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Acylated cyanidin 3-sambubioside-5-glucosides from the purple-violet flowers of *Matthiola longipetala* subsp. *bicornis* (Sm) P. W. Ball. (Brassicaceae)

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ABSTRACT

A novel acylated cyanidin 3-sambubioside-5-glucoside was isolated from the purple-violet flowers of *Matthiola longipetala* subsp. *bicornis* (Sm) P. W. Ball. (family: Brassicaceae), and determined to be cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-glucopyranoside]-5-*O*-[6-*O*-(malonyl)-β-glucopyranoside] by chemical and spectroscopic methods. In addition, two known acylated cyanidin 3-sambubioside-5-glucosides, cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-sinapoyl)-β-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-glucopyranoside]-5-*O*-[6-*O*-(malonyl)-β-glucopyranoside] and cyanidin 3-*O*-[2-*O*-(β-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-glucopyranoside]-5-*O*-[6-*O*-(malonyl)-β-glucopyranoside] were identified in the flowers.

1. Introduction

Matthiola longipetala subsp. *bicornis* is an ornamental plant with deliciously scented purple-violet flowers, which open in the evening (common name: Night Scented Stocks). In the limited flavonoid studies reported for this species, flavonol glycosides have been characterized from other subsp. *livida* (Marzouk et al., 2008). Moreover, floral anthocyanins from the genus *Matthiola* have only been characterized from the flowers of *M. incana* (Saito et al., 1995, 1996; Tatsuzawa et al., 2012a) and not from the flowers of *M. longipetala*.

Recently, the ornamental plants in the Brassicaceae are known as the source of complicated acylated anthocyanins in the flowers of *Arabis blepharophylla* (Ito et al., 2013), *Aubrieta* × *cultorum* (Tatsuzawa et al., 2012b), *Cheiranthus cheiri*, *Lobularia maritima*, *Lunaria annua* (Tatsuzawa et al., 2006, 2007, 2010), *Heliophila coronopifolia* (Saito et al., 2011), *Hesperis matronalis* (Tatsuzawa, 2012), *Iberis umbellata* (Saito et al., 2008), *Ionopsidium acaule* (Tatsuzawa et al., 2014), *Malcolmia maritima* (Tatsuzawa et al., 2008a), *Matthiola incana* (Saito et al., 1995, 1996; Tatsuzawa et al., 2012a), *Moricandia ramburii* (Tatsuzawa et al., 2012c), *Moricandia arvensis* (Tatsuzawa et al., 2013), *Orychophragmus violaceus* (Honda et al., 2005), and *Raphanus sativus* (Tatsuzawa et al., 2008b). In this study, the structure elucidation of a new acylated cyanidin 3-sambubioside-5-glucoside together with known ones from the purple-violet flowers of *M. longipetala* subsp. *bicornis* was reported.

2. Results and discussion

More than 10 anthocyanins were detected in a methanol–acetic acid–water (MAW) (4:1:5, v/v/v, 100 mL) extract from dried purple-violet flowers of *Matthiola longipetala* subsp. *bicornis* (1 g) by high performance liquid chromatography (HPLC) analysis (Fig. 1). The percentage of major three pigments **1**–**3**, calculated as the total anthocyanin content by HPLC vis peak area at 530 nm, was 40.1%, 30.4%, and 10.1%, respectively.

The major pigments were extracted from the purple-violet flowers with 5% HOAc, followed by isolation using Diaion HP-20 (Mitsubishi Chemical's Ion Exchange Resins, aromatic type adsorbent) column chromatography (CC), preparative HPLC and thin layer chromatography (TLC) (Tatsuzawa et al., 2012a).

Acid hydrolysis of **1**–**3** yielded cyanidin as their anthocyanidin (Harborne, 1984), while also indicating glucose, xylose, hydroxycinnamic acid and malonic acid moieties. Moreover, *trans*-ferulic

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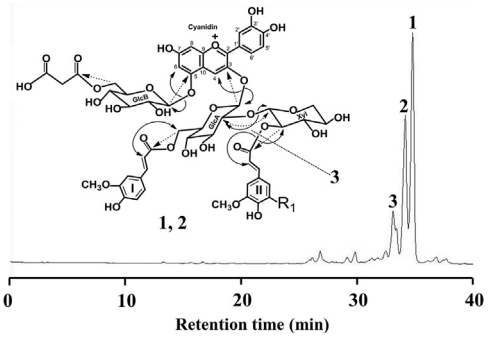

Fig. 1. HPLC profile (530 nm) and structure of acylated anthocyanins isolated from the purple-violet flowers of *Matthiola longipetala* subsp. *bicornis*. Observed NOEs are indicated by arrows. Observed HMBCs are indicated by dotted arrows. **1**: pigment **1**, R_1=H, **2**: pigment **2**, R_1=OCH_3.

acid, *trans*-sinapic acid and *trans*-ferulic acid, and *trans*-ferulic acid were detected in the hydrolysates of **1**, **2**, and **3** by TLC, respectively, with malonic acid found in all pigments (Harborne, 1984).

Alkaline hydrolysis of **1**–**3** yielded cyanidin 3-sambubioside-5-glucoside. The deacyl anthocyanin structures tentatively identified *via* co-TLC and co-HPLC with authentic cyanidin 3-sambubioside-5-glucoside (see Section 4.4.1), prepared from *Matthiola incana* (Tatsuzawa et al., 2012a) by alkaline hydrolysis.

The structures of **1**–**3** were confirmed based on the analyses of their ^{1}H (400 MHz), ^{13}C (100 MHz) and 2D (COSY, NOESY, ^{1}H–^{13}C HMQC and ^{1}H–^{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* 1181 ($C_{55}H_{57}O_{29}$), indicating a cyanidin structure with two molecules each of glucose and ferulic acid, and one molecule each of xylose and malonic acid. The elemental components of pigment **1** were confirmed by measuring its high resolution FAB-MS (see Section 4.4.2).

The chemical shifts of the 12 aromatic protons of the cyanidin and ferulic acid moieties with their coupling constants were assigned as shown in Table 1. Six protons were assigned to the two methoxyl groups of ferulic acids. Two sets of two pairs of doublet resonances, assigned to the four olefinic proton signals of the ferulic acid moieties, indicated *trans* configuration for the acids based on their coupling constants (J = 15.8 Hz each) (Table 1). The chemical shifts of the sugar moiety protons were observed in the region of δ 5.70–3.21, with the three anomeric proton resonances at δ 5.70 (*d*, J = 7.6 Hz, Glc A), 5.18 (*d*, J = 7.3 Hz, Glc B), and 5.15 (*d*, J = 8.0 Hz, Xyl). Based on the observed coupling constants (Table 1), these three sugars were assumed to be in the 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 the NOESY experiment, NOEs between H-1 of Glc A and H-4 (δ 8.75) of cyanidin, H-1 of Glc B and H-6 (δ 6.97) of cyanidin, and H-2 (δ 4.04) of Glc A and H-1 of Xyl were observed (Fig. 1), supporting glycosylation of the C-3 and C-5 cyanidin hydroxyl groups with Glc A and Glc B, respectively, while also indicating a sambubiose structure between Glc A and Xyl.

Five characteristic downfield shifted proton signals were assigned to the methylene protons of Glc A (δ 4.22 and 4.41, H-6a and b) and Glc B (δ 3.98 and 4.40, H-6a and b), and to a methine proton (δ 4.67, *t*, J = 8.7 Hz, H-2) of Xyl, indicating acylation of C-6 OH (Glc A and Glc B) and C-2 OH (Xyl) with three acid molecules. In the NOESY spectrum, the weak correlations between H-6a,b of Glc A and H-α (δ 6.30) of ferulic acid (I) and H-2 of Xyl and H-α (δ 6.52) of ferulic acid (II) were observed, establishing the acylation acid at C-6 OH (Glc A) and C-2 OH (Xyl) as ferulic acid (I) and ferulic acid (II), respectively.

In the HMBC spectrum, the correlations between the anomeric proton of Glc A and C-3 carbon (δ 144.5) of cyanidin, the anomeric proton of Glc B and C-5 carbon (δ 154.9) of cyanidin, the anomeric proton of Xyl and C-2 carbon (δ 77.3) of Glc A, methylene protons of Glc A and COOH carbon (δ 166.8) of ferulic acid (I), methylene protons of Glc B and COOH carbon (δ 167.0) of malonic acid, methine proton of H-2 of Xyl and COOH carbon (δ 166.3) of ferulic acid (II), and methine proton of H-2 of Glc A and C-1 carbon (δ 101.4) of Xyl were observed, establishing the glycosylation or acylation groups at C-3 OH (cyanidin), C-5 OH (cyanidin), C-2 OH (Glc A), COOH (ferulic acid (I)), COOH (malonic acid), COOH (ferulic acid (II)), and C-1 OH (Xyl) as Glc A, Glc B, Xyl, Glc A, Glc B, Xyl, and Glc A, respectively (Fig. 1). Consequently, the structure of pigment **1** was determined to be cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-xylosyl)-6-*O*-(*trans*-feruloyl)-β-glucoside]-5-*O*-[6-*O*-(malonyl)-β-glucoside], which is a new anthocyanin in plants (Andersen and Jordheim, 2006; Harborne and Baxter, 1999; Honda and Saito, 2002; Veitch and Grayer, 2008, 2011).

2.2. Pigment 2

The molecular ion $[M]^+$ of **2** was observed at *m*/*z* 1211 ($C_{56}H_{59}O_{30}$), indicating a cyanidin structure with two molecules of glucose, and one molecule each of ferulic acid, sinapic acid and malonic acid. The elemental components of pigment **2** were confirmed by measuring its high resolution FAB-MS (see Section 4.4.3).

The ^{1}H NMR spectrum of **2** was similar to that of **1**, with **2** containing a sinapic acid moiety (II) instead of a ferulic acid moiety (I) (Table 1). The two aromatic proton signals of sinapic acid (II) were observed at δ 7.00 (H-2 and H-6). The olefinic proton signals [δ 6.54 (*d*, J = 15.8 Hz, H-α) and 7.58(*d*, J = 15.8 Hz, H-β)] indicating a *trans*-sinapic acid moiety establishing **2** as cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-sinapoyl)-β-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-glucopyranoside]-5-*O*-[6-*O*-(malonyl)-β-glucopyranoside], which is a new anthocyanin in species *M. longipetala*, although this pigment has been found in *Matthiola incana* (family: Brassicaceae) (Saito et al., 1995; Tatsuzawa et al., 2012a). This structure was also confirmed by analysis of its ^{13}C, including ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC NMR spectra (Table 1, Fig. 1).

2.3. Pigment 3

The molecular ion $[M]^+$ of **3** was observed at *m*/*z* 1005 ($C_{45}H_{49}O_{26}$), indicating a cyanidin structure with two molecules of glucose, and one molecule each of ferulic acid and malonic acid. The elemental components of pigment **3** were confirmed by measuring its high resolution FAB-MS (see Section 4.4.4).

The ^{1}H NMR spectrum of pigment **3** was similar to that of pigment **1** except for the absence of the signals corresponding to ferulic acid (II). The linkages and/or positions of the attachments of the sugar and acyl groups in this pigment were confirmed by using 2D COSY and NOESY experiments. Therefore, the structure of pigment **3** was determined to be cyanidin 3-*O*-[2-*O*-(β-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-glucopyranoside]-5-*O*-[6-*O*-(malonyl)-β-glucopyranoside], which is a new anthocyanin in species *Matthiola longipetala*, although this pigment has been found in *Lunaria annua* and *Matthiola incana* (family: Brassicaceae) (Tatsuzawa et al., 2006, 2012a). This structure was also confirmed by analysis of its ^{13}C, including ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC NMR spectra (Table 1, Fig. 1).

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Table 1
NMR spectroscopic data of anthocyanins from the flowers of *Matthiola longipetala* in DMSO-d_6/CF_3COOD (9:1).

	1			2			3		
	^{1}H δ(ppm)		^{13}C δ(ppm)	^{1}H δ(ppm)		^{13}C δ(ppm)	^{1}H δ(ppm)		^{13}C δ(ppm)
Cyanidin									
2			162.3			162.5			162.6
3			144.5			144.7			144.7
4	8.75	s	131.5	8.47	s	130.6	8.79	s	131.7
5			155.2			155.4			155.1
6	6.97	brs	104.6	6.97	brs	104.6	6.98	brs	104.9
7			167.4			167.8			167.6
8	7.03	brs	96.2	7.01	brs	96.4	7.06	brs	96.4
9			154.9			155.1			155.4
10			111.6			111.9			111.8
1′			119.7			119.9			119.8
2′	8.01	d(2.3)	117.5	7.99	d(2.2)	117.4	8.06	d(2.2)	117.9
3′			146.7			146.9			146.6
4′			155.5			155.8			155.5
5′	7.07	d(8.8)	116.7	7.06	d(8.7)	116.8	7.08	d(8.8)	116.9
6′	8.50	dd(2.3, 8.8)	128.8	8.47	dd(2.2, 8.7)	129.0	8.40	dd(2.2, 8.8)	128.4
Glucose A									
1	5.70	d(7.6)	98.0	5.68	d(7.2)	98.2	5.72	d(7.6)	98.6
2	4.04	t(8.2)	77.3	4.03	t(7.8)	77.7	4.04	t(8.0)	80.7
3	3.63	t(8.8)	77.2	3.63	t(8.9)	77.4	3.78	t(9.0)	76.8
4	3.44	t(9.0)	70.7	3.46	m	71.1	3.50	t(9.3)	70.0
5	3.97	ddd(2.4, 7.2, 10.2)	73.6	3.96	m	73.8	4.04	m	74.1
6a	4.22	dd(6.8, 11.7)	63.2	4.20	dd(6.8, 11.5)	63.5	4.27	dd(7.0, 11.9)	63.4
6b	4.41	m		4.42	brd(11.5)		4.50	dd(1.8, 11.9)	
Glucose B									
1	5.18	d(7.3)	101.7	5.16	d(8.3)	101.9	5.18	d(7.6)	102.0
2	3.51	t(8.4)	73.3	3.54	t(8.5)	73.5	3.57	t(8.3)	73.4
3	3.44	t(9.0)	75.7	3.43	m	76.0	3.43	t(9.0)	76.0
4	3.21	t(9.3)	69.7	3.22	t(9.2)	70.0	3.25	t(9.3)	69.9
5	3.78	m	74.3	3.77	m	74.5	3.78	m	74.5
6a	3.98	m	64.1	3.90	m	64.3	3.96	dd(6.6, 11.7)	64.3
6b	4.40	brd(10.5)		4.38	brd(11.0)		4.43	dd(1.2, 11.7)	
Xylose									
1	5.15	d(8.0)	101.4	5.14	d(8.6)	101.5	4.75	d(7.8)	104.9
2	4.67	t(8.7)	73.9	4.66	t(8.4)	74.2	3.05	t(8.0)	74.5
3	3.41	m	74.8	3.43	m	75.0	3.18	t(8.8)	76.8
4	3.44	m	70.1	3.46	m	70.3	3.27	m	69.7
5a	3.90	m	66.4	3.90	m	66.6	3.57	m	66.3
5b	3.21	t(10.0)		3.22	m		3.00	t(11.0)	
Ferulic acid (I)									
1			126.1			126.3			125.7
2	7.03	brs	111.8	6.95	brs	111.8	7.06	brs	111.8
3			147.9			148.1			148.0
4			149.5			149.7			149.6
5	6.74	d(8.0)	115.6	6.73	d(8.3)	116.0	6.76	d(8.3)	115.7
6	6.95	dd(1.4, 8.0)	123.0	6.91	brd(8.3)	123.1	6.97	brd(8.3)	123.2
α	6.30	d(15.8)	114.1	6.27	d(15.8)	114.3	6.33	d(15.8)	114.3
β	7.40	d(15.8)	145.1	7.42	d(15.8)	145.7	7.42	d(15.8)	145.7
$-OCH_3$	3.74	s	55.7	3.72	s	55.9	3.76	s	55.8
COOH			166.8			167.1			166.9
Ferulic or Sinapic acid (II)									
1			125.6			125.8			
2	7.32	d(1.4)	111.6	7.00	s	106.6			
3			148.2			148.6			
4			149.5			138.9			
5	6.85	d(8.0)	115.8			148.6			
6	7.16	dd(1.4, 8.0)	123.2	7.00	s	106.6			
α	6.52	d(15.8)	115.4	6.54	d(15.8)	115.8			
β	7.59	d(15.8)	145.5	7.58	d(15.8)	145.7			
$-OCH_3$	3.87	s	55.9	3.83	s	56.5			
$-OCH_3$				3.83	s	56.6			
COOH			166.3			166.5			
Malonic acid									
$-CH_2$	3.39	s	41.2	3.36	s	41.5	3.41	s	41.1
COOH			167.0			167.2			167.1
COOH			168.2			168.5			168.3

Coupling constants (J in Hz) in parentheses.

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3. Concluding remarks

A new acylated cyanidin glycoside was isolated from the purple-violet flowers of *M. longipetala* subsp. *bicornis*. From the chemotaxonomical point of view, there are two C-3 OH glycosidic patterns for anthocyanidins found in the flowers of the Brassicaceae, namely acylated 3-sambubioside from *Arabis blepharophylla*, *Aubrieta* x *cultorum*, *Cheiranthus cheiri*, *Heliophila coronopifolia*, *Hesperis matronalis*, *Ionopsidium acaule*, *Lobularia maritima*, *Lunaria annua*, *Malcolmia maritima*, *M. incana* and *Orychophragmus violaceus* (Honda et al., 2005; Ito et al., 2013; Saito et al., 1995, 1996, 2011; Tatsuzawa, 2012; Tatsuzawa et al., 2006, 2007, 2008a, 2010, 2012a,b, 2014) and acylated 3-sophoroside from *Iberis umbellata*, *Moricandia arvensis*, *Moricandia ramburii* and *Raphanus sativus* (Saito et al., 2008; Tatsuzawa et al., 2008b, 2012c, 2013). Therefore, the floral anthocyanins of *M. longipetala* subsp. *bicornis* are grouped into the former pattern.

The FAB mass spectrum of an unknown anthocyanin from the purple-violet flower cultivars of *M. incana* (Saito et al., 1995, anthocyanin C) exhibited its molecular ion at 1181 *m/z*, corresponding to $C_{55}H_{57}O_{29}$. This unknown anthocyanin C is identical with pigment **1** which has been isolated and identified as a major anthocyanin in the flowers of *M. longipetala* subsp. *bicornis* in the present study. In addition, the distribution of the pigments **2** and **3** from the flowers of *M. longipetala* subsp. *bicornis* has been reported in the violet, purple-violet, and red-purple flower cultivars of *M. incana* (Saito et al., 1995; Tatsuzawa et al., 2012a). The distribution of 28 acylated cyanidin 3-sambubioside-5-glucosides has been reported from petals of 10 genera of the Brassicaceae (Honda et al., 2005; Ito et al., 2013; Saito et al., 1995, 1996, 2011; Tatsuzawa, 2012; Tatsuzawa et al., 2006, 2007, 2010, 2012a ,b, 2014). Among them, the distribution of cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-xylosyl)-6-*O*-(*trans*-feruloyl)-β-glucoside]-5-*O*-[6-*O*-(malonyl)-β-glucoside] (**1**) and cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-sinapoyl)-β-xylosyl)-6-*O*-(*trans*-feruloyl)-β-glucoside]-5-*O*-[6-*O*-(malonyl)-β-glucoside] (**2**) has only been reported in the genus *Matthiola*.

4. Experimental

4.1. General procedures

TLC was carried out on plastic coated cellulose sheets (Merck) using eight mobile phases: BAW (*n*-BuOH-HOAc-H_2O, 4:1:2, v/v/v), BuHCl (*n*-BuOH-2 N HCl, 1:1, v/v, upper layer), AHW (HOAc-HCl-H_2O, 15:3:82, v/v/v), 1% HCl for anthocyanins, Forestal (HOAc-HCl-H_2O, 30:3:10, v/v/v) for anthocyanidin and BAW, EAA (EtOAc-HOAc-H_2O, 3:1:1, v/v/v), ETN (EtOH-NH_4OH-H_2O, 16:1:3, v/v/v) and EFW (EtOAc-HCOOH-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 mm × 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% HOAc, 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., 2013b) (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 (JEOL) operating in the positive ion mode using 1:1 mixture of dithiothreitol and 3-nitrobenzyl alcohol as a matrix. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were measured on a JEOL JNM-AL-400 NMR spectrometer (JEOL) using CF_3COOD-DMSO-d_6 (1:9) as a solvent. Chemical shifts are reported on the d-scale from tetramethylsilane as the internal standard, and coupling constants (*J*) are in Hz.

4.2. Plant materials

Seeds of purple-violet flowers of *M. longipetala* subsp. *bicornis* were purchased from Thompson & Morgan. Ltd (UK). Seeds were sown in October, 2012 and plants were grown in a greenhouse of Iwate University. Flowers with a purple-violet color [Purple-Violet N80B by Royal Horticultural Society (R.H.S) Color Chart and $b^*(-8.03)/a^*(13.41) = -0.60$, $L^* = 62.29$ by a CM-700d Spectro Color Meter (Konica-Minolta Co., Ltd.)] were collected in spring in 2013. The petals were trimmed from flowers by hand and dried by air for 1 day at 45 °C. Then they were kept at −20 °C until used.

4.3. Isolation and purification of anthocyanins

Dried flowers (*ca.* 100 g) of *M. longipetala* subsp. *bicornis* were immersed in 5% HOAc (10 l) at room temperature for 5 h and extracted. Three anthocyanin pigments was isolated and purified by Diaion HP-20 (Mitsubishi Chemical's Ion Exchange Resins) column (90 mm × 150 mm) chromatography, paper chromatography and preparative HPLC from the extract as described previously (Tatsuzawa et al., 2006, 2007). The purified three anthocyanins were obtained from the flowers as follows; pigment **1** (*ca.* 35 mg), pigment **2** (*ca.* 23 mg), and pigment **3** (*ca.* 12 mg).

4.4. Chemical and spectroscopic analyses of purified anthocyanins

Acid hydrolyses of pigments **1**–**3** (*ca.* 0.5 mg, each) were carried out with 2 N HCl (1 ml) at 100 °C for 2 h, and resulted in cyanidin, glucose, xylose, ferulic acid and malonic acid. Moreover, sinapic acid was detected in the hydrolysates of **1**. These compounds were confirmed by direct comparison of TLC and/or HPLC with the authentic samples.

By alkaline hydrolysis, pigments **1**–**3** yielded cyanidin 3-sambubioside-5-glucoside as their deacyl anthocyanin. The deacyl anthocyanin structure was identified in direct comparison by the analyses of co-TLC and co-HPLC with authentic cyanidin 3-sambubioside-5-glucoside which was prepared from *Lunaria annua* (Tatsuzawa et al., 2006).

4.4.1. Deacyl anthocyanin of pigments 1–3 (cyanidin 3-sambubioside-5-glucoside)

UV–VIS (in 0.1% HCl–MeOH): λ_{max} 527, 278 nm, E_{440}/E_{527} (%) = 13, $AlCl_3$ shift + TLC: (R_f-values) BAW 0.28, BuHCl 0.05, 1% HCl 0.45, AHW 0.67; HPLC: R_t (min) 13.2.

4.4.2. Pigment 1

Dark red powder; for UV–VIS (in 0.1% HCl–MeOH): λ_{max} 530,327,296,284 nm, E_{327}/E_{530} (%) = 117, E_{440}/E_{530} (%) = 15, $AlCl_3$ shift + TLC: (R_f-values) BAW 0.71, BuHCl 0.54, 1% HCl 0.32, AHW 0.72, HPLC: R_t (min) 34.9, NMR spectrum, see Table 1; HR-FABMS calc. for $C_{55}H_{57}O_{29}$: 1181.2986. Found: 1181.3014.

4.4.3. Pigment 2

Dark red powder; for UV–VIS (in 0.1% HCl–MeOH): λ_{max} 530,324,298,284 nm, E_{324}/E_{530} (%) = 132, E_{440}/E_{530} (%) = 16, $AlCl_3$ shift + TLC: (R_f-values) BAW 0.68, BuHCl 0.49, 1% HCl 0.36, AHW 0.74, HPLC: R_t (min) 30.4, NMR spectrum, see Table 1; HR-FABMS calc. for $C_{56}H_{59}O_{30}$: 1211.3091. Found: 1211.3065.

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4.4.4. Pigment 3

Dark red powder; for UV–VIS (in 0.1% HCl–MeOH): λ_{max} 530,327,295,281 nm, E_{327}/E_{530} (%) = 88, E_{440}/E_{530} (%) = 13, $AlCl_3$ shift + TLC: (R_f-values) BAW 0.48, BuHCl 0.22, 1% HCl 0.35, AHW 0.69, HPLC: R_t (min) 33.2, NMR spectra, see Table 1; HR-FABMS calc. for $C_{45}H_{49}O_{26}$: 1005.2512. Found: 1005.2555.

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