic acid; UV-VIS λ_{max} 246 nm, R_f values (TLC) BAW 0.78, 0.61; 6% HOAc 0.77, 0.35; H_2O 0.59, ^1H NMR data (in DMSO-d_6) were identical with the data of [14].

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