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FOUR ACYLATED CYANIDIN 3-SAMBUBIOSIDE-5-GLUCOSIDES FROM THE PURPLE-VIOLET FLOWERS OF *LOBULARIA MARITIMA*

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Abstract – Four acylated cyanidin 3-sambubioside-5-glucosides were isolated from the purple-violet flowers of *Lobularia maritima* (L.) Desv. 'Easter Bonnet Violet' along with six known anthocyanins. These new pigments were determined by chemical and spectroscopic methods to be cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-D-xylopyranosyl)-6-*O*-(4-*O*-(β-D-glucopyranosyl)-*trans*-*p*-coumaroyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside, cyanidin 3-*O*-[2-*O*-(β-D-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside, cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-caffeoyl)-β-D-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside or cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-D-xylopyranosyl)-6-*O*-(*trans*-caffeoyl)- β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside, and cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-D-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside.

INTRODUCTION

Lobularia maritima (L.) Desv. is a popular garden plant with white, pale yellow, pink, red, purple or violet flowers. The occurrence of five acylated cyanidin 3-sambubioside-5-glucosides and also cyanidin 3-sambubioside-5-glucoside have been already reported in the purple-violet flowers of *L. maritima* 'Easter Bonnet Violet' by us.[1] However, it was observed by the analysis of HPLC that the structurally

unsolved acylated anthocyanins were still present in its purple-violet flowers (Figure 1).

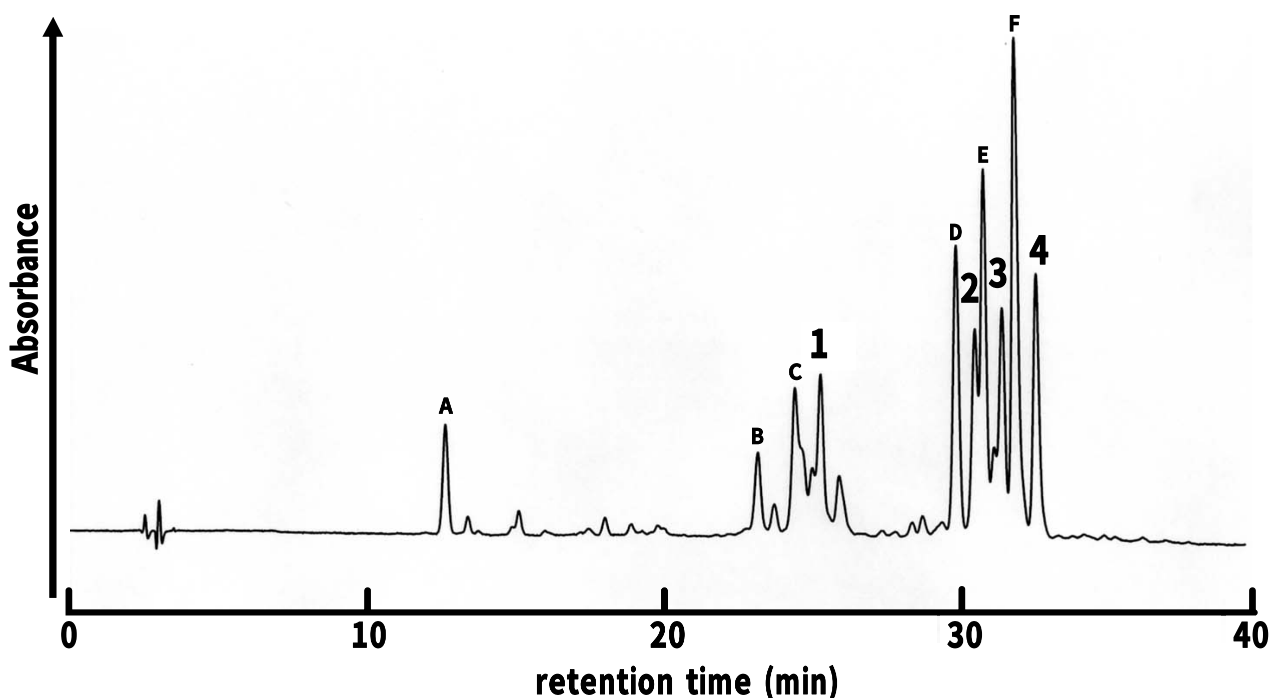

Figure 1. HPLC analysis of the pigments in the flowers of *Lobularia maritima*.

New anthocyanins:
1: cyanidin 3-[2-(2-(*trans*-feruloyl)xylosyl)-6-(4-glucosyl-*trans*-*p*-coumaroyl)glucoside]-5-glucoside
2: cyanidin 3-[2-(xylosyl)-6-(*trans*-feruloyl)-glucoside]-5-glucoside
3: cyanidin 3-[2-(2-(*trans*-caffeoyl)xylosyl)-6-(*trans*-feruloyl)glucoside]-5-glucoside
or cyanidin 3-[2-(2-(*trans*-feruloyl)xylosyl)-6-(*trans*- caffeoyl)glucoside]-5-glucoside
4: cyanidin 3-[2-(2-(*trans*-feruloyl)xylosyl)-6-(*trans*-feruloyl)glucoside]-5-glucoside

Known anthocyanins[1]:
A: cyanidin 3-sambubioside-5-glucoside
B: cyanidin 3-[2-(xylosyl)-6-(4-glucosyl-*trans*-*p*-coumaroyl)glucoside]-5-glucoside
C: cyanidin 3-[2-(2-(*trans*-caffeoyl)xylosyl)-6-(4-glucosyl-*trans*-*p*-coumaroyl)glucoside]-5-glucoside
D: cyanidin 3-[2-(xylosyl)-6-(*trans*-*p*-coumaroyl)glucoside]-5-glucoside
E: cyanidin 3-[2-(2-(*trans*-caffeoyl)xylosyl)-6-(*trans*-*p*-coumaroyl)glucoside]-5-glucoside
F: cyanidin 3-[2-(2-(*trans*-feruloyl)xylosyl)-6-(*trans*-*p*-coumaroyl)glucoside]-5-glucoside

For the purpose of understanding the relationship between the flower color and its pigment composition of *L. maritima* 'Easter Bonnet Violet', the isolation and structure investigation of anthocyanins was further carried out in the purple-violet flowers. The four acylated cyanidin 3-sambubioside-5-glucosides were freshly isolated from the purple-violet flowers of *L. maritima* 'Easter Bonnet Violet'. In this paper we wish to report the structure elucidation of these new anthocyanins.

RESULTS AND DISCUSSION

Six anthocyanins (**A** – **F**) from these anthocyanin peaks, have been isolated and determined to be cyanidin

3-sambubioside-5-glucosides (ranging from 3.4 % of the total anthocyanin contents calculated from the HPLC peak area at 530 nm, **A**), cyanidin 3-[2-(xylosyl)-6-(glucosyl-*trans*-*p*-coumaroyl)-glucoside]-5-glucoside (2.6 %, **B**), cyanidin 3-[2-(2-*trans*-caffeoyl)-xylosyl]-6-(glucosyl-*trans*-*p*-coumaroyl)-glucoside]-5-glucoside (7.9 %, **C**), cyanidin 3-[2-(xylosyl)-6-(*trans*-*p*-coumaroyl)-glucoside]-5-glucoside (10.2 %, **D**), cyanidin 3-[2-(2-*trans*-caffeoyl)-xylosyl]-6-(*trans*-*p*-coumaroyl)-glucoside]-5-glucoside (12.3 %, **E**), cyanidin 3-[2-(2-*trans*-feruloyl)-xylosyl]-6-(*trans*-*p*-coumaroyl)-glucoside]-5-glucoside (18.8 %, **F**) as mentioned above.[1] In addition to these six anthocyanins, four new anthocyanins (**1**: 6.2 %, **2**: 7.2 %, **3**: 8.0 %, **4**: 9.3 %) were detected in the extract as shown in Figure 1.

Acid hydrolysis of mixed pigments **1** – **4** (*ca*. 3 mg) was carried out with 2N HCl (15 mL) at 100 ^{o}C for 1 h, and resulted in cyanidin, glucose, xylose, *p*-coumaric acid, caffeic acid and ferulic acid. Those compounds were confirmed by direct comparison of TLC and HPLC with the authentic samples obtained from the hydrolysates of pigments **A** - **F**. The alkaline hydrolysis of mixed pigments **1** – **4** (*ca*. 3 mg) was carried out with 2N NaOH solution (20 mL) at ambient temperature for 15 min, and resulted in only one deacylated anthocyanin, whose structure was identified to be cyanidin 3-sambubioside-5-glucoside in comparison with the authentic sample obtained from *Matthiola* violet anthocyanin by analyses of TLC and HPLC.[2] 4-Glucosyl-*p*-coumaric acid was also obtained from the alkaline hydrolysate of pigment **1**, and identified in comparison with the authentic sample obtained from ternatins of *Clitoria ternatea* by the same process of alkaline hydrolysis.[3]

The FAB mass spectra of pigments **1** – **4** gave their molecular ions at 1227, 919, 1081 and 1095 *m/z*, respectively, in agreement with the masses calculated for $C_{57}H_{63}O_{30}$, $C_{42}H_{47}O_{23}$, $C_{51}H_{53}O_{26}$ and $C_{52}H_{55}O_{26}$, and their elemental components were confirmed by measuring their high-resolution FAB MS; Pigment **1**: calc. for $C_{57}H_{62}O_{30}Na$ requires: 1249.3224. Found: 1249.3179; Pigment **2**: calc. for $C_{42}H_{47}O_{23}$ requires: 919.2508. Found: 919.2532; Pigment **3**: calc. for $C_{51}H_{53}O_{26}$ requires: 1081.2825. Found: 1081.2794; Pigment **4**: calc. for $C_{52}H_{55}O_{26}$ requires: 1095.2982. Found: 1095.2985. Furthermore, the structure elucidations of these pigments were carried out by NMR spectral analyses as follows.

Pigment **1**: The chemical shifts of thirteen aromatic protons of cyanidin, ferulic acid and *p*-coumaric acid moieties were assigned as shown in Table 1. The four chemical shifts at δ 6.52, 7.59, 6.37 and 7.41 were also assigned to the olefinic protons of *trnas*-*p*-coumaric acid and *trans*-ferulic acid with the large coupling constants (*J*=15.9, 15.9, 15.9 and 15.9 Hz). The characteristic signals of four anomeric protons appeared at δ 5.71 (d, *J*=7.3 Hz, Glc A), δ 5.07 (d, *J*=8.0 Hz, Glc B), δ 4.98 (d, *J*=7.3 Hz, Glc C) and δ 5.14 (d, *J*=7.7 Hz, xylose). Based on the observed coupling constants (Table 1), four sugars were assumed to have β-pyranose forms. The linkages and/or positions of the attachments of the sugar and acyl groups in this pigment were mainly determined by using 2D COSY, NOESY and NOEDIF (negative difference NOE) experiments as shown in Figure 2. A proton signal (δ 4.03, *m*) shifting to a lower magnetic field

was assigned to H-2 of Glc A by the analysis of its 2D COSY spectrum indicating that xylose was linked to the OH-2 of Glc A due to forming sambubiose at the OH-3 of cyanidin. This bonding was confirmed by NOEDIF experiment as described previously.[4] In fact, strong NOEs were observed between H-2 of Glc A and H-1 of xylose by irradiation of each proton. Two characteristic proton signals (δ 4.31 and 4.36) shifted to a lower magnetic field were also assigned to the methylene protons of Glc A supporting the acylation with *p*-coumaric acid at OH-6 of Glc A. This bonding was also confirmed by the observation of rather week NOEs between the signal H-1 of Glc A and those of H-2,6 and H-3,5 for *p*-coumaric acid moiety by NOEDIF experiment. Furthermore, strong NOEs were observed at H-3,5 of *p*-coumaric acid by irradiation at H-1 of Glc C, indicating that Glc C was attached to OH-4 of *p*-coumaric acid through a glycosidic bond. On alkaline hydrolysis, 4-glucosyl-*p*-coumaric acid [Rt (min) 8.0] was detected in the hydrolysate of pigment **1** by HPLC analysis. Therefore, the OH-2 of xylose moiety was acylated with ferulic acid on account of the H-2 signal of xylose moiety being shifted to a lower field at δ 4.67 (t, *J* = 8.4 Hz) (Figure 2). Consequently, pigment **1** was determined to be cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-D-xylopyranosyl)-6-*O*-(4-*O*-(β-D-glucopyranosyl)-*trans*-*p*-coumaroyl)-β-D-gluco-pyranoside]-5-*O*-(β-D-glucopyranoside) (Figure 2), which is a new anthocyanin in plants.[5,6]

Pigment **2**: The chemical shifts of nine aromatic protons of cyanidin and ferulic acid moieties were assigned as shown in Table 2. The two chemical shifts at δ 6.36 and 7.42 were also assigned to the olefinic protons of *trans*-ferulic acid with the large coupling constants (*J*=15.9 and 15.9 Hz). The characteristic signals of three anomeric protons appeared at δ 5.72 (d, *J*=7.3 Hz, Glc A), δ 5.10 (d, *J*=7.67 Hz, Glc B), and δ 4.75 (d, *J*=7.6 Hz, xylose). Based on the observed coupling constants (Table 2), three sugars were assumed to have β-pyranose forms. The linkages and/or positions of the attachments of the sugar and acyl groups in this pigment were determined by 2D COSY and NOESY experiments.

A proton signal (δ 4.04, dd, *J*=7.3, 8.3 Hz) shifting to a lower magnetic field was assigned to H-2 of Glc A by the analysis of its 2D COSY spectrum, indicating that xylose was linked to the OH-2 of Glc A due to forming sambubiose at the OH-3 of cyanidin. This bonding was confirmed by the experiments of NOEDIF and NOESY as described previously.[4] Two characteristic proton signals (δ 4.34 and 4.44) shifted to a lower magnetic field were also assigned to the methylene protons of Glc A supporting the acylation with ferulic acid at OH-6 of Glc A. Therefore, pigment **2** was determined to be cyanidin 3-*O*-[2-*O*-(β-D-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside] (Figure 2) , which is a new anthocyanin in plants. [5,6]

Pigment **3**: The ^{1}H NMR spectrum of pigment **3** was very similar to that of pigment **2**, with the additional signals of caffeic acid moiety in its ^{1}H NMR spectrum (Table 1).

Table 1. ^{1}H NMR spectral data of pigments 1-4 of *Lobularia maritima*.*

	1		2		3		4	
Cyanidin								
4	8.72	s	8.80	s	8.76	s	8.74	s
6	6.99	br d (1.8)	6.99	d (1.9)	6.98	br d (1.9)	6.96	br d (1.9)
8	7.01	br d (1.8)	7.04	d (1.9)	7.04	br d (1.9)	7.01	br d (1.9)
2'	8.02	d (2.5)	8.07	d (2.5)	8.03	d (2.5)	8.00	d (2.4)
5'	7.07	d (8.9)	7.08	d (8.8)	7.09	d (8.9)	7.07	d (8.6)
6'	8.49	dd (2.5,8.9)	8.39	dd (2.5, 8.8)	8.50	dd (2.5, 8.9)	8.48	dd (2.4, 8.6)
Glucose A								
1	5.71	d (7.3)	5.72	d (7.3)	5.71	d (8.0)	5.69	d (7.3)
2	4.03	m	4.04	dd (7.3, 8.3)	4.03	t (8.1)	4.02	dd (7.3, 8.9)
3	3.65	m	3.77	t (8.9)	3.63	t (8.7)	3.61	t (8.9)
4	3.40	m	3.53	m	3.47	m	3.46	m
5	3.95	m	4.02	m	3.95	m	3.91	m
6a	4.31	m	4.34	dd (5.8, 11.9)	4.30	dd (6.0, 11.7)	4.28	m
6b	4.36	d (11.6)	4.44	br d (11.9)	4.37	d (11.7)	4.33	brd (10.4)
Glucose B								
1	5.07	d (8.0)	5.10	d (7.6)	5.10	d (7.6)	5.09	d (7.4)
2	3.51	m	3.53	m	3.53	m	3.49	m
3	3.35	m	3.41	t (9.0)	3.41	t (9.0)	3.38	m
4			3.28	t (9.0)	3.27	t (9.2)	3.24	m
5	3.30 - 3.90		3.50 - 3.60		3.50 - 3.60		3.35 - 3.54	
6a			3.59	m				
6b			3.79	m	3.81	m	3.77	m
Glucose C								
1	4.98	d (7.3)						
2	3.30	m						
3	3.26	m						
4	3.35	m						
5	3.49	m						
6a	3.72	m						
6b	4.04	m						
Xylose								
1	5.14	d (7.7)	4.75	d (7.6)	5.16	d (8.3)	5.14	d (8.2)
2	4.67	t (8.4)	3.05	dd (8.4, 8.0)	4.67	dd (8.3, 8.9)	4.65	dd (8.2, 8.9)
3	3.41	m	3.18	t (8.9)	3.43	m	3.40	m
4	3.41	m	3.26	m	3.45	m	3.43	m
5a	3.89	m	3.55	m	3.91	m	3.19	m
5b	3.22	m	2.99	t (11.0)	3.20	m	3.88	m
Cinnamic acid I								
2	7.51	d (8.8)	7.13	d (1.5)	7.10	br s	7.10	br s
3	7.04	d (8.8)						
5	7.04	d (8.8)	6.82	d (8.3)	6.82	d (8.3)	6.80	d (8.3)
6	7.51	d (8.8)	7.05	dd (1.5, 8.3)	7.02	dd (1.5, 8.3)	7.03	dd (1.6, 8.3)
α	6.52	d (15.9)	6.36	d (15.9)	6.34	d (15.9)	6.32	d (15.9)
β	7.59	d (15.9)	7.42	d (15.9)	7.40	d (15.9)	7.39	d (15.9)
OMe			3.80	s	3.80	s	3.78	s
Cinnamic acid II								
2	7.32	d (1.9)			7.13	d (2.1)	7.32	br d (1.5)
3								
5	6.85	d (8.2)			6.83	d (8.0)	6.84	d (8.2)
6	7.16	d (1.9, 8.2)			7.02	dd (2.1, 8.0)	7.15	dd (1.5, 8.2)
α	6.37	d (15.9)			6.38	d (15.9)	6.52	d (15.9)
β	7.41	d (15.9)			7.53	d (15.9)	7.58	d (15.9)
OMe	3.87	s					3.86	s

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

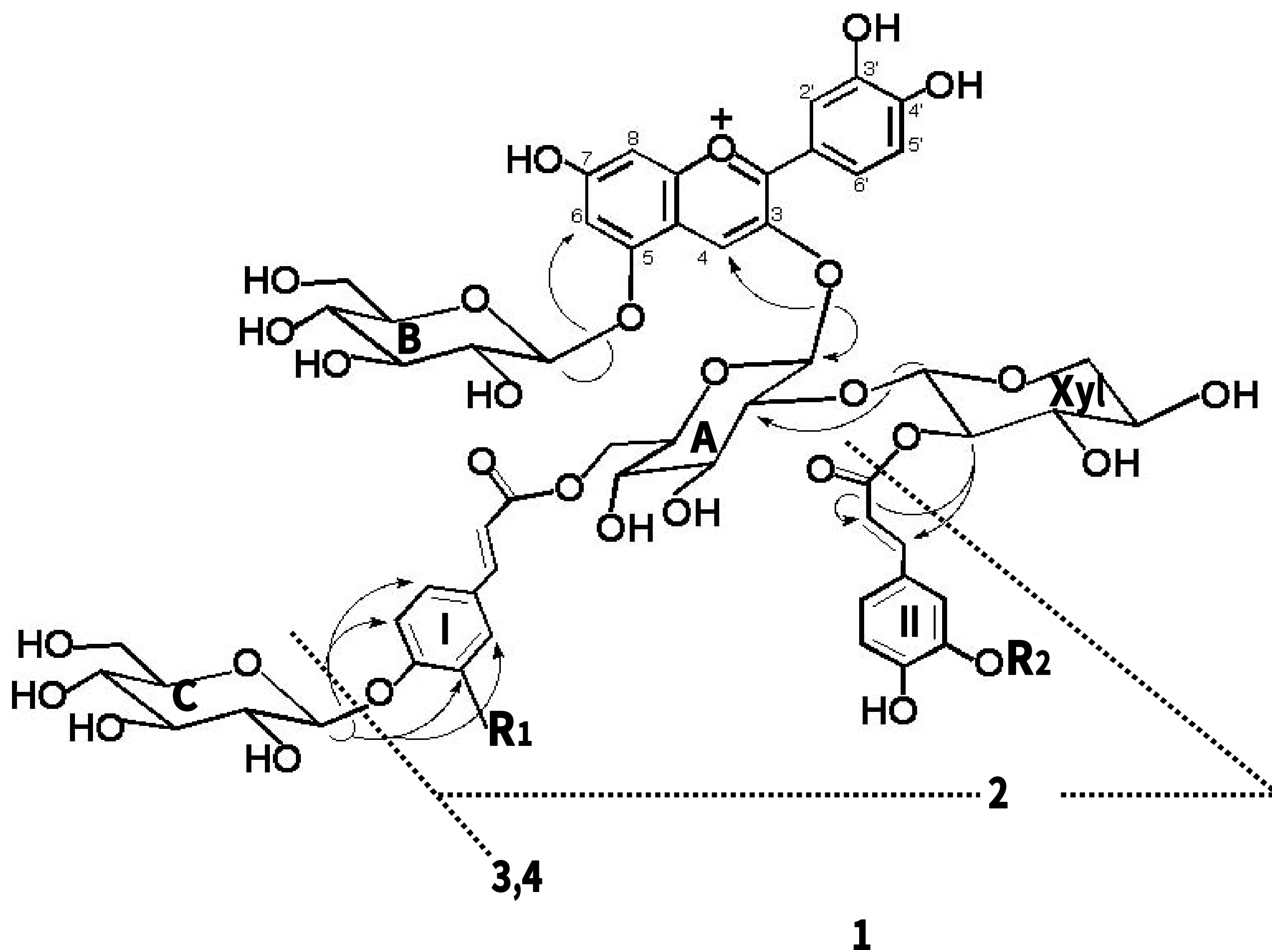

Figure 2. New acylated anthocyanins (**1** – **4**) from the flowers of *Lobulalia maritima*. Observed NOE's are indicated by arrows.

Pigment **1:** R_1=H, R_2=CH_3
Pigment **2:** R_1=OCH_3
Pigment **3:** R_1= OCH_3, R_2=H or R_1=OH, R_2=CH_3
Pigment **4:** R_1= OCH_3, R_2=CH_3

By the analysis of its 2D COSY spectrum, the chemical shifts of twelve aromatic protons of cyanidin, caffeic acid and ferulic acid were assigned as shown in Table 1. The four chemical shifts at δ 6.34, 6.38, 7.40, and 7.53 were also assigned to be olefinic protons of *trans*-ferulic acid and *trans*-caffeic acid with the large coupling constants (*J* = 15.9, 15.9, 15.9 and 15.9 Hz). Three characteristic proton signals (δ 4.30, 4.37 and 4.67) shifted to lower magnetic fields were assigned to the methylene protons of Glc A and H-2 proton of xylose supporting the acylation with hydroxycinnamic acids at OH-6 of Glc A and OH-2 of xylose. Although the linkages between sugars and hydroxycinnamic acids in pigment **3** could not be confirmed by its NOESY and NOEDIF experiments, unfortunately, the structure of pigment **3** was tentatively identified to be cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-caffeoyl)-β-D-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside) or cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-

β-D-xylopyranosyl)-6-*O*-(*trans*-caffeoyl)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside) (Figure 2), which is a new anthocyanin in plants.[5,6]

Pigment **4**: The ^{1}H NMR spectrum of pigment **4** was quite identical with that of pigment **3** except for the three proton signals (δ 3.86, *s*) of OCH_3 at the 3 position of ferulic acid (hydroxycinnamic acid II) being additionally observed (Table 1). Moreover, two strong proton signal sets (δ 3.78 and 3.86) of methyl group (OCH_3) were correlated to the H-2 signals (δ 7.10 and 7.32) of ferulic acid I and II by the analysis of 2D COSY spectrum, respectively. Therefore, the structure of pigment **4** was determined to be cyanidin 3-*O*-[2-*O*-(2-*O*-(*trans*-feruloyl)-β-D-xylopyranosyl)-6-*O*-(*trans*-feruloyl)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside) (Figure 2), which is a new anthocyanin in plants. [5,6]

As brief account for the flower pigments of *L. maritima*, its purple-violet flowers contained at least more than ten acyl cyanidin 3-sambubioside-5-glucosides, which were acylated with two molecules of hydroxycinnamic acids as their main anthocyanins like the flowers of *Matthiola*.[2] In these pigments it may be considered that three kinds of hydroxycinnamic acid groups play as the intracopigments in the bluing effect and stabilization of this flower color.[6]

EXPERIMENTAL

General procedures

TLC was carried out on plastic coated cellurose sheets (Merck) using ten mobile phases: BAW (*n*-BuOH/HOAc/H_2O. 4:1:2, v/v/v), BuHCl (*n*-BuOH/2N HCl, 1:1, v/v, upper layer), AHW (HOAc/HCl/H_2O, 15:3:82, v/v/v), and 1% HCl and Forestal (AcOH/HCl/H_2O, 30:3:10, v/v/v) for anthocyanins, and BAW, APW (EtOAc/pyridine/H_2O, 15:7:5, v/v/v), EAA (EtOAc/HCOOH/H_2O, 5:2:1, v/v/v), EFW (Et_2O/HCO_2H/H_2O, 5:2:1, v/v/v) and 15% AcOH-H_2O for sugars and organic acid with UV light and aniline hydrogen phthalate spray regent.[1]

Analytical HPLC was performed LC 10A system (Shimadzu), using a Waters C18 (4.6 ϕ x 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). UV-Vis spectra were recorded on a MPS-2400 (Shimadzu) in 0.1% HCl-MeOH (from 200 to 700 nm). FAB mass spectra were obtained in the positive ion mode using the magic bullet (5:1 mixture of dithiothreitol and dithioerythritol) as a matrix. NMR spectra ware recorded at 500 MHz for ^{1}H spectra in DMSO-d_6-CF_3COOD (9:1). Chemical shifts are reported relative to a TMS internal standard (δ), and coupling constants (*J*) are in Hz.

Plant materials

Seeds of purple-violet flowers of *Lobularia maritima* 'Easter bonnet violet' were purchased from Takii

Co. Ltd (Kyoto). These seeds were sown in August, 2006, and the plants were grown in a greenhouse of Minami-Kyushu University. The flowers exhibited purple-violet (Purple-Violet 81A and b*/a* = -0.52) were collected in December, 2006 and dried overnight at 40 ^oC, and kept in a refrigerator about 4 ^oC.

Extraction and purification of anthocyanins

Dried flower (*ca*. 100 g) of *L. maritima* was immersed in 5% AcOH-H_2O at rt for 5 h and extracted. The extract was passed through a Diaion HP-20 resin (Mitsubishi Chemical's Ion Exchange Resins) column (90 x 150 mm), on which acylated anthocyanins were absorbed. The column was thoroughly washed with H_2O (2 L) and eluted 5% AcOH-MeOH (500 mL) to recover the anthocyanins. After concentration, the eluates were separated and purified with paper chromatography using BAW. The separated pigments were further purified with preparative HPLC. Preparative HPLC was performed on a Waters C18 (19 ϕ x 150 mm) column at 40 ^oC with a flow rate of 4 mL/min^{-1} and monitoring at 530 nm. The solvent used was as follows: a linear gradient elution for 18 min from 55 to 80 % solvent B in solvent A. Finally, four new anthocyanins were obtained as follows: pigments **1** (*ca*.5 mg), **2** (*ca* 5 mg), **3** (*ca*.7 mg), and **4** (*ca*. 7 mg).

Pigment **1**, cyanidin 3-[2-(2-(*trans*-feruloyl)xylosyl)-6-(4-(glucosyl)-*trans*-*p*-coumaroyl)-glucoside]-5-glucoside; UV-VIS (in 0.1% HCl-MeOH): λmax 532, (318), (298), 279 nm, E_{acyl}/E_{max} (%) = 95, E_{440}/E_{max} = 13, $AlCl_3$ shift +; TLC R_f-values BAW 0.29, BuHCl 0.00, 1% HCl 0.32, AHW 0.68; HPLC: R_t(min) 26.1.

Pigment **2**, cyanidin 3-[2-(xylosyl)-6-(*trans*-feruroyl)glucoside]-5-glucoside; UV-VIS (in 0.1% HCl-MeOH): λmax 530, 322, (296), 280 nm, E_{acyl}/E_{max} (%) = 75, E_{440}/E_{max} = 14, $AlCl_3$ shift +; TLC R_f-values BAW 0.30, BuHCl 0.03, 1% HCl 0.19, AHW 0.53; HPLC: R_t(min) 31.4.

Pigment **3**, cyanidin 3-[2-(2-(*trans*-caffeoyl)xylosyl)-6-(*trans*-feruloyl)glucoside]-5-glucoside or cyanidin 3-[2-(2-(*trans*-feruloyl)xylosyl)-6-(*trans*-caffeoyl)glucoside]-5-glucoside; UV-VIS (in 0.1% HCl-MeOH): λmax 531, 327, (296), 281 nm, E_{acyl}/E_{max} (%) = 109, E_{440}/E_{max} = 13, $AlCl_3$ shift +; TLC R_f-values BAW 0.36, BuHCl 0.05, 1% HCl 0.20, AHW 0.57; HPLC: R_t(min) 32.5.

Pigment **4**, cyanidin 3-[2-(2-(*trans*-feruloyl)xylosyl)-6-(*trans*-feruloyl)glucoside]-5-glucoside; UV-VIS (in 0.1% HCl-MeOH): λmax 531, 326, (295), 281 nm, E_{acyl}/E_{max} (%) = 112, E_{440}/E_{max} = 12, $AlCl_3$ shift +; TLC R_f-values BAW 0.41, BuHCl 0.08, 1% HCl 0.26, AHW 0.67; HPLC: R_t(min) 33.8.

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.

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