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ACYLATED PEONIDIN 3-RUTINOSIDE-5-GLUCOSIDES FROM COMMERCIAL PETUNIA CULTIVARS WITH PINK FLOWERS

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Abstract – Two new acylated peonidin glycosides and three known acylated or non-acylated peonidin glycosides were identified in the flowers of 17 commercial pink petunias: peonidin 3-*O*-[6-*O*-(4-*O*-(4-*O*-(6-*O*-(*trans*-caffeoyl)- β -D-glucopyranosyl)-*trans*-*p*-coumaroyl)- α -L-rhamnopyranosyl)- β -D-glucopyranoside]-5-*O*-[β -D-glucopyranoside], peonidin 3-*O*-[6-*O*-(4-*O*-(4-*O*-(β -D-glucopyranosyl)-*trans*-*p*-coumaroyl)- α -L-rhamnopyranosyl)- β -D-glucopyranoside]-5-*O*-[β -D-glucopyranoside], peonidin 3-*p*-coumaroylrutinoside-5-glucoside, peonidin 3-caffeoylrutinoside-5-glucoside, and peonidin 3-rutinoside-5-glucoside.

Recently, the floral anthocyanins in many species and cultivars of the genus *Petunia* (Solanaceae) have been intensively investigated using NMR spectroscopy and other techniques. From these plants, 32 different anthocyanins have been unambiguously determined, including 15 new di- and monoacylated delphinidin, petunidin and malvidin compounds.¹⁻⁷ We have previously investigated the anthocyanins that are present in the flowers of selected commercial petunia lines with cultivar names containing the word “Red” or “Scarlet”.⁸ In the present study, we analyzed commercial petunias with the words “Pink” or “Rose” in their names, hereafter referred to as pink petunias. We found that the major anthocyanins in these cultivars were five peonidin glycosides, and we determined the structures of two novel acylated peonidin 3-rutinoside-5-glucosides from these plants.

An HPLC⁹ survey of the floral anthocyanins in 17 pink petunias revealed at least 15 different anthocyanin peaks. The five major peaks were isolated and determined to be pigments (1) (ranging from 0-38.2% of the total anthocyanin content, as calculated from the HPLC peak area), (2) (0-22.3%), (3) (13.4-76.8%), (4) (4.0-19.6%) and (5) (0.5-2.1%) (Table 1).

Table 1. Distribution of floral anthocyanins in the commercial petunias having the word, Pink or Rose, in the cultivar name.

Cultivars	Company	Anthocyanins (%)					
		1	2	3	4	5	Total
Baccara Cherry Rose	Sakata Seed Co.	1.0	1.5	66.2	7.2	0.8	76.7
Baccara Pink	Sakata Seed Co.	–	1.2	62.5	16.5	1.8	82.0
Baccara Pink Morn	Sakata Seed Co.	0.5	1.0	68.9	9.0	0.6	80.0
Baccara Rose	Sakata Seed Co.	0.6	0.4	62.5	10.1	0.7	74.3
Baccara Rose Morn	Sakata Seed Co.	0.2	0.4	67.8	10.5	1.0	79.9
Carpet, Pink	PanAmerican Seed Co.	0.2	0.5	71.2	5.2	0.8	77.9
Carpet, Rose	PanAmerican Seed Co.	2.3	1.8	63.0	5.4	0.6	73.1
Celebrity Hot Pink	Bodger Seeds Ltd.	0.1	0.6	54.7	19.6	1.3	76.3
Celebrity Pink Morn	Bodger Seeds Ltd.	1.6	1.0	76.8	6.2	1.0	86.6
Fantasy Rose Picotee	Goldsmith Seeds Co.	2.9	1.3	72.2	6.0	0.9	83.3
Fulcon Deep Rose	Sakata Seed Co.	0.5	0.5	66.8	9.5	1.7	79.0
Fulcon Pink Morn	Sakata Seed Co.	–	0.1	72.3	7.8	1.0	81.2
Fulcon Rose	Sakata Seed Co.	–	–	69.0	18.8	0.5	88.3
Madness, Double Rose	PanAmerican Seed Co.	1.1	0.9	52.5	12.5	2.1	69.1
Madness, Rose	PanAmerican Seed Co.	0.1	0.3	70.3	10.5	0.8	82.0
Madness, Rose Star	PanAmerican Seed Co.	3.0	4.0	59.8	6.1	0.6	73.5
PrimeTime Pink Morn	Goldsmith Seeds Co.	38.2	22.3	13.4	4.0	0.6	78.5

The above anthocyanins were isolated from the dried corolla limbs (*ca.* 300 g) of PrimeTime Pink Morn and Baccara Pink (= Merlin Pink) using MAW solvent (MeOH-HOAc-H₂O, 2:1:7) and purified using Diaion-HP-20 column chromatography (CC), preparative HPLC,⁹ and TLC. Finally, pigments (1)¹⁰ (*ca.* 10 mg), (2)¹¹ (*ca.* 15 mg), (3)¹² (*ca.* 5 mg), (4)¹³ (*ca.* 1 mg) and (5)¹⁴ (*ca.* 3 mg) were obtained, respectively. Pink petunias produce at least two types of HPLC profiles: the Baccara Pink and PrimeTime Pink Morn types (Figure 1). PrimeTime Pink Morn is the only cultivar of the latter type that exhibits a complex HPLC profile.

Alkaline hydrolysis of **1-4** yielded only one pigment (**5**) as the corresponding deacyl anthocyanin, and **1** also gave caffeic acid and glucosyl *p*-coumaric acid; **2** gave glucosyl *p*-coumaric acid, **3** gave *p*-coumaric

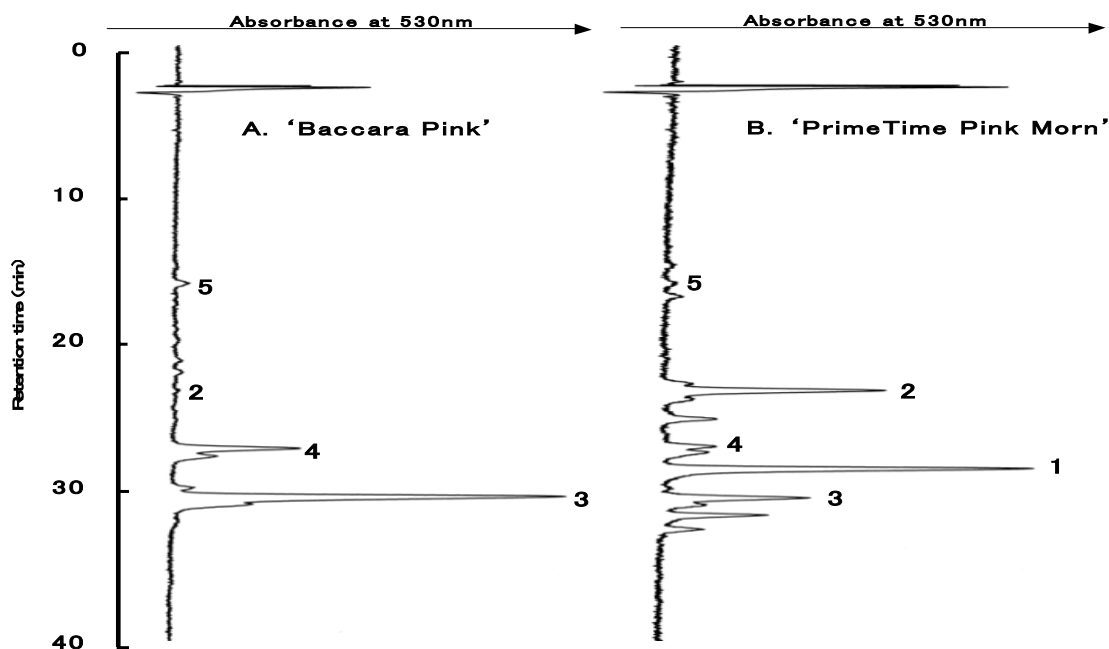


Figure 1. HPLC profiles for the floral anthocyanins of the commercial petunia cultivars Baccara Pink and PrimeTime Pink Morn.

- 1: peonidin 3-caffeoylglucosyl*p*-coumaroylrutinoside-5-glucoside
- 2: peonidin 3-glucosyl*p*-coumaroylrutinoside-5-glucoside
- 3: peonidin 3-*p*-coumaroylrutinoside-5-glucoside
- 4: peonidin 3-caffeoylrutinoside-5-glucoside
- 5: peonidin 3-rutinoside-5-glucoside

acid and **4** gave caffeic acid. Acid hydrolysis of **5** gave peonidin, glucose and rhamnose, and H_2O_2 degradation of **5** yielded rutinose. Alkaline and acid hydrolysis and H_2O_2 degradation products were analysed by TLC¹⁵ in comparison with authentic samples. The FAB MS spectrum of pigment (**1**) showed a molecular ion peak $[\text{M}]^+$ at 1241 *m/z*, corresponding to the molecular formula $\text{C}_{58}\text{H}_{65}\text{O}_{30}$. This MS value was confirmed by the measurement of its high resolution FAB-MS [Calcd for $\text{C}_{58}\text{H}_{65}\text{O}_{30}$ requires: 1241.3561. Found: 1241.3567]. Full assignment of the COSY, ^1H - ^1H NOESY, ^1H - ^{13}C HMQC and ^1H - ^{13}C HMBC spectra has been carried out (Table 2). The ^1H NMR spectrum of pigment (**1**) demonstrated the presence of three molecules of glucose and one molecule each of peonidin, rhamnose, *p*-coumaric acid and caffeic acid. The aromatic protons of peonidin, *p*-coumaric acid and caffeic acid in this pigment were assigned by analysis of the COSY spectrum. The ^1H NMR spectral data of **1** were

similar to those of malvidin 3-caffeoylglucosyl-*p*-coumaroyl-rutinoside-5-glucoside,¹ except for the malvidin moiety. All four olefinic proton signals of the *p*-coumaric and caffeic acid moieties of pigment (1) had large coupling constants ($J = 15.7$ and 15.6 Hz), indicating that these two hydroxycinnamic acids are in the *trans* configuration. The signals of four anomeric protons of pigment (1) appeared at δ 5.78 (*d*, $J = 7.8$ Hz, glucose A; Figure 1), δ 5.15 (*d*, $J = 7.5$ Hz, glucose B), δ 5.07 (*d*, $J = 7.6$ Hz, glucose C) and δ 4.63 (*s*, rhamnose), and the assigned glucose protons had coupling constants of $J = 7.5$ -11.0 Hz, suggesting that these glucose residues comprise a β -D-glucopyranose. In the rhamnose moiety, one singlet signal from an anomeric proton (δ 4.63) and doublet signals of methyl protons (δ 0.84, *d*, $J =$

Table 2. NMR spectral data for two pigments isolated from the flowers of commercial petunia 'PrimeTime Pink Morn'. *

	1		2	
	δ C	δ H	δ C	δ H
Peonidin				
2	162.0		162.0	
3	144.3		144.3	
4	133.2	9.08 <i>s</i>	133.3	9.09 <i>s</i>
5	155.0		155.1	
6	103.5	7.13 <i>br s</i>	103.5	7.13 <i>br s</i>
7	167.3		167.4	
8	96.4	7.35 <i>br s</i>	96.4	7.34 <i>br s</i>
9	154.6		154.6	
10	111.5		111.5	
1'	119.1		119.1	
2'	114.0	8.14 <i>br s</i>	114.0	8.17 <i>br s</i>
3'	147.9		147.9	
4'	155.0		155.1	
5'	116.4	7.25 <i>d</i> (8.8)	116.5	7.27 <i>d</i> (8.7)
6'	127.9	8.28 <i>br d</i> (9.1)	127.9	8.30 <i>br d</i> (8.9)
-OCH ₃	55.8	3.92 <i>s</i>	55.8	3.95 <i>s</i>
Glucose A				
1	100.6	5.78 <i>d</i> (7.8)	100.7	5.77 <i>d</i> (7.8)
2	75.9	3.53 <i>t</i> (8.4)	76.9	3.56 <i>m</i>
3	76.0	3.34 <i>t</i> (8.4)	76.3	3.38 <i>t</i> (8.9)
4	69.1	3.30 <i>t</i> (9.1)	69.1	3.23 <i>t</i> (9.2)
5	75.2	3.44 <i>t</i> (8.9)	76.0	3.41 <i>m</i>
6	65.1	3.66 <i>m</i>	65.3	3.68 <i>m</i>
		3.83 <i>br d</i> (11.0)		3.84 <i>br d</i> (12.0)
Glucose B				
1	100.9	5.15 <i>d</i> (7.5)	100.9	5.15 <i>d</i> (7.6)
2	75.9	3.50 <i>t</i> (8.3)	76.0	3.53 <i>m</i>
3	75.4	3.44 <i>t</i> (8.9)	76.2	3.34 <i>t</i> (8.9)
4	69.6	3.32 <i>t</i> (8.6)	72.9	3.29 <i>t</i> (8.4)
5	76.9	3.40 <i>t</i> (8.9)	76.6	3.42 <i>m</i>
6	60.1	3.55 <i>m</i>	60.3	3.55 <i>m</i>
		3.75 <i>br d</i> (11.0)		3.70 <i>br d</i> (12.0)
Glucose C				
1	99.4	5.07 <i>d</i> (7.6)	99.6	5.00 <i>d</i> (7.6)
2	72.8	3.33 <i>t</i> (8.3)	73.0	3.29 <i>t</i> (8.4)
3	76.0	3.33 <i>t</i> (8.3)	76.0	3.36 <i>t</i> (8.9)
4	72.6	3.42 <i>t</i> (8.9)	72.6	3.45 <i>t</i> (8.9)
5	75.9	3.79 <i>m</i>	75.4	3.51 <i>m</i>
6	63.0	4.23 <i>dd</i> (11.0, 5.6)	60.1	3.57 <i>m</i>
		4.40 <i>br d</i> (11.0)		3.76 <i>br d</i> (12.0)
Rhamnose				
1	100.0	4.63 <i>s</i>	100.0	4.63 <i>s</i>
2	69.9	3.69 <i>m</i>	69.9	3.69 <i>m</i>
3	67.8	3.67 <i>m</i>	67.9	3.67 <i>m</i>
4	73.6	4.79 <i>t</i> (9.4)	73.6	4.80 <i>t</i> (9.4)
5	65.7	3.62 <i>m</i>	65.7	3.62 <i>m</i>
-CH ₃	17.1	0.84 <i>d</i> (5.6)	17.1	0.86 <i>d</i> (6.0)
<i>p</i> -Coumaric acid				
1	127.5		127.5	
2,6	129.7	7.59 <i>d</i> (8.4)	129.8	7.62 <i>d</i> (8.4)
3,5	116.3	7.07 <i>d</i> (8.4)	116.3	7.08 <i>d</i> (8.4)
4	158.6		158.9	
a	115.7	6.26 <i>d</i> (15.7)	115.6	6.35 <i>d</i> (15.8)
b	145.1	7.45 <i>d</i> (15.7)	144.3	7.56 <i>d</i> (15.8)
C=O	166.0		166.1	
Caffeic acid				
1	125.2			
2	114.3	7.12 <i>brs</i>		
3	147.8			
4	144.0			
5	115.7	6.82 <i>d</i> (8.0)		
6	121.4	6.97 <i>d</i> (8.0)		
a	113.6	6.25 <i>d</i> (15.6)		
b	145.2	7.48 <i>d</i> (15.6)		
C=O	166.3			

*13 C NMR (150.80 MHz) and 1 H NMR (600.05 MHz), in DCl-DMSO-*d*₆ (1:9), at 25 °C, an internal standard of TMS. Coupling constants (J in Hz) in parentheses.

5.6) at C-5 suggested the presence of α -L-rhamnopyranose. The proton signals of the H-4 of rhamnose (δ 4.79, *t*, J = 9.4 Hz) and the H-6a (δ 4.23, *dd*, J = 11.0, 5.6 Hz) and H-6b (δ 4.40, *br d*, J = 11.0 Hz) of glucose C were shifted downfield (Table 2), indicating that the OH-4 of rhamnose and the OH-6 of glucose C are acylated with hydroxycinnamic acids. NOESY and HMBC spectra were used to distinguish the sites of attachment of the cinnamic acid moieties, the sugar and the peonidin aglycone (Figure 2). The signal of the anomeric proton of glucose A correlated to the signal of the H-4 proton in the NOESY spectrum of peonidin. The signal of the anomeric proton of glucose B correlated to the signal of the C-5 carbon in the HMBC spectra, and to the signal of the proton H-6 in the NOESY spectrum of peonidin. These characteristic features revealed that the peonidin 3 and 5 positions are both bound to glucose molecules. The signal of the anomeric proton of the rhamnose at δ 4.63 correlated to the signal of the C-6 carbon of glucose A at δ 65.1 in the HMBC spectra, and to the signals from protons H-6a and 6b in the NOESY spectrum of glucose A. A NOE effect was observed between the anomeric proton of glucose C and the protons H-3/H-5 of the *p*-coumaric acid moiety, and a cross-peak in the HMBC spectra between this same anomeric proton and the carbon C-4 of *p*-coumaric acid moiety, indicating that glucose C was glycosylated on OH-4 of the *p*-coumaric acid. The caffeic acid unit was determined to be bonded to the 6-OH glucose C, based on the downfield shift of the methylene protons of glucose C. Therefore, pigment (1) is peonidin 3-*O*-[6-*O*-(4-*O*-(4-*O*-*trans*-(6-*O*-(*trans*-caffeoyl)- β -D-glucopyranosyl)-*p*-coumaroyl)- α -L-rhamnopyranosyl)- β -D-glucopyranoside]-5-*O*-[β -D-glucopyranoside] (Figure 2), a novel anthocyanin.¹⁶⁻¹⁸

The FAB MS spectrum of pigment (2) had a molecular ion $[M]^+$ at 1079 *m/z*, which was in good agreement with the mass calculated for C₄₉H₅₉O₂₇. This MS value was confirmed by the measurement of its high resolution FAB-MS [Calcd for C₄₉H₅₉O₂₇ requires: 1079.3244. Found:1079.3232]. Full assignment of the COSY, NOESY, HMQC and HMBC spectra has been carried out (Table 2). The ¹H NMR spectrum of pigment (2) showed the presence of three molecules of glucose and one molecule each of peonidin, rhamnose and *p*-coumaric acid. The structure of 2 was almost the same as 1, except for the

absence of the terminal caffeic acid moiety. A downfield shift of proton H-4 of the rhamnose and a correlation between this signal and the carbonyl carbon of the *p*-coumaric acid moiety in the HMBC spectrum revealed their connection site. Analysis of the ^1H NMR and COSY spectra revealed that the methylene protons of glucose C (δ 3.57, H-6a and 3.76, H-6b) were shifted upfield, as compared to those of **1** (δ 4.23, H-6a and 4.40, H-6b). The other proton signals of **2** were assigned as for **1**, and were in good agreement with those expected for **1** without the terminal caffeic acid moiety. Therefore, pigment (**2**) is peonidin 3-*O*-[6-*O*-(4-*O*-(4-*O*-*trans*-(β -D-glucopyranosyl)-*p*-coumaroyl)- α -L-rhamnopyranosyl)- β -D-glucopyranoside]-5-*O*-[β -D-glucopyranoside] (Figure 2), another novel anthocyanin.¹⁶⁻¹⁸

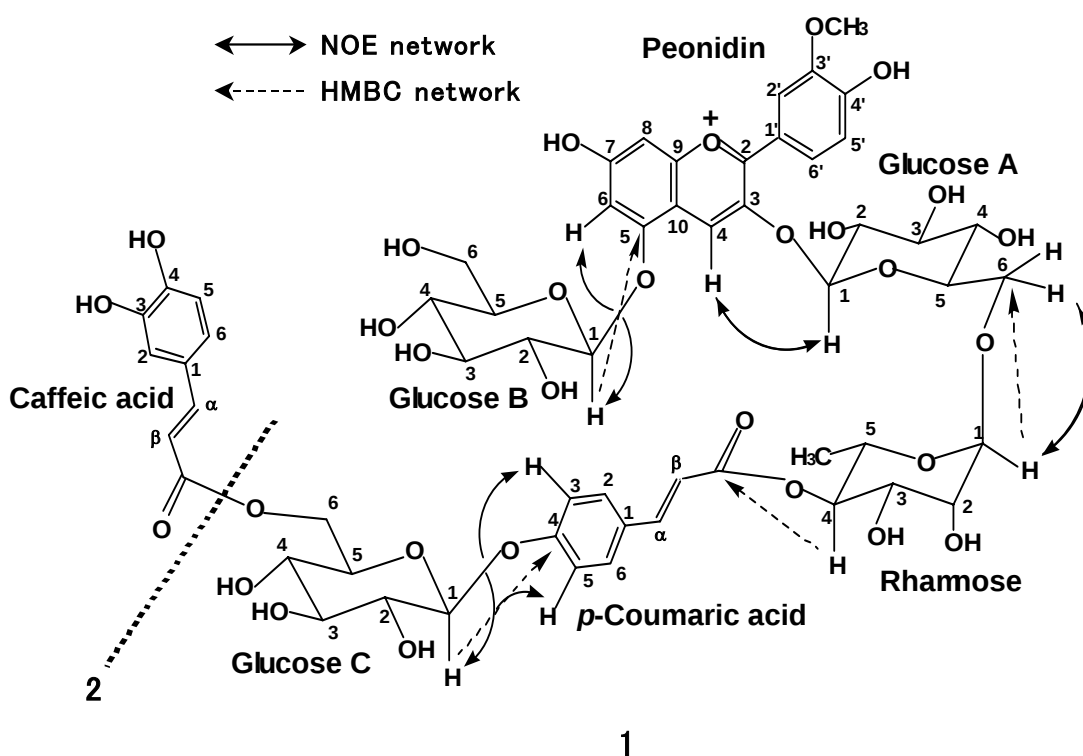


Figure 2. Peonidin 3-caffeoylglucosyl*p*-coumaroylrutinoside-5-glucoside (**1**) and peonidin 3-glucosyl*p*-coumaroylrutinoside-5-glucoside (**2**) from the flowers of pink petunias. Arrows highlight important NOE and HMBC networks.

The FAB MS spectra of pigments (**3**) and (**4**) gave their molecular ions $[\text{M}]^+$ at 917 and 933 *m/z*, which were in good agreement with the masses calculated for $\text{C}_{43}\text{H}_{49}\text{O}_{22}$ and $\text{C}_{43}\text{H}_{49}\text{O}_{23}$. These values indicate that **3** is composed of peonidin containing two glucose molecules and one molecule each of *p*-coumaric acid and rhamnose, and that **4** is composed of peonidin with two glucose molecules and one molecule

each of caffeic acid and rhamnose. Upon alkaline hydrolysis, both anthocyanins gave peonidin 3-rutinoside-5-glucoside, and **3** and **4** also yielded *p*-coumaric acid and caffeic acid, respectively. The ¹H NMR spectral data of **3** were similar to those of malvidin 3-*p*-coumaroylrutinoside-5-glucoside,³ except for the malvidin moiety.¹² Therefore, the structure of **3** is peonidin 3-*trans-p*-coumaroylrutinoside-5-glucoside. This molecule has been reported to be a major pigment in several petunia strains,¹⁹⁻²⁰ as well as in commercial cultivars such as Pink Magic and Pink Grandball.²¹

Determination of the structure of pigment (**4**) was not possible due to the small amount available for analysis. Based on the above findings, the structure of **4** is assumed to be peonidin 3-caffeoylrutinoside-5-glucoside. This pigment has been reported to exist in flowers of the commercial petunia cultivars Pink Magic, Pink Grandball and others.²¹

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9. Preparative HPLC was run on a Waters C18 (19 x 150 mm) column at 40 °C with flow rate of 4 mL min⁻¹ and monitored at 530 nm for anthocyanins. A Solvent system used was linear gradient elution for 15 min from 60 to 80 % solvent B (1.5% H₃PO₄, 20% HOAc, 25% MeCN in H₂O) in solvent A (1.5% H₃PO₄ in H₂O). Analytical HPLC was run on a Waters C18 (4.6 x 250 mm) column at 40 °C with flow rate of 1 mL min⁻¹ and monitored at 530 nm for anthocyanins. A Solvent system used was linear gradient elution for 40 min from 20 to 85 % solvent B in solvent A.
10. Pigment (1) (peonidin 3-caffeoylglucosyl-*p*-coumaroylrutinoside-5-glucoside): UV-VIS: $\lambda_{\max}$ 531, 327, 279 nm, $E_{\text{acyl}}/E_{\max}$ (%) = 68, $E_{440}/E_{\max}$ (%) = 13, AlCl₃ shift 0; TLC: R_f -values BAW (BuOH-HOAc-H₂O, 4:1:2) 0.25, BuHCl (BuOH-2N HCl, 1:1) 0.03, 1% HCl 0.10, AHW (HOAc-HCl-H₂O, 15:3:82) 0.34; HPLC: R_t (min) 28.5.
11. Pigment (2) (peonidin 3-glucosyl-*p*-coumaroylrutinoside-5-glucoside): UV-VIS: $\lambda_{\max}$ 529, 309, 281 nm, $E_{\text{acyl}}/E_{\max}$ (%) = 56, $E_{440}/E_{\max}$ (%) = 12, AlCl₃ shift 0; TLC: R_f -values BAW 0.16, BuHCl 0.01, 1% HCl 0.52, AHW 0.76; HPLC: R_t (min) 23.2.
12. Pigment (3) (peonidin 3-*p*-coumaroylrutinoside-5-glucoside): UV-VIS: $\lambda_{\max}$ 531, 310, 281 nm, $E_{\text{acyl}}/E_{\max}$ (%) = 60, $E_{440}/E_{\max}$ (%) = 12, AlCl₃ shift 0; TLC: R_f -values BAW 0.52, BuHCl 0.14, 1% HCl 0.23, AHW 0.61; HPLC: R_t (min) 30.6. ¹H NMR (600 MHz, DCl-DMSO-*d*₆, 1 : 9) δ : peonidin 9.09 (1H, s, H-4), 8.30 (1H, *dd*, J =8.7, 1.6 Hz, H-6'), 8.17 (1H, *br d*, J =2.0 Hz, H-2'), 7.35 (1H, *br s*, H-8), 7.25 (1H, *d*, J =8.7 Hz, H-5'), 7.13 (1H, *br s*, H-6), 3.94 (3H, s, -OCH₃); glucose A 5.76 (1H, *d*, J =7.7 Hz, H-1), 3.83 (1H, *br d*, J =11.0 Hz, H-6b), 3.67 (1H, *dd*, J =11.0, 3.2 Hz, H-6a), 3.55 (1H, *m*, H-5), 3.53 (1H, *m*, H-2), 3.38 (1H, *m*, H-3), 3.34 (1H, *t*, J =9.2 Hz, H-4); glucose B 5.15 (1H, *d*, J =7.6 Hz, H-1), 3.76 (1H, *br d*, J =11.3 Hz, H-6b), 3.57 (1H, *m*, H-6a), 3.52 (1H, *m*, H-5), 3.51 (1H, *t*, J =8.1 Hz, H-2), 3.45 (1H, *t*, J =9.0 Hz, H-3), 3.35 (1H, *m*, H-4); rhamnose 4.79 (1H, *t*, J =9.5 Hz, H-4), 4.62 (1H, s, H-1), 3.67 (1H, *m*, H-2), 3.66 (1H, *m*, H-3), 3.60 (1H, *m*, H-5), 0.85 (3H, *d*, J =6.1 Hz, -CH₃); *p*-coumaric acid 7.51 (2H, *d*, J =8.6 Hz, H-2, 6), 7.50 (1H, *d*, J =16.0 Hz, H-b), 6.87 (2H, *d*, J =8.6 Hz, H-3, 5), 6.25 (1H, *d*, J =16.0 Hz, H-a); HR-FAB MS Calcd for C₄₃H₄₉O₂₂: 917.2715. Found:

917.2793.

13. Pigment (4) (peonidin 3-caffeoylrutinoside-5-glucoside): UV-VIS: $\lambda_{\max}$ 531, 326, 278 nm, $E_{\text{acyl}}/E_{\max}$ (%) = 58, $E_{440}/E_{\max}$ (%) = 13, AlCl_3 shift 0; TLC: R_f -values BAW 0.40, BuHCl 0.09, 1% HCl 0.20, AHW 0.51; HPLC: R_t (min) 27.1.
14. Pigment (5) (peonidin 3-rutinoside-5-glucoside): UV-VIS: $\lambda_{\max}$ 529, 278 nm, $E_{440}/E_{\max}$ (%) = 12, AlCl_3 shift 0; TLC: R_f -values BAW 0.15, BuHCl 0.01, 1% HCl 0.62, AHW 0.72; FAB MS m/z 771; HPLC: R_t (min) 16.4. ^1H NMR δ : peonidin 8.95 (1H, s, H-4), 8.34 (1H, *br d*, $J=10.2$ Hz, H-6'), 8.22 (1H, *br s*, H-2'), 7.27 (1H, *br s*, H-8), 7.17 (1H, *d*, $J=10.2$ Hz, H-5'), 7.09 (1H, *br s*, H-6), 3.97 (3H, s, $-\text{OCH}_3$); glucose A 5.54 (1H, *d*, $J=8.9$ Hz, H-1), 3.53 (1H, *m*, H-2), 2.90 – 3.90 (5H, H-3, 4, 5, 6a, 6b); glucose B 5.14 (1H, *d*, $J=8.9$ Hz, H-1), 3.49 (1H, *m*, H-2), 2.90 – 3.90 (5H, H-3, 4, 5, 6a, 6b); rhamnose 4.96 (1H, s, H-1), 3.31 (1H, *m*, H-2), 2.90 – 3.90 (3H, H-3, 4, 5), 1.04 (3H, *m*, $-\text{CH}_3$).
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