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Acylated cyanidin 3-sambubioside-5-glucosides in the purple flowers of *Hesperis matronalis* L. (Brassicaceae)

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1. Subject and source

Recently, the flowers of plants in the Brassicaceae are known as the sources of complicated anthocyanins, such as acylated 3-sambubioside-5-glucosides of pelargonidin, cyanidin and delphinidin in *Cheiranthus cheiri*, *Heliophila coronopifolia*, *Lobularia maritima*, *Lunaria annua*, *Matthiola incana* and *Orychophragmus violaceus* (Honda et al., 2005; Saito et al., 1995, 1996, 2011; Tatsuzawa et al., 2006, 2007, 2010a,b, 2012a), acylated 3-(3^{X}-glucosylsambubioside)-5-glucosides of cyanidin in *Malcolmia maritima* (Tatsuzawa et al., 2008a) and acylated 3-sophoroside-5-glucosides of pelargonidin, cyanidin and peonidin in *Raphanus sativus*, *Iberis umbellata*, *Moricandia ramburii* (Saito et al., 2008; Tatsuzawa et al., 2008b, 2010b, 2012b).

In the present study, I report two new acylated cyanidin 3-sambubioside-5-glucosides from flowers of *Hesperis matronalis* (Sweet rocket) together with two known acylated cyanidin 3-sambubioside-5-glucosides.

The seeds of *H. matronalis* were purchased from the Johnsons seeds Co. Ltd. (UK), and grown in the experimental farm of Iwate University. Fresh purple flowers [Purple 78C by RHS colour chart and its chromaticity values ($L^* = 57.14$, $b^*/a^* = -27.01/40.24 = -0.67$) by NR-1 color difference meter (Nippon Denshoku Co. Ltd., Japan)] were collected in summer. Voucher specimens are deposited at Iwate University Museum (IUM).

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2. Previous work

There are no previous reports on acylated anthocyanins from the genus *Hesperis*.

3. Present study

3.1. Isolation and identification of anthocyanins

By the analysis of HPLC [HPLC was performed on an LC 10A system (Shimadzu), using a Waters C18 (4.6 × 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)], 4 major anthocyanin peaks (**1**: ranging from 16.4% of the total anthocyanin contents observed from the HPLC peak area at 530 nm, **2**: 22.6%, **A**: 19.3%, **B**: 25.2%) were observed in the MAW (MeOH–HOAc–H_2O, 4:1:5, v/v/v) extract from the purple flowers of *H. matronalis* (Fig. 1).

Dried purple petal of *H. matronalis* (10 g) were immersed in 5% HOAc–MeOH (acetic acid–methanol, 5:95, v/v, 500 ml), kept at 4 °C for 1 day and extracted. The extract was concentrated to 50 ml. Anthocyanin pigments in the concentrated extract were purified by prep. HPLC [HPLC was performed on an LC 10A system (Shimadzu), using a Waters C18 (19 × 150 mm) column at 40 °C with a flow rate of 4 ml/min, the eluate was monitored at 530 nm. The eluant was applied to a linear gradient elution for 10 min from 60 to 70 % 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.* 5 mg), **2** (*ca.* 7 mg), **A** (*ca.* 8 mg) and **B** (*ca.* 17 mg) were obtained as the major anthocyanins.

Acid hydrolysis of pigments **1**, **2**, **A** and **B** gave cyanidin as their anthocyanidin (Harborne, 1984). These anthocyanins also showed glucose and xylose as their sugar component and *p*-coumaric acid and malonic acid as their acid by acid hydrolysis. Moreover, caffeic acid was detected in the hydrolysates of **1**, ferulic acid was detected in those of **2** and sinapic acid was detected in those of **B** by HPLC, respectively.

By alkaline hydrolysis, pigments **1**, **2**, **A** and **B** yielded cyanidin 3-sambubioside-5-glucoside as their deacyl anthocyanin. The deacyl anthocyanin structure was identified in direct comparison by the analyses of co-TLC and co-HPLC with authentic cyanidin 3-sambubioside-5-glucoside which was prepared from *Lunaria annua* (Tatsuzawa et al., 2006).

The pigments **A** and **B** were easily identified to be cyanidin 3-[2-(xylosyl)-6-(*trans*-*p*-coumaroyl)-glucoside]-5-[6-(malonyl)-glucoside] and cyanidin 3-[2-(2-(*trans*-sinapoyl)-xylosyl)-6-(*trans*-*p*-coumaroyl)-glucoside]-5-[6-(malonyl)-glucoside] with authentic samples obtained from *M. incana* and *L. annua* (Saito et al., 1995; Tatsuzawa et al., 2006, 2012a) by co-TLC, co-HPLC, UV–VIS spectrometry. Moreover, elemental components of these pigments were confirmed by measuring their high resolution fast atom bombardment mass spectra (HR-FABMS) and the structures of these pigments were confirmed by analysis of their ^{1}H NMR measurements [400 MHz for ^{1}H spectra in CF_3COOD-DMSO-d_6 (1:9)] including ^{1}H–^{1}H correlation

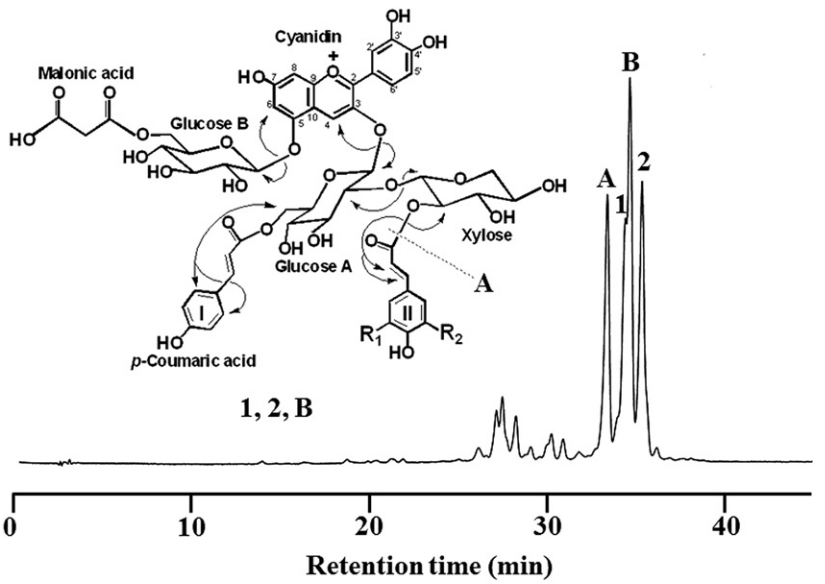

Fig. 1. HPLC profile (530 nm) and structure of acylated anthocyanins isolated from the purple flowers of *Hesperis matronalis*. Observed main NOEs are indicated by arrows. **1**: pigment **1**, $R_1 = OH$, $R_2 = H$; **2**: pigment **2**, $R_1 = OCH_3$, $R_2 = H$; **B**: pigment **B**, $R_1 = OCH_3$, $R_2 = OCH_3$.