Biochemical Systematics and Ecology 39 (2011) 220–224

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Malonylated anthocyanidin 3,5-diglucosides in the flowers of the genus *Disa* (Orchidaceae)

Fumi Tatsuzawa [a,*], Kazumitsu Miyoshi [b], Tomohisa Yukawa [c], Koich Shinoda [d], Kenjiro Toki [e], Norio Saito [f], Atsushi Shigihara [f], Toshio Honda [f]

[a] *Faculty of Agriculture, Iwate University, Morioka, Iwate 020-8550, Japan*
[b] *Faculty of Bioresource Sciences, Akita Pref. University, Akita 010-0195, Japan*
[c] *Tsukuba Botanical Garden, National Museum of Nature and Science, Tsukuba, Ibaraki 305-0005, Japan*
[d] *National Agricultural Research Center for Hokkaido Region, Sapporo, Hokkaido 062-8555, Japan*
[e] *Faculty of Horticulture, Minami-Kyushu University, Takanabe, Miyazaki 884-0003, Japan*
[f] *Faculty of Pharmaceutical Sciences, Hoshi University, Shinagawa, Tokyo 142-8501, Japan*

ARTICLE INFO

Article history:
Received 14 December 2010
Accepted 20 February 2011
Available online 21 March 2011

Keywords:
Disa cultivars
Orchidaceae
Cyanidin 3,5-diglucoside
Pelargonidin 3,5-diglucoside
Malonic acid

1. Subject and source

Recently we have detected the occurrence of pelargonidin and cyanidin in the flowers of *Disa* hybrids (Tatsuzawa et al., 2010a). In the present study, we further investigated the detailed structures of anthocyanins in the red-purple, red, and orange-red flowers of *Disa* cultivars, grown by Hokkaisankyo Co. Ltd (Hokkaido, Japan), and identified them as acylated and non-acylated pelargonidin and cyanidin 3,5-diglucosides. The distribution of these anthocyanins in Orchidaceae was discussed along with the classification by phylogenetic analysis of Orchidaceae.

Voucher specimens are deposited at Tsukuba Botanical Garden, National Museum of Nature and Science (TNS).

2. Previous work

There are only two previous reports on flavonoids from the genus *Disa*. Flavon *C*-glycosides were detected in leaf material of *Disa uniflora* Berg. (Williams, 1979). More recently, we have reported the distribution of cyanidin and pelargonidin as the aglycones of anthocyanins in the flowers of cultivars of the given genus (Tatsuzawa et al., 2010a).

* Corresponding author. Tel./fax: +81 19 621 6145.
E-mail address: fumi@iwate-u.ac.jp (F. Tatsuzawa).

0305-1978/$ – see front matter
doi:10.1016/j.bse.2011.02.006

3. Present study

3.1. Isolation and identification of anthocyanins

By the analysis of HPLC [HPLC was performed on a LC 10A system (Shimadzu), using a Waters C18 (4.6 φ × 250 mm) column at 40 °C with a flow rate of 1 ml/min, the eluate was monitored at 530 nm. The eluant was applied to 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)], more than 20 anthocyanin peaks were observed in the extract from the flowers of red cultivar *Disa* Transvaal 'Dawn Angel' (Fig. 1). Anthocyanins **1**–**3** were easily identified to be cyanidin 3,5-di-glucoside, pelargonidin 3,5-di-glucoside and cyanidin 3-(6-malonyl)-glucoside-5-glucoside (Fig. 2) with authentic samples obtained from the pink and purple flowers of *Centaurea cyanus* (Takeda et al., 1988; Goto and Kondo, 1991) by co-TLC, co-HPLC and UV–VIS spectrometry (Tatsuzawa and Shinoda, 2005) (See in Sections 3.1.1–3.1.3.). Pigment **5** was identified by the analysis of FAB-MS and ^{1}H NMR measurement (Section 3.2.). Pigment **4** was identified by the analysis of partial acid hydrolysis of pigment **5** and FAB-MS (Section 3.3.). Moreover, pigment **6** was identified by the analysis of FAB-MS (Section 3.4.).

Dried corolla mixture of *Disa* red cultivars (60 g) were immersed in 5% HOAc–MeOH (acetic acid-methanol, 5:95, v/v, 500 ml), kept at 4 °C for 1 h and extracted. The extract was concentrated to 50 ml. Anthocyanin pigments in the concentrated extract were purified by prep. HPLC [HPLC was performed on a LC 10A system (Shimadzu), using a Waters C18 (19 φ × 150 mm) column at 40 °C with a flow rate of 1 ml/min, the eluate was monitored at 530 nm. The eluant was applied to a linear gradient elution for 40 min from 20 to 85% solvent B in solvent A] after thin layer and paper chromatography (BAW: BuOH–HOAc–H_2O, 4:1:2, v/v/v and 15% HOAc). Finally, pigments **1** (*ca.* 0.5 mg), **2** (*ca.* 0.5 mg), **3** (*ca.* 0.5 mg), **4** (*ca.* 3 mg), **5** (*ca.* 5 mg) and **6** (*ca.* 2 mg) were obtained as the major anthocyanins.

On hydrolysis of pigments **4** and **5** with 2 N HCl for 3 days at 25 °C, cyanidin 3,5-diglucoside was obtained in its hydrolysate. Similar hydrolysis of pigment **6** afforded pelargonidin 3,5-diglucoside. Moreover, malonic acid was detected in both of the hydrolysates.

3.1.1. Cyanidin 3,5-diglucoside (*1*)

UV–VIS in 0.1% HCl–MeOH; λ_{max} 526,270 nm, $E_{440}/E_{max}(\%) = 16$, $AlCl_3$ shift +, TLC; R_f-values BAW (*n*-BuOH–HOAc–H_2O, 4:1:2, v/v/v) 0.02, BuHCl (*n*-BuOH–-2 N HCl, 1:1, v/v, upper phase) 0.01, 1%HCl 0.05, AHW (HOAc–HCl–H_2O, 15:3:82, v/v/v) 0.19, HPLC; t_R(min) 13.0.

3.1.2. Pelargonidin 3,5-diglucoside (*2*)

UV–VIS in 0.1% HCl–MeOH; λ_{max} 507,267 nm, $E_{440}/E_{max}(\%) = 21$, $AlCl_3$ shift 0, TLC; R_f-values BAW 0.07, BuHCl 0.04, 1%HCl 0.13, AHW 0.35, HPLC; t_R(min) 14.9.

3.1.3. Cyanidin 3-(6-malonyl)-glucoside-5-glucoside (*3*)

UV–VIS in 0.1% HCl–MeOH; λ_{max} 526,278 nm, $E_{440}/E_{max}(\%) = 16$, $AlCl_3$ shift +, TLC; R_f-values BAW 0.08, BuHCl 0.08, 1%HCl 0.04, AHW 0.12, HPLC; t_R(min) 16.6.

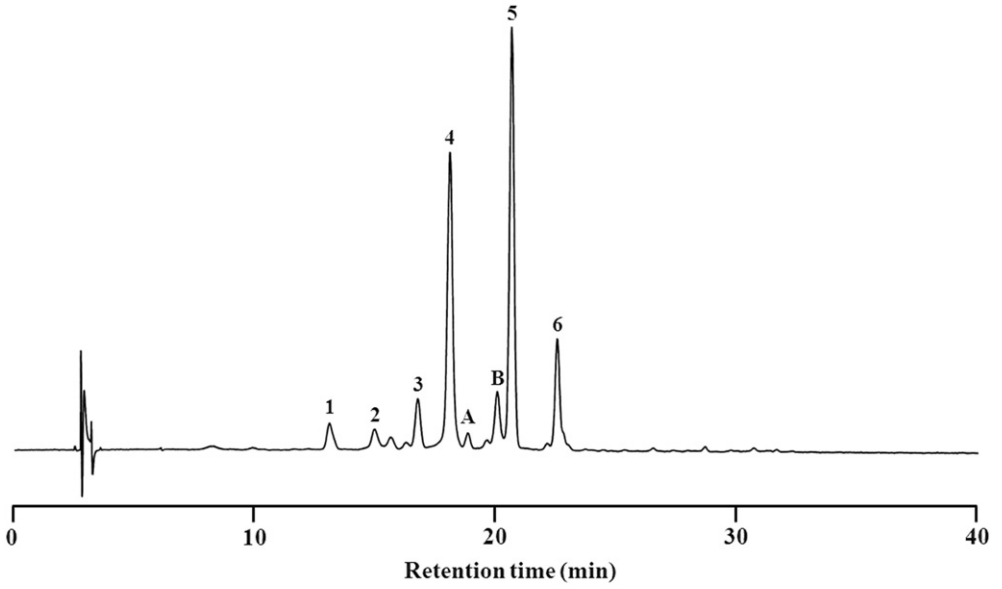

Fig. 1. HPLC profile for anthocyanins (530 nm) in the red flower extract of *Disa* Sid Cywes 'Marlene'. Pigments **1**–**6** are purified. Pigments **A** and **B** are not purified.