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ACYLATED PELARGONIDIN GLYCOSIDES IN RED–PURPLE FLOWERS OF *IPOMOEA PURPUREA*

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Key Word Index—*Ipomoea purpurea*; Convolvulaceae; red–purple flower colour; acylated anthocyanins; di- and tricaffeyl pelargonidin 3-sophoroside-5-glucoside.

Abstract—Four acylated pelargonidin glycosides were isolated from the red–purple flowers of *Ipomoea purpurea*. The acylated anthocyanins were all based on pelargonidin 3-sophoroside-5-glucoside, acylated with caffeic acid and/or glucosylcaffeic acids. Three novel anthocyanins were elucidated to be pelargonidin 3-*O*-[2-*O*-(6-*O*-(*trans*-3-*O*-(β-D-glucopyranosyl)-caffeyl)-β-D-glucopyranosyl)-6-*O*-(*trans*-4-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranosyl)-caffeyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside], pelargonidin 3-*O*-[2-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranosyl)-6-*O*-(*trans*-4-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranosyl)-caffeyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside] and pelargonidin 3-*O*[2-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranosyl)-6-*O*-(*trans*-caffeyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyosdide]. Another anthocyanin was pelargonidin 3-*O*-[2-*O*-(glucosylcaffeylglucosyl)-6-*O*-(caffeyl)-glucoside]-5-gluoside, which was reported to be present in the red–purple flowers of *Pharbitis nil*. Apart from the second pigment cited, these are analogous pigments of the violet–blue *Ipomoea* anthocyanins which are composed of acylated cyanidin glycosides, instead of pelargonidin. Copyright © 1996 Elevier Science Ltd

INTRODUCTION

In the course of an investigation of the flower colour variation in *Ipomoea purpurea*, we found six acylated cyanidin glycosides in the violet–blue flowers of this species [1]. These anthocyanins were substituted by the complicated caffeyl residues at the 3-OH of anthocyanidin in the same manner as those of *Pharbitis* anthocyanins in the flowers of *P. nil* [2–7]. As part of our ongoing programme, we have isolated three new acylated pelargonidin glycosides from the red–purple flowers of *I. purpurea* along with one known acylated pelargonidin glycoside. In the present paper, we wish to report the results of a structural study of these new acylated pelargonidin glycosides.

RESULTS AND DISCUSSION

Four anthocyanin peaks were observed in the extract of the red–purple flowers of *I. purpurea* by HPLC. Their relative frequency of occurrence was 58.7% (pigment **1**), 5.1% (pigment **2**), 7.3% (pigment **3**) and 5.1% (pigment **4**). The isolation of individual anthocyanins was performed by procedures similar to those previously reported [1–7]. The four anthocyanins were extracted from the red–purple flowers with MAW (methanol–acetic acid–water, 10:1:9), isolated and purified using Diaion HP-20 column chromatography, paper chromatography (*n*-butanol–acetic acid–water, 4:1:5; BAW) and HPLC. The chromatographic and spectral properties of the anthocyanins are summarized in Table 1. Acid hydrolysis of all four anthocyanins gave pelargonidin, glucose and caffeic acid; alkaline hydrolysis yielded only one deacylanthocyanin (**6**), whose structure was elucidated to be pelargonidin 3-sophoroside-5-glucoside by TLC and HPLC comparison with authentic samples of deacylated *Pharbitis* red anthocyanins [3]. Furthermore, this structure was confirmed by the analysis of ^{1}H NMR and ^{1}H–^{1}H COSY spectra (Table 2).

The FAB mass spectra of **1**–**4** gave $[M]^+$ **1** *m/z* 1567 ($C_{72}H_{79}O_{39}$), **2** *m/z* 1405 ($C_{66}H_{69}O_{34}$), **3** *m/z* 1243 ($C_{57}H_{63}O_{31}$) and **4** *m/z* 1081 ($C_{51}H_{53}O_{26}$). In order to elucidate their structures, ^{1}H NMR and ^{1}H–^{1}H COSY spectra were measured in CF_3CO_2D–DMSO-d_6 (1:9) and analysed as those previously reported [1–7]. Analysis of these spectra revealed that the molecular ratios of anthocyanin chemical composition (aglycone,

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Table 1. Chromatographic and spectral properties of anthocyanins from the red–purple flowers of *Ipomoea purpurea*

Anthocyanin*	R_f values (×100)				Spectral data in 0.1% HCl-MeOH				R_t† (min)	FAB-MS $[M]^+$
	BAW	BuH	1% HCl	AHW	λ_{max} (nm)	E_{acyl}/E_{max} (%)	E_{440}/E_{max} (%)	$AlCl_3$		
1	43	3	16	33	512,319,287	106	24	0	19.0	1567
2	49	3	7	28	511,325,289	113	24	0	21.4	1405
3	47	5	35	48	511,323,287	98	25	0	15.6	1243
4	58	12	25	41	510,329,289	94	28	0	17.4	1081
5	48	5	41	51	507,323,284	82	33	0	9.9	919
6 (Deacyl)	35	7	71	66	507,—,286	—	26	0	5.7	757

For key to abbreviations, see Experimental.

*1: Pelargonidin 3-[2-(glucosylcaffeylglucosyl)-6-(caffeylglucosylcaffeyl)-glucoside]-5-[glucoside].

2: Pelargonidin 3-[2-(caffeylglucosyl)-6-(caffeylglucosylcaffeyl)-glucoside]-5-[glucoside].

3: Pelargonidin 3-[2-(glucosylcaffeylglucosyl)-6-(caffeyl)-glucoside]-5-[glucoside].

4: Pelargonidin 3-[2-(caffeylglucosyl)-6-(caffeyl)-glucoside]-5-[glucoside].

5: Pelargonidin 3-[2-(glucosyl)-6-(caffeyl)-glucoside]-5-[glucoside].

6: Deacylanthocyanin: pelargonidin 3-sophoroside-5-glucoside.

†*HPLC retention time (min).

Table 2. NMR spectral data for *Ipomoea purpurea* red–purple anthocyanins (400 M Hz, CF_3CO_2D-DMSO-d_6, 1:9 at 25°)*

	1	2	3	4	5	6 (deacyl pigment)
Pelargonidin						
4	8.92 *s*	8.92 *s*	8.90 *s*	8.91 *s*	8.88 *s*	9.01 *s*
6	6.95 *br s*	6.98 *br s*	6.95 *d* (1.6)	6.97 *br s*	7.04 *d* (1.7)	7.10 *d* (2.0)
8	7.02 *br s*	7.00 *br s*	7.03 *d* (1.6)	7.01 *br s*	7.12 *d* (1.7)	7.28 *d* (2.0)
2′,6′	8.55 *d* (9.2)	8.56 *d* (9.2)	8.58 *d* (9.1)	8.57 *d* (9.2)	8.62 *d* (9.1)	8.63 *d* (9.1)
3′,5′	7.07 *d* (9.2)	7.08 *d* (9.2)	7.09 *d* (9.1)	7.09 *d* (9.2)	7.13 *d* (9.1)	7.16 *d* (9.1)
Caffeic acid †‡						
(I)						
2	7.01 *d* (1.5)	7.00 *br s*	6.97 *d* (1.6)	6.96 *br s*	6.98 *d* (2.0)	
5	7.07 *d* (9.2)	7.07 *d* (8.5)	6.76 *d* (8.4)	6.77 *d* (8.8)	6.77 *d* (8.4)	
6	6.85 *m*	6.84 *br d* (8.5)	6.84 *dd* (1.9,8.4)	6.86 *br d* (8.8)	6.89 *dd* (2.0,8.4)	
α	6.03 *d* (16.2)	6.02 *d* (15.9)	6.14 *d* (15.5)	6.15 *d* (16.1)	6.19 *d* (15.8)	
β	7.22 *d* (16.2)	7.22 *d* (15.9)	7.31 *d* (15.5)	7.32 *d* (16.1)	7.34 *d* (15.8)	
(II)						
2	7.50 *br s*	6.96 *br s*	7.33 *br s*	6.89 *d* (1.5)		
5	6.84 *d* (8.1)	6.76 *d* (8.1)	6.82 *d* (8.4)	6.74 *d* (8.6)		
6	7.17 *br d* (8.1)	6.68 *br d* (8.1)	7.02 *br d* (8.4)	6.80 *dd* (1.8,8.6)		
α	6.43 *d* (16.1)	6.15 *d* (15.9)	6.11 *d* (15.9)	5.94 *d* (16.1)		
β	7.52 *d* (16.1)	7.32 *d* (15.9)	7.26 *d* (15.9)	7.21 *d* (16.1)		
(III)						
2	6.95 *d* (1.5)	7.05 *br s*				
5	6.76 *d* (8.1)	6.77 *d* (8.1)				
6	6.85 *m*	6.96 *m*				
α	6.15 *d* (16.1)	6.27 *d* (15.9)				
β	7.32 *d* (16.1)	7.47 *d* (15.9)				
Glucose †‡						
(A)						
1	5.63	5.63	5.61	5.61	5.72	5.66
2	4.00	4.01	4.00	4.00	4.10	4.00
3	3.75	3.75	3.74	3.74	3.76	3.68
4	3.47	3.49	3.49	3.50	3.53	3.38
5	3.90	3.91	3.90	3.89	4.02	3.49
6 a,b	4.32,4.42	4.30,4.42	4.31,4.41	4.30,4.42	4.34,4.43	3.56,3.75
(B)						
1	5.13	5.13	5.10	5.12	5.13	5.12
2	3.55	3.56	3.54	3.55	3.55	3.50
3	3.40	3.41	3.40	3.39	3.39	3.40
4	3.29	3.31	3.27	3.29	3.30	3.29
5	3.50	3.54	3.52	3.53	3.54	3.48
6 a,b	3.57,3.81	3.57,3.82	3.57,3.81	3.58,3.81	3.58,3.82	3.56,3.72
(C)						
1	4.58	4.85	4.84	4.84	4.76	4.77
2	3.17	3.16	3.17	3.15	3.02	2.97
3	3.34	3.37	3.29	3.29	3.14	3.14
4	] 3.28–3.31	] 3.31	3.35	3.43	3.08	3.08
5			3.29	3.29	2.88	2.84
6 a,b	4.08,4.08	4.08,4.08	4.06,406	4.08,4.08	3.28,3.42	3.29,3.36
(D)						
1	4.90	4.90				
2	3.39	3.41				
3	3.30	3.3 1				
4	3.47	3.39				
5	3.75	3.75				
6 a,b	4.29,4.47	4.29,4.47				
(E)						
1	4.82		4.81			
2	3.34		3.33			
3	3.30		3.27			
4	3.17		3.19			
5	3.38		3.40			
6 a,b	3.43, 3.46		3.50,3.75			

*Anthocyanin number as in Table 1.

†Assigned by ^1H–^1H COSY.

‡Assigned by DIFNOE coupling constant (J in Hz) are given in parentheses.

glucose and caffeic acid) for **1**–**4** were as follows; **1** was one molecule of pelargonidin, five molecules of glucose and three molecules of caffeic acid; **2** one of pelargonidin, four of glucose and three of caffeic acid; **3** one of pelargonidin, four of glucose and two of caffeic acid; **4** one of pelargonidin, three of glucose and two of caffeic acid (as shown in Table 2). All these glucose units were determined to be in the β-glucopyranoside form based on the J values (J 1, 2, J 2, 3, J 3, 4 and J 4, 5 = 7.0–10.0). Also, all the caffeic acid units were determined to be of the *trans*-configuration because of α- and β-protons had large coupling constants (J = 15.5–16.2 Hz).

The chemical shifts of the five anomeric protons (A–E) of pigment **1** appeared at δ 5.36–4.82 with large coupling constants (J = 7.0–7.7 Hz). The six methylene protons of Glc A at δ 4.32, 4.42, Glc C at 4.08, 4.08 and Glc D at 4.29, 4.47, being shifted to a lower magnetic field, were assigned by the analysis of ^{1}H–^{1}H COSY and negative NOE difference (DIFNOE) spectra as described previously [2]. Therefore, the three 6-OHs of these glucose units (A, C and D; Fig. 1) were acylated with three *trans*-caffeic acid units (I, II, and III), which exhibited large coupling constants (J = 16.2, 16.1 and 16.1 Hz) for these pairs of their olefinic protons, respectively. All aromatic protons of pelargonidin and caffeic acid moieties were assigned by ^{1}H–^{1}H COSY and confirmed by DIFNOE spectra (Table 2). The application of the DIFNOE spectrum method made it possible to determine the linkages and/or the position of attachments of glucose and caffeic acid units in the molecule (Fig. 1). Thus, these glucose units (A, B and C) were found to be attached to the 3-OH and 5-OH of pelargonidin, and the 2-OH of Glc A, respectively, by irradiations at each anomeric proton of these glucose units. Also, the Glc D and E were determined to be bonded to the 4-OH of caffeic acid I and the 3-OH of caffeic acid II, respectively, by irradiations of H-1 of Glc D and E. By irradiation at H-4 of pelargonidin, NOEs were observed at the α-, β-, 2- and 6-protons of caffeic acid I, as well as the strong NOE at H-1 of Glc A. Therefore, Glc A was deduced to be acylated with caffeic acid I at the 6-OH of Glc A [1–4]. Irradiation of H-1 of Glc D gave a DIFNOE spectrum in which NOEs were observed with a strong doublet signal (δ 7.07, J = 9.2 Hz) of H-5 of caffeic acid I and rather weak signals of the α- and β-H of caffeic acid I. Furthermore, weak NOEs were also observed at α-, β-, 2- and 6-H of caffeic acid III. Therefore, Glc D is attached to the 4-OH of caffeic acid I by a glycosidic bond and esterified with caffeic acid III at the 6-OH of Glc D. Similar irradiation of H-1 of Glc E exhibited NOEs to H-2, H-α and H-β signals of caffeic acid II. Thus, Glc E was attached to the 3-OH of caffeic acid II. As the methylene proton signals of Glc E (δ 3.43 and 3.46) were not shifted to lower field, Glc E was determined to be a terminal residue in this pigment. Therefore, **1** is pelargonidin 3-*O*-[-2-*O*-(6-*O*-(*trans*-3-*O*-(β-D-glucopyranosyl)-caffeyl)-β-D-glucopytanosyl)-6-*O*-(*trans*-4-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranosyl)-caffeyl)-β-D-glucopyranoside-5-*O*-[β-D-glucopyranoside], which is a new pigment [8, 9]. Its structure was also confirmed by the analysis of ^{13}C NMR, HMQC and HMBC spectra (Table 2 and

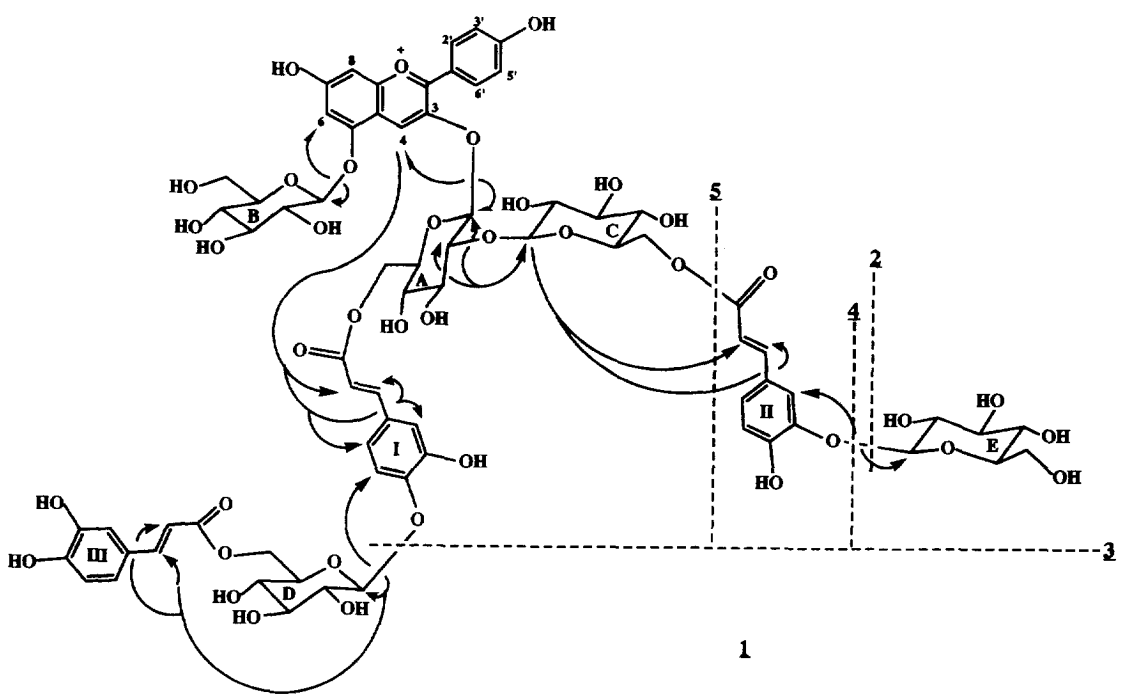

Fig. 1. Anthocyanins isolated from the red–purple flowers of *Ipomoea purpurea*. Observed NOEs are indicated by arrows.

Experimental). We have already reported the structural determination of an analogous cyanidin pigment, present in the violet–blue flowers of *I. purpurea* as a major anthocyanin [1].

The ^{1}H NMR spectrum of pigment **2** was superimposable on that of **1**, except for the signals of Glc E moiety of **1** (Fig. 1 and Table 2). The detailed structure of **2** was elucidated by ^{1}H NMR including ^{1}H–^{1}H COSY and DIFNOE spectral methods as described for the structural determination of **1**. The six characteristic protons being shifted to a lower magnetic field were assigned to be the three pairs of methylene protons of glucose units (A δ 4.32, 4.42; C δ 4.08, 4.08; and D δ 4.29, 4.47) indicating that the 6-OH of these glucose units are esterified with three caffeic acid units (**I**–**III**). Therefore, the Glc B unit was not acylated with caffeic acid but free from acid. Irradiation of H-1 of Glc D gave a DIFNOE spectrum in which NOEs to a strong doublet signals (δ 7.07, $J = 8.5$ Hz) of H-5 of caffeic acid-I moiety were observed. Therefore, Glc D is attached to the 4-OH of caffeic acid I. Irradiation of H-1 of Glc C gave a strong NOE to H-2 of Glc A and also rather weak NOEs at the α- and β-H of caffeic acid III. Thus, pigment **2** is determined to be pelargonidin 3-*O*-[2-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranosyl)-6-*O*-(*trans*-4-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranosyl)caffeyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside], which is a new pigment in plants [8, 9].

The chromatographic and spectral properties of pigment **3** were identical with those of *Pharbitis* red anthocyanin **4** (Table 1) [4]. Also, the chemical shifts of protons of **3** were similar to those of *Pharbitis* red anthocyanin **4** (Table 2). The four characteristic protons, being shifted to a lower magnetic field at δ 4.31, 4.41 (Glc A) and δ 4.06, 4.06 (Glc C), were assigned to be methylenes of glucose units A and C by analysis of the ^{1}H–^{1}H COSY and DIFNOE spectra of **3**, indicating that the 6-OH of Glc A and Glc C are esterified with caffeic acids (Fig. 1). Irradiation of H-1 of Glc E gave a strong NOE to the H-2 signals and also rather weak NOEs to the signals of H-α and -β of caffeic acid II. Thus, Glc E was attached to the 3-OH of caffeic acid II. By irradiation of H-1 of Glc C, NOEs to signals of H-2, -3 and -6 of Glc C, as well as H-1 and -2 of Glc A, were observed and also NOEs were found at the signals of H-α, -β, -2, -5 and -6 of caffeic acid II by the same irradiation. Therefore, **3** is pelargonidin 3-*O*-[2-*O*-(6-*O*-(*trans*-3-*O*-(β-D-glucopyranosyl)-caffeyl-β-D-glucopyranosyl)-6-*O*-(*trans*-caffeyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside). This pigment is also reported to be present in the red–purple flowers of *P. nil* [4].

The chemical shifts of the protons of pigment **4** were assigned by ^{1}H NMR, including ^{1}H–^{1}H COSY and DIFNOE spectral methods (Table 2). By application of DIFNOE [2], three anomeric protons at δ 5.61, 5.12 and 4.84 were assigned to those of glucoses A, B and C, respectively. Two caffeic acid units were determined to be bonded to the 6-OH of Glc A and Glc C, because of the low-field shifts of both their methylene protons (Glc A: δ 4.30, 4.42 and Glc C: δ 4.08, 4.08). Moreover, the glycosidic linkage of sophorose (Glc A and Glc C) was deduced by the observation of a low-field shift (H-2, δ 4.00) of Glc A, suggesting that the C-1 of Glc C was bonded to C-2 of Glc A. Therefore, **4** is determined to be pelargonidin 3-*O*-[2-*O*-(6-*O*-(*trans*-caffeyl)-β-D-glucopyranosyl)-6-*O*-(*trans*-cafeyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside], which is a new pigment [8, 9].

A monoacylated anthocyanin (pigment **5**) was obtained as a product of alkaline hydrolysis of **1**–**4**. This pigment was purified by the same process as used for **1**–**4** and identified as pelargonidin 3-*O*-[2-*O*-β-D-glucopyranosyl)-6-*O*-(*trans*-caffeyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside] (Table 2, Pigment **5**). The occurrence of this pigment from the red–purple flowers of *P. nil* has been reported previously [4].

EXPERIMENTAL

Plant material. Red–purple strains of *I. purpurea* were grown on the farm of Chiba University and also in the private garden of one of us (K.K.). These flowers exhibited mostly similar red–purple colours (Red-Purple 62D, 74A by R.H.S. colour chart, chromaticity value (b/a) = 0.28, −0.20). Fresh corollas were collected in August–October 1993 and 1994.

Isolation of anthocyanins. Fresh corollas (1 kg) were extracted with MAW (10:1:9, 10 *l*). The extract was concd to 500 ml. The concd extract was purified by Diaion HP-20 CC, PC and HPLC as described previously [1–7]. Solvents used were 15% HOAc, BAW 4:1:5), 5% HOAc–MeOH and MAW for CC and PC. Prep. HPLC was run on a Waters C_{18} (19ϕ × 150 mm) column at 40° at 4 ml min^{-1} and monitored at 530 nm for anthocyanins. Solvent systems used were as follows; a linear gradient elution for 30 min from 40 to 85% solvent B (1.5% H_3PO_4, 20% HOAc, 25% MeCN in H_2O) in solvent A (1.5% H_3PO_4 in H_2O). The pigment frs were evapd *in vacuo* to dryness. The evapn residues were dissolved in a small vol. of 5% HOAc–EtOH followed by addition of excess Et_2O, and then dried to give pigment powder (pigment **1**, *ca* 40 mg, pigment **2**, *ca* 20 mg, pigment **3**, *ca* 30 mg and pigment **4**, 20 mg).

Analysis of anthocyanins. Fresh corolla limbs (*ca* 0.02 g) of each strain were extracted with 20% MeOH containing 1.5% H_3PO_4 or MAW. Quantitative analysis was performed by HPLC on a Waters C_{18} (4.6ϕ × 250 mm) column at 40° with a flow rate of 1 ml min^{-1}, monitoring at 530 nm for anthocyanins. Solvent system employed was: linear gradient elution for 30 min from 40 to 85% solvent B in solvent A. Characterization of pigments was carried out by PC, TLC and UV–VIS spectrometry. Solvents used were BAW, BUH (*n*-BuOH-2–N HCl, 1:1), 1% HCl and AHW (HOAc–HCL–H_2O), 15:3:82) for anthocyanins, and *n*-BuOH–HOAc–H_2O (4:1:2), EtOAc–HOAc–H_2O (3:1:1)

and EtOAc–HCO_2H–H_2O (5:2:1) for organic acids and sugars. Acid hydrolysis, alkaline deacylation, H_2O_2 oxidation and partial acid hydrolysis of anthocyanins were performed according to standard procedures [10, 11].

FAB MS and NMR measurements. FAB MS were recorded in positive mode using the magic bullet and in negative mode in glycerol. NMR were recorded at 400 and 500 MHz for ^1H and at 100 and 125 NHz for ^{13}C in DMSO-d_6-CF_3CO_2D (9:1). Chemical shifts are reported relative to TMS int. standard (δ) and coupling constants are reported in Hz.

^{13}C NMR of pigment **1**. Pelargonidin: 162.5 (C-2), 144.1 (C-3), 135.1 (C-4), 155.5 (C-5), 104.9 (C-6), 168.3 (C-7), 96.5 (C-8), 155.4 (C-8a), 111.9 (C-4a), 118.7 (C-1′), 135.1 (C-2′, -6′), 117.2 (C-3′, -5′); caffeic acid (I): 128.8 (C-1), 115.3 (C-2), 145.6 (C-3), 147.5 (C-4), 117.2 (C-5), 121.5 (C-6), 115.8 (C-α), 144.5 (C-β), 166.2 (CO); (II): 126.0 (C-1), 116.4 (C-2), 147.0 (C-3), 149.6 (C-4), 116.1 (C-5), 124.5 (C-6), 115.0 (C-α), 145.2 (C-β), 166.7 (CO); (III): 125.6 (C-1), 115.3 (C-2), 145.7 (C-3), 148.5 (C-4) 116.0 (C-5), 120.8 (C-6), 113.7 (C-α), 145.8 (C-β), 166.7 (CO); glucose (A): 100.2 (C-1), 81.0 (C-2), 76.1 (C-3), 69.8 (C-4), 74.2 (C-5), 63.2 (C-6); (B): 101.9 (C-1), 73.4 (C-2), 75.8 (C-3), 69.8 (C-4), 77.8 (C-5), 60.9 (C-6); (C): 104.2 (C-1), 74.6 (C-2), 76.1 (C-3), 70.0 (C-4), 76.3 (C-5), 63.4 (C-6); (D): 101.6 (C-1), 73.4 (C-2), 76.1(C-3), 69.8 (C-4), 74.2 (C-5), 63.0 (C-6), (E): 102.1 (C-1), 73.5 (C-2), 76.3 (C-3), 70.2 (C-4), 77.5 (C-5), 61.1 (C-6).

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