PERGAMON

Phytochemistry 55 (2000) 937–939

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Cyclic malyl anthocyanins in *Dianthus caryophyllus*

Masayoshi Nakayama [a], Masaji Koshioka [a,*], Hiroyuki Yoshida [b], Yukiko Kan [c], Yuko Fukui [d], Akira Koike [e,1], Masa-atsu Yamaguchi [e]

[a] *Department of Floriculture, National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, 360 Kusawa, Ano, Mie 514-2392, Japan*

[b] *Applied Plant Research Laboratory, Japan Tobacco Inc., 1900 Idei, Oyama, Tochigi 323-0808, Japan*

[c] *Suntory Institute for Bioorganic Research, 1-1-1 Wakayamadai, Shimamoto, Osaka 618-8503, Japan*

[d] *Institute for Fundamental Research, Suntory Ltd., 1-1-1 Wakayamadai, Shimamoto, Osaka 618-8503, Japan*

[e] *Minami-Kyushu University, Takanabe, Miyazaki 884-0003, Japan*

Received 19 January 2000; received in revised form 5 June 2000

Abstract

3, 5-Di-*O*-(β-glucopyranosyl) pelargonidin 6″-*O*-4, 6‴-*O*-l-cyclic malate and a previously reported cyanidin equivalent, 3, 5-di-*O*-(β-glucopyranosyl) cyanidin 6″ -*O*-4, 6‴-*O*-l-cyclic malate were identified from petals of deep pink and red-purple flower cultivars of *Dianthus caryophyllus,* respectively.

Keywords: Dianthus caryophyllus; Caryophyllaceae; 3, 5-Di-*O*-(β-glucopyranosyl) pelargonidin 6″-*O*-4, 6‴-*O*-1-cyclic malate; 3, 5-Di-*O*-(β-glucopyranosyl) cyanidin 6″-*O*-4, 6‴-*O*-1-cyclic malate; Cyclic malyl anthocyanins

1. Introduction

While variously acylated anthocyanins have been identified as natural compounds (Harborne and Grayer, 1988; Strack and Wray, 1994), anthocyanins acylated with malic acid have been detected only in *Dianthus* plants, *D. barbatus* (Yamaguchi, 1986), *D. caryophyllus* (Terahara et al., 1986; Terahara and Yamaguchi, 1986; Bloor, 1998), *D. chinensis* (Yamaguchi, 1986; Nadanasabapathy and Sayeed, 1991) and *D. deltoides* (Terahara et al., 1986). Two malyl monoester anthocyanins, 3-*O*-(β-glucopyranosyl) pelargonidin 6″-*O*-malate and 3-*O*-(β-glucopyranosyl) cyanidin 6″-*O*-malate, have been identified from the red and purplish-red *D. caryophyllus* (Terahara et al., 1986; Terahara and Yamaguchi, 1986). Furthermore, 3, 5-di-*O*-(β-glucopyranosyl) cyanidin (6″-, 6‴-malyl diester) was recently isolated from *D. caryophyllus* as a macrocyclic anthocyanin (Bloor, 1998).

We detected two anthocyanins in the deep pink and red-purple flowers of *D. caryophyllus,* and identified them as the new anthocyanin 3, 5-di-*O*-(β-glucopyranosyl) pelargonidin (6″-, 6‴-malyl diester) (**1**), and the previously isolated cyanidin equivalent (**2**) (Bloor, 1998) whose orientation of the malyl group had not been determined. In this paper, we determined the orientation of the malyl groups and clarified the complete structures of anthocyanins **1** and **2**.

2. Results and discussion

Anthocyanins **1** and **2** were extracted from *D. caryophyllus* petals and purified successively by XAD-7 column, paper chromatography, Sephadex LH-20 chromatography and ODS-HPLC. The purified anthocyanin **1** was analyzed by ^{1}H and ^{13}C NMR spectroscopy (Table 1) and FABMS. The proton signals in the region of δ 6.91–8.83 were assigned to pelargonidin and the proton signals in the region of δ 3.36–5.62 except δ 4.42 were assigned to two hexoses, based on the chemical shifts and the integration data in the ^{1}H NMR spectrum, and the DQF-COSY and TOCSY data. The two anomeric protons at δ 5.36 (H-1‴) and δ 5.62 (H-1″) had large

* Corresponding author. Tel.: +81-59-268-4663; fax: +81-59-268-1339.

E-mail address: masaji@nivot.affrc.go.jp (M. Koshioka).

[1] Present address: Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-0862, Japan.

0031-9422/00/$ - see front matter
PII: S0031-9422(00)00263-6

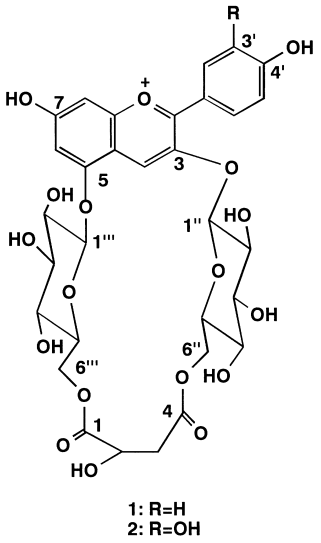

Fig. 1. Structures of cyclic 5–3 malyl anthocyanins **1** and **2** isolated from *Dianthus caryophyllus*.

coupling constants (*d*, $J = 7.6$ Hz) and the coupling constants of the other protons in each sugar ring were in the range $J = 8$–10 Hz, indicating that both hexoses were β-glucopyranosides. The other proton signals were assigned to the methylene protons (δ 2.51 and δ 2.80) and the methine proton (δ 4.42) of malate.

The HMBC spectrum showed cross-peaks between the H-1″ of glucose (δ 5.62) and the C-3 of the pelargonidin (δ 146.0) and between the H-1‴ of glucose (δ 5.36) and the C-5 of the pelargonidin (δ 156.6). This indicated that each glucose was attached to the OH-3 and the OH-5 of the pelargonidin through the glucosidic bonds, respectively. The methylene proton signals of the H-6″ of the 3-*O*-glucoside and the H-6‴ of the 5-*O*-glucoside were shifted to lower fields at δ 4.32 and δ 4.31, and δ 5.03 and δ 4.27, respectively. The HR-FABMS spectrum of anthocyanin **1** gave a molecular ion at *m/z:* 693.1696, corresponding to $C_{31}H_{33}O_8$ (calcd. 693.1655). These suggested that two hydroxyl groups at the C-6″ position (in the 3-*O*-glucoside) and the C-6‴ position (in the 5-*O*-glucoside) should be bound with two carboxyl groups of a malic acid through esterifing dehydration. The HMBC spectrum showed cross-peaks between the H_2-6″ of the 3-*O*-glucose and the carbonyl C-4 of the malate (δ 172.0), and between the H_2-6‴ of the 5-*O*-glucose and the carbonyl C-1 of the malate (δ 174.8). The assignments of the ^{13}C NMR spectra of the malate are referred to spectrum No. 6831C of the Sadtler standard spectra (Philadelphia: Sadtler Research Laboratories, 1979). Therefore, the structure of anthocyanin **1** was determined to be 3, 5-di-*O*-(β-glucopyranosyl) pelargonidin 6″-*O*-4, 6‴-*O*-1-cyclic malate (Fig. 1).

The HR-FABMS analysis of anthocyanin **2** gave a molecular ion at *m/z* 709.1650, corresponding to $C_{31}H_{33}O_{19}$ (calcd. 709.1623). This suggested that the anthocyanin **2** might be a cyanidin equivalent of anthocyanin **1.** The ^1H and ^{13}C NMR spectra were analyzed in a similar fashion to that of anthocyanin **1** (Table 1). Finally, the anthocyanin **2** was identified as 3, 5-di-*O*-(β-glucopyranosyl) cyanidin 6″-*O*-4, 6‴-*O*-1-cyclic malate (Fig. 1). The UV-Vis λ_{max} of these anthocyanins, recorded by on-line HPLC, were at 265 and 505 nm (**1**, R_t 25.2 min), and 275 and 520 nm (**2**, R_t 22.9 min).

The occurrence of anthocyanin **2** in red or mauve flower cultivars of *D. caryophyllus* has been already reported by Bloor (1998) although the orientation of malyl group had not been clarified. He suggested that the linkage between the 6‴-*O* of the 5-*O*-glucoside and the carbonyl C-1 of the malate of anthocyanin **2** is

Table 1
NMR spectral data of **1** and **2** in MeOH-d_4-TFA-*d* (9:1)

	δ_H(ppm)		δ_C(ppm)	
	1	**2**	**1**	**2**
Anthocyanidins				
2			163.9	164.8
3			146.0	146.4
4	8.83 (*s*)[a]	8.77 (*s*)	133.3	133.0
5			156.6	156.9
6	6.91 (*d*, 2)	6.89 (*d*, 2)	105.0	105.2
7			170.0	170.1
8	7.04 (*d*, 2)	6.99 (*d*, 2)	97.8	98.0
9			157.4	157.5*[b]
10			113.2	114.0
1′			121.0	118.6†
2′	8.54 (*d*, 9)	7.96 (*d*, 2)	136.5	119.3
3′	7.02 (*d*, 9)		118.6	148.4
4′			167.9	157.6*
5′	7.02 (*d*, 9)	6.96 (*d*, 9)	118.6	118.5†
6′	8.54 (*d*, 9)	8.18 (*dd*, 2, 9)	136.5	129.7
3-o-*Glucose*				
1″	5.62 (*d*, 7.6)	5.63 (*d*, 7.7)	101.2	101.4
2″	3.70 (*t*, 10)	3.75 (*t*, 9)	75.1	75.4
3″	3.60 (*t*, 9)	3.63 (*t*, 9)	78.8	79.0
4″	3.36 (*t*, 9)	3.37 (*t*, 9)	71.9	72.3
5″	3.93 (*ddd*, 3, 9, 10)	3.93 (*ddd*, 3, 9, 10)	76.7	77.0
6″	4.31 (*dd*, 10, 12)	4.30 (*dd*, 10, 12)	66.1	66.4
	4.32 (*dd*, 3, 12)	4.32 (*dd*, 3, 12)		
5-o-*Glucose*				
1‴	5.36 (*d*, 7.6)	5.37 (*d*, 7.6)	101.8	102.0
2‴	3.69 (*t*, 10)	3.69 (*dd*, 8, 10)	74.9	75.3
3‴	3.60 (*t*, 9)	3.61 (*t*, 9)	77.1	77.4
4‴	3.78 (*t*, 9)	3.78 (*t*, 9)	70.3	70.6
5‴	3.76 (*dd*, 3, 9)	3.75 (*brt*, 9)	76.7	77.0
6‴	4.27 (*d*, 12)	4.27 (*d*, 12)	62.4	62.7
	5.03 (*dd*, 3, 12)	5.05 (*dd*, 3, 12)		
Malyl				
1			174.8	175.2
2	4.42 (*dd*, 3, 11)	4.42 (*dd*,3,11)	70.0	70.4
3	2.80 (*dd*, 11, 17)	2.80 (*dd*,11,17)	41.7	42.0
	2.50 (*dd*, 3, 17)	2.50 (*dd*,3,17)		
4			172.0	172.3

[a] Multiplicity and *J* (Hz) in parentheses.
[b] *†Values may be exchangeable between the same symbols.

readily cleaved by hydrolysis. Anthocyanin **1** also showed instability, which might be due to the same hydrolysis cleavage.

We propose to call these compounds cyclic malyl anthocyanins, so that anthocyanins **1** and **2** could be simply described as cyclic malyl pelargonidin and cyclic malyl cyanidin. While malyl anthocyanins seem to be widely distributed in *Dianthus* spp. (Yamaguchi, 1986), malyl anthocyanins have not been detected in outside the *Dianthus* genus. Cyclic malyl anthocyanins seem to be peculiar to *Dianthus* spp. as well.

3. Experimental

3.1. Plant materials

Petals were obtained from plants grown either in the greenhouses at Japan Tobacco Inc., Oyama, Tochigi, Japan or National Research Institute of Vegetables, Ornamental Plants and Tea, Ano, Mie, Japan.

3.2. General

The ^{1}H and ^{13}C NMR spectra consisting of DQF-COSY, TOCSY, HSQC-editing and HMBC methods were analyzed in MeOH-d_4-TFA-*d* (9:1) on a Bruker DMX-500 system. The FABMS spectra were analyzed by JEOL-DX-300/DA-5000 system using a glycerol matrix. UV-Vis absorption spectra were recorded by on-line HPLC analysis using the following conditions: column, Inertsil ODS-2 (250×4.6 min, i.d., GL Science); solvent, linear gradient of 20–100% of HCO_2H–H_2O–MeCN–phosphoric acid (40:107:50:3) in H_2O-phosphoric acid (197:3) for 40 min; flow rate, 0.6 ml/min; column temperature, 40°C; monitoring, UV-Vis 240–600 nm using a photodiode array detector.

3.3. Isolation of anthocyanins

The anthocyanins **1** and **2** were respectively extracted from petals of *D. caryophyllus* cv. "Symphony Rose" (deep pink, 300 g dry weight) and cv. "Perfume" (red-purple, 380 g dry weight) with HCO_2H–H_2O (10:90, vol/vol). Each extract was diluted twice and applied to an Amberlite XAD-7 column which was eluted with EtOH–HCO_2H (95:5). Preparative paper chromatography was successively performed on Advantec-Toyo No. 526 filter paper developing with *n*-BuOH–HCO_2H–H_2O (4:1:2) and HCO_2H–H_2O (1:9). Anthocyanin fractions were subjected to Sephadex LH-20 chromatography by eluting with EtOH–HCO_2H–H_2O (10:1:9). The constituents of interest were purified further by ODS–HPLC (250×20 mm i.d., Wakosil-II 5C18 AR Preparative column) at 35°C eluted with either 23% of HCO_2H–H_2O–MeCN (10:50:40) in HCO_2H–H_2O (10:90) at a flow rate of 7.5 ml/min (**1**) or 22% of HCO_2H-H_2O–MeCN (10:50:40) in HCO_2H–H_2O (10:90) at a flow rate of 9 ml/min (**2**). The anthocyanins were eluted at R_t 27.4 min (**1**) and R_t 23.2 min (**2**) with several impurities. The impurities were excluded by the second ODS–HPLC using the same column with a linear-gradient solvent system of 22–55% of HCO_2H–H_2O–MeCN–TFA (20:99:80:1) in HCO_2H–H_2O–TFA (20:179:1) for 22 min at 35°C at a flow rate of 9 ml/min. The anthocyanins were isolated at R_t 17.6 min (**1**, 50 mg) and R_t 11.6 min (**2**, 60 mg).

Acknowledgements

We are grateful to Dr. L. Milne (Royal Society of Chemistry) and Dr. L. N. Mander (Australian National University) for advice about the nomenclature of the compounds and to Mr. T. Fujita (Suntory Institute for Bioorganic Research) for technical assistance. This work was supported by grant-aid from the Ministry of Agriculture, Forestry and Fisheries of Japan.

References

Bloor, S.J., 1998. A macrocyclic anthocyanin from red/mauve carnation flowers. Phytochemistry 49, 225–228.

Harborne, J.B., Grayer, R.J., 1988. The anthocyanins. In: Harborne, J.B. (Ed.), The Flavonoids: Advance in Research since 1980. Chapman and Hall, London, pp. 1–20.

Nadanasabapathy, S., Sayeed, S.A., 1991. Identification and characterization of malylated anthocyanins in *Dianthus chinensis* L. Philippine Journal of Science 120, 391–396.

Strack, D., Wray, V., 1994. The anthocyanins. In: Harborne, J.B. (Ed.), The Flavonoids: Advance in Research since 1986. Chapman and Hall, London, pp. 1–22.

Terahara, N., Takeda, K., Harborne, J.B., Self, R., Yamaguchi, M., 1986. Anthocyanins acylated with malic acid in *Dianthus caryophyllus* and *D. deltoides*. Phytochemistry 25, 1715–1717.

Terahara, N., Yamaguchi, M., 1986. ^{1}H NMR spectral analysis of the malylated anthocyanins from *Dianthus*. Phytochemistry 25, 2906–2907.

Yamaguchi, M., 1986. Studies of acylated anthocyanins (II): Distribution and identification of malyl anthocyanins in *Dianthus* spp. Abstract of Spring Meeting of Japanese Society for Horticultural Science (in Japanese), 304–305.