HETEROCYCLES, Vol. 63, No. 6, 2004, pp. 1449 - 1454
Received, 17th March, 2004, Accepted, 15th April, 2004, Published online, 16th April, 2004

AN ACYLATED PELARGONIDIN 3-SOPHOROSIDE FROM THE PALE-BROWNISH RED FLOWERS OF *IPOMOEA NIL*

Kenjiro Toki,[†] Norio Saito,[††] Yasumasa Morita,[#] Atsushi Hoshino,[#] Shigeru Iida,[#] Atsushi Shigihara,[‡] and Toshio Honda[‡]*

[†]Department of Floriculture, Minami-Kyusyu University, Takanabe, Miyazaki, Japan; [††]Chemical Laboratory, Meiji-Gakuin University, Totsuka, Yokohama, Japan; [#]Divison of Gene Expression and Regulation I, National Institute for Basic Biology, Okazaki, Japan; [‡]Faculty of Pharmaceutical Sciences, Hoshi University, Shinagawa, Tokyo, Japan

Abstract – A structurally new acylated anthocyanin was isolated from the pale-brownish red flowers of a *duskish-2* mutant in the Japanese morning glory (*Ipomoea nil* or *Pharbitis nil*) as a major pigment together with a known anthocyanin. The new pigment was determined as pelargonidin 3-*O*-[2-*O*-(6-*O*-(*trans*-3-*O*-(β-glucopyranosyl)caffeoyl)-β-glucopyranosyl)-β-glucopyranoside], and the known anthocyanin was identified to be pelargonidin 3-sophoroside.

The Japanese morning glory (*Ipomoea nil* or *Pharbitis nil*) has been domesticated well as a floricultural plant, and its various spontaneous mutations exhibiting many different flower pigmentation have been isolated.[1] Of these, four recessive mutations, *dusky*, *duskish-1*, *duskish-2* and *dingy* designated by Hagiwara,[2] confer dull-colored flowers. In our continuing effort to elucidate flower color variation due to the altered production and accumulation of anthocyanins in the flowers of various *Ipomoea nil* mutants, we have reported the anthocyanin constituents in the dull-colored mutants such as the maroon,[3] slate[4] and dingy[5] lines which are believed to carry the *dusky* and *dingy* mutations. The maroon and slate flowers of the *dusky* mutants contained anthocyanidin 3-monoglycosides or 3,5-diglycosides as their deacylanthocyanins.[3,4] On the other hand, the flowers of the *dingy* mutant contained anthocyanidin 3-sophoroside-5-glucoside as their deacylanthocyanin. This deacylanthocyanin was found to be present as a major pigment, pelargonidin 3-*O*-[2-*O*-(glucosyl)-6-*O*-(4-*O*-glucosylcaffeoyl)glucoside]-5-*O*-glucoside

by acylation with one molecule of glucosylcaffeic acid.[5] In the previous report of the *dingy* mutant, the anthocyanin constituents of the other dull-colored flowers of the *duskish-1* and *-2* mutants were estimated to be pelargonidin 3-glucoside and its 3-sophoroside as their main pigments by the analysis of HPLC.[5]

In this paper, we would like to report more detail distribution of anthocyanin pigments of the *duskish-2* mutant;[6] being pelargonidin 3-sophoroside and an acylated pelargonidin 3-sophoroside as its main anthocyanin pigment.

Dry flowers (15 g) of the *duskish-2* mutant strain,[6] Q854, their fresh flower color being pale-brownish red of *Ipomoea nil* were immersed in 5% AcOH for 24 h at room temperature to leave the red extract, in which major five anthocyanin peaks were observed in its HPLC spectrum[7] as described previously.[5] From the mixed anthocyanin extract, pigment A (*ca*. 3.5 mg),[8] pigment B (*ca*. 1.2 mg),[9] pigment C (*ca*. 18.3 mg),[10] and pigment D (*ca*. 2.0 mg)[11] were obtained, respectively, by the process described previously.[3-5]

By acid hydrolysis, pigments A, C, and D gave pelargonidin and glucose. The similar treatment of pigment B afforded peonidin as its aglycone. By alkaline hydrolysis, pigment C gave a deacylanthocyanin, caffeic acid, and glucosylcaffeic acid. The deacylanthocyanin obtained here was identical with pigment A by the analysis of their HPLC.

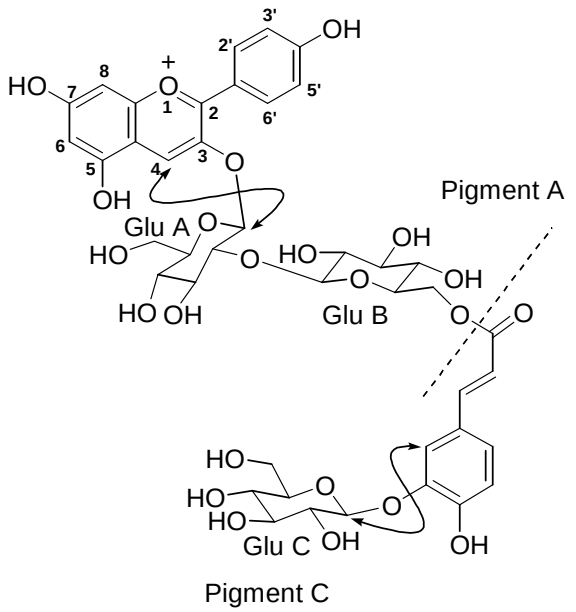

Figure 1. Duskish flower anthocyanins
(Observed NOE's are indicated by arrows).

The structure of pigment A was elucidated to be pelargonidin 3-sophoroside by direct comparison of HPLC, TLC, and spectral properties with the authentic sample obtained from the flowers of *Papaver rhoeas*.[12]

Table 1. NMR spectral data for the pale-brownish red flower anthocyanins of *Ipomoea nil*.
(500 MHz, in CF_3CO_2D-DMSO-d_6 (1:9). TMS as an internal standard, *J* Hz in parentheses)

	Pigment C		Pigment A
	δC	δH	δH
Pelargonidin			
2	162.5		
3	143.6		
4	137.9	9.03 s	8.99 s
5	156.3		
6	102.5	6.71 d (2.1)	6.75 d (1.8)
7	169.1		
8	94.5	6.90 d (2.1)	7.00 d (1.8)
9	158.0		
10	112.2		
1'	119.5		
2'	117.1	8.59 d (9.2)	8.60 d (9.2)
3'	134.9	7.10 d (9.2)	7.12 d (9.2)
4'	164.9		
5'	134.9	7.10 d (9.2)	7.12 d (9.2)
6'	117.1	8.59 d (9.2)	8.60 d (9.2)
Caffeic acid (Caf)			
1	125.9		
2	116.8	7.35 d (1.8)	
3	149.7		
4	145.7		
5	116.4	6.82 d (8.6)	
6	124.2	7.01 dd (1.8, 8.6)	
α	114.7	6.11 d (15.9)	
β	144.9	7.27 d (15.9)	
C=O	166.0		
Glucose A (GluA)			
1	101.1	5.46 d (7.6)	5.57 d (7.7)
2	81.2	3.88 t (8.5)	3.88 t (8.9)
3	76.4	3.65 t (8.9)	3.67 t (9.2)
4	69.5	3.35 m	3.36 t (8.6)
5	77.8	3.50 m	3.60 m
6	61.1	3.52 dd (11.3, 6.1)	3.55 dd (11.3, 5.2)
		3.71 br d (11.3)	3.76 br d (11.3)
Glucose B (GluB)			
1	104.3	4.85 d (7.9)	4.75 d (7.9)
2	74.7	3.12 t (8.0)	3.00 t (8.6)
3	76.5	3.30 m	3.15 t (8.9)
4	69.9	3.39 m	3.09 t (9.5)
5	73.6	3.34 m	2.85 m
6	63.3	4.06 dd (11.6, 4.6)	3.27 dd (11.3, 5.2)
		4.14 br d (11.6)	3.38 br d (11.3)
Glucose C (GluC)			
1	102.2	4.83 d (7.3)	
2	74.3	3.36 m	
3	70.2	3.34 m	
4	76.4	3.21 t (9.6)	
5	77.5	3.43 ddd (9.6, 5.2, 1.8)	
6	60.8	3.49 dd (11.6, 5.2)	
		3.76 dd (11.6, 1.8)	

The FAB-MS spectrum of pigment A gave its molecular ion $[M]^+$ at 595 *m/z*, in agreement with the mass calculated for $C_{27}H_{31}O_{15}$, which was composed of pelargonidin with two molecules of glucose.

The structure was confirmed by the analysis of its ^1H NMR spectrum as shown in Figure 1. Seven aromatic proton signals of pelargonidin were observed and assigned as shown in Table 1.

Fourteen proton signals of sugar moiety were assigned by its ^1H-^1H COSY spectrum. OH-3 of pelargonidin is bonded with Glu A through the glycosidic bond, and also OH-2 of Glu A is bonded with Glu B. The linkages were confirmed by the analyses of negative NOE difference (DIFNOE)[13] and 2D COSY spectra. Thus, this pigment is determined to be pelargonidin 3-sophoroside, and identical to the pigment of the peak 3 in the extracts of the *duskish-1* and *-2* mutant flowers, as described previously.[5]

The FAB MS of pigment C gave its molecular ion $[M]^+$ at 919 *m/z* in agreement with the mass calculated for $C_{42}H_{47}O_{23}$, which was composed of pelargonidin with three molecules of glucose and one molecule of caffeic acid. The elemental components of pigment C were further confirmed by the high resolution FAB-MS [Calcd for $C_{42}H_{47}O_{23}$ requires 919.2508. Found 919.2516]. In order to determine the structure of pigment C, its ^1H and ^{13}C NMR spectral measurements including ^1H-^1H COSY, DIFNOE,[13] HMQC, and HMBC spectra were carried out in CF_3COOD-DMSO-d_6 (1 : 9), and the chemical shifts of H and C were assigned as shown in Table 1. Ten aromatic proton signals of pelargonidin and caffeic acid were assigned by analysis of its ^1H-^1H COSY spectrum as shown in Table 1. Two olefinic proton signals of caffeic acid in this pigment had large coupling constants (J = 15.6 Hz), indicating caffeic acid to have *trans* configuration (Figure 1). Regarding the sugar moieties of this pigment, the signals of three anomeric protons were observed at δ 5.46 (d, J = 7.6 Hz, Glu A), 4.85 (d, J = 7.9 Hz, Glu B), and 4.83 (d, J = 7.3 Hz, Glu C), and the assigned glucose protons had coupling constants (J = 7.3 ~ 11.7 Hz), suggesting that all the glucose units must be β-glucopyranose form (Figure 1). Two characteristic proton signals shifted to the lower magnetic field at δ 4.06 and 4.14 were assigned to methylene protons ($-CH_2-$) of Glu B by the analysis of its ^1H-^1H COSY spectrum, indicating that Glu B was acylated at OH-6 with caffeic acid. The linkages and/or the positions of attachment of glucoses and caffeic acid were determined by the measurements of DIFNOE and HMBC[14] spectra as the process described previously.[5] By irradiation at H-1 of Glu A, the appearance of negative NOE signal at H-4 of pelargonidin indicated Glu A to be attached to OH-3 of pelargonidin through a glucosidic bond. Furthermore, Glu A was confirmed to link with Glu B at OH-2 by its 2D COSY, DIFNOE, and HMBC spectral analyses. Glu C is attached to the OH-3 of caffeic acid through a glucosidic bond by exhibiting NOEs between H-2 of caffeic acid and H-1 of Glu C in the DIFNOE spectra. Therefore, pigment C is determined to be pelargonidin 3-*O*-[2-*O*-(6-*O*-(3-*O*-(β-glucopyranosyl)caffeoyl)-β-glucopyranosyl)-β-glucopyranoside], which is a new caffeoylpelargonidin glycoside. Pigment C is identical with the pigment of the peak 4 in the *duskish-1* and *2* extracts by the HPLC analysis described previously.[5] Structures of the other pigments (B and D)

could not be determined at present unfortunately, because of their small amounts obtained. Further study will be continued to determine their structures.

REFERENCES AND NOTES

*Corresponding author: Tel. +81-3-5498-5791; Fax. +81-3-3787-0036; E-mail: honda@hoshi.ac.jp.

1. S. Iida, Y. Morita, J.-D. Choi, K.-I. Park, and A. Hoshino, *Adv. Biophys.*, 2004, **39,** 141.
2. T. Hagiwara, *Bull. Res. College Agr. Vet. Sci., Nihon Univ*., 1954, **3**, 1.
3. N. Saito, T. S. Lu, M. Akaizawa, M. Yokoi, A. Shigihara, and T. Honda, *Phytochemistry*, 1994, **35**, 407.
4. N. Saito, F. Tatsuzawa, K. Kasahara, M. Yokoi, S. Iida, A. Shigihara, and T. Honda, *Phytochemistry*, 1996, **41**, 1607.
5. K. Toki, N. Saito, S. Iida, A. Hoshino, A. Shigihara, and T. Honda, *Heterocycles*, 2001, **55**, 2261.
6. The seed was obtained from Dr. E. Nitasaka (Kyushu University). The plant was grown in the farm of Minami-Kyushu University, and fresh corollas of this plant were collected in July to October, 2003.
7. HPLC was run on Inertsil ODS-2 column (4.6 x 250 mm for analysis, and 20x250 mm for prep.) at 35ºC, and monitoring at 520 nm. Solvent systems for analysis were as follows: a linear gradient elution for 40 min from 40 - 85% solvent B (1.5% H_3PO_4, 20% AcOH, 25% MeCN) in solvent A (1.5% H_3PO_4).
8. Pigment A (pelargonidin 3-sophoroside): UV λ max (0.1% HCl-MeOH) 508, 432 (sh), 280 nm. TLC R*f*-values (x100): BAW (n-butanol:acetic acid:water = 4:1:5): 59, BuHCl (n-BuOH:2M HCl = 1:1): 35, 1% HCl: 47, AHW (acetic acid:conc. HCl:water = 15:3:82): 75. HPLC R*t* (min) 14.07. FAB MS $[M]^+$ m/z 595.
9. Pigment B; UV λ max (0.1% HCl-MeOH) 518, 320, 283 nm. TLC R*f*-values (x100): BAW: 57, BuHCl: 27, 1% HCl: 42, AHW: 76. HPLC R*t* (min) 15.78.
10. Pigment C (pelargonidin 3-*O*-[2-*O*-(6-*O*-(3-*O*-(β-glucosyl)caffeoyl)-β-glucosyl)-β-glucoside]): UV λ max 507, 321, 283 nm. TLC R*f*-values (x100) BAW: 56, BuHCl: 28, 1% HCl: 32, AHW: 68. HPLC R*t* (min) 18.12. FAB MS $[M]^+$ m/z 919.
11. Pigment D: UV λ max (0.1% HCl-MeOH) 507, 321, 285 nm. TLC R*f*-values (x100) BAW: 78, BuHCl: 49, 1% HCl: 18, AHW: 52. HPLC R*t* (min) 20.67.
12. J. B. Harborne, "*Comparative Biochemistry of the Flavonoids*", 1967, Academic Press, London and New York.
13. T. S. Lu, N. Saito, M. Yokoi, A. Shigihara, and T. Honda, *Phytochemistry*, 1991, **30**, 2387.
14. Observed major HMBC correlations (δ values of ^{13}C are in Table 1): H-4 of pelargonidin (pel): C-2, C-3, C-5, and C-9; H-6 of pel: C-8 and C-10; H-8 of pel: C-10; H-2' and 6' of pel: C-2, C-3', C-4',

and C-5’; H-3’ and 5’ of pel: C-1’, C-2’, and C-6’; H-2 of caffeic acid (Caf): C-4 of Caf; H-5 of Caf: C-3 of Caf; H-6 of Caf: C-2 of Caf and C-5 of Caf; H-α of Caf: C-COOH; H-β of Caf: C-1 of Caf; H-1 of Glu A: C-3 of pel; H-2 of Glu A: C-1 of Glu B, C-1 of Glu A, and C-3 of Glu A; H-3 of Glu A: C-2 of Glu A and C-4 of Glu A; H-1 of Glu B: C-2 of Glu A.