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ACYLATED CYANIDIN 3-SAMBUBIOSIDE-5-GLUCOSIDES IN *MATTHIOLA INCANA*

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Key Word Index—*Matthiola incana*; Cruciferae; purple–violet flowers; acylated cyanidin 3-sambubioside-5-glucoside; malonic acid; sinapic acid; ferulic acid; caffeic acid; *p*-coumaric acid.

Abstract—Four acylated cyanidin 3-sambubioside-5-glucosides were isolated from purple–violet flowers of *Matthiola incana* and their structures were determined by chemical and spectroscopic methods. Three acylated anthocyanins were cyanidin 3-*O*-(6-*O*-acyl-2-*O*-(2-*O*-sinapyl-β-D-xylopyranosyl)-β-D-glucopyranosides)-5-*O*-(6-*O*-malonyl-β-D-glucopyranosides), in which the acyl group is *p*-coumaryl, caffeyl or ferulyl, respectively. The remaining pigment is free from malonic acid and was identified as cyanidin 3-*O*-(6-*O*-*trans*-ferulyl-2-*O*-(2-*O*-*trans*-sinapyl-β-D-xylopyranosyl)-β-D-glucopyranoside)-5-*O*-(β-D-glucopyranoside). Analysis of the anthocyanin constituents in 16purple–violet cultivars revealed that they contained the above triacylated anthocyanins in variable amounts as main pigments. An aromatic pair of pigments containing sinapic and ferulic acids are considered to produce an important intramolecular effect, making bluish colours in these flowers.

INTRODUCTION

Matthiola incana is a popular ornamental plant with white, pale yellow, pink, red, red–purple, purple or violet flowers. The occurrence of several acylated 3-glycosides and 3, 5-diglycosides of pelargonidin and cyanidin were observed in the flowers of *M. incana* by Seyffert in 1960 [1]. Two main anthocyanins of red or red–purple flowers were identified as pelargonidin 3-glucoside and pelargonidin 3-ferulyl *p*-coumaryl sambubioside-5-glucoside, matthiolanin, by Harborne in 1964 [2, 3]. Recently Teusch *et al.* (1987) reported the presence of cyanidin 3-glucoside, 3-((caffeyl) glucoside), 3-((*p*-coumaryl) glucoside), 3-((caffeyl) sambubioside) and 3-((*p*-coumaryl) sambubioside) in some lines of this plant [4]. However, up to now, there have been no detailed reports on the purple–violet flowers of this plant [5, 6]. As a part of our continuing work on flower colour variation owing to acylated anthocyanins [7–13], we examined purple or purple–violet flowers of *M. incana*. Four novel acylated anthocyanins were isolated as purple–violet pigments. We report the occurrence and the structure determination of these anthocyanins.

RESULTS AND DISCUSSION

In a survey of 16 purple–violet cultivars of *M. incana* by HPLC analysis, seven major anthocyanin peaks were observed in the purple–violet flowers of these cultivars (Table 1). Among these peaks, four major anthocyanins were isolated and identified as **1** (frequency 26.5–63.4%), **2** (12.7 – 33.6%), **3** (– 18.5) and **4** (– 12.2%). These anthocyanins were isolated from the flowers of these cultivars with MAW (methanol–acetic acid–water, 9:1:10) solvent and purified using Diaion HP-20 column C, PC and HPLC. R_f values, R_t (min) and spectral data are shown in Table 2. These four anthocyanins yielded only one pigment (**5**) as their deacyl anthocyanins by alkaline hydrolysis with NaOH under N_2. As acyl moieties of these pigments (**1**–**4**), two kinds of hydroxycinnamic acids were produced among four hydroxycinnamic acids from each acylated anthocyanin by hydrolysis: **1** (sinapic acid and ferulic acid), **2** (sinapic acid and *p*-coumaric acid), **3** (sinapic acid and caffeic acid) and **4** (sinapic acid and ferulic acid). In addition, pigments **1**–**3** yielded malonic acid as the third acyl group.

Deacylanthocyanin (**5**). R_f values, R_t and spectral data of **5** are shown in Table 2. On acid hydrolysis **5** gave cyanidin, glucose and xylose. Partial acid hydrolysis gave rise to 3- and 5-glucoside, 3-sambubioside and 3, 5-diglucoside. Sambubiose was obtained by H_2O_2 degradation of **5** (Table 3). The structure of **5** is cyanidin 3-sambubioside-5-glucoside from these results. This structure was confirmed by FAB-mass, 1D and 2D ^{1}H NMR spectral measurements (Tables 2 and 4). The FAB-mass spectral measurement gave $[M]^+$ at 743 m/z (calcd for $C_{32}H_{39}O_{20}$ 743. 203). ^{1}H NMR and ^{1}H–^{1}H COSY spectra also indicated that **5** is cyanidin 3-*O*-(2-*O*-(β-D-xylopyranosyl)-β-D-glucopyranoside)-5-*O*-β-D-glucopyranoside (Table 4).

Table 1. Distribution of anthocyanins in the purple–violet flower extracts of *Matthiola incana*

Cultivar	Floral colour*	Anthocyanin (as %)‡							
		b/a†	A	3	B	4	2	1	C
Wase Seien	P76A	−0.89	—	6.6	+	5.7	25.5	53.6	+
Awanami	P76B	−0.68	+	7.1	+	+	19.4	59.5	—
Kaze no Mai	P76B	−0.86	—	+	+	+	18.1	71.7	—
Suzuka no Kaori	P76A	−0.84	—	5.8	+	6.4	21.6	57.7	+
Bōda Lavender	P76A	−0.80	—	5.1	+	6.9	17.6	50.7	—
Kurokawa Purple	P-V81A	−0.73	+	5.4	+	6.6	33.8	32.5	+
Murasaki Doruse	P-V81A	−0.74	+	+	+	6.7	—	34.8	12.1
Shihō	P-V82A	−0.77	+	5.5	+	7.2	37.8	33.2	+
Aki no Murasaki	P-V82A	−0.73	—	+	+	10.9	38.5	25.0	+
Sosei	V84A	−0.87	—	+	+	+	18.7	62.4	+
Suzuka no Sola	V84A	−0.92	—	5.2	+	5.6	27.6	56.0	—
Spray Lavender	V84C	−0.88	—	+	+	5.9	19.4	61.7	—
Okinami	V84A	−0.80	—	6.0	+	+	27.5	56.3	—
Seikai	V84A	−0.88	+	5.6	+	7.2	25.6	46.5	+
Seiha	V84C	−0.77	+	+	+	+	43.6	42.4	—

— Not detected, + under 5%.

*RHS Colour Chart.

† Hunter values (hue).

‡ Anthocyanin no. and HPLC condition were the same as Table 2. Unidentified anthocyanins (R_t min): A (14.3), B (19.0), C (22.2).

Per cent of total absorbance of all detected anthocyanins at 530 nm in HPLC analysis.

Table 2. Chromatographic and spectral properties of anthocyanins from flowers of *Matthiola incana*

Anthocyanin	R_f values (×100)				Spectral data in 0.1% HCl–MeOH			R_t (min)	FAB-MS $[M]^+$
	BAW	BuHCl	1%HCl	AHW	λ_{max} (nm)	E_{acyl}/E_{max} (%)	$AlCl_3$		
1	40	34	30	68	528, 326, 281	115	+	21.5	1211
2	41	38	29	67	529, 317, 281	115	+	20.7	1181
3	36	29	27	61	531, 321, 281	132	+	16.3	1197
4	43	32	23	58	531, 325, 281	120	+	19.6	1125
Deacyl (5)	4	6	34	52	527, —, 277	—	+	5.1	743

For key to abbreviations, see Experimental.

The FAB-mass spectrum of *Matthiola* anthocyanin **1** indicated an $[M]^+$ 1211 *m/z*. Its high resolution-mass spectrometry gave $C_{56}H_{59}O_{30}$, 1211.3103 (calcd for 1211.3092), which was composed of cyanidin with two molecules of glucose and one each of xylose, sinapic acid, ferulic acid and malonic acid. In order to determine the structure, the ^1H and ^{13}C NMR, and ^1H–^1H, and ^1H–^{13}C COSY spectra of **1** were measured at 400 MHz with the solvent (CF_3CO_2D–DMSO-d_6, 1:9). The proton signals of the sugar moiety were observed in the region of δ3.18–5.65 (Table 4). The signals of three anomeric protons appeared at δ5.65 (1H, *d*, J = 7.7 Hz, GlcA) and δ5.10 (2H, *d*, J = 7.3 Hz, Glc B and Xyl), and the assigned sugar protons had coupling constants J = *ca* 7.7–11.1 Hz. Therefore, these two glucoses and xylose must be β-D-glucopyranoses and β-D-xylopyranose. The characteristic five protons shifted in the lower magnetic field were assigned to two methylenes ($-CH_2-$) of glucose A (δ4.41 and 4.15) and glucose B (δ4.36 and 3.86), and also H-2 of xylose (δ4.46). Therefore, it revealed that the two OH-6 of Glc A and B, and the OH-2 of xylose were acylated by three acid residues (malonic, sinapic and ferulic acids). Also, a proton (δ4.01, *t*, J = 8.6 Hz) being shifted to lower field was easily correlated to the proton H-1 of Glc A. Thus, this proton was assigned to be the H-2 of Glc A. These results suggested that xylose is attached to OH-2 of Glc A through a glucosidic bond, and formed a sambubiose unit. In order to determine the linkages and positions between sugars and acids in pigment **1**, the negative NOE difference (DIFNOE) spectra were measured [11, 14]. Observed negative DIFNOE between H-1 of Glc A and cyanidin H-4 indicated that Glc A is attached to OH-3 of cyanidin through a glucosidic bond (Fig. 1). Glc B was determined to be glucosylated at OH-5 of cyanidin, because of the presence of NOEs between H-6 and/or H-8 of cyanidin and H-1 of Glc B. By the

Table 3. Chromatographic data of acyl groups and H_2O_2 oxidation products of Matthiola anthocyanins

Sample	TLC ($R_f \times 100$)				Colour by	
	BAW	APW	BEW	15% HOAc	UV	AHP
Ferulylsinapylsambubiose (1, 4)*	14	10	14	84	BB	Br
p-Coumarylsinapylsambubiose (2)*	16	11	16	86	BB	Br
Caffeylsinapylsambubiose (3)*	13	9	12	82	BB	Br
Sambubiose (5)*	16	15	14	80	ND	Br
Ferulic acid	87	91	89	46, 65	BB	ND
p-Coumaric acid	91	96	93	49, 74	Va	ND
Caffeic acid	79	86	80	36, 53	BB	ND
Sinapic acid	83	89	82	40, 56	BB	ND
Xylose	31	39	28	88	ND	RB
Glucose	23	25	21	86	ND	Br

BAW(*n*-BuOH–HOAc–H_2O, 4:1:2), APW (EtOAc–pyridine–H_2O, 15:7:5), BEW (*n*-BuOH–EtOH–H_2O, 4:1:2.2), 15% HOAc (15% acetic acid).

Va = Violet absorption, BB = bright blue, Br = brown, RB = reddish brown, ND = not detected.

* 1 Obtained from pigment 1, 2 obtained from pigment 2, 3 obtained from pigment 3, 4 obtained from pigment 4 and 5 obtained from deacyl *Matthiola* anthocyanins.

irradiation of cyanidin H-4, NOEs were observed at H-α, H-β, H-2 and H-6 of ferulic acid as well as protons of Glc A, but would not be detected at proton signals of sinapic acid. Furthermore, the presence of NOEs between H-1 of Glc A and H-6 of ferulic acid was observed (as shown in Fig. 1). Ferulic acid was attached to the H-6 of Glc A through an ester bond. By H_2O_2 degradation, ferulylsinapylsambubiose was obtained (Table 3). Ferulyl *p*-coumarylsambubiose was previously obtained from matthiolanin isolated from red culativars of this plant by Harborne [2]. Therefore, sinapic acid was attached to the OH-2 of xylose, and malonic acid was attached to the OH-6 of Glc B. The proton signals of cyanidin, ferulic acid and sinapic acid were assigned by ^1H–^1H COSY and DIFNOE spectra as shown in Table 4. Those of malonic acid were assigned by ^1H–^{13}C COSY spectra (Table 4). The ^{13}C signals of **1** were correlated with proton signals of **1**, respectively, and assigned by ^1H–^{13}C COSY and COLOC spectra (as shown in the Experimental). Thus, pigment **1** is cyanidin 3-*O*-(6-*O*-(*trans*-feruloyl)-2-*O*-(2-*O*-(*trans*-sinapoyl)-β-D-xylopyranosyl)-β-D-glucopyranoside)-5-*O*-(6-*O*-(malonyl)-β-D-glucopyranoside)-5-*O*-(6-*O*-(malonyl)-β-D-glucopyranoside), which is new [5, 6].

From analysis of the ^1H NMR spectrum and FAB mass spectra, *Matthiola* anthocyanin **2** consisted of one molecule of cyanidin, two molecules of glucose, one molecule of xylose, and three molecules of acids (malonic, *p*-coumaric and sinapic acids). By high resolution mass measurement the $[M]^+$ and its elemental composition were determined to be 1181.3009 (calcd for 1181.2985) and $C_{55}H_{57}O_{29}$. These results indicated that the structure of **2** was very similar to that of **1**, but differed from **1** by *p*-coumaric acid instead of ferulic acid as acid moiety. Thus the same analytic process of **2** was performed as for **1**. The ^1H NMR and ^1H–^1H COSY spectra showed identical proton signals except for the protons of the *p*-coumaroyl moiety (Table 4). The proton signals of cyanidin, *p*-coumaric acid, sinapic acid and malonic acid moieties were mainly assigned by analysis of the ^1H–^1H COSY spectrum of **2** (Table 4). The linkages of sugars and acids were determined by means of ^1H–^1H COSY and DIFNOE spectral methods as described for **1** (Fig. 1). Also, the presence of *p*-coumarylsinapylsambubiose was confirmed by H_2O_2 degradation at the OH-3 of cyanidin (Table 3). Consequently, **2** was cyanidin 3-*O*-(6-*O*-(*trans*-p-coumaryl)-2-*O*-(2-*O*-(*trans*-sinapoyl)-β-D-xylopyranosyl)-β-D-glucopyranoside)-5-*O*-(6-*O*-(malonyl)-β-D-glucopyranoside). Cyanidin 3-*p*-coumarylsambubioside-5-glucoside has already been isolated from *Sambucus canadensis* by Johansen *et al.* [15], but this pigment is a new one.

For *Matthiola* anthocyanin **3** the FAB-mass spectrum gave the $[M]^+$ at 1197 m/z, in good agreement with the mass calculated for $C_{55}H_{57}O_{30}$ (1197.2933). Analysis of the ^1H NMR and ^1H–^1H COSY spectra indicated that the structure was composed of one molecule of cyanidin, two molecules of glucose, one molecule of xylose and one molecule each of three acids (malonic, caffeic and sinapic acids) (Table 4). The structure of **3** was similar to **1**, with caffeic acid instead of ferulic. The ^1H NMR and ^1H–^1H COSY spectra of **3** were compared with those of **1** and **2**, and the proton signals of **3** were assigned easily as shown in Table 4. The linkages and positions of sugars and acids were also confirmed by H_2O_2 degradation as described for **1**. Therefore, **3** is cyanidin 3-*O*-(6-*O*-(*trans*-caffeyl)-2-*O*-(2-*O* (*trans*-sinapyl)-β-D-xylopyranosyl)-β-D-glucopyranoside)-5-*O*-(6-*O*-(malonyl)-β-D-glucopyranoside), which is new.

For *Matthiola* anthocyanin **4**, the FAB-mass spectrum gave the $[M]^+$ at 1125 m/z, in good agreement with the mass calculated for $C_{53}H_{57}O_{27}$ (1125.3086). The ^1H NMR spectrum showed the presence of one molecule of cyanidin, two molecules of glucose, one molecule of xylose and two molecules of acids (ferulic and sinapic acids). Thus **4** was similar to **1** except that malonic acid was absent from **1**. By analysis of ^1H NMR and ^1H–^1H COSY

Table 4. ^{1}H NMR spectral data of *Matthiola* purple anthocyanins (400 MHz in 10% TFA-*d*–DMSO-d_6 at 25°)

H	1	2	3	4	5
Cyanidin*					
4	8.71 *s*	8.67 *s*	8.68 *s*	8.71 *s*	8.68 *s*
6	6.91 *br s*†	6.95 *br s*†	6.95 *br s*†	6.92 *br s*†	7.01 *br s*†
8	6.94 *br s*	6.96 *br s*	6.99 *br s*	7.03 *br s*	7.13 *br s*
2'	7.97 *br s*	7.96 *br* 2	7.97 *d*(2.0)	7.96 *d*(2.0)	8.10 *d*(2.0)
5'	7.04 *d*(8.2)	7 02 *d*(8.1)	7.03 *d*(8.6)	7.03 *d*(8.6)	7.08 *d*(8.6)
6'	8.43 *br d*(8.2)	8.45 *br d*(8.1)	8.44 *dd*(8.6, 2.0)	8.44 *dd*(8.6, 2.0)	8.40 *dd*(8.6, 2.0)
Glucose A*					
1	5.65†	5.66†	5.65†	5.66†	5.66†
2	4.01	3.99	3.99	4.00	3.96
3	3.60	3.59	3.58	3.59	3.69
4	3.39	3.37	3.40	3.46	3.40
5	3.94	3.93	3.89	3.87	3.52
6a	4.15	4.21	4.23	4.24	3.58
6b	4.41	4.32	4.31	4.32	3.78
Glucose B*					
1	5.10†	5.13†	5.12†	5.04†	5.14†
2	3.51	3.50	3.48	3.48	3.51
3	3.39	3.37	3.40	3.22	2.95
4	3.18	3.23	3.22	3.08	3.30
5	3.70	3.75	3.75	3.37	3.40
6a	3.86	4.01	4.05	3.49	3.59
6b	4.36	4.35	4.39	3.72	3.75
Xylose*					
1	5.10	5.10	5.10	5.10	4.72
2	4.64	4.63	4.62	4.63	3.00
3	3.39	3.40	3.38	3.40	3.15
4	3.18	3.17	3.16	3.19	3.26
5a, b	3.86	3.89	3.85	3.87	3.40
Hydroxycinnamic acid*					
(I)†					
2	}	}	}	}	
6	} 6.96 *s*	} 6.99 *s*	} 6.99 *s*	} 6.98 *s*	
3	—	—	—	—	
5	—	—	—	—	
α	6.51 *d*(15.8)	6.52 *d*(15.8)	6.52 *d*(15.8)	6.52 *d*(16.2)	
β	7.54 *d*(15.8)	7.55 *d*(15.8)	7.55 *d*(15.8)	7.55 *d*(16.2)	
OMe-3	}	}	}	}	
OMe-5	} 3.79 *s*	} 3.81 *s*	} 3.80 *s*	} 3.80 *s*	
(II)					
2	6.91 *d*(2.0)	}	6.84 *br s*	6.95 *br s*	
6	6.86 *dd*(8.1, 2.0)	} 7.25 *d*(8.5)	6.83 *br d*(8.6)	6.97 *br d*(7.7)	
3	—	}	—	—	
5	6.69 *d*(8.1)	} 6.67 *d*(8.5)	6.67 *d*(8.6)	6.76 *d*(7.7)	
α	6.23 *d*(15.8)	6.19 *d*(15.8)	6.52 *d*(15.8)	6.26 *d*(15.8)	
β	7.32 *d*(15.8)	7.30 *d*(15.8)	7.27 *d*(15.8)	7.33 *d*(15.8)	
OMe-3	3.68 *s*	—	—	3.73 *s*	
Malonic acid					
$-CH_2-$	3.32	3.30	3.32	—	

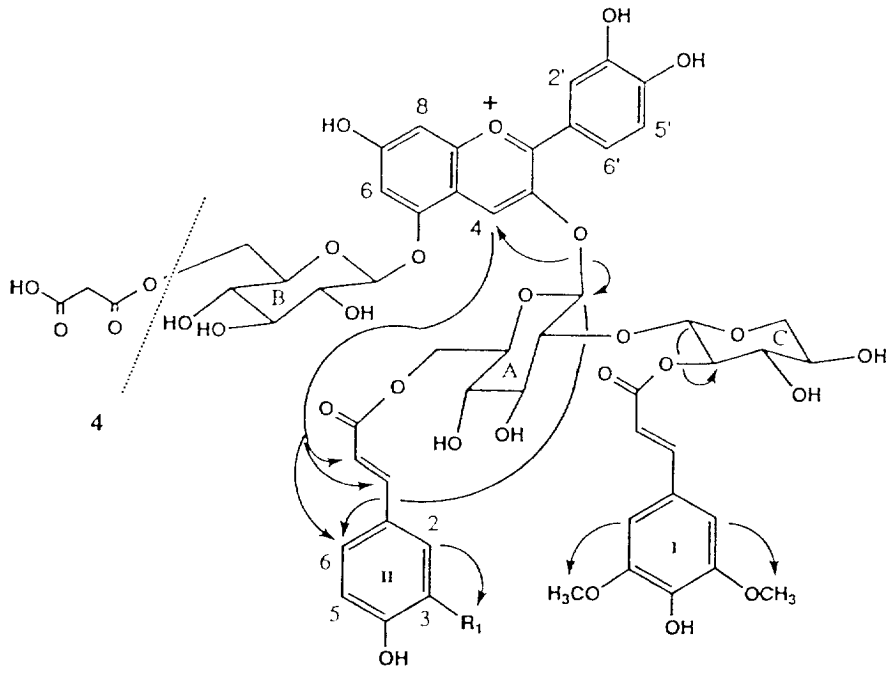
* Assigned by ^{1}H–^{1}H COSY

† Assigned by DIFNOE.

Coupling constants (*J* in Hz) in parentheses.

Hydroxycinnamic acid: I, **1**, **2**, **3** and **4** = sinapic acid; II, **1** and **4** = ferulic acid, **2** = *p*-coumaric acid, **3** = caffeic acid.

spectra, it was revealed that the methylene protons (δ3.49, H-6a and 3.72, H-6b) of Glc **B** were shifted to higher magnetic field than those of pigment **1** (δ3.86 and 4.36). Furthermore, other proton signals of **4** were assigned by the same process as **1** and were in good agreement with those of **1** without the malonic moiety (Table 4). Therefore, **4** was cyanidin 3-*O*-(6-*O*-(*trans*-feruloyl)-2-*O*-(2-*O*-(*trans*-sinapyl)-β-D-xylopyranosyl)-β-



1 - 3

Fig. 1. Acylated cyanidin glycosides **1**–**4** isolated from the purple–violet flowers of *Matthiola incana*.
1 R_1 = O, 2 R_1 = H, 3 R_1 = OH, 4 R_1 = O
Observed NOEs are indicated by arrows.

D-glucopyranoside)-5-*O*-(*β*-D-glucopyranoside), which is new.

This study reveals that *Matthiola* purple–violet anthocyanins are based on cyanidin 3-sambubioside-5-glucoside and, furthermore, contain two molecules of characteristic hydroxycinnamic acids in addition to one molecule of malonic acid. In particular, two pairs, sinapic and ferulic acids, and sinapic and *p*-coumaric acids were important to make the bluish colour in these pigments.

EXPERIMENTAL

Plant material. Purple–violet flowers of 15 cultivars of *Matthiola incana*; 'Awanami', 'Aki no Murasaki', 'Bōda Lavender', 'Kurokawa Purple', 'Murasaki Doruse', 'Seikai', 'Shihō', 'Sosei', 'Spray Lavender', 'Wase Seien', 'Suzukano Kaori' and 'Suzuka no Sora' collected from plants at the farm of Mr H. Kurokawa, Tateyama, Chiba, Japan in spring 1993; and 'Okinami' and 'Kaze no Mai' collected from plants at the farm of Mr S. Bōda, Tateyama, Chiba, Japan in spring 1992. Fresh flowers of these cultivars were dried overnight at 40°.

Isolation of anthocyanins. Mixed dried purple–violet flowers (200 g) were extracted with MAW (MeOH–HOAc–H_2O, 9:1:10) (10 l) at room temp. for 6 hr. The extract was concd to 500 ml. The reddish purple concd extract was purified by Diaion HP-20 CC, PC and HPLC as described previously [9, 11]. Solvents used were BAW (*n*-BuOH–HOAc–H_2O, 4:1:5), 5% HOAc–MeOH, 3% and 15% HOAc for CC and PC. Prep. HPLC was run on Waters C_{18} (19ϕ × 150 mm) column at 40° with a flow rate 4 ml min^{-1} monitoring at 530 mm for anthocyanins. Solvent systems used were; linear gradient elution for 30 min from 40 to 85% solvent B (1.5% H_3PO_4, 20% HOAc, 25% MeCN in H_2O) in solvent A (1.5% H_3PO_4 in H_2O). The pigment frs were evapd *in vacuo* to dryness. The evapn residues were dissolved in a small vol. of 5% HOAc–MeOH followed by addition of excess Et_2O and then drying to give 5 pigment powders; pigment **1**, *ca* 32 mg; **2**, *ca* 15 mg; **3**, *ca* 10 mg; **4**, *ca* 6 mg.

Distribution of anthocyanins in the purple–violet flowers of 16 cultivars. Dried petal (*ca* 0.02 g) of each cultivar were extracted with 50% MeOH–solvent containing 1.5% H_3PO_4. TLC and HPLC of these extracts were carried out [11, 16]. TLC solvents used were BAW, BuH (*n*-BuOH–2 M HCl, 1:1), 1% HCl and AHW (HOAc–HCl–H_2O, 15:3:82). The quantitative analysis of anthocyanins was performed by HPLC, Waters C_{18} (4.6ϕ × 250 mm) column at 40° with a flow rate of 1 ml min^{-1} monitoring at 530 nm. Solvent systems used were; a linear gradient elution for 30 min from 40 to 85% solvent B in solvent A.

Standard analysis. Pigment identifications were carried out by standard procedures involving H_2O_2 oxidation, deacylation with alkaline and hydrolysis with acid [3, 9, 11]. The NMR spectra were measured in

CF_3CO_2D–DMSO–d_6 (1:9) and recorded at 400 for ^1H NMR and 100.6 MHz for ^{13}C NMR spectra. Chemical shifts were given in δ values relative to TMS. The mass spectra were performed to obtain the positive mode with a magic bullet matrix and the negative mode with a glycerol matrix.

^{13}C NMR of pigment **1**. Cyanidin: 162.9 (C-2), 147.0 (C-3), 133.2 (C-4), 155.9 (C-5), 105.4 (C-6), 168.0 (C-7), 96.7 (C-8), 155.7 (C-9), 112.2 (C-10), 120.2 (C-1'), 118.1 (C-2'), 149.9 (C-3'), 155.5 (C-4'), 117.5 (C-5'), 129.1 (C-6'), sinapic acid; 126.2 (C-1), 107.0 (C-2), 148.9 (C-3), 139.1 (C-4), 148.9 (C-5), 107.0 (C-6), 116.3 (C-α), 145.8 (C-β), 166.6 (CO), 56.7 (Me-2); ferulic acid: 125.5 (1), 112.3 (2), 148.3 (3), 145.0 (4), 116.1 (5), 123.2 (6), 114.7 (α), 145.8 (β), 167.2 (CO), 56.1 (– Me); malonic acid: 74.5 (CH_2), 167.3 (CO), 168.5 (CO); Glc A: 98.8 (C-1), 78.1 (C-2), 77.5 (C-3), 71.5 (C-4), 74.3 (C-5), 63.7 (C-6); Glc B: 102.5 (C-1), 73.7 (C-2), 75.3 (C-3), 70.5 (C-4), 74.9 (C-5), 64.6 (C-6); xylose: 101.8 (C-1), 74.5 (C-2), 76.3 (C-3), 70.2 (C-4), 66.8 (C-5).

Acylated sugars. Four pigments (**1**–**4**) were dissolved in H_2O and oxidized with H_2O_2 [2]. The resulting solutions were chromatographed in BAW and bands containing the acylated sugars were cut out, eluted and purified by TLC. *p*-Coumarylferulylsambubiose was obtained from matthiolanin isolated from the red flowers of *M. incana* as the authentic sample [2].

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