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ACYLATED PELARGONIDIN 3,7-GLYCOSIDES FROM PINK FLOWERS OF *SENECIO CRUENTUS*

KENJIRO TOKI, NORIO SAITO,* HARUMITSU KUWANO,† ATSUSHI SHIGIHARA‡ and TOSHIO HONDA‡

Laboratory of Floriculture, Minami-Kyushu University, Takanabe, Miyazaki 884, Japan; *Chemical Laboratory, Meiji-Gakuin University, Totsuka, Yokohama 224, Japan; †Institute of Science and Technology, Inc., Shinagawa, Tokyo 140, Japan; ‡Institute of Medicinal Chemistry, Hoshi University, Shinagawa, Tokyo 142, Japan

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Key Word Index—*Senecio cruentus*; Compositae; flower colour; acylated anthocyanins; pelargonidin 3,7-diglucoside; caffeic and malonic acids.

Abstract—Two new acylated pelargonidin glycosides, and pelargonidin 3-malonylglucoside were isolated from the pink flowers of *Senecio cruentus*. The structure of the main pigment was determined to be pelargonidin 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside)-7-*O*-(6-*O*-(4-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranosyl)-*trans*-caffeyl)-β-D-glucopyranoside), and the second new pigment was identified as pelargonidin 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside)-7-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranoside).

INTRODUCTION

Cineraria, *Senecio cruentus*, is a popular ornamental plant, and shows in flower colour white, pink, orange-red, purple-red, red-purple, mauve, purple and purple-blue. To date, delphinidin and cyanidin glycosides have been isolated from the purple-blue and red-purple flowers of this plant [1–6]. First, Yoshitama *et al.* isolated an acylated delphinidin 3,7,3′-triglucoside from the purple-blue flowers and named it cinerarin [1, 2]. Later, Goto *et al.* determined the structure of this compound unambiguously by spectroscopic analyses [3]. Three acylated cyanidin glucosides were then isolated from the red-purple and purple-red cultivars by two groups who also elucidated their structures [4–6]. However, there is no report on acylated pelargonidin glycosides in this species. We have now isolated two new pelargonidin glycosides from pink flowers of cineraria, and elucidated their structures by chemical and spectroscopic means.

RESULTS AND DISCUSSION

We isolated three anthocyanins from the pink flowers of *Senecio cruentus* cv. 'Jupitar Pink-White' by extraction with 5% acetic acid, and purification with Diaion HP-20 and Sephadex LH-20 CC, PC and HPLC. The main anthocyanin (**1**) and two minor ones (**2** and **3**) were obtained as orange-red powders. TLC, HPLC, UV-VIS and FAB-mass spectral data are shown in Table 1. Acid hydrolysis of **1** and **2** gave pelargonidin, glucose, caffeic acid and malonic acid.

Alkaline hydrolysis of **1** gave deacylanthocyanin, caffeic acid, glucosylcaffeic acid and malonic acid. Deacylanthocyanin was identified as pelargonidin 3,7-diglucoside by standard analysis according to the previous reports [6, 7]. The FAB-mass spectrum of **1** gave a $[M]^+$ at m/z 1167, in good agreement with the mass calculated for $C_{54}H_{55}O_{29}$ (1167.283), which was composed of pelargonidin with three molecules of glucose, two of caffeic acid and one of malonic acid. The detailed structure was elucidated by ^{1}H NMR spectrometry including ^{1}H–^{1}H COSY and DIFNOE [8, 9]. In the ^{1}H NMR spectrum of **1**, seven characteristic proton signals at δ8.88(*s*, H-4), 8.56 (*d*, J = 9.0 Hz, H-2′, H-6′), 7.39 (*br s*, H-8), 7.04 (*d*, J = 9.0 Hz, H-3′, H-5′) and 6.86 (*br s*, H-6) showed aromatic protons of pelargonidin nucleus as shown in Table 2. Two pairs of doublet signals (δ 7.37, 6.23 and δ7.54, 6.33) with large coupling constants (J = 15.4 and 15.8 Hz) indicated the presence of the *trans*-olefinic protons of caffeic acids I and II. Six protons of two caffeic acid nuclei were also observed. The proton signals of the sugar part were observed in the region of δ3.29–5.38 and signals of three anomeric protons were assigned to Glc A (δ5.38, *d*, J = 7.3 Hz), Glc B (δ5.33, *d*, J = 7.3 Hz) and Glc C (δ4.86, *d*, J = 7.3 Hz) by analysis of ^{1}H–^{1}H COSY and DIFNOE. The vicinal coupling constants of three glucose A–C protons (J = 7.7–13.2 Hz) showed that these glucose moieties have the β-configuration. All methylene proton signals of three glucosyl moieties were shifted to the lower magnetic field (Glc A, δ4.30, 4.52; Glc B, δ4.18, 4.71; Glc C, δ4.27, 4.46) indicating all the OH-6 groups of three glucose units to be acylated with three acids. In

Table 1. Chromatographic and spectral properties of anthocyanins from the pink flowers of *Senecio cruentus*

Anthocyanin*	R_f values (×100)†				R_t† (min)	Spectral data in 0.1% HCl–MeOH			FAB-MS [M]†
	BAW	BuH	1% HCl	HOAc–HCl		λ_{max}(nm)	E_{acyl}/E_{max}(%)	E_{440}/E_{max}(%)	
1	40	13	6	22	29.5	284, 324, 513	97	34	1167
2	56	30	23	49	21.1	282, 331, 508	66	36	843
3	63	52	14	41	21.0	269, 510	—	40	519
Related anthocyanins									
Pg3G7G	38	7	40	65	5.1	279, 503	—	43	—
Pg3G	59	36	13	34	14.6	270, 510	—	40	—

*1, Pelargonidin 3-malonylglucoside-7-caffeylglucosylcaffeylglucoside; **2**, pelargonidin 3-malonylglucoside-7-caffeylglucoside; **3**, pelargonidin 3-malonylglucoside; Pg3G7G, pelargonidin 3,7-diglucoside (deacylated **1** or **2**); Pg3G, pelargonidin 3-glucoside.

†BAW, *n*-BuOH–HOAc–H_2O (4:1:5); BuH, *n*-BuOH–2M HCl (1:1); HOAc-HCl, AcOH–HCl–H_2O (15:3:82); R_t retention time; analytical condition, see Experimental.

order to determine the linkages and/or the position of attachments of glucose and acid units in the molecule, DIFNOE spectra were measured (Fig. 1). By irradiation at the H-4 of pelargonidin the observation of a negative NOE signal at H-1 of Glc A showed Glc A to be bonded at the OH-3 of pelargonidin through a glycosidic bond. Also Glc B was indicated to be glucosylated at the OH-7 of pelargonidin, because of the presence of NOEs between H-1 of Glc B and H-6 and/or H-8 of pelargonidin. Furthermore, the occurrence of NOE was observed at the signals of H-2, H-6, H-5 and H-α of caffeic acid I by irradiation of H-1 of Glc B. Therefore, caffeic acid I was bonded to the OH-6 of Glc B. Similarly, Glc C was indicated to be glycosylated at the OH-4 of caffeic acid I, because of the occurrence of NOE between H-1 of Glc C and H-5 of caffeic acid. By H_2O_2 degradation of **1** malonylglucose was produced and identified with TLC. Therefore, Glc C was esterified at its OH-6 with caffeic acid II, as the methylene protons of Glc C were shifted to the lower magnetic field (Table 2). The structure of **1** was thus pelargonidin 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside)-7-*O*-(6-*O*-(4-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranosyl)-caffeyl)-β-D-glucopyranoside), which is a new anthocyanin [10, 11].

Alkaline hydrolysis of **2** gave pelargonidin 3,7-diglucoside, caffeic acid and malonic acid. The FAB-mass spectrum of **2** gave $[M]^+$ at 843 *m/z*, which is identical with the mass calculated for $C_{39}H_{39}O_{21}$ (843.198). The ^{1}H NMR spectrum indicated that **2** was composed of pelargonidin with two glucose, one caffeic and malonic acids. By analysis of the ^{1}H–^{1}H COSY spectrum of **2**, seven proton signals of pelargonidin and five proton signals of caffeic acid were assigned as shown in Table 2. The proton signals of sugar and malonyl units were determined by COSY and HOHAHA spectra [12] (Table 2). The ^{13}C signals of **2** were correlated with proton signals by HMQC spectrum [13], and assigned as shown in the Experimental. The proton signals of the sugar units were observed in the region of δ3.30–5.45, and the vicinal coupling constants of two glucose moieties were 7.3–12.2 Hz. Signals of the two anomeric protons appeared at δ5.45 (*d*, J = 7.3, Glc A) and δ5.33 (*d*, J = 7.3, Glc B). Therefore, all the glucose units must be β-glucopyranoside. The four characteristic protons at δ4.51, 4.20 (Glc A) and δ4.56, 4.23 (Glc B) were assigned to the methylenes of Glc A and B by ^{1}H–^{1}H COSY, HOHAHA and HMQC spectra, indicating caffeic and malonic acids to be attached to both OH-6 of Glc A and B. To elucidate the linkages and/or the position of attachments of glucose and acid units, the DIFNOE and HMBC [14] spectra of **2** were measured (Figs 1 and 2). Glc A was attached to the OH-3 of pelargonidin and Glc B to the OH-3 through the glycosidic bonds. By analysis of the HMBC spectrum of **2** it was revealed that the carbonyl group of caffeic acid was bonded to the OH-6 of Glc B, and one of the carbonyl groups in the malonic acid unit was also linked to the OH-6 of Glc A. Therefore, **2** was pelargonidin 3-*O*-(6-*O*-(malonyl)-β-D-glucopyranoside)-7-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranoside), which is a new pigment [10, 11]. The structure of **3** was also identified as pelargonidin 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside) by the analysis of FAB-mass and NMR spectra (Tables 1, 2 and Experimental).

EXPERIMENTAL

Plant material. We obtained seeds of cineraria cultivar 'Jupitar Pink and White' from Sakata Seed Co., Ltd, Yokohama, and cultivated it in a greenhouse of the garden of Minami-Kyushu University. Flower petals were collected and air-dried at 45°.

Isolation of anthocyanins. The dried petals (50 g) were extracted with 5% HOAc at room temp. for 24 hr and filtered. In the crude extract one major and 13 minor anthocyanins were detected with HPLC. The filtered extract was adsorbed on Diaion HP-20 resin column, and successively washed with 1% HOAc. The pigments were eluted with HOAc–MeOH–H_2O (1:14:5). After concn, the eluate was fractionated over Sephadex LH-20 CC using HOAc–MeOH–H_2O (1:6:12). The orange-red frs were further purified by prep. PC (BAW and 15% HOAc) and HPLC (HOAc solvent system). Pigments **1** (300 mg), **2** (20 mg) and **3** (10 mg) were obtained.

Analysis. Characterization of these anthocyanins was carried out with UV-VIS, FAB-mass and ^{1}H (400 MHz)

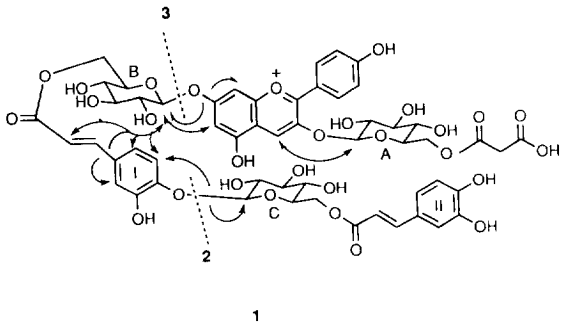
Table 2. ^{1}H NMR spectral data of acylated pelargonidin glycosides in the pink flowers of *Senecio cruentus* (CF_3CO_2D–DMSO-d_6, 1:9 at 25°)

H	1	2	3
Pelargonidin			
4	8.88 *s*	8.91 *s*	8.93 *s*
6	6.86 *br s**†	6.92 *d* (2.0)	6.83 *d* (2)
8	7.39 *br s**†	7.42 *d* (2.0)	7.02 *dd* (1, 2)
2',6'	8.56 *d* (9.0)*	8.62 *d* (9.3)	8.61 (9.3)
3',5'	7.04 *d* (9.0)*	7.07 *d* (9.3)	7.10 (9.3)
Caffeic acid*†			
(I)			
2	6.97 *br s*	6.92 *d* (2.0)	
5	6.87 *d* (7.7)	6.67 *d* (7.8)	
6	6.58 *br d* (7.7)	6.80 *dd* (2.0, 7.8)	
α	6.23 *d* (15.4)	6.25 *d* (15.6)	
β	7.37 *d* (15.4)	7.42 *d* (15.6)	
(II)			
2	7.12 *br s*		
5	6.83 *d* (8.6)		
6	7.04 *d* (8.6)		
α	6.33 *d* (15.8)		
β	7.54 *d* (15.8)		
Glucose*†			
(A)			
1	5.38 *d* (7.3)	5.45 *d* (7.3)	5.42 *d* (7.8)
2	3.64 *t* (8.6)	3.56 *t* (9.3)	3.52 *t* (7.8–8)
3	3.45 *m*	3.46 *t* (9.3–10.4)	3.43 *m*
4	3.36 *m*	3.30 *t* (9.5)	3.28 *t* (9.3)
5	3.92 *m*	3.90 *dd* (6.8, 9.8)	3.85 *dd* (7.8, 9.3)
6a	4.30 *m*	4.20 *br d* (10.3)	4.16 *dd* (7.8, 12)
6b	4.52 *d* (13.2)	4.51 *d* (10.3)	4.49 *d* (1.5, 12)
(B)			
1	5.33 *d* (7.3)	5.33 *d* (7.3)	
2	3.47 *m*	3.43 *m*	
3	3.41 *m*	3.43 *m*	
4	3.36 *m*	3.38 *m*	
5	3.92 *m*	3.90 *dd* (6.8, 9.8)	
6a	4.18 *m*	4.23 *dd* (6.8, 12.2)	
6b	4.71 *d* (11.1)	4.56 *d* (12.2)	
(C)			
1	4.86 *d* (7.3)		
2	3.43 *m*		
3	3.39 *m*		
4	3.29 *m*		
5	3.78 *m*		
6a	4.27 *m*		
6b	4.46 *d* (12.4)		
Malonic acid			
$-CH_2-$	3.50–3.44	3.43	3.43

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

and ^{13}C (100.53 MHz) NMR spectrometry, HPLC and TLC involving alkaline deacylation, acid hydrolysis, partial acid hydrolysis and H_2O_2 degradation [15]. Analytical HPLC was carried out on an Inertsil ODS-2 column (4.6ϕ × 250 mm) at 35°, using a 40 min gradient elution 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) at flow rate 0.8 ml min^{-1}, monitoring at 520 nm. FAB-mass spectra were recorded on JEOL JMS SX-102A (positive mode in magic bullet; negative mode in glycerol), and ^{1}H and ^{13}C NMR spectra on JEOL GX-400 in DMSO-d_6–CF_3CO_2D (9:1) with internal standard TMS.

^{13}C NMR *spectra data.* Pigment **2**: pelargonidin δ164.2 (C-2), 145.7 (C-3), 133.9 (C-4), 112.7 (C-4a), 157.0 (C-5), 103.0 (C-6), 165.3 (C-7), 94.5 (C-8), 155.3 (C-8a), 119.3 (C-1'), 135.5 (C-2' and C-6'), 117.2 (C-3' and C-5'),



1

Fig. 1. Acylated anthocyanins in the pink flowers of *Senecio cruentus*. Observed NOE's are indicted by arrows.

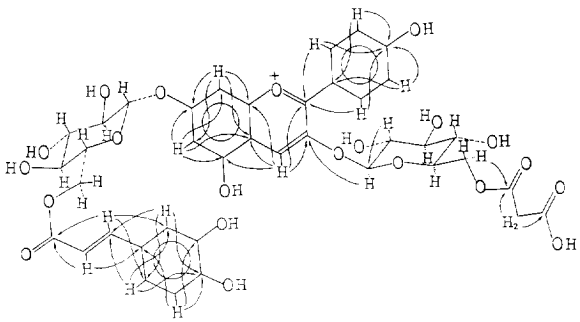


Fig. 2. HMBC correlations in Pigment **2**.

167.9 (C-4′); caffeic acid δ166.7 (CO_2H), 113.9 (C-α), 145.6 (C-β), 125.6 (C-1), 115.1 (C-2), 145.6 (C-3), 148.5 (C-4), 116.0 (C-5), 121.3 (C-6); malonic acid δ167.1 (CO_2H), 41.2 (C-1), 168.1 (CO_2H); glucose A δ101.9 (C-1), 72.9 (C-2), 76.2 (C-3), 70.0 (C-4), 74.4 (C-5), 64.6 (C-6); glucose B δ100.0 (C-1), 73.1 (C-2), 76.5 (C-3), 69.8 (C-4), 74.4 (C-5), 63.2 (C-6). Pigment **3**: pelargonidin δ162.2 (C-2), 144.1 (C-3), 135.6 (C-4), 112.2 (C-4a), 157.7 (C-5), 102.5 (C-6), 168.8 (C-7), 94.5 (C-8), 156.2 (C-8a), 119.4 (C-1′), 134.8 (C-2′ and C-6′), 117.0 (C-3′ and C-5′), 164.9 (C-4′); malonic acid δ167.1 (CO_2H), 41.2 (C-1), 168.1 (CO_2H); glucose δ102.1 (C-1), 73.1 (C-2), 76.2 (C-3), 69.8 (C-4), 74.4 (C-5), 64.4 (C-6).

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