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Acylated Pelargonidin 3-sambubioside-5-glucosides from the Red-purple Flowers of *Lobularia maritima*

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Six acylated pelargonidin 3-*O*-sambubioside-5-*O*-glucosides were isolated from red-purple flowers of *Lobularia maritima* (L.) Desv. 'Easter Bonnet Deep Rose'. These pigments were determined by chemical and spectroscopic methods to be pelargonidin 3-*O*-[2-*O*-(2-*O*-(acyl-II)-β-xylopyranosyl)-6-*O*-(acyl-I)-β-glucopyranoside]-5-*O*-β-glucopyranoside, in which the acyl-I group was replaced by 4-*O*-glucosyl-*p*-coumaric acid, *p*-coumaric acid or ferulic acid, and acyl-II by caffeic acid or ferulic acid, respectively. In comparison with the floral anthocyanins of purple-violet flowers in *L. maritima* cultivar 'Easter Bonnet Violet', the molecular composition of organic acids and sugars of 'Easter Bonnet Deep Rose' were identical, however, aglycones of both cultivars were different, and pelargonidin is the floral anthocyanin of 'Easter Bonnet Deep Rose' and cyanidin is that of 'Easter Bonnet Violet'. Variations of the flower colors of these cultivars are responsible for the aglycone component in their floral anthocyanins. In this paper, the relation between flower color and anthocyanins in *L. maritima* cultivars is discussed.

Key Words: acylated pelargonidin 3-*O*-sambubioside-5-*O*-glucoside, caffeic acid, ferulic acid, *Lobularia maritima* (L.) Desv. 'Easter Bonnet Deep Rose', *p*-coumaric acid.

Introduction

Lobularia maritima (L.) Desv., (Sweet alyssum in English) is a perennial plant species, native to the Mediterranean region, and usually cultivated as a popular annual garden plant with white, lemon yellow, apricot, salmon, red, red-purple, purple-violet, and violet flowers. In the Cruciferae, *Lobularia* cultivars are major and important ornamentals with *Matthiola.* As part of our continuing work on flower color variation due to acylated anthocyanins in ornamental plants in this family, we previously reported the isolation of 41 acylated anthocyanins from flowers of *Cheiranthus cheiri* L., *Iberis umbellata* L., *Lunaria annua* L., *Malcolmia maritima* (L.) R. Br., *Matthiola incana* (L.) R. Br., *Raphanus sativus* L., and *Orychophragmus violaceus* (L.) O. E. Schulz as well as the presence of nine acylated cyanidin glycosides in the purple-violet flowers of *L. maritima* cultivar. However, little attention has been given to floral anthocyanins in the red-purple flowers of *L. maritima* cultivars. As an extension of our work, we investigated floral anthocyanins in the red-purple flowers of *L. maritima* 'Easter Bonnet Deep Rose' and found six new acylated anthocyanins. In this paper, we wish to report the structure elucidation of their floral anthocyanins and discuss the flower colors responsible for the floral anthocyanins in *L. maritima*, and also their distribution in the Cruciferae.

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Materials and Methods

General procedures

Characterization of anthocyanins was carried out with UV-VIS, FAB mass and NMR spectrometry, and also thin layer chromatography (TLC) and high performance liquid chromatography (HPLC), involving alkaline and acid hydrolysis and H_2O_2 degradation, as described previously (Tatsuzawa et al., 2006, 2007)

Plant materials

Seeds of red-purple flowers of *Lobularia maritima* 'Easter Bonnet Deep Rose' were purchased from Takii Co., Ltd. (Kyoto, Japan). Seeds were sown in August, 2006 and plants were grown in a greenhouse of Minami-Kyushu University. Flowers with a red-purple color [Red-Purple 74A by Royal Horticultural Society (R.H.S) Colour Chart and chromaticity value (b*/a* = –0.19)] were collected in December, 2006, dried overnight at 40°C, and kept in a refrigerator at about 4°C until needed.

Isolation and purification of anthocyanins

Dried flowers (*ca.* 100 g) of *L. maritima* 'Easter Bonnet Deep Rose' were immersed in 5% HOAc at room temperature for 5 h and extracted with 5% HOAc. The extract was purified by Diaion HP-20 Ion exchange resins, (Mitsubishi Chemical's, Tokyo, Japan) column (90 × 150 mm) chromatography, paper chromatography, and preparative HPLC as described previously (Tatsuzawa et al., 2006, 2007). Finally, the following six new anthocyanins were obtained: **1** (*ca.* 8 mg), **2** (*ca.* 10 mg), **3** (*ca.* 6 mg), **4** (*ca.* 3 mg), **5** (*ca.* 7 mg), and **6** (*ca.* 3 mg).

Alkaline hydrolysis

Mixed crude pigments (*ca.* 30 mg) were dissolved in 2N NaOH (3 mL) using a degassed syringe to stir for 15 min. The solution was then acidified with 2N HCl and evaporated in vacuo to dryness. The resulting residue was dissolved in 1% HCl-MeOH and subjected to TLC (BAW) to yield a deacylanthocyanin (*ca.* 5 mg) and 4-*O*-glucosyl-*p*-coumaric acid (*ca.* 1 mg), as described previously (Saito et al., 2008).

1. Deacyl anthocyanin

Pelargonidin 3-*O*-sambubioside-5-*O*-glucoside; high-resolution FAB mass spectra (HR-FAB MS) calc. for $C_{32}H_{39}O_{19}$: 727.2086. found: 727.2091; UV-VIS (in 0.1% HCl-MeOH): λmax 284, 507 nm, E_{440}/E_{507} (%) = 19, $AlCl_3$ shift 0; TLC: (R_f-values) BAW 0.26, BuHCl 0.07, 1% HCl 0.46, AHW 0.65; HPLC: R_t (min) 15.5.

2. 4-O-Glucosyl-p-coumaric acid

TLC: (R_f-values) BAW 0.76, EAA 0.82, EFW 0.79, HPLC: R_t (min) 8.0.

Partial acid hydrolysis

1–6 (each 0.5 mg) were each dissolved in 2N HCl (0.5 ml) and hydrolyzed by heating in a water bath (*ca.* 90°C) for 10 min. The partial hydrolysates were immediately analyzed by HPLC with authentic samples. As authentic samples, pelargonidin 3-*O*-[2-*O*-(xylosyl)-6-*O*-(*p*-coumaroyl)-glucoside]-5-*O*-glucoside and pelargonidin 3-*O*-[2-*O*-(xylosyl)-6-*O*-(feruloyl)-glucoside]-5-*O*-glucoside were obtained from *Matthiola* red flowers by the process of demalonylation described previously (Saito et al., 1996).

1. Pelargonidin 3-O-[2-O-(xylosyl)-6-O-(p-coumaroyl)-glucoside]-5-O-glucoside

UV-VIS (in 0.1% HCl-MeOH): λmax 269sh, 279sh, 286, 316, 509 nm, E_{316}/E_{509} (%) = 86, E_{440}/E_{509} (%) = 19, $AlCl_3$ shift 0; TLC: (R_f-values) BAW 0.31, BuHCl 0.32, 1% HCl 0.30, AHW 0.59, *R*t (min) 33.4.

2. Pelargonidin 3-O-[2-O-(xylosyl)-6-O-(feruloyl)-glucoside]-5-O-glucoside

UV-VIS (in 0.1% HCl-MeOH): λmax 270sh, 279, 285sh, 327, 509 nm, E_{327}/E_{509} (%) = 64, E_{440}/E_{509} (%) = 19, $AlCl_3$ shift 0; TLC: (R_f-values) BAW 0.28, BuHCl 0.23, 1% HCl 0.29, AHW 0.58, *R*t (min) 34.0.

H_2O_2 Degradation of pigment 4

4 (*ca.* 1 mg) was dissolved in H_2O (200 μL) and oxidized with H_2O_2 (200 μL) (Saito et al., 1995). The resulting solution was chromatographed on cellulose using BAW, and its acylated sugar band was excised, eluted, and purified by TLC. 2-*O*-(2-*O*-(Caffeoyl)-xylosyl)-6-*O*-(feruloyl)-glucose was obtained from pigment 3 of *L. maritima* 'Easter Bonnet Violet' by a similar procedure described above (Tatsuzawa et al., 2007).

1. 2-O-(2-O-(Caffeoyl)-xylosyl)-6-O-(feruloyl)-glucose;

TLC: (R_f-values) BAW 0.54, 15% HOAc 0.59, 0.73; Color Under UV-Blue.

Results and Discussion

Identification of the anthocyanins

1. Anthocyanin analysis in red-purple flowers of L. maritima

Six major anthocyanin peaks (**1**: ranging from 12.1% of the total anthocyanin contents observed from the HPLC peak area at 530 nm, **2**: 17.5%, **3**: 12.3%, **4**: 9.9%, **5**: 19.4%, **6**: 10.1%) were detected in the 5% HOAc extract from fresh red-purple flowers of *L. maritima* 'Easter Bonnet Deep Rose' as shown in Figure 1. These anthocyanins (**1–6**) were isolated from dried flowers and purified according to the procedure described previously (Tatsuzawa et al., 2006, 2007). The chromatographic and spectroscopic properties of these pigments are summarized in the Table 1. In the UV-VIS spectra of **1–6** in 0.1% HCl-MeOH (Table 1), visible maximum ($E_{vis.max}$) was observed at 510–512 nm. Also, the percentages of the absorbance ratios at 440 nm and the visible maximum ($E_{440}/E_{vis.max}$) were 19–21%. On the addition of $AlCl_3$, **1–6** did not exhibit a bathochromic shift, indicating that they lack a vicinal hydroxyl group in the B-ring, therefore, these data suggested that **1–6** may have a pelargonidin 3,5-diglycosyl type structure (Tatsuzawa and Shinoda, 2005). The percentage $E_{acyl}/E_{vis.max}$ ratio of **1–6** was 113–146% suggesting that **1–6** had two acyl units, respectively (Tatsuzawa et al., 2006, 2007).

Acid hydrolyses of the mixed **1–6** (*ca.* 3 mg) were carried out with 2N HCl (15 mL) at 100°C for 1 h, resulting in the isolation of pelargonidin, glucose, xylose,

p-coumaric acid, caffeic acid, and ferulic acid. These compounds were confirmed by comparing TLC and HPLC behaviors with those of authentic samples. Alkaline hydrolysis of the mixed **1–6** (*ca.* 3 mg) yielded only one deacylated anthocyanin, the structure of which was identified to be pelargonidin 3-*O*-sambubioside-5-*O*-glucoside by direct comparison of TLC and HPLC behavior with those of an authentic sample obtained from *Matthiola* red anthocyanins (Saito et al., 1996). Moreover, the structure of the deacylanthocyanin was confirmed by analysis of its ^{1}H NMR and ^{13}C spectra [500 MHz for ^{1}H and 125.78 MHz for ^{13}C spectra in CF_3COOD-DMSO-d_6 (1:9)], including ^{1}H-^{1}H correlation spectroscopy (COSY), nuclear Overhauser enhancement and exchange spectroscopy (NOESY), heteronuclear multiple quantum coherence spectroscopy (HMQC) and heteronuclear multiple bond correlation spectroscopy (HMBC) spectra. 4-*O*-Glucosyl-*p*-coumaric acid was also obtained from the alkaline hydrolysate of **1** and **2**, and its structure was identified by comparison with an authentic sample that was obtained from a purple-violet cultivar of *L. maritima* 'Easter Bonnet Violet' by the same process as that for alkaline hydrolysis (Tatsuzawa et al., 2007).

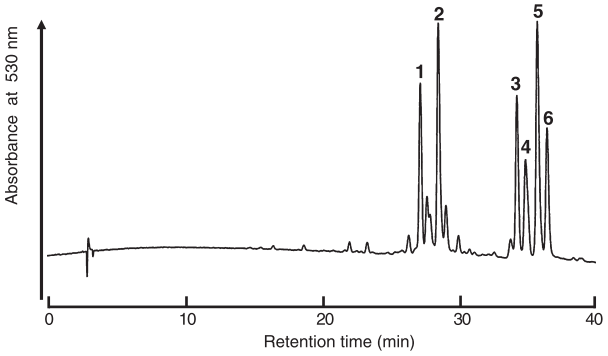

Fig. 1. HPLC analysis of anthocyanins in red-purple flowers of *L. maritima*.
1: pelargonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-caffeoyl)-xylosyl)-6-*O*-(4-glucosyl-*trans*-*p*-coumaroyl)-glucoside]-5-*O*-glucoside (**1**)
2: pelargonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-xylosyl)-6-*O*-(4-glucosyl-*trans*-*p*-coumaroyl)-glucoside]-5-*O*-glucoside (**2**)
3: pelargonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-caffeoyl)-xylosyl)-6-*O*-(*trans*-*p*-coumaroyl)-glucoside]-5 *O*-glucoside (**3**)
4: pelargonidin 3-*O*-[2-*O*-(caffeoyl)-xylosyl)-6-*O*-(feruloyl)-glucoside]-5-*O*-glucoside (**4**)
5: pelargonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-xylosyl)-6-*O*-(*trans*-*p*-coumaroyl)-glucoside]-5-*O*-glucoside (**5**)
6: pelargonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-xylosyl)-6-*O*-(*trans*-feruloyl)-glucoside]-5-*O*-glucoside (**6**)

Fast atom bombardment (FAB) mass measurements of **1–6** gave their molecular ions $[M]^+$ (*m/z*) at 1197 (**1**), 1211 (**2**), 1035 (**3**), 1065 (**4**), 1049 (**5**), and 1079 (**6**), respectively. Elemental components of **1–6** were confirmed by measuring their HR-FAB MS. The chemical and molecular compositions of **1–6** based on high resolution FAB mass spectra (HR-FABMS) are summarized in Table 1. To obtain information on the linkages of the aglycone, sugars, and/or acids and also to investigate the structural similarity of these pigments, partial hydrolysis of **1–6** was carried out by the procedure described previously (Saito et al., 2008). Pelargonidin 3-*O*-sambubioside-5-*O*-glucoside, pelargonidin 3-*O*-[2-*O*-(xylosyl)-6-*O*-(feruroyl)-glucoside]-5-*O*-glucoside and pelargonidin 3-*O*-[2-*O*-(xylosyl)-6-*O*-(*p*-coumaroyl)-glucoside]-5-*O*-glucoside were detected in their hydrolysates as intermediary pigment products

Table 1. Chromatographic and spectroscopic data for anthocyanins of *L. maritima*.

Anthocyanins	R_f values (× 100)				Spectral data in 0.1% HCl-MeOH			
	BAW	BuHCl	1% HCl	AHW	λmax (nm)	$E_{acyl}/E_{vis.max}$ (%)	$E_{440}/E_{vis.max}$ (%)	$AlCl_3$
1	42	17	39	68	271sh, 279sh, 288, 309, 512	113	19	0
2	44	14	48	69	272sh, 279sh, 288, 313, 512	115	19	0
3	66	56	30	58	272sh, 280sh, 289, 319, 511	146	20	0
4	62	47	26	60	269sh, 281sh, 289, 327, 511	141	21	0
5	73	53	35	61	271sh, 279sh, 290, 321, 511	126	20	0
6	64	43	34	65	272sh, 280sh, 289, 327, 510	132	20	0

Anthocyanins	HPLC R_t (min)	HR FAB-MS[z]			Chemical composition[y]					
		Mf	calc.	$[M]^+$ found	Pel	Glc	Xy	pC	Caf	Fer
1	27.2	$C_{56}H_{61}O_{29}$	1197.3299	1197.3307	1	3	1	1	1	
2	28.4	$C_{57}H_{63}O_{29}$	1211.3455	1211.3453	1	3	1	1		1
3	33.9	$C_{50}H_{51}O_{24}$	1035.2770	1035.2748	1	2	1	1	1	
4	34.6	$C_{51}H_{53}O_{25}$	1065.2876	1065.2843	1	2	1		1	1
5	35.4	$C_{51}H_{53}O_{24}$	1049.2927	1049.2911	1	2	1	1		1
6	36.1	$C_{52}H_{55}O_{25}$	1079.3032	1079.3020	1	2	1			2

Anthocyanin numbers are the same as those in Figure 1.
[z] $[M]^+$ and Mf are molecular ion mass values observed, and estimated molecular formulae as flavylium forms of anthocyanins isolated from red-purple flowers of *L. maritima* based on HR-FAB mass data.
[y] Pel = pelargonidin, Glc = glucose, Xy = xylose, pC = *p*-coumaric acid, Caf = caffeic acid, Fer = ferulic acid.

(Table 2). These intermediates were confirmed by comparison of HPLC and TLC behaviors with those of authentic deacylated and demalonylated anthocyanins obtained from *Matthiola* red anthocyanins 4 and 5 (Saito et al., 1996). **3** was also detected in the hydrolysate of **1** as another major intermediary pigment product. Similarly, **5** was detected in the hydrolysate of **2** (Table 2). From the above results, the structures of **1–6** were presumed to be based on pelargonidin 3-*O*-sambubioside-5-*O*-glucoside, and **1–3** and **5** were assumed to be acylated with *p*-coumaric acid at OH-6 of 3-glucosyl residues. On the other hand, **4** and **6** were acylated with ferulic acid at OH-6 of 3-glucosyl residues. The structures of **1–6** were further elucidated on the basis of analyses of their ^{1}H and ^{13}C NMR spectra, including COSY, NOESY, HMQC, and HMBC spectra.

2. Deacylanthocyanin

The ^{1}H NMR spectrum of deacylanthocyanin exhibited seven aromatic protons and their chemical shifts were identical with the values of pelargonidin (Table 3). Three anomeric protons of the sugar moieties were assigned at δ 5.63 (*d*, *J* = 7.7 Hz, glucose (Glc) A), δ 5.17 (*d*, *J* = 7.7 Hz, Glc B), and δ 4.82 (*d*, *J* = 8.0 Hz, Xylose), and Glc A and B and xylose were determined to be in β-pyranose forms based on their observed *J* values. Long-range NOEs between H-1 of Glc A and H-4 (δ 8.97) of pelargonidin, H-1 of Glc B and H-6 (δ 7.04) of pelargonidin, and H-2 (δ 3.92) of Glc A and H-1 of xylose were observed in an NOESY experiment, indicating that OH-3 and OH-5 of pelargonidin were glycosylated with Glc A and Glc B, respectively, and also that OH-2 of Glc A was bonded with xylose to form sambubiose. Therefore, the deacylanthocyanin was determined to be pelargonidin 3-*O*-[2-*O*-(β-xylopyranosyl)-β-glucopyranoside]-5-*O*-β-glucopyranoside, i.e., pelargonidin 3-*O*-sambubioside-5-*O*-glucoside. This structure was also confirmed by analysis of its ^{13}C NMR spectrum (Table 3).

Table 2. Main intermediary anthocyanin products of *Loburalia* red-purple anthocyanins by partial acid hydrolysis.

Anthocyanins	Main intermediary products*				
	Pg3S5G	Pg3PS5G	Pg3FS5G	3	5
1	+	+		+	
2	+	+			+
3	+	+			
5	+	+			
4	+		+		
6	+		+		

Anthocyanins **1–6** are the same as in Figure 1 and Table 1.
* + = presence:
Pg3S5G: pelargonidin 3-*O*-sambubioside-5-*O*-glucoside,
Pg3PS5G: pelargonidin 3-*O*-[2-*O*-(xylosyl)-6-*O*-(*p*-coumaroyl)-glucoside]-5-*O*-glucoside,
Pg3FS5G: pelargonidin 3-*O*-[2-*O*-(xylosyl)-6-*O*-(feruloyl)-glucoside]-5-*O*-glucoside.

*3. Pigments **3**, **5** and **6***

As shown in Tables 1 and 2, **3** was composed of a deacylanthocyanin (pelargonidin 3-*O*-sambubioside-5-*O*-glucoside) with one molecule each of *p*-coumaric acid and caffeic acid, whereas **5** was composed of the same deacylanthocyanin with one molecule each of *p*-coumaric acid and ferulic acid, and **6** was composed of the same anthocyanin with two molecules of ferulic acid. These results were confirmed by analysis of their ^{1}H NMR spectra (Table 3).

The ^{1}H NMR spectra of the three pigments exhibited very similar signals, except for the signals of their hydroxycinnamic acid moieties (Table 3). Regarding the resonances of their sugar moieties in three pigments, three signals for H-6a and H-6b of Glc A (δ 4.31–4.38) and H-2 of xylose (δ 4.61–4.71) were shifted to a lower field than those (H-6a and H-6b, δ 3.62 and 3.80; H-2, δ 3.04) of the deacylanthocyanin, indicating that the OH-6 groups of Glc A and OH-2 of xylose were acylated with two molecules of hydroxycinnamic acids in **3**, **5**, and **6**, respectively. All olefinic protons of the six hydroxycinnamic acids in **3**, **5**, and **6** showed large coupling constants (*J* = 15.9 Hz), suggesting a *trans*-configuration (Table 3). The observed coupling constants of sugar moieties in **3**, **5**, and **6** were 11.9–7.4 Hz, indicating that the nine sugars were in β-pyranose forms; therefore, **6** was determined to be pelargonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-glucopyranoside]-5-*O*-β-glucopyranoside, which is a new anthocyanin in plants (Andersen and Jordheim, 2006; Harborne and Baxter, 1999; Honda and Saito, 2002).

As shown in Table 2, pelargonidin 3-*O*-[2-*O*-(xylosyl)-6-*O*-(*p*-coumaroyl)-glucoside]-5-*O*-glucoside was detected as the main intermediary pigment product in hydrolysates of **3** and **5** by partial hydrolysis experiments (Table 2); therefore, the OH-2 groups of the xylose moiety in these pigments were acylated with hydroxycinnamic acids, such as caffeic acid in **3** and ferulic acid in **5**. Thus, **3** and **5** were determined to be pelargonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-caffeoyl)-β-xylopyranosyl)-6-*O*-(*trans*-*p*-coumaroyl)-β-glucopyranoside]-5-*O*-β-glucopyranoside and pelargonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-xylopyranosyl)-6-*O*-(*trans*-*p*-coumaroyl)-β-glucopyranoside]-5-*O*-β-glucopyranoside, respectively. These pigments are new anthocyanins in plants (Andersen and Jordheim, 2006; Harborne and Baxter, 1999; Honda and Saito, 2002).

*4. Pigments **1** and **2***

The ^{1}H NMR spectrum of **1** was identical to that of **3** except for signals of the Glc C moiety. Similarly, the ^{1}H NMR spectrum of **2** was identical to that of **5** except for signals of the Glc C moiety (Table 3). The resonances of both anomeric protons of Glc C in **1** and **2** appeared at δ 5.00 and δ 4.99 and their coupling constants were *J* = 7.6 Hz and 7.3 Hz. Thus, both Glc C in **1** and **2** must be of β-glucopyranose form. Analysis of their NOESY

Table 3. NMR[z] spectral data of anthocyanins of *L. maritima*.

	1			2			3		5		6		Deacyl		
	^{1}H		^{13}C	^{1}H		^{13}C	^{1}H		^{1}H		^{1}H		^{1}H		^{13}C
Pelargonidin															
2			162.8			162.8									163.1
3			145.4			145.3									144.6
4	8.78	s	131.6	8.79	s	132.5	8.78	s	8.79	s	8.82	s	8.97	s	134.2
5			155.4			155.5									155.8
6	7.02	brs	105.1	7.02	brs	105.2	7.01	brs	7.01	d (1.5)	7.00	brs	7.04	brs	104.5
7			167.8			167.9									168.2
8	7.10	brs	96.6	7.11	brs	96.6	7.09	brs	7.08	d (1.5)	7.10	brs	7.21	brs	96.5
9			155.4			155.5									155.5
10			112.3			112.2									112.1
1'			119.5			119.6									119.6
2',6'	8.72	d (9.2)	135.8	8.73	d (9.2)	135.7	8.73	d (9.2)	8.72	d (9.2)	8.73	d (8.9)	8.73	d (8.9)	135.7
3',5'	7.11	d (9.2)	117.1	7.12	d (9.2)	117.2	7.11	d (9.2)	7.12	d (9.2)	7.12	d (8.9)	7.12	d (8.9)	117.3
4'			165.7			165.8									165.7
Cinnamic acid I															
1			126.3			126.3									
2	7.52	d (8.6)	130.4	7.52	d (8.9)	130.4	7.40	d (8.6)	7.39	d (8.9)	7.10	brs			
3	7.05	d (8.6)	116.7	7.05	d (8.9)	116.8	6.79	d (8.6)	6.79	d (8.3)					
4			159.5			159.5									
5	7.05	d (8.6)	116.7	7.05	d (8.9)	116.8	6.79	d (8.6)	6.79	d (8.3)	6.82	d (7.9)			
6	7.52	d (8.6)	130.4	7.52	d (8.9)	130.4	7.40	d (8.6)	7.39	d (8.9)	7.04	brd (7.9)			
7α	6.39	d (15.9)	115.1	6.39	d (15.9)	115.6	6.29	d (15.9)	6.28	d (15.9)	6.34	d (15.9)			
8β	7.42	d (15.9)	144.3	7.42	d (15.9)	144.3	7.36	d (15.9)	7.36	d (15.9)	7.40	d (15.9)			
9 (CO)			166.8			166.8									
OMe											3.79	s			
Cinnamic acid II															
1			127.9			127.9									
2	7.12	brs	112.1	7.33	brs	111.9	7.14	brs	7.33	brs	7.33	brs			
3			148.6			149.7									
4			145.9			148.3									
5	6.83	d (8.3)	116.1	6.86	d (8.3)	116.0	6.84	d (7.9)	6.86	d (8.3)	6.86	d (8.3)			
6	7.04	m	121.8	7.17	brd (8.3)	123.3	7.05	brd (8.3)	7.17	d (8.3)	7.17	brd (8.3)			
7α	6.39	d (15.9)	115.6	6.55	d (15.9)	115.5	6.39	d (15.9)	6.55	d (15.9)	6.55	d (15.9)			
8β	7.53	d (15.9)	144.9	7.61	d (15.9)	144.9	7.54	d (15.9)	7.61	d (15.9)	7.61	d (15.9)			
9 (CO)			166.4			166.4									
OMe				3.89	s	56.1			3.88	s	3.88	s			
Glucose A															
1	5.72	d (8.0)	97.9	5.74	d (7.7)	98.0	5.73	d (7.9)	5.73	d (7.6)	5.72	d (8.0)	5.63	d (7.7)	99.6
2	3.99	m	77.0	4.01	t* (8.0)	77.0	3.99	m	4.01	t* (8.3)	4.01	t* (8.6)	3.92	t* (8.2)	80.1
3	3.64	t* (8.6)	77.4	3.66	t* (8.9)	77.4	3.63	t* (8.9)	3.65	t* (8.9)	3.65	t* (8.5)	3.72	t* (9.2)	77.0
4	3.39	t* (9.2)	71.0	3.39	m	71.0	3.46	t* (8.9)	3.46	t* (9.2)	3.48	m	3.39	t* (9.2)	69.8
5	3.99	m	73.9	3.99	m	73.9	3.99	m	3.98	m	3.96	m	3.45	m	77.9
6a	4.35	m	63.5	4.32	m	63.5	4.34	m	4.32	m	4.31	m	3.62	m	60.9
6b	4.35	m		4.36	m		4.34	m	4.36	d (10.7)	4.38	d (10.5)	3.80	d (12.5)	
Glucose B															
1	5.09	d (7.6)	102.8	5.10	d (7.7)	102.8	5.11	d (7.3)	5.10	d (7.7)	5.11	d (7.4)	5.17	d (7.7)	104.5
2	3.55	t* (8.9)	78.0	3.55	t* (7.7)	78.0	3.55	t* (9.2)	3.55	m	3.53	m	3.53	t* (8.0)	77.8
3	3.36	m	77.1	3.42	m	77.1	3.41	t* (8.9)	3.43	m	3.46	m	3.44	t* (8.9)	77.4
4	3.25	m	70.1	3.26	t* (9.5)	70.0	3.29	m	3.28	m	3.28	m	3.36	m	69.9
5	3.55	m	73.6	3.56	m	73.5	3.57	m	3.55	m	3.55	m	3.60	m	74.5
6a	3.81	m	61.0	3.82	m	61.0	3.83	m	3.83	m	3.83	m	3.62	m	60.8
6b	3.77	m		3.77	d (10.4)		3.78	m	3.80	d (10.4)	3.78	m	3.78	d (11.9)	
Glucose C															
1	4.99	d (7.3)	100.3	5.00	d (7.6)	100.3									
2	3.30	m	73.6	3.30	m	73.5									
3			76.2			76.2									
4			70.3			70.2									
5	3.24–3.60		—	3.25–3.57		—									
6a			61.2			61.2									
6b															
Xylose															
1	5.21	d (8.2)	101.5	5.22	d (7.9)	101.5	5.22	d (8.2)	5.21	d (8.0)	5.22	d (8.0)	4.82	d (8.0)	101.9
2	4.69	t* (8.6)	74.1	4.71	t* (8.9)	74.0	4.69	t* (8.7)	4.71	t* (8.9)	4.71	t* (8.9)	3.04	t* (8.5)	73.4
3	3.46	t* (9.2)	75.0	3.46	t* (8.6)	75.0	3.37	m	3.41	t* (9.2)	3.41	t* (9.2)	3.21	t* (8.9)	76.2
4	3.50	m	70.0	3.54	m	70.0	3.53	m	3.54	m	3.52	m	3.34	m	69.5
5a	3.27	m	66.8	3.28	m	67.0	3.31	m	3.29	m	3.29	m	3.09	m	66.6
5b	3.99	m		4.00	m		3.99	m	3.98	m	3.98	m	3.70	m	

[z] 125.78 MHz for ^{13}C NMR and 500 MHz for ^{1}H NMR, CF_3CO_2D-DMSO-d_6 (1:9), at 25°C, an internal standard of TMS; Coupling constants (J in Hz) in parentheses.

s-singlet, brs = broad singlet, d = doublet, t* = distorted triplet, m = multiplet.

spectra established strong long-range NOEs between H-1 of Glc C and H-3 and H-5 of *p*-coumaric acid in both **1** and **2**, indicating that the OH-4 groups of both *p*-coumaric acids were glycosylated with Glc C in **1** and **2**. In partial acid hydrolysis experiments of **1** and **2**, **3** was detected in the hydrolysate of **1** as well as the deacylanthocyanin and pelargonidin 3-*O*-[2-*O*-(xylosyl)-6-*O*-(*p*-coumaroyl)-glucoside]-5-*O*-glucoside as its intermediary pigment products (Table 2). **5** was also detected in the hydrolysate of **2**, as well as the deacylanthocyanin and pelargonidin 3-*O*-[2-*O*-(xylosyl)-6-*O*-(*p*-coumaroyl)-glucoside]-5-*O*-glucoside as its intermediary pigment products (Table 2).

Consequently, **1** was determined to be pelargonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-caffeoyl)-β-xylopyranosyl)-6-*O*-(4-*O*-(β-glucopyranosyl)-*trans*-*p*-coumaroyl)-β-glucopyranoside]-5-*O*-β-glucopyranoside, which is a new anthocyanin in plants, and **2** was determined to be pelargonidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-xylopyranosyl)-6-*O*-(4-*O*-(β-glucopyranosyl)-*trans*-*p*-coumaroyl)-β-glucopyranoside]-5-*O*-β-glucopyranoside, which is also a new anthocyanin in plants (Andersen and Jordheim, 2006; Harborne and Baxter, 1999; Honda and Saito, 2002).

5. Pigment 4

The HR-FAB MS spectrum of **4** gave its $[M]^+$ at *m/z* 1065.2843 ($C_{51}H_{53}O_{25}$) as shown in Table 1, indicating that this pigment was composed of pelargonidin with one molecule each of caffeic acid, ferulic acid and xylose and two molecules of glucose. On alkaline hydrolysis, **4** gave pelargonidin 3-*O*-sambubioside-5-*O*-glucoside, caffeic acid and ferulic acid. As shown in Table 2, pelargonidin 3-*O*-[2-*O*-(xylosyl)-6-*O*-(feruloyl)-glucoside]-5-*O*-glucoside was detected in its partial acid hydrolysate as an intermediary pigment product as well as pelargonidin 3-*O*-sambubioside-5-*O*-glucoside. Moreover, 2-*O*-(2-*O*-(caffeoyl)-xylosyl)-6-*O*-(feruloyl)-glucoside was obtained by H_2O_2 degradation of **4**. Based on the above findings, **4** was preliminarily determined to be pelargonidin 3-*O*-[2-*O*-(2-*O*-(caffeoyl)-xylosyl)-6-*O*-(feruloyl)-glucoside]-5-*O*-glucoside. This pigment is also a new anthocyanin in plants (Andersen and Jordheim, 2006; Harborne and Baxter, 1999; Honda and Saito, 2002). Because of the small amounts obtained, further structure confirmation of **4** could not be carried out.

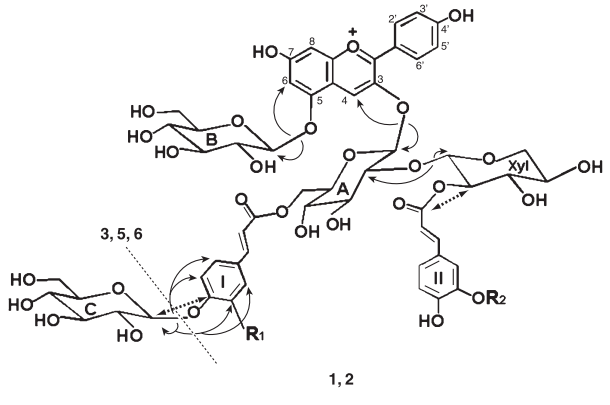

Fig. 2. Acylated pelargonidin 3-*O*-sambubioside-5-*O*-glucosides from red-purple flowers of *L. maritima*. NOEs are indicated by arrows. HMBC of 1 is indicated by dotted arrows.
1: $R_1 = R_2 = H$, **2**: $R_1 = H$, $R_2 = CH_3$, **3**: $R_1 = R_2 = H$, **5**: $R_1 = H$, $R_2 = CH_3$, **6**: $R_1 = OCH_3$, $R_2 = CH_3$

Flower colors and the distribution of anthocyanins in L. maritima.

As reported previously, nine acylated cyanidin 3-*O*-sambubioside-5-*O*-glucosides were isolated from the purple-violet flowers of *L. maritima* 'Easter Bonnet Violet' as floral anthocyanin pigments (Tatsuzawa et al., 2006, 2007). In this study, six acylated pelargonidin 3-*O*-sambubioside-5-*O*-glucosides were isolated from the red-purple flowers of *L. maritima* 'Easter Bonnet Deep Rose', and their structures were elucidated to be pelargonidin 3-*O*-[2-*O*-(2-*O*-(acyl-II)-β-xylopyranosyl)-6-*O*-(acyl-I)-β-glucopyranoside]-5-*O*-β-glucopyranoside, in which the acyl-I group was replaced by 4-*O*-glucosyl-*p*-coumaroyl, *p*-coumaroyl or feruloyl, and acyl-II by caffeoyl or feruloyl. These anthocyanins were to be analogous acylated pigments of the purple-violet flowers of 'Easter Bonnet Violet' bearing cyanidin as the aglycone (Tatsuzawa et al., 2006, 2007). Thus, the red-purple flower color must depend on the pelargonidin core instead of cyanidin for the purple-violet flower color in *L. maritima*. It may also be considered that the presence of two molecules of hydroxycinnamic acids bonded in these floral anthocyanins contribute to stabilization as intramolecular co-pigmentation in these cultivars (Honda and Saito, 2002). There is a large number of cultivars in *Matthiola* and *Lobularia*, and both *Matthiola* and *Lobularia* are major ornamental crops in the Cruciferae. To the best of our knowledge, four acylated cyanidin glycosides and ten acylated pelargonidin glycosides were reported to be present in the flowers of *Matthiola* cultivars (Saito et al., 1995, 1996), and in the flowers of *Lobularia maritima* cultivars, nine acylated cyanidin glycosides and six pelargonidin glycosides were present (Tatsuzawa et al., 2006, 2007).

From the chemotaxonomic point of view, there are two typical glycoside patterns in the Cruciferae at OH-3 position of anthocyanins, such as acylated 3-sambubioside (Bloor and Abrahams, 2002; Honda et al., 2005; Saito et al., 1995, 1996; Takeda et al., 1988; Tatsuzawa et al., 2006, 2007, 2008a) and 3-sophoroside (Fuleki, 1969; Giusti et al., 1998; Harborne, 1964; Hrazdina et al., 1977; Idaka et al., 1987a, b; Igarashi et al., 1990; Ikeda et al., 1987; Ishikura and Hayashi, 1962, 1963; Mori et al., 2006; Nakatani et al., 1987; Otsuki et al., 2002; Saito et al., 2008; Suzuki et al., 1997; Tanchev and Timberlake, 1969; Tatsuzawa et al., 2008b); therefore, the floral anthocyanins of *L. maritima* are grouped into the former pattern.

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