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The first isolation of *C*-glycosylanthocyanin from the flowers of *Tricyrtis formosana*

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Abstract—A novel *C*-glycosylanthocyanin was isolated from the flowers of *Tricyrtis formosana* cultivar Fujimusume, and its structure was elucidated to be 8-*C*-β-D-glucopyranosylcyanidin 3-*O*-(6-*O*-malonyl)-β-D-glucopyranoside by chemical and spectroscopic methods.

More than four hundred anthocyanidin *O*-glycosides have been isolated as flower color pigments from higher plants. To the best of our knowledge, however, no report for the occurrence of *C*-glycosylanthocyanin in plants has appeared to date,[1,2] although the presence of a number of *C*-glycosylflavonoids have been widely recognized. Since anthocyanins are biosynthetically derived from chalcone as well as flavonoids, the occurrence of *C*-glycosylanthocyanin has long been expected from the biosynthetic point of view. In this paper, we would like to report the first isolation and structure determination of natural *C*-glycosylanthocyanin from the purple flowers of *Tricyrtis formosana* cultivar Fujimusume (Liliaceae), that is a common ornamental plant in Japan.

Dried purple perianth lobes (ca. 80 g) with dark spots of *T. formosana* cultivar Fujimusume were immersed overnight in the solvent (MeOH/H_2O/HOAc, 4:5:1) at room temperature. The extract was concentrated and purified by successive chromatography on Diaion HP-20 column chromatography (CC) using MeOH, HOAc and H_2O as eluents, thin-layer chromatography (BAW; BuOH/OHAc/H_2O, 4:1:2) and preparative HPLC as described previously.[3] A novel anthocyanin **1** (a dark-red powder, ca. 10 mg) was obtained as one of the major anthocyanins.[4]

Acid hydrolysis of **1** with 2N HCl for 1 h afforded an anthocyanidin **2** as the aglycone[5] together with glucose and with malonic acid.[6,7] Fast atom bombardment (FAB) MS spectrum of **1** showed its $[M]^+$ peak at m/z 697, corresponding to the molecular formula $C_{30}H_{33}O_{19}$, which is presumed to be composed of one molecule each of *C*-hexsosylcyanidin, glucose and malonic acid. The elemental components of **1** were further confirmed by HR-FABMS. The structure including the stereochemistry of **1** was determined on the basis of ^1H and ^{13}C NMR spectroscopy. The ^1H NMR spectrum of **1** exhibited two anomeric protons at 4.83 ppm (d, $J=9.8$ Hz, Glc A) and 5.40 ppm (d, $J=7.6$ Hz, Glc B), respectively, in addition to twelve other proton signals of two sugars. Since all the vicinal coupling constants of the two sugars were observed to be 7.6–9.8 Hz, both sugars must take β-glucopyranoside form.[8] A typical H-4 signal of the anthocyanidin nucleus was observed at 8.9 ppm as a singlet,[8,9] and four other signals corresponding to the 6-, 2′-, 5′- and 6′-H were assigned by 2D COSY spectral analysis, as shown in Table 1, supporting the cyanidin nucleus as the basic aglycone structure.[8] However, the H-8 signal which usually appeared in the region of 6.0–8.0 ppm was missing in the ^1H NMR spectrum.[8] Furthermore, the down-field shift (ca. 9 ppm) of the C-8 signal appeared at 105.1 ppm and the up-field shift (ca. 27 ppm) of the C-1″ signal of Glc A appeared at 73.7 ppm in comparison with those of cyanidin and its *O*-glycosides[9] were observed in its ^{13}C NMR spectrum suggesting the presence of the carbon–carbon bond between C-8 of anthocyanidin and C-1″ of Glc A. Thus, Glc A was

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determined to be bonded to C-8 of anthocyanidin, directly. This linkage was further confirmed by the observation of the HMBC correlations between H-1″ of Glc A and C-8, 8a and 7 of cyanidin as depicted in Figure 1.

Table 1. ^{1}H and ^{13}C NMR data for **1** and **2** (500 and 125.78 MHz, DMSO-d_6–CF_3COOD, TMS as an internal standard)

	8-*C*-glycosylanthocyanin **1**		8-*C*-glycosylcyanidin **2**	
	^{13}C (δ/ppm)	^{1}H (δ/ppm)	^{13}C (δ/ppm)	^{1}H (δ/ppm)
Cyanidin				
2	161.7		160.2	
3	143.8		145.1	
4	135.3	8.90 s	133.5	8.73 s
4a	112.6		111.9	
5	156.9		154.1	
6	101.9	6.90 s	100.1	6.94 s
7	167.2		169.1	
8	105.1		109.9	
8a	154.4		152.4	
1'	120.0		120.8	
2'	118.6	8.21 d (2.0)	118.8	8.30 br s
3'	146.3		145.9	
4'	154.5		153.5	
5'	117.0	7.08 d (8.5)	117.1	7.10 d (8.9)
6'	127.2	8.27 dd (2.0, 8.5)	125.9	8.23 br d (8.9)
C-Glucose (Glc A)				
1''	73.7	4.83 d (9.8)	73.4	4.83 d (9.5)
2''	70.8	3.84 t (9.2)	70.9	3.86 t (9.5)
3''	70.7	3.56 t (9.2)	70.6	3.86 t (9.8)
4''	82.4	3.39 m	82.4	3.55 t (9.0)
5''	78.6	3.38 m	77.0	3.39 t (9.0)
6''	61.5	3.73 m 3.85 m	61.5	3.49 m 3.71 br d (11.0)
O-Glucose (Glc B)				
1'''	102.3	5.40 d (7.6)		
2'''	73.1	3.61 t (8.5)		
3'''	76.3	3.44 m		
4'''	70.0	3.30 t (8.6)		
5'''	74.6	3.84 m		
6'''	64.6	4.18 dd (10.7, 7.3) 4.52 br d (10.7)		
Malonic acid				
CO	167.3			
CH_2	41.5	3.39 - 3.48 m		
CO	168.3			

Coupling constants are indicated in parentheses.

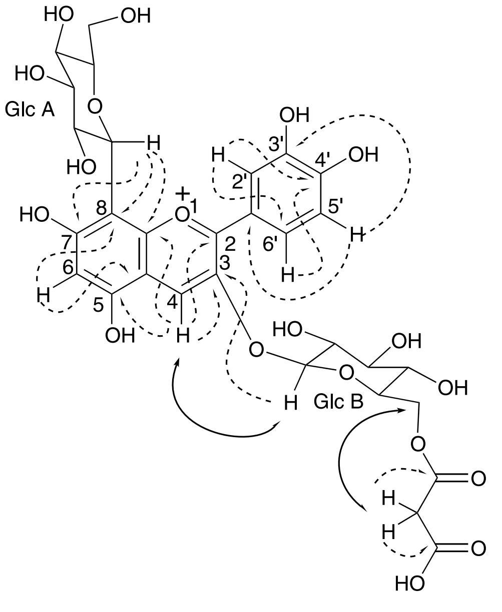

Figure 1. Structure of 8-*C*-glucosylcyanidin 3-*O*-(6-*O*-malonyl)glucoside **1** from *Tricyrtis formosana*. Observed major HMBC correlations are indicated by dashed arrows. Observed NOE's are indicated by arrows.

By irradiation at H-4 of the cyanidin nucleus and at H-1‴ of Glc B, intensive NOE's were observed between H-1‴ of Glc B and H-4 of cyanidin, respectively, supporting that Glc B was attached to the oxygen at the 3-position of cyanidin through the *O*-glycosidic bond. As two characteristic protons being shifted to the lower magnetic field at 4.18 and 4.52 ppm were assigned to C-6‴ methylene protons of Glc B, the malonyl group must be esterified with 6-OH of Glc B.[8,9] Both linkages were further confirmed by NOE and HMBC correlations as shown in Figure 1. Regarding the stereochemistry of the sugar moiety, it is well established that D-glucose is usually incorporated into anthocyanins based on the biosynthetic pathway. Therefore, the structure of **1** was determined to be 8-*C*-β-D-glucopyranosylcyanidin 3-*O*-(6-*O*-malonyl)-β-D-glucopyranoside, unambiguously, which is a new anthocyanin pigment in a plant, and this is the first report for the isolation of a *C*-glycosylated anthocyanin.

The anthocyanidin **2** obtained by acid hydrolysis of **1** exhibited the $[M]^+$ peak at m/z 449, which supported its structure to be 8-*C*-glucosylcyanidin, $C_{21}H_{21}O_{11}$, by the measurement of FABMS spectroscopy (Fig. 2). This structure was confirmed by analysis of ^{1}H and ^{13}C NMR spectra as shown in Table 1.

The isolation of **2** as the aglycone provided further confirmation of the structure of **1** as depicted in Figure 1.

This aglycone **2** exhibited its λ_{max} (0.1% HCl/MeOH) at 541 nm, in UV–vis spectrum, which is shifted into the longer wavelength region than that of cyanidin (at 535 nm), and is almost similar to that of delphinidin (at 546 nm). Therefore, 8-*C*-glycosylation may be considered as another new bluing factor in producing flower color, such as the hydroxylation, acylation, metal complexation, and so on.[10]

In conclusion, we have disclosed the isolation and structure determination of a new pigment from *Tricyrtis formosana* cultivar Fujimusume (Liliaceae). The isolation of *C*-glycosylated anthocyanin has not been reported to date, and this will be the first report for the occurrence of *C*-glycosylanthocyanin in nature.

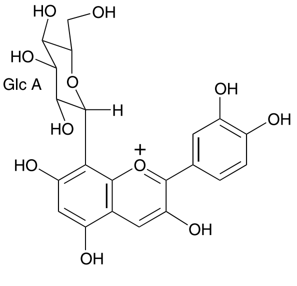

Figure 2. Structure of 8-*C*-glucosylcyanidin **2**.

Study on the biosynthesis of *C*-glycosylanthocyanin will be a future subject in this field of chemistry.

Acknowledgements

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4. 8-*C*-Glucosylcyanidin 3-*O*-(6-*O*-malonyl)glucoside: UV–vis λ_{max} (0.1% HCl–MeOH) 286, 531 nm; TLC R_f values BAW 0.22, BuHCl (BuOH/2 M–HCl, 1:1) 0.06, 1% HCl 0.35, AHW (HOAc/HCl/H_2O, 15:3:85) 0.60; HPLC t_R (min) 11.9. HRFABMS: Calcd for $C_{30}H_{33}O_{19}$ requires 697.1616. Found 697.1581.
5. 8-*C*-Glucosylcyanidin: UV–vis λ_{max} (0.1% HCl–MeOH) 276, 541 nm; TLC R_f values BAW 0.25, BuHCl 0.11, 1% HCl 0.04, AHW 0.13, Forestal (HOAc/HCl/H_2O, 30:3:10) 0.43; HPLC t_R (min) 11.1. HPLC was run on Inertsil ODS-2 column (4.6×250 mm for analysis and 20×250 mm for prep.) at 40°C, with a flow rate of 1.0 ml/min and 4 ml/min, and monitoring at 530 nm. Solvent system for analysis was as follows; 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). HRFABMS: Calcd for $C_{21}H_{21}O_{11}$ requires 449.1093. Found 449.1076.
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