Phytochemistry 76 (2012) 73–77

Contents lists available at SciVerse ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Triacylated peonidin 3-sophoroside-5-glucosides from the purple flowers of *Moricandia ramburii* Webb.

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ARTICLE INFO

Article history:
Received 28 July 2011
Received in revised form 11 November 2011
Available online 20 January 2012

Keywords:
Moricandia ramburii Webb.
Brassicaceae
Purple flower color
Acylated peonidin 3-sophoroside-5-glucoside

ABSTRACT

Triacylated peonidin 3-sophoroside-5-glucosides were isolated from the purple flowers of *Moricandia ramburii* Webb. (Family: Brassicaceae), and determined to be peonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-glucosyl)-6-*O*-(*trans*-*p*-coumaroyl)-glucoside]-5-*O*-[6-*O*-(malonyl)-glucoside] (**1**), peonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-glucosyl)-6-*O*-(*cis*-*p*-coumaroyl)-glucoside]-5-*O*-[6-*O*-(malonyl)-glucoside] (**2**) and peonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-sinapoyl)-glucosyl)-6-*O*-(*trans*-*p*-coumaroyl)-glucoside]-5-*O*-[6-*O*-(malonyl)-glucoside] (**3**), respectively, by chemical and spectroscopic methods. In addition, one known acylated cyanidin glycoside, cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-glucosyl)-6-*O*-(*trans*-*p*-coumaroyl)-glucoside]-5-*O*-[6-*O*-(malonyl)-glucoside] (**4**), was also identified in the flowers. Peonidin glycosides have not been reported hitherto in floral tissues in to Brassicaceae.

1. Introduction

Moricandia DC. (Violet Cabbage) species (Brassicaceae) are native to the Mediterranean region, and cultivated as ornamental plants with purple, violet and white flowers in Europe and America. In the limited flavonoid work reported for this genus, flavonol glycosides have been characterized (Braham et al., 2005). To date, 17 acylated 3-sophoroside-5-glucosides of pelargonidin and cyanidin isolated from 2 genera and 45 acylated 3-sambubioside-5-glucosides of pelargonidin, cyanidin and delphinidin isolated from 7 genera, have been unambiguously identified from flowers of Brassicaceae (Honda et al., 2005; Saito et al., 1995a,b, 1996, 2008, 2011; Tatsuzawa et al., 2006, 2007, 2008a,b, 2010). Distribution of 3-sophoroside-5-glucosides of anthocyanidin is, however, restricted to the flowers of the genera *Raphanus* and *Iberis* (Saito et al., 2008; Tatsuzawa et al., 2008b), while the occurrence of peonidin has not been reported in flowers of Brassicaceae species. In this paper, the structure elucidation of three new triacylated peonidin 3-sophoroside-5-glucosides from the purple flowers of *Moricandia ramburii*, is reported.

2. Results and discussion

Four major anthocyanin pigments (**1**–**4**) were found in the methanol–acetic acid–water (MAW) (4:1:5, v/v/v) extracts from purple flowers of *M. ramburii* by high performance liquid chromatography (HPLC) UV–Vis analysis (Fig. 1). The percentage of pigments **1**–**4**, calculated as the total anthocyanin content by HPLC vis peak area at 530 nm, was 35.9%, 6.2%, 12.9% and 19.2% respectively, with the MAW extract also containing numerous small pigment peaks.

The four pigments (**1**–**4**) were extracted from the purple flowers with 5% AcOH, followed by isolation using Diaion HP-20 column chromatography, preparative HPLC and thin layer chromatography (Tatsuzawa et al., 2010).

Acid hydrolysis of pigments **1**–**3** and **4** yielded peonidin and cyanidin as their anthocyanidins, respectively (Harborne, 1984), while also affording glucose, hydroxycinnamic acid and malonic acid moieties. Moreover, *trans*-*p*-coumaric acid and *trans*-ferulic acid, *cis*-*p*-coumaric acid and *trans*-ferulic acid, and *trans*-*p*-coumaric acid and *trans*-sinapic acid were detected in the hydrolysates of **1** and **4**, **2**, and **3** by TLC, respectively, with malonic acid found in all pigments (Harborne, 1984).

Alkaline hydrolysis of **1**–**3** yielded peonidin 3-sophoroside-5-glucoside, and alkaline hydrolysis of **4** yielded cyanidin 3-sophoroside-5-glucoside. The deacyl anthocyanin structures were tentatively identified via co-TLC and co-HPLC with authentic peonidin 3-sophoroside-5-glucoside and cyanidin 3-sophoroside-5-glucoside, with the latter being prepared from *Ipomoea tricolor* (Kondo et al., 1987) and *Ipomoea purpurea* (Saito et al.,1995b), respectively, by alkaline hydrolysis.

The structures of **1**–**4** were confirmed based on the analyses of their ^{1}H (500 MHz), ^{13}C (126 MHz) and 2D (COSY, NOESY, ^{1}H–^{13}C

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doi:10.1016/j.phytochem.2011.12.019

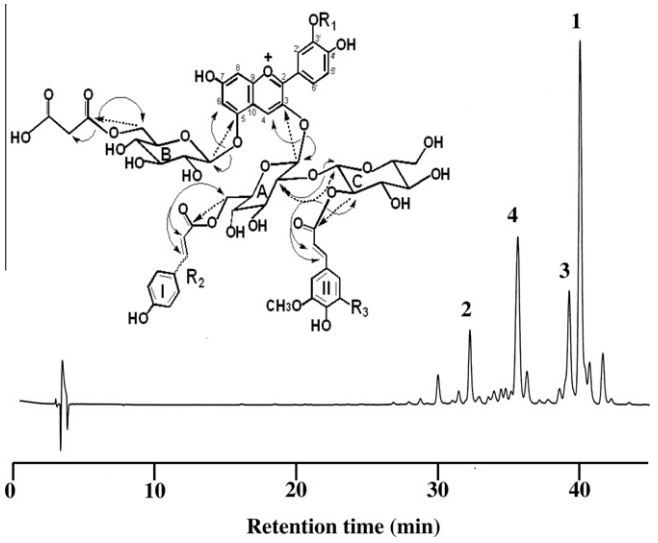

Fig. 1. HPLC profile (530 nm) and structure of acylated anthocyanins isolated from the purple flowers of *Moricandia ramburii*. Observed main NOEs are indicated by arrows. Observed HMBCs of **1** are indicated by dotted arrows. **1**: $R_1 = CH_3$, R_2 = *trans*, $R_3 = H$; **2**: $R_1 = CH_3$, R_2 = *cis*, $R_3 = H$; **3**: $R_1 = CH_3$, R_2 = *trans*, $R_3 = OCH_3$; **4**: $R_1 = H$, R_2 = *trans*, $R_3 = H$.

HMQC and ^1H–^{13}C HMBC) NMR spectra in DMSO-d_6-CF_3COOD (9:1), as well as their fast atom bombardment mass spectra (FABMS).

2.1. Pigment 1

The molecular ion $[M]^+$ of **1** was observed at *m/z* 1195.3160 ($C_{56}H_{59}O_{29}$), indicating a peonidin structure with three molecules of glucose, and one molecule each of *p*-coumaric acid, ferulic acid and malonic acid.

The chemical shifts of the 13 aromatic protons of the peonidin, *p*-coumaric acid and ferulic acid moieties, together with their coupling constants, were assigned as shown in Table 1. Six protons were assigned to the two methoxyl groups of peonidin and ferulic acid. Two sets of two pairs of doublet resonances, assigned to the four olefinic proton signals of the *p*-coumaric and ferulic acid moieties, indicated a *trans* configuration for the acids based on their coupling constants (J = 16.0 Hz each) (Table 1). The chemical shifts of the sugar moiety protons were observed in the region of δ_H 5.74–3.17, with the three anomeric proton resonances at δ_H 5.74 (*d*, J = 7.5 Hz, Glc A), 5.14 (*d*, J = 7.5 Hz, Glc B), and 5.15 (*d*, J = 8.5 Hz, Glc C). Based on the observed coupling constants (Table 1), these three sugars were assumed to be in their β-pyranose forms. The linkages and/or positions of the attachments of the sugar and acyl groups were determined based on 2D COSY and NOESY experiments.

By application of a NOESY experiment, NOEs between H-1 of Glc A and H-4 (δ_H 8.73) of peonidin, H-1 of Glc B and H-6 (δ_H 6.94) of peonidin, and H-2 (δ_H 4.04) of Glc A and H-1 of Glc C were observed (Fig. 1), supporting glycosylation of the C-3 and C-5 peonidin hydroxyl groups with Glc A and Glc B, respectively, while also indicating a sophorose structure between Glc A and Glc C.

Five characteristic downfield shifted proton signals were assigned to the methylene protons of Glc A (δ_H 4.12 and 4.35, H-6a and b) and Glc B (δ_H 4.06 and 4.37, H-6a and b), and to a methine proton (δ_H 4.68, *t*, J = 8.8 Hz, H-2) of Glc C, indicating acylation of C-6 OH (Glc A and Glc B) and C-2 OH (Glc C) with three acid molecules. In the NOESY spectrum, the correlations between H-6a,b of Glc A and H-α,β of *p*-coumaric acid, H-6a,b of Glc B and -CH_2- of malonic acid, and H-2 of Glc C and H-α,β of sinapic acid were observed, establishing acylation group at C-6 OH (Glc A and Glc B) and C-2 OH (Glc C) as *p*-coumaric acid, malonic acid and ferulic acid, respectively. Consequently, the structure of pigment **1** was determined to be peonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-glucosyl)-6-*O*-(*trans*-*p*-coumaroyl)-glucoside]-5-*O*-[6-*O*-(malonyl)-glucoside], which is a new anthocyanin in plants (Andersen and Jordheim, 2006; Harborne and Baxter, 1999; Honda and Saito, 2002). This structure was also confirmed by analysis of its ^{13}C, ^1H–^{13}C HMQC and ^1H–^{13}C HMBC NMR spectra. (Table 1, Fig. 1).

2.2. Pigment 2

The molecular ion $[M]^+$ of **2** was observed at *m/z* 1195.3142 ($C_{56}H_{59}O_{29}$) indicating a peonidin structure with three molecules of glucose, and one molecule each of *p*-coumaric acid, ferulic acid and malonic acid, similar to **1**. The ^1H NMR spectrum of **2** was similar to that of **1**, except for the signals of the *p*-coumaric acid moiety (Table 1). The olefinic protons of **2** were shifted upfield to δ_H 5.63 and 6.34 (*d*, J = 12.9 Hz each) in comparison with those of **1**, establishing **2** as peonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-glucosyl)-6-*O*-(*cis*-*p*-coumaroyl)-glucoside]-5-*O*-[6-*O*-(malonyl)-glucoside], which is a new anthocyanin in plants. This structure was also confirmed by analysis of its ^{13}C, including ^1H–^{13}C HMQC and ^1H–^{13}C HMBC NMR spectra. (Table 1).

2.3. Pigment 3

The molecular ion $[M]^+$ of **3** was observed at *m/z* 1225.3224 ($C_{57}H_{61}O_{30}$), indicating a peonidin structure with three molecules of glucose, and one molecule each of *p*-coumaric acid, sinapic acid and malonic acid. The ^1H NMR spectrum of **3** was similar to that of **1**, with **3** containing a sinapic acid moiety instead of a ferulic acid moiety (Table 1). The two aromatic proton signals of sinapic acid (II) were observed at δ_H 6.76 (H-2 and H-6). The olefinic proton signals [δ_H 6.31 (*d*, J = 16.0 Hz, H-α) and (*d*, J = 16.0 Hz, H-β)] indicating a *trans*-sinapic acid moiety establishing **3** as peonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-sinapoyl)-glucosyl)-6-*O*-(*trans*-*p*-coumaroyl)-glucoside]-5-*O*-[6-*O*-(malonyl)-glucoside], which is a new anthocyanin in plants. This structure was also confirmed by analysis of its ^{13}C, including ^1H–^{13}C HMQC and ^1H–^{13}C HMBC NMR spectra. (Table 1).

2.4. Pigment 4

The molecular ion $[M]^+$ of **4** was observed at *m/z* 1181.2991 ($C_{55}H_{57}O_{29}$), indicating a cyanidin structure with three molecules of glucose, and one molecule each of *p*-coumaric acid, ferulic acid and malonic acid. The ^1H NMR spectrum of **4** was similar to that of **1**, with 4 containing a cyanidin moiety instead of a peonidin moiety, establishing (Table 1), **4** as cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-glucosyl)-6-*O*-(*trans*-*p*-coumaroyl)-glucoside]-5-*O*-[6-*O*-(malonyl)-glucoside], which has been found in *Iberis umbellata* (Family: Brassicaceae) (Saito et al., 2008). This structure was also confirmed by analysis of its ^{13}C, ^1H–^{13}C HMQC and ^1H–^{13}C HMBC NMR spectra (Table 1).

3. Concluding remarks

Three new acylated peonidin glycosides were isolated along with one known acylated cyanidin glycoside from the purple flowers of *M. ramburii*. From a chemotaxonomical point of view, this report is the first to describe the distribution of peonidin glycosides in the flowers of Brassicaceae (Harborne, 1967; Timberlake and Bridle, 1975; Hrazdina, 1982; Harborne and Grayer, 1988; Strack and Wray, 1994; Andersen and Jordheim, 2006). Moreover,

Table 1
^{1}H and ^{13}C NMR spectroscopic data of acylated anthocyanins from the flowers of *Moricandia ramburii* in DMSO-d_6/CF_3CO_2D (9:1).

	1		2		3		4	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Anthocyanidin								
2		162.4		161.7		162.4		162.8
3		144.5		144.3		144.5		144.8
4	8.73 s	132.7	8.53 s	131.8	8.74 s	132.7	8.69 s	131.1
5		155.3		155.0		155.2		155.3
6	6.94 s	105.0	6.81 brs	103.9	6.95 brs	105.0	6.94 d(1.5)	104.9
7		168.0		168.3		168.4		167.1
8	6.93 s	96.8	6.72 brs	96.3	6.92 brs	96.8	6.89 d(1.5)	96.4
9		155.4		155.1		155.4		155.7
10		111.9		111.9		112.0		114.1
1′		119.9		119.8		119.9		120.0
2′	7.95 d(2.0)	114.5	7.86 d(1.9)	113.9	7.94 d(1.9)	114.1	7.91 d(2.2)	117.5
3′		148.8		148.9		148.8		146.9
4′		156.3		156.1		156.3		156.3
5′	7.13 d(9.0)	117.2	7.17 d(8.8)	116.9	7.13 d(8.8)	117.2	7.11 d(9.0)	117.3
6′	8.30 dd(2.0, 9.0)	129.6	8.25 dd(1.9, 8.8)	129.4	8.29 dd(1.9, 8.8)	129.6	8.32 dd(2.2, 9.0)	128.8
$-OCH_3$	3.93 s	56.3	3.96 s	56.0	3.93 s	56.3		
p-Coumaric acid (I)								
1		125.4		125.6		125.4		125.3
2,6	7.23 d(8.5)	130.7	7.16 d(8.8)	132.5	7.22 d(8.5)	130.7	7.23 d(8.5)	130.7
3,5	6.68 d(8.5)	116.0	6.41 d(8.8)	114.9	6.68 d(8.5)	116.0	6.67 d(8.5)	116.0
4		160.3		159.3		160.3		160.2
α	6.16 d(16.0)	114.1	5.63 d(12.9)	115.0	6.16 d(16.1)	114.1	6.39 d(15.8)	114.7
β	7.28 d(16.0)	145.5	6.34 d(12.9)	143.3	7.27 d(16.1)	145.6	7.29 d(15.8)	145.4
COOH		167.1		166.5		167.1		166.4
Ferulic acid or Sinapic acid (II)								
1		126.0		125.9		124.9		126.3
2	7.06 brs	111.4	7.01 brs	111.3	6.76 s	106.5	7.20 d(1.3)	111.6
3		148.4		148.3		148.5		148.4
4		149.8		149.8		138.8		149.8
5	6.71 d(8.0)	115.9	6.70 d(8.5)	115.9		148.5	6.77 d(8.5)	116.2
6	6.98 brd(8.0)	123.5	6.86 brd(8.5)	123.4	6.76 s	106.5	7.03 dd(1.3, 8.5)	123.4
α	6.25 d(16.0)	115.2	6.22 d(15.8)	115.0	6.31 d(16.0)	115.6	6.18 d(15.8)	115.5
β	7.35 d(16.0)	145.3	7.31 d(15.8)	144.3	7.34 d(16.0)	145.5	7.47 d(15.8)	145.3
COOH		166.2		166.1		166.2		167.3
$-OCH_3$	3.75 s	56.1	3.74 s	55.8	3.74 s	56.5	3.80 s	56.1
$-OCH_3$					3.74 s	56.5		
Malonic acid								
$-CH^2-$	3.30 s	41.5	3.42 s	41.7	3.30 s	41.6	3.31 s	41.5
COOH		167.3		167.4		167.3		167.7
COOH		168.4		167.9		168.0		168.0
Glucose (A)								
1	5.74 d(7.5)	98.3	5.71 d(7.9)	97.4	5.75 d(7.6)	98.2	5.65 d(7.6)	98.8
2	4.04 t(7.8)	78.3	4.07 t(8.5)	77.9	4.05 t(8.2)	78.3	4.13 t(8.0)	77.8
3	3.72 t(8.5)	75.1	3.71 t(9.2)	75.9	3.72 m	75.8	3.64 t(9.2)	76.7
4	3.44 t(9.0)	71.0	3.31 t(8.8)	70.4	3.42 t(9.5)	70.8	3.37 t(9.5)	70.9
5	3.95 m	74.1	3.90 ddd(2.2, 8.2, 10.4)	74.7	3.95 m	74.1	3.95 m	74.0
6a	4.21 dd(7.5, 12.0)	63.5	4.39 dd(7.6, 11.5)	63.3	4.21 dd(7.3, 12.0)	63.5	4.22 dd(7.3, 12.5)	63.5
6b	4.35 brd(12.0)		4.24 brd(11.5)		4.35 m		4.36 brd(12.5)	
Glucose (B)								
1	5.14 d(7.5)	102.3	5.15 d(7.6)	101.2	5.14 d(7.3)	102.2	5.14 d(7.6)	101.0
2	3.56 t(8.5)	73.5	3.55 t(8.5)	73.4	3.56 t(8.4)	73.5	3.54 t(8.5)	73.5
3	3.42 t(9.0)	74.7	3.44 t(8.8)	75.9	3.42 t(9.5)	76.1	3.39 t(9.1)	76.1
4	3.27 t(9.5)	69.9	3.28 t(9.1)	70.4	3.27 t(9.3)	69.9	3.24 t(9.2)	69.9
5	3.78 m	74.7	3.81 ddd(2.6, 6.5, 9.1)	74.6	3.78 m	74.7	3.75 m	74.6
6a	4.06 dd(7.0, 12.0)	64.4	4.31 dd(7.6, 11.5)	64.4	4.06 dd(5.9, 11.8)	64.4	4.03 dd(6.2, 11.9)	64.4
6b	4.37 brd(12.0)		4.46 brd(11.5)		4.37 m		4.38 brd(11.9)	
Glucose (C)								
1	5.15 d(8.5)	99.2	5.19 d(8.2)	99.0	5.15 d(7.9)	99.2	5.15 d(7.6)	100.1
2	4.68 t(8.8)	74.2	4.66 t(8.8)	74.3	4.69 t(8.8)	74.2	4.65 t(9.0)	74.4
3	3.40 t(7.7)	75.1	3.38 t(8.8)	74.9	3.40 t(9.8)	75.1	3.32 m	75.1
4	3.18 t(8.0)	70.8	3.16 t(9.5)	70.9	3.19 t(9.8)	71.0	3.16 t(8.8)	71.0
5	3.17 m	77.8	3.17 m	77.9	3.16 m	77.8	3.04 m	77.8
6a	3.44 dd(7.0, 11.0)	61.6	3.46 dd(7.3, 11.7)	61.6	3.46 m	61.6	3.42 m	61.6
6b	3.70 brd(11.0)		3.72 m		3.71 brd(10.7)		3.66 m	

there are two C-3 OH glycosidic patterns for anthocyanidins found in the flowers of Brassicaceae species, namely acylated 3-sophoroside from *Iberis umbellata* and *Raphanus sativus* (Saito et al., 2008; Tatsuzawa et al., 2008b) and acylated 3-sambubioside from *Cheiranthus cheiri*, *Heliophila coronopifolia*, *Lobularia maritima*, *Lunaria annua*, *Malcolmia maritima*, *Matthiola incana* and *Orychophragmus violaceus* (Honda et al., 2005; Saito et al., 1995a, 1996, 2011; Tatsuzawa et al., 2006, 2007, 2008a, 2010). Therefore, the floral anthocyanins of *M. ramburii* are grouped into the former pattern.

4. Experimental

4.1. General procedures

TLC was carried out on plastic coated cellulose sheets (Merck) using eight mobile phases: BAW (*n*-BuOH–AcOH–H_2O, 4:1:2, v/v/v), BuHCl (*n*-BuOH-2N HCl, 1:1, v/v, upper layer), AHW (AcOH–HCl–H_2O, 15:3:82, v/v/v), 1% HCl for anthocyanins, Forestal (AcOH–HCl–H_2O, 30:3:10, v/v/v) for anthocyanidin and BAW, EAA (EtOAc–AcOH–H_2O, 3:1:1, v/v/v), ETN (EtOH–NH_4OH–H_2O, 16:1:3, v/v/v) and EFW (EtOAc–HCO_2H–H_2O, 5:2:1, v/v/v) for sugars and organic acids with UV light and aniline hydrogen phthalate spray reagent (Harborne, 1984).

Analytical HPLC was performed on a LC 10A system (Shimadzu), using a Waters C18 (4.6 × 250 mm) column at 40 °C with a flow rate of 1 mL/min and monitoring at 530 nm. The eluant was applied as a linear gradient elution for 40 min from 20 to 85% solvent B (1.5% H_3PO_4, 20% AcOH, 25% MeCN in H_2O) in solvent A (1.5% H_3PO_4 in H_2O) with 5 min of re-equilibration at 20% solvent B, for anthocyanins, anthocyanidins and hydroxycinnamic acids (method 1). The other eluant for malonic acid was applied as an isocratic elution of solvent A for 10 min and monitoring at 210 nm (Tatsuzawa et al., 2009) (method 2).

UV–Vis spectra were recorded on UV–Vis Multi Purpose Spectrophotometer (MPS-2450, Shimadzu) in 0.1% HCl–MeOH (from 200 to 700 nm).

High resolution FAB mass (FABMS) spectra were determined on a JEOL JMS-700 Mass spectrometer operating in the positive ion mode using 1:1 mixture of dithiothreitol and 3-nitrobenzyl alcohol as a matrix. ^1H (500 MHz) and ^{13}C (126 MHz) NMR spectra were measured on a Bruker Avance III 500 MHz NMR spectrometer using CF_3COOD-DMSO-d_6 (1:9) as a solvent. Chemical shifts are reported relative to DMSO (2.5 ppm), and coupling constants (*J*) are in Hz.

4.2. Plant materials

The seeds of *M. ramburii* Webb. (accession number: MOR-RAM-1) were obtained from Prof. T. Nishio at the Tohoku University *Brassica* Seed Bank, and grown in greenhouses at the Laboratory of Olericultural and Floricultural Science, Faculty of Agriculture, Iwate University. The flowers exhibited a purple coloration [purple 78B by Royal Horticultural Society color chart and chromaticity value, $b^*(-29.36)/a^*(78.66)$]. Flowers were collected from winter to spring seasons in Iwate, Japan and dried overnight at 40 °C, and kept in a refrigerator at 4 °C. Chromaticity values were recorded on a NR-3000 handy colorimeter (Nippon Denshoku Industries Co., Ltd.).

4.3. Isolation of anthocyanin

Dried flowers (100 g) of *M. ramburii* were immersed in AcOH–H_2O (3L; AcOH–H_2O, 1:19) at room temperature for 5 h. and extracted. The extract was passed through a Diaion HP-20 (Mitsubishi Chemical's Ion Exchange Resins, aromatic type adsorbent) column (90 × 150 mm), on which acylated anthocyanins were adsorbed. The column was thoroughly washed with H_2O (2L) and eluted with 5% AcOH:MeOH (500 mL, 5:95, v/v) to recover the anthocyanins. After concentration, the residue was separated and purified with paper chromatography (PC) using BAW. The separated pigments were further purified by TLC (15% AcOH) and prep. HPLC. With the latter performed on a Waters C18 (19 × 150 mm) column at 40 °C with a flow rate of 4 mL/min and monitoring at 530 nm. The solvent used was as follows: a linear gradient elution for 16 min from 60 to 80% solvent B in solvent A. Each fraction was transformed to a Diaion HP-20 column, on which pigments were adsorbed. Anthocyanin pigments were eluted with 5% AcOH–MeOH followed by addition of excess of Et_2O, and then dried. The purified pigments from the flowers were obtained as follows; pigment **1** (ca. 95 mg), pigment **2** (ca. 13 mg), pigment **3** (ca. 23 mg) and pigment **4** (ca. 55 mg).

4.4. Analyses of anthocyanins

The identification of anthocyanins was carried out by standard procedures and both alkaline and acid hydrolyses (Harborne 1984). Acid hydrolysis of pigments (ca. 1 mg each) was carried out with 2N HCl (1 mL) at 100 °C for 1 h. Alkaline hydrolysis of pigments (ca. 1 mg each) was carried out with 2N NaOH solution (1 mL) under degassed syringe allowed to stand for 15 min. The solution was next acidified with 2N HCl (1.1 mL) and evaporated in vacuo to dryness. The data of TLC (R_f values), HPLC (R_t-min, method 1), UV–Vis (λ_{max}), and FABMS spectra are shown in Section 4.4.1.–4.4.4.

4.4.1. Pigment 1

Dark purple-red powder; UV–Vis (in 0.1% HCl-MeOH): λ_{max} 533, 318, 295, 283 nm, E_{acyl}/E_{max} = 109%, E_{440}/E_{max} = 13%, $AlCl_3$ shift 0; TLC: R_f-values (100×) BAW 61, BuHCl 84, 1% HCl 33, AHW 78; HPLC: R_t (min) 39.8; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HR-FABMS calc. for $C_{56}H_{59}O_{29}$: 1195.3142. Found: 1195.3160.

4.4.2. Pigment 2

Dark purple-red powders; UV–Vis: λ_{max} 538, 320, 295, 283 nm, E_{acyl}/E_{max} = 87%, E_{440}/E_{max} = 14%, $AlCl_3$ shift 0; TLC: R_f-values (100×) BAW 55, BuHCl 85, 1% HCl 41, AHW 82; HPLC: R_t (min) 31.9; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HR-FABMS calc. for $C_{56}H_{59}O_{29}$: 1195.3142. Found: 1195.3105.

4.4.3. Pigment 3

Dark purple-red powders; UV–Vis: λ_{max} 533, 318, 296, 283 nm, E_{acyl}/E_{max} = 107%, E_{440}/E_{max} = 12%, $AlCl_3$ shift 0; TLC: R_f-values (100×) BAW 53, BuHCl 78, 1% HCl 36, AHW 86; HPLC: R_t (min) 39.0; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HR-FABMS calc. for $C_{57}H_{61}O_{30}$: 1225.3248. Found: 1225.3224.

4.4.4. Pigment 4

Dark purple-red powders; UV–Vis: λ_{max} 530, 318, 296, 284 nm, E_{acyl}/E_{max} = 112%, E_{440}/E_{max} = 12%, $AlCl_3$ shift 0; TLC: R_f-values (100×) BAW 53, BuHCl 83, 1% HCl 28, AHW 71; HPLC: R_t (min) 35.3; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HR-FABMS calc. for $C_{55}H_{57}O_{29}$: 1181.2986. Found: 1181.2991.

4.5. Analyses of hydrolysates (deacylanthocyanins, anthocyanidins, sugar and acids)

The identification of hydrolysates by alkaline and acid, was carried out by standard procedures (Harborne, 1984).

4.5.1. Peonidin 3-sophoroside-5-glucoside

UV–Vis (in 0.1% HCl–MeOH): λ_{max} 524, 278 nm, E_{440}/E_{max} = 13%, $AlCl_3$ shift 0; TLC: R_f-values (100×) BAW 29, BuHCl 13, 1% HCl 50, AHW 82; HPLC (method 1): R_t (min) 15.8.

4.5.2. Cyanidin 3-sophoroside-5-glucoside

UV–Vis: λ_{max} 525, 279 nm, E_{440}/E_{max} = 13%, $AlCl_3$ shift +; TLC: R_f-values (100×) BAW 20, BuHCl 10, 1% HCl 45, AHW 75 HPLC (method 1): R_t (min) 12.5.

4.5.3. Peonidin

UV–Vis: λ_{max} 537, 275 nm, E_{440}/E_{max} = 32%, $AlCl_3$ shift 0; TLC: R_f-values (100×) Forestal 60; HPLC(method 1): R_t (min) 32.6.

4.5.4. Cyanidin

UV–Vis: λ_{max} 536, 273 nm, E_{440}/E_{max} = 44%, $AlCl_3$ shift +; TLC: R_f-values (100×) Forestal 42; HPLC (method 1): R_t (min) 25.3.

4.5.5. Glucose

TLC: R_f-values (100×) BAW 24, EAA 18, ETN 62, EFW 49; Color (AHP) Brown.

4.5.6. p-Coumaric acid

TLC: R_f-values (100×) BAW 91, EAA 96, EFW 85; Color (under UV) Violet; HPLC (method 1): R_t (min) 17.2 (*trans*), 16.2 (*cis*).

4.5.7. Ferulic acid

TLC: R_f-values (100×) BAW 87, EAA 92, EFW 78; Color (under UV) Bright Blue; HPLC (method 1): R_t (min) 20.6.

4.5.8. Sinapic acid

TLC: R_f-values (100×) BAW 83, EAA 88, EFW 73; Color (under UV) Bright Blue; HPLC (method 1): R_t (min) 22.1.

4.5.9. Malonic acid

HPLC (method 2): R_t (min) 4.1.

Acknowledgement

This work was supported in part by a Grant-in-Aid for Science Research (C) [No. 22580024, to F.T.] from the Japan Society for the Promotion of Science (JSPS).

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