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FIVE ACYLATED PELARGONIDIN GLUCOSIDES IN THE RED FLOWERS OF *HYACINTHUS ORIENTALIS*

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Key Word Index—*Hyacinthus orientalis*; Liliaceae; red flowers; acylated pelargonidin 3,5-diglucosides; *p*-coumaric acid; caffeic acid; ferulic acid; malonic acid; acetic acid.

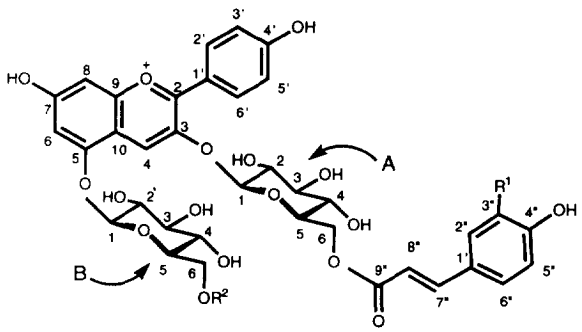
Abstract—Two novel anthocyanins, pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-acetyl-β-D-glucoside) and pelargonidin 3-*O*-(6-*O*-feruloyl-β-D-glucoside)-5-*O*-(6-*O*-malonylglucoside), have been isolated from the red flowers of *Hyacinthus orientalis* cv Holly Hock. Three known acylated anthocyanins, pelargonidin 3-*O*-(6-*O*-caffeoyl-β-D-glucoside)-5-*O*-β-D-glucoside, pelargonidin 3-*O*-(6-*O*-feruloyl-β-D-glucoside)-5-*O*-β-D-glucoside and pelargonidin 3-*O*-(6-*O*-caffeoyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside), were also obtained. Their complete structures were unambiguously elucidated principally by 1D and 2D NMR techniques.

INTRODUCTION

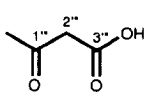
Hyacinthus orientalis L. (Liliaceae) is a popular ornamental plant with blue, red, pink, yellow or white flowers. We have reported about anthocyanins of the flowers of *H. orientalis* L. cv Delft Blue [1] and cv Holly Hock [2]. Acyl groups in the anthocyanins, which were contained in the flowers of Holly Hock, were *p*-coumaric acid and/or malonic acid. Continuing this work on Holly Hock, we obtained five anthocyanins that contained acyl groups other than *p*-coumaric acid or malonic acid and report the complete structure determination of two novel and three known acylated anthocyanins.

RESULTS AND DISCUSSION

The anthocyanins (**1**–**5**) of red flowers of *H. orientalis* were isolated by Amberlite XAD-7 column chromatography, followed by preparative HPLC. UV–Vis and FAB-mass spectra are shown in Table 1. In the FAB-mass spectra of all five anthocyanins, the fragment peak at *m/z* 271 was observed, indicating the presence of pelargonidin as aglycone. In the UV–Vis spectra, $E_{440}/E_{vis,max}$ were near 0.20 for all five anthocyanins, indicating the presence of pelargonidin 3,5-diglycosides [3]. In addition, all five anthocyanins exhibited characteristic absorption bands in the UV between 316 and 332 nm and $E_{acyl}/E_{vis,max}$ were between 0.47 and 0.60, suggesting acylation with one molecule of hydroxycinnamic acid [3].



malonyl ;



acetyl ;

	R^1	R^2
1	OH	H
2	OCH_3	H
3	H	acetyl
4	OCH_3	malonyl
5	OH	malonyl

The FAB-mass spectrum of **5** gave its molecular ion at *m/z* 843, in good agreement with the mass calculated for $C_{39}H_{39}O_{21}$. The fragment peaks were also observed at *m/z* 595 $[M - 248$ (malonylhexose)$]^+$, 519 $[M - 324$ (caffeoylhexose)$]^+$ and 271 [pelargonidin]$^+$, which indicated that **5** was composed of pelargonidin, malonylhexose and caffeoylhexose. Analysis of the ^{1}H NMR spectrum of **5** revealed the presence of pelargonidin, two glucoses, malonic acid and caffeic acid (Table 2). In the

Table 1. Spectral properties of anthocyanins from the red flowers of *Hyacinthus*

Anthocyanin	UV–Vis (0.1% HCl–MeOH) $\lambda_{vis.max}$ (nm)	$\lambda_{acyl.max}$ (nm)	$E_{acyl}/E_{vis.max}$	$E_{440}/E_{vis.max}$	$AlCl_3$ shift	FAB-MS $[M]^+$ and fragment ions
1	509	332	0.48	0.20	0	757, 595, 433, 271
2	508	329	0.51	0.19	0	771, 609, 433, 271
3	509	316	0.56	0.18	0	783, 579, 475, 271
4	508	328	0.60	0.18	0	857, 609, 519, 271
5	509	331	0.47	0.19	0	843, 595, 519, 271

Table 2. ^1H NMR spectra of anthocyanins from the red flowers of *Hyacinthus* (in methanol-d_4 containing 10% TFA-*d*)

	1 δH *J*(ppm) (Hz)	**2** δH *J*(ppm) (Hz)	**3** δH *J*(ppm) (Hz)	**4** δH *J*(ppm) (Hz)	**5** δH *J*(ppm) (Hz)
Aglycone					
4	8.95 *s*	9.00 *s*	8.94 *s*	8.92 *s*	8.94 *s*
6	6.99 *d* 1.9	6.98 *d* 1.6	6.97 *br s*	6.94 *br s*	6.96 *d* 1.7
8	6.92 *br s*	6.94 *d* 1.6	6.96 *br s*	6.92 *br s*	6.94 *d* 1.7
2′	8.56 *d* 9.2	8.55 *d* 9.2	8.55 *d* 9.1	8.50 *d* 8.7	8.54 *d* 9.2
3′	7.04 *d* 9.2	7.03 *d* 9.2	7.03 *d* 9.1	7.00 *d* 8.7	7.02 *d* 9.2
5′	7.04 *d* 9.2	7.03 *d* 9.2	7.03 *d* 9.1	7.00 *d* 8.7	7.02 *d* 9.2
6′	8.56 *d* 9.2	8.55 *d* 9.2	8.55 *d* 9.1	8.50 *d* 8.7	8.54 *d* 9.2
Glucose A					
1	5.38 *d* 7.8	5.35 *d* 7.6	5.43 *d* 7.9	5.41 *d* 7.8	5.40 *d* 7.9
2	3.71 *dd* 9.0, 7.8	3.70 *dd* 9.0, 7.6	3.72 *dd* 8.9, 7.9	3.72 *dd* 8.5, 7.8	3.71 *dd* 9.0, 7.9
3	3.58 *dd* 9.1, 9.0	3.58 *dd* 9.0, 8.7	3.61 *dd* 9.1, 8.9	3.62 *dd* 9.1, 8.5	3.60 *dd* 9.2, 9.0
4	3.50 *dd* 9.5, 9.1	3.55 *dd* 9.0, 8.7	3.49 *dd* 9.5, 9.1	3.55 *dd* 9.7, 9.1	3.50 *dd* 9.6, 9.2
5	3.90 *ddd* 9.5, 6.4, 3.8	3.85 *ddd* 9.0, 6.5, 2.8	3.95 *ddd* 9.5, 8.0, 3.0	3.94 *ddd* 9.7, 7.4, 2.8	3.90 *ddd* 9.6, 7.5, 2.9
6	4.45–4.52 *m*	4.44 *dd* 12, 6.5	4.44 *dd* 12, 8.0	4.39 *dd* 12, 7.4	4.42 *dd* 12, 7.5
	4.45–4.52 *m*	4.52 *dd* 12, 2.8	4.50 *dd* 12, 3.0	4.57 *dd* 12, 2.8	4.52 *dd* 12, 2.9
Glucose B					
1	5.18 *d* 7.9	5.15 *d* 7.8	5.21 *d* 7.8	5.17 *d* 7.8	5.18 *d* 7.9
2	3.77 *dd* 9.3, 7.9	3.73 *dd* 9.3, 7.8	3.79 *dd* 9.2, 7.8	3.75 *dd* 9.2, 7.8	3.76 *dd* 9.1, 7.9
3	3.57 *dd* 9.3, 9.1	3.55 *dd* 9.3, 9.2	3.58 *dd* 9.3, 9.2	3.58 *dd* 9.2, 9.2	3.58 *dd* 9.3, 9.1
4	3.43 *dd* 9.7, 9.1	3.42 *dd* 9.2 8.4	3.47 *dd* 9.4, 9.3	3.41 *dd* 9.8, 9.2	3.46 *dd* 9.6, 9.3
5	3.62 *ddd* 9.7, 6.5, 2.2	3.55–3.58 *m*	3.77–3.80 *m*	3.77 *ddd* 9.8, 6.0, 1.9	3.79 *ddd* 9.6, 6.2, 2.0
6	3.75 *dd* 12, 6.5	3.69 *dd* 12, 5.9	4.16 *dd* 12, 6.1	4.11 *dd* 12, 6.0	4.25 *dd* 12, 6.2
	4.02 *dd* 12, 2.2	3.94 *dd* 12, 2.3	4.43 *dd* 12, 2.1	4.52 12, 1.9	4.56 *dd* 12, 2.0
Aromatic acid moiety					
2″	6.92 *s*	6.91 *d* 1.6	7.19 *d* 8.5	6.82 *d* 1.9	6.79 *d* 1.9
3″			6.69 *d* 8.5		
5″	6.70 *s*	6.74 *d* 8.2	6.69 *d* 8.5	6.66 *d* 8.1	6.67 *d* 8.2
6″	6.70 *s*	6.89 *dd* 8.2, 1.6	7.19 *d* 8.5	6.78 *d* 8.1, 1.9	6.71 *dd* 8.2, 1.9
7″	7.27 *d* 16	7.37 *d* 16	7.34 *d* 16	7.34 *d* 16	7.27 *d* 16
8″	6.17 *d* 16	6.21 *d* 16	6.23 *d* 16	6.21 *d* 16	6.16 *d* 16
3″-OMe		3.80 *s*		3.73 *s*	
Malonic/acetic acid moiety					
2‴			1.99 *s*	3.41 *s*	nd*

*nd = not detected.

caffeic acid moiety, H-7″ and H-8″ had a large coupling constant (J = 16 Hz); thus, the olefinic part of the caffeic acid moiety has a *trans* configuration. The signals of two hexoses were observed in the region of δ3.46–5.40 and all vicinal coupling constants of two hexose moieties were at 7.9–9.6 Hz. The chemical shifts and the large coupling constants of two anomeric protons appeared at δ5.40 (*d*, J = 7.9 Hz, glucose A) and δ5.18 (*d*, J = 7.9 Hz, glu-

cose B). Therefore, both hexose units must be β-D-glucopyranoside. By analysis of the proton network of the glucose moieties, the anomeric protons (δ5.40 and 5.18) of glucose A and B were finally correlated to non-equivalent methylene protons of C-6 at δ4.42 and 4.52, and δ4.25 and 4.56, respectively. The downfield shift of these methylenes indicated that the caffeoyl/malonyl moieties were attached to 6-OH of the glucoses.

In order to confirm the position of the ester linkage, the heteronuclear multiple-bond correlation (HMBC) spectrum was determined. A correlation between H-6 (δ4.42 and 4.52) of glucose A and a carbonyl carbon (δ169.2) of caffeic acid was observed, indicating that caffeic acid was attached to 6-OH of glucose A through an ester bond. Similarly, malonic acid is attached to the 6-OH of glucose B, shown by the presence of the correlation between H-6 (δ4.25 and 4.56) of glucose B and one of the carbonyl carbons (δ168.6) of malonic acid. The position of the glucosidic linkage was determined by HMBC and nuclear Overhauser effect (NOE) difference spectra. The correlation between the anomeric proton (δ5.40) of glucose A and C-3 (δ145.7) of pelargonidin was observed, indicating that glucose A was attached to the 3-OH of pelargonidin. Similarly, glucose B was attached to the 5-OH of pelargonidin, shown by the presence of the correlation between the anomeric proton (δ5.18) of glucose B and C-5 (δ156.6) of pelargonidin. These connectivities were also confirmed by the NOE experiments, in which negative NOE of H-4 (δ8.94) and H-6 (δ6.96) of pelargonidin to anomeric protons of glucoses A and B were observed, respectively. Thus, **5** is pelargonidin 3-*O*-(6-*O*-caffeoyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside).

The anthocyanin **4** gave a molecular ion at *m/z* 857 by FAB-MS, which is 14 mass units (methylene) larger than that of **5**, in good agreement with the mass calculated for $C_{40}H_{41}O_{21}$. Fragment peaks were also observed at *m/z* 609 [M − 248 (malonylhexose)]$^+$, 519 (M − 338 (feruloylhexose)]$^+$ and 271 [pelargonidin]$^+$. In the ^{1}H NMR spectrum, the singlet of methoxyl (δ3.73) was found. The position of the methyl was determined by HMBC. A correlation between the methyl protons (δ3.73) and C-3″ (δ149.0) of the 1,2,4-trisubstituted benzene ring was observed, indicating that the aromatic moiety should be ferulate.

The anthocyanin **3** gave a molecular ion at *m/z* 783 by FAB-MS, in good agreement with the mass calculated for $C_{38}H_{39}O_{18}$. Fragment peaks were also observed at *m/z* 579 [M − 204 (acetylhexose)]$^+$, 475 [M − 308 (coumaroylhexose)]$^+$ and 271 [pelargonidin]$^+$. The ^{1}H NMR spectra of **3** were similar to those of **5**, except for the signals of acyl moieties. An acyl moiety was determined to be acetic acid by ^{1}H and ^{13}C NMR. In the HMBC, a correlation between the methylene protons (δ4.16 and 4.43) of glucose B and a carbonyl carbon (δ172.9) of acetic acid were observed, which indicated that acetic acid was present instead of malonate as shown in other anthocyanins. In the ^{1}H NMR spectrum of the aromatic acyl moiety, the signals of the 1,2,4-trisubstituted benzene ring found in **5** were not observed and the presence of a 1,4-disubstituted benzene ring was demonstrated instead of caffeate (Table 2). It was indicated that *trans-p*-coumaric acid was present instead of caffeate.

The anthocyanins **1** and **2** gave molecular ions at *m/z* 757 and 771, respectively, by FAB-MS. Each molecular ion was in good agreement with the mass calculated for $C_{36}H_{37}O_{18}$ and $C_{37}H_{39}O_{18}$, respectively. The ^{1}H NMR spectra of **1** and **2** were similar to those of **5** and **4**, except for the malonate region. Fragment peaks were also observed at *m/z* 595 [M − 162(hexose)]$^+$, 433 [M − 324 (caffeoylhexose)]$^+$ and 271 [pelargonidin]$^+$ for **1** and *m/z* 609 [M − 162 (hexose)]$^+$, 433 [M − 338 (feruloylhexose)]$^+$ and 271 [pelargonidin]$^+$ for **2**. These data suggested that **1** and **2** corresponded to the demalonyl derivatives of **5** and **4**, respectively.

The structures of anthocyanins **1**–**4** were hence deduced to be pelargonidin 3-*O*-(6-*O*-caffeoyl-β-D-glucoside)-5-*O*-β-D-glucoside, pelargonidin 3-*O*-(6-*O*-feruloyl-β-D-glucoside)-5-*O*-β-D-glucoside, pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-acetyl-β-D-glucoside) and pelargonidin 3-*O*-(6-*O*-feruloyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside), respectively. The complete assignments of the ^{1}H and ^{13}C signals deduced with 1D and 2D techniques are shown in Tables 2 and 3.

The anthocyanins **3** and **4** are novel anthocyanins. The anthocyanin **3** contains acetic acid as an acylglucosyl moiety. So far, this type of acetylated anthocyanin has been reported in the fruit of *Eurya japonica* [4], the flower of *Verbena hybrida* [5–7] and the flower of *Tibouchina urvilleana* [8]. Anthocyanidins acylated with feruloyl glucoside at 3-OH and with malonylglucoside at 5-OH group have only been reported as a cyanidin derivative in cell cultures of *Perilla frutescens* var. *crispa* [9], but the corresponding pelargonidin derivative, **4**, has not previously been identified.

EXPERIMENTAL

Plant material. Bulbs (*ca* 6 cm in diameter) of *H. orientalis* L. cv Holly Hock were obtained from Heiwaen Co., in Sept. 1991, and then grown in the experimental field. Fresh red flowers were collected from March to April 1992, and freeze-dried.

Isolation of anthocyanins. Freeze-dried flowers (25 g) were extracted with EtOH–HOAc–H_2O (10:1:9) at 4°. The concd extract was adsorbed on an Amberlite XAD-7 column and washed with 5% HOAc. The anthocyanins were eluted by 50% MeOH containing 5% HOAc. For further purification, the crude anthocyanins were purified by prep. HPLC using a Chromatorex-ODS (Fuji Silysia Chemical Ltd) column with a flow rate of 5–8 ml min^{-1} monitoring at 510 nm for anthocyanins. Two solvent systems were used for elution: 25–60% MeCN–HOAc–H_2O (5:4:11) containing 0.5% TFA and then 10–25% MeOH containing 15% HOAc and 0.5% TFA. To replace the counter anion of anthocyanins with trifluoroacetate, the concd frs were adsorbed on activated

Table 3. ^{13}C NMR spectra of anthocyanins from the red flowers of *Hyacinthus* (in methanol-d_4 containing 10% TFA-*d*)

	1 δ_C(ppm)	**2** δ_C(ppm)	**3** δ_C(ppm)	**4** δ_C(ppm)	**5** δ_C(ppm)
Aglycone					
2	165.3	165.6	165.2	165.0	165.2
3	145.6	145.7	145.8	145.8	145.7
4	136.3	137.0	135.6	135.8	136.1
5	156.8	156.9	156.6	156.6	156.6
6	106.0	106.1	106.1	106.2	106.3
7	170.1	170.1	169.9	169.8	170.0
8	97.4	97.6	97.4	97.5	97.4
9	157.2	157.4	157.1	157.0	157.2
10	113.4	113.5	113.3	113.3	113.4
1′	120.4	120.6	120.6	120.5	120.6
2′	136.1	136.2	136.2	136.1	136.1
3′	118.1	118.1	118.2	118.1	118.1
4′	167.3	167.4	167.4	167.4	167.3
5′	118.1	118.1	118.2	118.1	118.1
6′	136.1	136.2	136.2	136.1	136.1
Glucose A					
1	103.1	103.4	102.5	102.9	102.8
2	74.5	74.6	74.4	74.5	74.5
3	77.9	78.0	78.2	78.1	78.1
4	71.3	71.7	72.3	71.9	72.1
5	79.0	75.8	75.5	75.5	75.6
6	62.7	64.1	64.5	64.4	64.4
Glucose B					
1	102.8	103.1	102.8	102.7	102.8
2	74.8	74.8	74.8	74.7	74.7
3	78.1	77.9	77.7	77.7	77.7
4	72.1	71.2	71.1	71.1	71.0
5	75.6	78.8	76.0	75.9	75.9
6	64.2	62.5	64.5	65.1	65.1
Aromatic acid moiety					
1″	127.3	127.4	126.7	127.3	127.4
2″	115.2	112.0	131.4	111.9	115.8
3″	146.5	149.2	116.8	149.0	146.6
4″	149.7	150.7	161.3	150.6	149.5
5″	116.4	116.5	116.8	116.4	116.4
6″	123.6	124.2	131.4	124.2	122.9
7″	147.4	147.3	147.1	147.3	147.4
8″	114.7	115.0	114.7	115.0	114.8
9″	169.2	169.1	169.2	169.1	169.2
3″-OMe		56.5		56.5	
Malonic acid/acetic acid moiety					
1‴			172.9	168.5	168.6
2‴			20.7	41.9	41.9
3‴				170.5	170.5

Sep-Pak tC18 (Waters Associates). The cartridge was washed with 0.5% aq. TFA and then eluted with MeOH containing 0.5% TFA. The purified anthocyanins were concd to dryness under N_2. The residue was dissolved in a small amount of 1% TFA and then freeze-dried to give 5 anthocyanins as powders (**1**, 2.1 mg; **2**, 3.5 mg, **3**, 11.6 mg, **4**, 21.2 mg, **5**, 11.1 mg).

Spectral analysis. UV–Vis spectra were measured in MeOH containing 0.1% HCl. FAB-mass spectra were obtained in a positive mode with glycerol as matrix. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained using 10% TFA-*d*–methanol-d_4 as a solvent.

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