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TWO MALONYLATED ANTHOCYANIDIN GLYCOSIDES IN *RANUNCULUS ASIATICUS*

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Key Word Index—*Ranunculus asiaticus*; Ranunculaceae; flower colour; acylated anthocyanins; delphinidin and cyanidin 3-malonylsambubiosides.

Abstract—Two new acylated anthocyanins were isolated from pink and purple flowers of *Ranunculus asiaticus* cultivars and identified as 3-*O*-[2-*O*-(β-D-xylopyranosyl)-6-*O*-(malonyl)-β-D-glucopyranosides] of delphinidin and cyanidin.

INTRODUCTION

Ranunculus asiaticus is a popular ornamental, and its garden hybrids have a wide range of flower colour (white, yellow, orange, red, pink and red–purple). As no information is available [1–4], we started to investigate the flower anthocyanins in these cultivars and found two new acylated anthocyanins along with two known ones. In this paper we report the structural elucidation of these anthocyanins.

RESULTS AND DISCUSSION

We isolated four anthocyanins (**1–4**) from the flowers of *R. asiaticus* cultivars using a process similar to that described previously [5]. Their relative concentrations were 67% (pigment **1**) and 13% (pigment **2**) in the extract of pink flowers by HPLC analysis, and 55% (pigment **3**) and 15% (pigment **4**) in the extract of red–purple flowers.

Pigments **1** and **2** were hydrolysed to give cyanidin, glucose and xylose, whereas **3** and **4** gave delphinidin and the same sugars by 2 M HCl. On alkaline hydrolysis, **1** and **3** changed into **2** and **4**, respectively, with production of malonic acid. UV–VIS spectral features of these compounds resembled each other and showed that these are cyanidin (**1** and **2**) or delphinidin (**3** and **4**) 3-glycosides free from phenolic acids (Table 1) [6]. Also, **2** and **4**, were identical with the standard 3-sambubiosides of cyanidin and delphinidin on TLC and HPLC, respectively (Table 1). On partial acid hydrolysis **2** (deacylated **1**) and **4** (deacylated **3**) converted into each of their aglycones with one intermediate anthocyanin. Furthermore, the FAB mass spectra of **1–4** gave $[M]^+$ at *m/z* 667, 581, 683 and 597, respectively (Table 1). These data show that the structures of **1–4** are cyanidin 3-malonylsambubioside, 3-sambubioside, delphinidin 3-malonylsambubioside and 3-sambubioside, respectively.

The detailed structures of these compounds were elucidated by ^{1}H NMR analyses including ^{1}H–^{1}H COSY and difference NOE as shown in Table 2. In the aromatic region the characteristic signals were easily correlated to cyanidin or delphinidin nuclear protons. The signals of sugar moieties were observed in the region of δ 5.67–2.72. Two anomeric protons appeared in these compounds, **1–4**, and had large coupling constants (J = 7.7 Hz), indicating all of these sugar

Table 1. Chromatographic and spectral properties of anthocyanins from the pink and purple flowers of *Ranunculus asiaticus*

	R_f values (×100)†				R_t†	Spectral data in 0.1% HCl–MeOH				FAB-MS
Anthocyanins*	BAW	BuH	1%HCl	HAc–HCl	(min)	λ_{max} (nm)	E_{440}/E_{max}	E_{310}/E_{max}	+$AlCl_3$	$[M]^+$
Cy3mGX (**1**)	53	48	40	69	19.7	283, 529	22	15	+	667
Cy3GX (**2**)	48	32	27	55	13.1	282, 529	22	12	+	581
Dp3mGX (**3**)	51	41	40	67	15.5	279, 541	17	15	+	683
Dp3GX (**4**)	42	25	27	53	9.2	278, 541	18	18	+	597

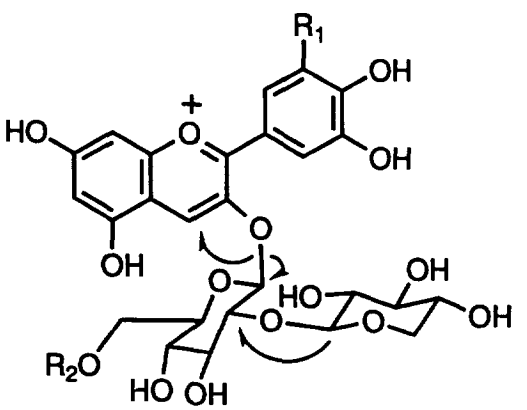
*1, Cyanidin 3-malonylsambubioside; 2, cyanidin 3-sambubioside; 3, delphinidin 3-malonylsambubioside; 4, delphinidin 3-sambubioside.
†See Experimental.

Table 2. ^{1}H NMR data for the anthocyanins from flowers of *Ranunculus asiaticus* (TFA–DMSO-d_6, 1:9, at 25°)

H	1	2	3	4
Anthocyanidin				
4	8.82 *s*	8.88 *s*	8.76 *s*	8.80 *s*
6	6.89 *d*(1.8)	6.91 *d*(1.5)	6.85 *br*	6.86 *d*(2.0)
8	7.01 *d*(1.8)	7.03 *d*(1.5)	6.99 *br*	6.99 *d*(2.0)
2′ or 2′ and 6′	7.97 *d*(2.3)	8.01 *d*(2.3)	7.75 *s*	7.77 *s*
5′	7.07 *d*(8.7)	7.09 *d*(8.6)		
6′	8.32 *dd*(2.3, 8.7)	8.31 *dd*(2.3, 8.6)		
Glucose*				
1	5.64 *d*(7.7)	5.60 *d*(7.7)	5.67 *d*(7.7)	5.64 *d*(7.7)
2	3.93 *t*(9.3)	3.86 *t*(8.3)	4.02 *t*(8.1)	3.97 *t*(8.1)
3	3.67 *t*(9.1)	3.63 *t*(8.8)	3.67 *t*(9.0)	3.64 *t*(9.0)
4	3.32 *t*(9.1)	3.32 *t*(8.6)	3.38 *t*(7.7)	3.36 *t*(9.4)
5	3.88 *t*(8.4)	3.52–3.56 *m*	3.95 *t*(9.6)	3.54 *dd*(5.6, 9.4)
6a	4.13 *dd*(7.6, 10.8)	3.54–3.60 *m*	4.17 *dd*(7.7, 11.6)	3.59 *m*
6b	4.41 *d*(10.8)	3.67 *d*(10.3)	4.41 *d*(11.6)	3.69 *d*(11.1)
Xylose*				
1	4.71 *d*(7.7)	4.68 *d*(7.7)	4.59 *d*(7.7)	4.56 *d*(7.7)
2	2.97 *t*(8.3)	2.95 *t*(8.2)	2.96 *t*(7.7)	2.95 *t*(8.6)
3	3.12 *t*(8.8)	3.12 *t*(8.8)	3.08 *t*(8.6)	3.07 *t*(8.6)
4	3.25 *dd*(5.4, 8.8)	3.22–3.28 *m*	2.73 *t*(10.7)	2.72 *t*(11.1)
5a	2.90–2.96 *m*	2.93 *dd*(10.5, 17)	3.14 *m*	3.12 *m*
5b	3.53 *dd*(5.4, 12)	3.47–3.53 *m*	3.22 *dd*(4.7, 10.7)	3.20 *dd*(4.7, 11.1)
Malonic acid				
CH_2	3.39 *d*(16)		3.35–3.36	
	3.34 *d*(16)			

*Assigned by ^{1}H–^{1}H COSY.
Coupling constants (*J* in Hz) in parentheses.

units to be β-forms. Also, as the vicinal coupling constants of their ring protons were $J = 4.7$–17 Hz, these units must be in the β-D-pyranose forms [7]. The position of attachment was confirmed by difference NOE (Fig. 1). The appearance of NOE signals at H-4 of cyanidin (**1** and **2**) or delphinidin (**3** and **4**) by irradiation at H-1 of glucoses of **1**–**4** indicated glucoses to be attached to OH-3 of each aglycone through glycosidic bonds. Also, the presence of NOEs was observed between H-1 of xylose and H-2 of glucose in each of the four compounds. Furthermore, in **1** and **3**, the signals of H-6a and 6b of glucose were shifted downfield in comparison with those in **2** and **4**, respectively, indicating that the malonyl groups are attached to OH-6 of glucoses in **1** and **3**, respectively. Therefore, the structures of **1**–**4** are cyanidin 3-*O*-[2-*O*-(β-D-xylopyranosyl)-6-*O*-(malonyl)-β-D-glucopyranoside], cyanidin 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-glucopyranoside], delphinidin 3-*O*-[2-*O*-(β-D-xylopyranosyl)-6-*O*-(malonyl)-β-D-glucopyranoside] and delphinidin 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-glucopyranoside], respectively.



1 $R_1 = H, R_2 = COCH_2CO_2H$
2 $R_1 = R_2 = H$
3 $R_1 = OH, R_2 = COCH_2CO_2H$
4 $R_1 = OH, R_2 = H$

Fig. 1. Anthocyanins **1**–**4** from *Ranunculus asiaticus*. Observed NOEs are indicated by arrows.

EXPERIMENTAL

Plant material. We obtained the pink and purple flower petals of *R. asiaticus* cv. 'Wander Land' from Aya Engei Co., Aya-cho, Miyazaki. Fresh petals were collected and air-dried at 45°.

Extraction and isolation. The dried petals were extracted with 5% HOAc at room temp. overnight. The filtered extract was adsorbed on Diaion HP-20, washed

with 1% HOAc and eluted with HOAc–MeOH–H_2O (1:14:6). The eluate was concd, and fractionated over Sephadex LH-20 CC using HOAc–EtOH–H_2O (1:6:12). The frs containing anthocyanins were further purified by PC (*n*-BuOH–HOAc–H_2O, 4:1:2, and 15% HOAc) and HPLC. Prep. HPLC was performed on a Hitachi 6200 system, using an Inertsil ODS-2 (20ϕ × 250 mm) column and HOAc or HCO_2H as solvent. We obtained **1** (*ca* 25 mg) and **2** (*ca* 12 mg) from the pink petals (30 g, dry wt), and **3** (46 mg) and **4** (16 mg) from the purple petals (50 g).

Analysis. Characterization of the 4 anthocyanins was carried out with standard procedures involving alkaline deacylation and acid hydrolysis [8]. They were also analysed with FAB-MS and ^{1}H NMR spectra including ^{1}H–^{1}H COSY and difference NOE [5, 7]. TLC was carried out on microcrystalline cellulose (Avicel SF, Funakoshi) using BAW (*n*-BuOH–HOAc–H_2O, 4:1:5), BuH(*n*-BuOH–2 M HCl, 1:1), 1% HCl and HAc–HCl(HOAc–HCl–H_2O, 15:3:82) for anthocyanins; HOAc–HCl–H_2O. (30:3:10) and HCO_2H–HCl–H_2O (5:2:3) for anthocyanidins; BAW, *i*-PrOH–*n*-BuOH–H_2O (7:1:2) and PhOH–H_2O (4:1) for sugars; and BAW, EtOAc–HOAc–H_2O (3:1:1) and EtOH–H_2O–NH_4OH (16:3:1) for aliphatic acids. HPLC was run on an Inertsil ODS-2 column (4.6ϕ × 250 mm) at 35°, with a flow rate of 0.8 ml min^{-1} and monitoring at 520 nm. Solvent systems used were as follows; linear gradient elution for 40 min from 25 to 85% solvent B (1.5% H_3PO_4, 20% HOAc, 25% MeCN) in solvent A (1.5% H_3PO_4). ^{1}H NMR spectra (400 MHz) were obtained with a JOEL JNM-GX 400 spectrometer in TFA–DMSO-d_6 (1:9).

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REFERENCES

1. Harborne, J. B. (1967) *Comparative Biochemistry of the Flavonoids*, p. 126. Academic Press, London.
2. Hrazdina, G. (1982) in *The Flavonoids. Advances in Research* (Harborne, J. B. and Mabry, T. J., eds), p. 135. Chapman & Hall, London.
3. Harborne, J. B. and Greyer, R. J. (1988) in *The Flavonoids, Advances in Research Since 1980* (Harborne, J. B., ed.), p. 1. Chapman & Hall, London.
4. Strack, D. and Wray, V. (1994) in *The Flavonoids, Advances in Research Since 1986* (Harborne, J. B. ed.), p. 1. Chapman & Hall, London.
5. Toki, K., Saito, N., Harada, K., Shigihara, A. and Honda, T. (1995) *Phytochemistry* **39**, 243.
6. Harborne, J. B. (1958) *Biochem. J.* **70**, 22.
7. Markham, K. R. and Geiger, H. (1994) in *The Flavonoids, Advances in Research Since 1986* (Harborne, J. B., ed.), p. 441. Chapman & Hall, London.
8. Harborne, J. B. (1984) *Phytochemical Methods*, 2nd Edn. Chapman & Hall, London.