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ACYLATED ANTHOCYANINS FROM THE BLUE-PURPLE FLOWERS OF *TRITELEIA BRIDGESII*

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Key Word Index—*Triteleia bridgesii*; Liliaceae; blue-purple flower colour; acylated anthocyanins; delphinidin and cyanidin 3-*p*-coumaroylglucoside-5-glucosides; *p*-coumaric acid; malonic acid; glucosyl-*p*-coumaric acid.

Abstract—Five acylated anthocyanins were isolated from the blue-purple flowers of *Triteleia bridgesii*. Three main components were determined to be 3-*trans*- and 3-*cis*-*p*-coumaroylglucoside-5-malonylglucosides of delphinidin, and delphinidin 3-*O*-[6-*O*-(*trans*-4-*O*-(β-D-glucopyranosyl)-*p*-coumaroyl)-β-D-glucopyranoside]-5-*O*-[6-*O*-(malonyl)-β-D-glucopyranoside]. Delphinidin 3-*trans*-*p*-coumaroylglucoside-5-glucoside and a triacylated cyanidin 3,5-diglucoside were also present as minor pigments.

INTRODUCTION

Triteleia bridgesii is distributed in North and South America and is a popular ornamental with blue-purple flowers. As no information on the chemistry of the anthocyanins is available [1–4], we investigated the structure determination of anthocyanins in the blue-purple flowers of this plant and found two new acylated anthocyanins along with three known compounds. In this paper we report the structural elucidation of these anthocyanins.

RESULTS AND DISCUSSION

Twenty anthocyanin peaks were observed in the extract of blue-purple flowers of *Triteleia bridgesii* by HPLC, and five (**1**–**5**) of them were isolated as amorphous powder. Their relative concentrations, performed by procedures similar to those reported previously [5, 6], were 4.8% (**1**), 35.9% (**2**), 15.4% (**3**), 18.3% (**4**), and 6.6% (**5**) respectively.

The chromatographic and spectral properties of these anthocyanins are shown in Table 1. On acid hydrolysis we found delphinidin (**1**–**4**), cyanidin (**5**), glucose and malonic acid (**2**–**5**) in addition to *p*-coumaric acid (**1**–**5**). On alkaline deacylation **1**–**4** gave delphinidin 3,5-diglucoside and **5** gave cyanidin 3,5-diglucoside. The pigments **4** and **5** gave the same glucosyl-*p*-coumaric acid [7].

Pigments **1**, **2**, *and* **3**

The FAB mass spectra of **1**, **2**, and **3** gave their molecular ions at 773, 859 and 859 *m/z*, respectively, in good agreement with the masses calculated for $C_{36}H_{37}O_{19}$, $C_{39}H_{40}O_{21}$ and $C_{39}H_{40}O_{21}$. Analysis of the ^{1}H NMR spectra of **1**, **2** and **3** revealed the presence of one mol of delphinidin, two of glucose and one of *p*-coumaric acid respectively, and the additional one mol of malonic acid in **2** and **3**. The aromatic proton signals of delphinidin and *p*-coumaric acid of **1**–**3** were assigned by ^{1}H-^{1}H COSY and negative NOE difference (DIFNOE) spectra (Table 2). Four olefinic proton signals of two *p*-coumaric acids of **1** and **2** had large coupling constants (J = 15.6 and 15.9 Hz), indicating both *p*-coumaric acids to have the *trans* configurations. On the other hand, the two olefinic proton signals of **3** had smaller coupling constants (J = 12.8 Hz) than those of **1** and **2** showing that *p*-coumaric acid in **3** has the *cis* configuration. The signals of the glucose moieties of **1**–**3** were observed in the region of δ 5.61–3.23 (Table 2). The signals of six anomeric protons appeared at δ 5.61 (*d*, J = 7.9 Hz, Glc A of **1** and **3**), δ 5.59 (*d*, J = 7.9 Hz, Glc A of **2**), δ 5.06 (*d*, J = 7 Hz, Glc B of **1**), δ 5.16 (*d*, J = 7.6, Glc B of **2**) and δ 5.18 (*d*, J = 7, Glc B of **3**), and the assigned sugar protons had coupling constants J = 7–12 Hz.

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Table 1. Chromatographic and spectral properties of anthocyanins from the blue-purple flowers of *Triteleia bridgesii*

Anthocyanins	R_f values (×100)*				R_t* (min)	Spectral data in 0.1% HCl–MeOH			FAB-MS $[M]^+$
	BAW	BuH	1% HCl	HAc–HCl		λ_{max} (nm)	E_{acyl}/E_{max} (%)	E_{440}/E_{max} (%)	
1	47	38	5	29	26.8	280, 309, 542	76	11	773
2	46	42	5	34	28.7	280, 309, 541	62	10	859
3	45	41	15	46	22.5	278, 305, 545	51	10	859
4	28	14	11	47	20.5	280, 297, 541	81	10	1021
5	53	52	10	47	31.1	282, 313, 528	77	12	1151

* See Experimental for solvent abbreviations.

Therefore, these six glucoses must be in the β-D-glucopyranose form.

The characteristic methylene proton peaks of glucoses were exhibited at 4.02 and 4.32 in Glc A of pigment **1**, δ 4.31 and 4.41 in Glc A of **2**, δ 4.04 and 4.38 in Glc B of **2**, δ 4.35 and 4.49 in Glc A of **3**, and δ 4.30 and 4.49 in Glc B of **3** in their ^{1}H NMR spectra. These results suggested that Glc A of **1** was bonded with *p*-coumaric acid at OH-6, and Glc A and B of **2** and **3** were acylated with both malonic and *p*-coumaric acids at each OH-6 of those sugars, respectively. Furthermore, by the irradiations of H-1 of Glc A of **1–3** NOEs were observed at H-α, β, -2 and -6 of *p*-coumaric acid, respectively, indicating that those *p*-coumaric acids were attached to the 6-OHs of Glc A through an ester bond in **1–3** (see Fig. 1). The H_2O_2 oxidation of **1–3** gave *p*-coumaroylglucose [5, 7]. Thus, the structures of **1–3** are determined to be 3-*O*-(6-*O*-(*trans*-*p*-coumaroyl)-β-D-glucopyranoside)-5-*O*-(β-D-glucopyranoside), 3-*O*-(6-*O*-(*trans*-*p*-coumaroyl)-β-D-glucopyranoside)-5-*O*-(6-*O*-malonyl-β-D-glucopyranoside) and 3-*O*-(6-*O*-(*cis*-*p*-coumaroyl)-β-D-glucopyranoside)-5-*O*-(6-*O*-malonyl-β-D-glucopyranoside) of delphinidin, respectively.

Pigment **4**

The FAB mass spectrum of **4** gave its molecular ion at 1021 *m/z* in good agreement with the mass calculated for $C_{45}H_{49}O_{27}$. Analysis of the ^{1}H NMR spectra including ^{1}H-^{1}H COSY spectrum indicated the presence of same molecular composition as for **2** with an additional glucose (Glc C) as shown in Table 2. The proton chemical shifts of **4** were assigned by a process similar to that described for **1** and **2**, and were identical with those of **2** except those of Glc C of **4** (Table 2). By alkaline hydrolysis of **4**, 4-glucosyl-*p*-coumaric acid was detected indicating that the 3-OH of delphinidin is bound with glucosyl-*p*-coumaroylglucoside. Therefore, the structure of **4** is delphinidin 3-*O*-(6-*O*-(*trans*-4-*O*-(β-D-glucopyranosyl)-*p*-coumaroyl)-β-D-glucopyranoside)-5-*O*-(6-*O*-malonyl-β-D-glucopyranoside), which is a new anthocyanin in plants [3, 4].

Pigment **5**

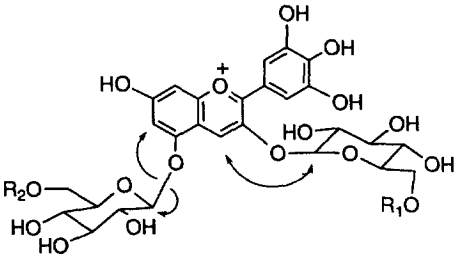
The FAB mass spectrum of **5** gave a molecular ion at 1151 *m/z*, in good agreement with the mass calculated for $C_{54}H_{55}O_{28}$, which was composed of cyanidin with three molecules of glucose, two molecules of *p*-coumaric acid and one molecule of malonic acid as shown in Table 2. The detailed chemical structure was elucidated by ^{1}H NMR including ^{1}H-^{1}H COSY spectral methods. Six proton signals of cyanidin moiety were observed as shown in Table 2. In *p*-coumaric acid moieties, two pairs of doublet signals (δ 6.27, 7.38 and δ 6.46, 7.58) with large coupling constants (J = 15.9 Hz) indicated the presence of *trans* olefinic protons of *p*-coumaric acid (I, II). Also two pairs of four aromatic proton signals of these *p*-coumaric acids and the proton signals of three glucose moieties (Glc A, B, C) were assigned as shown in Table 2. These three glucose units were determined as being β-D-glucopyranose by their coupling constants (J = 7.5–10.4 Hz). Six characteristic proton signals corresponding to three methylene groups of Glc A, B, C were shifted to the lower magnetic field at δ 4.21, 4.43 (Glc A), δ 4.04, 4.38 (Glc B) and δ 4.14, 4.32 (Glc C), indicating that all the three 6-OH of these glucose units (A, B, C) are acylated with two *p*-coumaric acid (I, II) and one of malonic acid, respectively. For a routine procedure, DIFNOE spectral measurement for **5** was attempted to determine the linkages and positions of these glucoses and acyl units; however, complete structure determination could not be achieved because of a small amount of **5**. Therefore, the structure of **5** was tentatively assigned to be cyanidin 3-*p*-coumaroyl-glucosyl-*p*-coumaroyl-glucoside-5-malonylglucoside. To date, there are only four papers describing the occurrence of di- or poly-acyl anthocyanins in Liliaceae, *Scilla pensylvanica* [8], *Hyacinthus orientalis* [9, 10] and *Hyacinthoides nonscipta* [8], in which only diacyl anthocyanins are present. In this study a triacyl anthocyanin is first found in the Liliaceae. On the conformation of hydroxycinnamic acid units in the acylated anthocyanins the *trans*-hydroxycinnamic acids are commonly present in the flowers [4, 11], whereas *cis*-isomers are rare. So far there are three reports on the isolation of the pigments

Table 2. ^{1}H NMR data of *Triteleia* anthocyanins (DCl–DMSO-d_6, 1:20 at 30°)

H	1	2	3	4	5
Delphinidin					
4	8.80 *s*	8.78 *s*	8.63 *s*	8.80 *s*	8.83 *s*
6	7.03 *br s*	7.01 *d* (1.5)	6.90 *d* (1.5)	7.01 *br s*	7.03 *d* (2.0)
8	7.12 *br s*	7.11 *d* (1.5)	6.97 *d* (1.5)	7.14 *br s*	7.16 *d* (2.0)
2′ or 2′, 6′	7.81 *s*	7.79 *s*	7.75 *s*	7.81 *s*	8.06 *d* (2.4)
5′					7.10 *d* (8.0)
6′					8.23 *dd* (2.4, 8.9)
p-Coumaric acid*					
(I)					
2, 6	7.42 *d* (7.9)	7.33 *d* (8.5)	7.30 *d* (8.5)	7.46 *d* (8.5)	7.35 *d* (8.6)
3, 5	6.79 *d* (7.9)	6.73 *d* (8.5)	6.52 *d* (8.5)	6.97 *d* (8.5)	6.72 *d* (8.6)
α	6.30 *d* (15.6)	6.27 *d* (15.9)	5.70 *d* (12.8)	6.38 *d* (15.9)	6.27 *d* (15.9)
β	7.39 *d* (15.6)	7.39 *d* (15.9)	6.48 *d* (12.8)	7.43 *d* (15.9)	7.38 *d* (15.9)
(II)					
2, 6					7.58 *d* (8.9)
3, 5					6.72 *d* (8.9)
α					6.46 *d* (15.9)
β					7.58 *d* (15.9)
Glucose*					
(A)					
1	5.61	5.59	5.61	5.63	5.58
2	3.69	3.70	3.70	3.69	3.60
3	3.50	3.53	3.49	3.49	3.47
4	3.23	3.39	3.28	3.37	3.38
5	3.38	4.00	3.95	4.00	3.98
6a	4.02	4.31	4.35	4.32	4.21
6b	4.32	4.41	4.49	4.36	4.43
(B)					
1	5.06	5.16	5.18	5.15	5.16
2	3.50	3.51	3.55	3.52	3.52
3	3.50	3.41	3.39	3.40	3.40
4	3.23	3.27	3.26	3.31	3.27
5	3.38	3.78	3.84	3.79	3.69
6a	3.50	4.04	4.30	4.11	4.04
6b	3.76	4.38	4.49	4.34	4.38
(C)					
1				4.96	5.02
2				3.24	3.76
3				}	}
4					3.62–3.17
5				3.53–3.17	
6a					4.14
6b				}	4.32
Malonic acid		3.33 *s*	3.44 *m*	3.68	3.60–3.30

* Assigned by DIFNOE and ^{1}H-^{1}H COSY spectra. Coupling constants (*J* in Hz) in parentheses.

1: delphinidin 3-*trans*-*p*-coumaroylglucoside-5-glucoside, **2**: delphinidin 3-*trans*-*p*-coumaroylglucoside-5-malonylglucoside, **3**: delphinidin 3-*cis*-*p*-coumaroylglucoside-5-malonylglucoside, **4**: delphinidin 3-(glucosyl)-*trans*-*p*-coumaroylglucoside-5-malonylglucoside, **5**: cyanidin 3-*p*-coumaroylglucosyl-*p*-coumaroylglucoside-5-malonylglucoside.



1. R_1 = *trans*-*p*-coumaric acid, R_2 = H
2. R_1 = *trans*-*p*-coumaric acid, R_2 = malonic acid
3. R_1 = *cis*-*p*-coumaric acid, R_2 = malonic acid
4. R_1 = *trans*-4-*O*-(glucosyl)-*p*-coumaric acid, R_2 = malonic acid

Fig. 1. Anthocyanins isolated from the blue-purple flowers of *Triteleia bridgesii*. Observed NOEs are indicated by arrows.

with *cis*-hydroxycinnamic acids in the flower of the Liliaceae [9, 10]. This finding of pigment **3** is the third report of *cis*-*p*-coumaric acid in the Liliaceae.

EXPERIMENTAL

Plant material

The bulbs of *Triteleia bridgesii* Greene were obtained from Takii Nursery Co., Ltd, Kyoto, Japan, and cultivated in the garden of Minami-Kyushu University. Fresh petals were collected and dried at 45°.

Extraction and isolation

The dried petals (80 g) were extracted with 5% HOAc at room temp. overnight. The filtered extract was adsorbed on Diaion HP-20 column, washed with ca 1% HOAc and then eluted with 5% HOAc in 75% MeOH. After concn, the eluate was fractionated with Sephadex LH-20 CC using HOAc–MeOH–H_2O (1:6:12). The red-purple frs were further purified with PC (*n*-BuOH–HOAc–H_2O, 4:1:2 and 15% HOAc) and HPLC. Prep. HPLC was performed on a Hitachi 6200 system, using an Inertsil ODS-2 (20 × 250 mm) column and HOAc solvent system. Pigment **1**, (4.3 mg), **2** (35.5 mg), **3** (10.6 mg), **4** (3.9 mg) and **5** (3.7 mg) were obtained.

Analysis

Pigment identification were carried out by standard procedures involving H_2O_2 oxidation, alkaline deacylation, demalonylation and hydrolysis with acid [1, 11, 12]. TLC was carried out on microcrystalline cellulose (Avicel SF, Funakoshi) using BAW (*n*-BuOH–HOAc–H_2O, 4:1:5), BuH (*n*-BuOH–2N HCl, 1:1), 1% HCl, HAc–HCl (HOAc–HCl–H_2O, 15:3:82) for anthocyanins; HOAc–HCl–H_2O (30:3:10) and HCO_2H–HCl–H_2O (5:2:3) for anthocyanidins; BAW, *i*-PrOH–*n*-BuOH–H_2O (7:1:2) and PhOH–H_2O (4:1) for sugars; and BAW, EtOAc–HOAc–H_2O (3:1:1) and EtOH–H_2O–NH_4OH (16:3:1) for acids. HPLC was run on an Inertsil ODS-2 column (4.6ϕ × 250 nm) at 35°, with a flow rate of 0.8 ml min^{-1} and monitoring at 520 nm. Solvent systems were as follows: linear gradient elution for 40 min from 25–85% solvent B (1.5% H_3PO_4, 20% HOAc, 25% MeCN) in solvent A (1.5% H_3PO_4). NMR spectra were measured in DCl–DMSO-d_6 (1:20) and recorded at 500 MHz for ^1H NMR. Chemical shifts are in δ values relative to TMS. Mass spectra were recorded to obtain the positive mode with a magic bullet and negative mode with a glycerol matrix.

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