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# MALONYLATED ANTHOCYANINS IN *VERBENA* FLOWERS

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**Key Word Index**—*Verbena hybrida*; Verbenaceae; malonic acid; delphinidin 3-*O*-(6″-*O*-malonyl-β-D-glucopyranoside); pelargonidin 3-*O*-(6″-*O*-malonyl-β-D-glucopyranoside); flower colour.

**Abstract**—The major anthocyanin in the maroon flowers of *Verbena hybrida* cv. 'Splendor' was identified as delphinidin 3-*O*-(6″-*O*-malonyl-β-D-glucopyranoside) by chromatographic and spectral methods. The major anthocyanin of scarlet cultivar 'Blaze' was determined to be pelargonidin 3-*O*-(6″-*O*-malonyl-β-D-glucopyranoside).

## INTRODUCTION

Scott-Moncrieff and Sturgess (1940) first reported delphinidin 3-glucoside in the maroon cultivar of *Verbena hybrida*, and delphinidin 3,5-diglucoside in the purple form [1]. From genetical studies of flower colours on *Verbena hybrida*, Beale *et al.* suggested that acylated anthocyanins occurred in the flowers of this plant [2]. Later, Harborne confirmed the presence of pelargonidin, cyanidin and delphinidin in a number of cultivars of *Verbena hybrida* [3]. Moreover, he showed the occurrence of the zwitterionic anthocyanins in this plant during an anthocyanin survey by electrophoresis [4], but the structures of the anthocyanins were not determined.

We, therefore reinvestigated the anthocyanin components in *Verbena* cultivars. This paper deals with the structural elucidation of malonylated pelargonidin 3-glucoside and delphinidin 3-glucoside from two cultivars of *Verbena hybrida*.

## RESULTS AND DISCUSSION

The major anthocyanin **1** was extracted from the maroon flowers of *Verbena hybrida* cv. 'Splendor', and the major anthocyanin **2** from the scarlet flowers of cultivar 'Blaze'. Both **1** and **2** extracted were purified by repeated prep. paper chromatography, and followed by Sephadex LH-20 gel column chromatography.

On acid hydrolysis, **1** and **2** gave delphinidin and pelargonidin, respectively, as the aglycones [cf-3]. In addition, TLC data of these hydrolysates in various solvents indicated that the sugar was glucose. UV-VIS spectral features of **1** and **2** were closely similar to those of delphinidin 3-glucoside (Dp 3-G) and pelargonidin 3-glucoside (Pg 3-G), respectively (Table 1), although, their $R_f$ values of TLC were clearly different. On electrophoretic analysis, pigments **1** and **2** moved towards the anode in buffer pH 4.4, whereas Dp 3-G and Pg 3-G remained at the origin. Furthermore, when both pigments **1** and **2** were dissolved in the HCl–MeOH solvent, they were gradually converted into Dp 3-G and Pg 3-G, respectively. IR spectra of **1** and **2** showed' characteristic carbonyl absorption bands (**1**; 1710 $cm^{-1}$ and **2**; 1730 $cm^{-1}$, respectively), indicating that **1** and **2** are acylated with aliphatic dicarboxylic acids [5, 6]. On alkaline hydrolysis of these pigments gave only malonic acid.

The deacylated anthocyanins of **1** and **2** were identified as Dp 3-G and Pg 3-G, respectively (Table 1). Partial acid hydrolysis of **1** and **2** suggested that they were composed of. anthocyanidin 3-glucoside and malonic acid at the molar ratio of 1:1. This information was confirmed by the

Table 1. Chromatographic and spectral properties of *Verbena hybrida* anthocyanins

| | $R_f \times 100$ in | | | | In 0.01% HCl–MeOH | | | |
|---|---|---|---|---|---|---|---|---|
| Pigments | BAW | BHW | 1% HCl | AHW† | $\lambda_{max}^{UV}$ | $\lambda_{max}^{VIS}$ | $E_{320}/E_{max}^{VIS}$ | $AlCl_3$ shift |
| **1*** | 33 | 19 | 03 | 13 | 276 | 544 | 27 | + |
| **2*** | 63 | 48 | 10 | 33 | 272 | 512 | 13 | – |
| Dp 3-G | 25 | 10 | 01 | 06 | 278 | 542 | 26 | + |
| Pg 3-G | 49 | 43 | 05 | 27 | 272 | 512 | 13 | – |

*Pigment **1**, **2** = 3-*O*-(6″-*O*-malonyl-β-D-glucopyranoside) of delphinidin and pelargonidin respectively; Dp 3-G, Pg 3-G = 3-glucoside of delphinidin and pelargonidin.

†TLC on microcrystalline cellulose; BAW = *n*-BuOH–HOAc–$H_2O$ (4:1:5), BHW = *n*-BuOH– 2M HCl (1:1), AHW = HOAc–HCl–$H_2O$ (15:3:82).

FAB-MS. The FAB-MS of each anthocyanin gave the molecular ion $[M]^+$ (as flavylium ion) peak at $m/z$ 551 or 519 with fragment of $[M-86]^+$ and aglycone ion, indicating **1** and **2** to be monomalonylated anthocyanins.

In order to confirm these structures, the 400 MHz proton FT-NMR spectra were measured in 10% $CF_3COOD$ and 90% DMSO-$d_6$. The NMR signals of both pigments clearly indicated the presence of malonyl $-CH_2-$ protons at near $\delta$3.4 ppm; **1** at $\delta$3.37 (*d*, $J=17$ Hz) and 3.40 (*d*, $J=17$ Hz), and **2** at $\delta$3.35 (*d*, $J=16$ Hz) and 3.40 (*d*, $J=16$ Hz) [6]. For the aglycone moiety, the large differences of shifts were observed at H-2', $\delta$8.59 (or $\delta$8.61 [7]) for Pg, 8.04 for Cy [6] and 7.72 for Dp, indicating a consistent effect due to increasing hydroxylation on the B-ring. The signal patterns of sugar protons of both pigments were similar [7]. The characteristic two downfield shift protons at $\delta$4.13, 4.46 ppm in **1** and 4.12, 4.46 in **2** with the geminal coupling ($J=12$ Hz) were assigned to glucose methylene protons, and this fact indicated that the malonyl residue in each pigment was attached to C-6'' of glucose moiety [5–7]. Furthermore, each proton resonated at the lower magnetic field with large coupling constant (**1**; $J=8$ Hz and **2**; $J=8$ Hz), and the sugar protons had also large $J$-values such about 9 Hz. Therefore the glucose was proved to have a $\beta$-D-pyranose form in each pigment.

Hence, the major anthocyanin **1** in the maroon flowers of the cultivar 'Splendor' is delphinidin 3-*O*-(6''-*O*-malonyl-$\beta$-D-glucopyranoside), which is a new malonylated pigment. The major pigment **2** in the scarlet 'Blaze' is pelargonidin 3-*O*-(6''-*O*-malonyl-$\beta$-D-glucopyranoside), which has been already identified by Takeda *et al.* [8]. However, it is the first time that the 'Blaze' pigment structure has been fully determined by FAB-MS and NMR.

## EXPERIMENTAL

*Plant material.* Flower petals of *Verbena hybrida* cv. Splendor and Blaze were collected at the farm of Minami-Kyusyu University and dried at 50° overnight.

*Isolation and purification.* Dried petals (*ca* 100 g) of each cultivar were extracted with EtOH–HOAc–$H_2O$ (10:1:10), and the extract concd to a small vol. at 40°. The concentrate was purified by prep. PC (Toyo No. 526 filter paper) in the solvents 15% HOAc and *n*-BuOH–HOAc–$H_2O$ (6:1:2), and followed by Sephadex LH-20 CC with 15% HOAc in order to separate flavonols and other phenolic components. Then, pigment **1** (*ca* 40 mg) and **2** (*ca* 60 mg) were obtained respectively.

*Standard analysis.* Characterization of pigment **1** and **2** was carried out by Avicel cellulose TLC, paper electrophoresis and UV-VIS spectrometry [4, 8]. Moreover, these pigments were analysed by TLC after the processes of acid hydrolysis, alkaline deacylation, and partial acid hydrolysis.

*IR and FAB-MS.* IR spectra were measured as the KBr discs. Positive FAB-MS spectra were recorded with glycerol as a matrix.

*Delphinidin* 3-O-(6''-O-*malonyl-β-*D-*glucopyranoside*) (**1**). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1710 ( C=O), 1625, 1380, 1060; FAB-MS $m/z$ (rel. int.): 551 $[M]^+$ (24) ($C_{24}H_{23}O_{15}$ requires 551, as flavylium ion), 465 $[M-86]^+$ (4) (loss of malonyl group), 303 [delphinidin]$^+$ (11); $^1$H NMR (400 MHz, 10% $CF_3COOD$ in DMSO-$d_6$): $\delta$ aglycone moiety: 8.77 (1H, *s*, H-4), 7.72 (2H, *s*, H-2' and 6'), 6.86 (1H, *d*, $J=2$ Hz, H-8), 6.72 (1H, *d*, $J=1$ Hz, H-6), glucose moiety: 5.44 (1H, *d*, $J=8$ Hz, H-1''), 4.46 (1H, *dd*, $J=12$, 2 Hz, H-6''a), 4.13 (1H, *dd*, $J=12$, 8 Hz, H-6''b), 3.86 (1H, *ddd*, $J=9$, 8, 2 Hz, H-5''), 3.60 (1H, *dd*, $J=9$, 8 Hz, H-2''), 3.43 (1H, *t*, $J=9$ Hz, H-3''), 3.26 (1H, *t*, $J=9$ Hz, H-4''), malonyl $-CH_2-$ moiety: 3.40 (1H, *d*, $J=17$ Hz), 3.37 (1H, *d*, $J=17$ Hz).

*Pelargonidin* 3-O-(6''-O-*malonyl-β-*D-*glucopyranoside*) (**2**). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1730 ( C=O), 1635, 1330, 1170, 1060; FAB-MS $m/z$ (rel. int.): 519 $[M]^+$ (66) ($C_{24}H_{23}O_{13}$ requires 519, as flavylium ion), 433 $[M-86]^+$ (5) (loss of malonyl group), 271 [pelargonidin]$^+$ (20); $^1$H NMR (400 MHz, 10% $CF_3CO$ OD in DMSO-$d_6$): $\delta$ aglycone moiety: 8.88 (1H, *s*, H-4), 8.59 (2H, *d*, $J=9$ Hz, H-2' and 6'), 7.07 (2H, *d*, $J=9$ Hz, H-3' and 5'), 6.99 (1H, *d*, $J=1$ Hz, H-8), 6.74 (1H, *d*, $J=2$ Hz, H-6), glucose moiety: 5.40 (1H, *d*, $J=8$ Hz, H-1''), 4.46 (1H, *dd*, $J=12$, 2 Hz, H-6'' a), 4.12 (1H, *dd*, $J=12$, 8 Hz, H-6''b), 3.82 (1H, *ddd*, $J=9$, 8, 2 Hz, H-5''), 3.49 (1H, *dd*, $J=9$, 8 Hz, H-2''), 3.42 (1H, *t*, 9 Hz, H-3''), 3.24 (1H, *t*, $J=9$ Hz, H-4''), malonyl $-CH_2-$ moiety: 3.40 (1H, *d*, $J=16$ Hz), 3.35 (1H, *d*, $J=16$ Hz).

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## REFERENCES

1. Scott-Moncrieff, R. and Sturgess, V. C. (1940) *Biochem. J.* **34**, 268.
2. Beale, G. H., Price, J. R. and Scott-Moncrieff, R. (1940) *J. Genet.* **41**, 65.
3. Harborne, J. B. (1976) in *Chemistry and Biochemistry of Plant Pigments* (Goodwin, T. W., ed.), pp. 736–778. Academic Press, London.
4. Harborne, J. B. (1986) *Phytochemistry*, **25**, 1887.
5. Goto, T., Kondo, T., Tamura, H. and Takase, S. (1983) *Tetrahedron Letters* **24**, 4863.
6. Bridle, P., Loeffler, R. S. T., Timberlake, C. F. and Self, R. (1984) *Phytochemistry* **23**, 2968.
7. Terahara, N. and Yamaguchi, M. (1986) *Phytochemistry* **25**, 2906.
8. Takeda, K., Harborne, J. B. and Self, R. (1986) *Phytochemistry* **25**, 1337.