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Differences in the floral anthocyanin content of violet–blue flowers of *Vinca minor* L. and *V. major* L. (Apocynaceae)

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ABSTRACT

Two novel delphinidin 3-(tri or di)-glycoside-7-glycosides were isolated from the violet–blue flowers of *Vinca minor* L. and *V. major* L. (Family: Apocynaceae), and determined to be delphinidin 3-*O*-[2-*O*-(β-xylopyranosyl)-6-*O*-(α-rhamnopyranosyl)-β-galactopyranoside]-7-*O*-(α-rhamnopyranoside) [= delpphinidin 3-(2^{G}-xylosylrobinobioside)-7-rhamnoside] as major floral anthocyanin of *V. minor* and delphinidin 3-*O*-[6-*O*-(α-rhamnopyranosyl)-β-galactopyranoside]-7-*O*-(α-rhamnopyranoside) [= delphinidin 3-robinobioside-7-rhamnoside] as major floral anthocyanin of *V. major* by chemical and spectroscopic methods. In addition, chlorogenic acid and kaempferol 3-*O*-[6-*O*-(α-rhamnopyranosyl)-β-galactopyranoside]-7-*O*-(α-rhamnopyranoside) [= kaempferol 3-robinobioside-7-rhamnoside (robinin)] were identified in these flowers. In this paper, the relation between the structure of floral anthocyanins and classification of *Vinca* species was discussed.

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1. Introduction

Vinca minor L. and *V. major* L. (Apocynaceae) are popular ornamentals as trailing ground cover plants. The main factors to distinguish between these species are the size of corollas (*V. minor* 25–30 mm, *V. major* 30–70 mm) and presence of minute hair around the margins of leaves and sepals (*V. minor* absent, *V. major* present) (Huxley et al., 1992). In the limited floral flavonoid studies reported for these species, anthocyanin glycosides from *V. major* flowers have been characterized as unusual in apparently having some sugars (Harborne, 1967), and delphinidin 3-robinobioside-5-rhamnoside (Vincanin A) has been identified as the major anthocyanin glycoside by chemical, spectral, and paper chromatographic analyses (Ishikura and Minekishi, 1978). However, Ishikura and Minekishi (1978) stated that a definition of 5-rhamnoside was not clear on their chromatograms. In the floral flavonol study of *Vinca*, kaempferol 3-robinobioside-7-rhamnoside (Robinin) and a myricetin derivative have been identified from *V. minor* and *V. major* by paper chromatography (Harborne, 1967). In addition, in a pharmacognostical study, kaempferol 3-rutinoside-7-glucoside, 2,3-dihydroxybenzoic acid, 3-glucosyloxy-2-hydroxybenzoic acid, and chlorogenic acid have been isolated from the leaves of *V. minor* (Nishibe et al., 1996).

Here, I wish to report the structural elucidation of two new delphinidin glycosides (one of which is a revised structure of Vincanin A) with chlorogenic acid and kaempferol 3-robinobioside-7-rhamnoside (Robinin) isolated from the violet–blue flowers of *V. minor* and *V. major*.

2. Results and discussion

One major peak each of **1** and **2** with some minor peaks at 530 nm and two major peaks (**3** and **4**) with many minor peaks at 350 nm were found in the 5%HOAc (acetic acid–water = 5:95, v/v) (2L) extracts from dried violet–blue flowers of *Vinca minor* (20 g) and *V. major* (20 g), respectively, by high performance liquid chromatography (HPLC) analysis (Fig. 1). The percentage of **1** and **2** of *V. minor* and **2** of *V. major*, calculated as the total content by HPLC vis peak area at 530 nm, was 77.2% (**1**) and 5.5% (**2**) for *V. minor* and 75.8% (**2**) for *V. major*, respectively (Fig. 1). Moreover, the percentage of **3** and **4** of *V. minor* and *V. major*, calculated as the total content by HPLC peak area at 350 nm, was 46.2% (**3**) and 35.7% (**4**) for *V. minor* and 46.6% (**3**) and 40.7% (**4**) for *V. major*, respectively (Fig. 1).

The major peaks were extracted from the violet–blue flowers with 5% HOAc, followed by isolation using Diaion HP-20 (Nippon Rensui Co., Tokyo, Japan) column chromatography (CC), Sephadex^{TM} LH-20 (GE Healthcare UK Ltd., UK) CC and paper chromatography (PC). The chromatographic and spectroscopic properties of **1**–**4** are summarized in Sections 4.3.1–4.3.4.

Acid hydrolysis of **1**,**2**, and **4** yielded delphinidin (**1** and **2**) (Forestal 0.32), and kaempferol (**4**) (Forestal 0.58) as their

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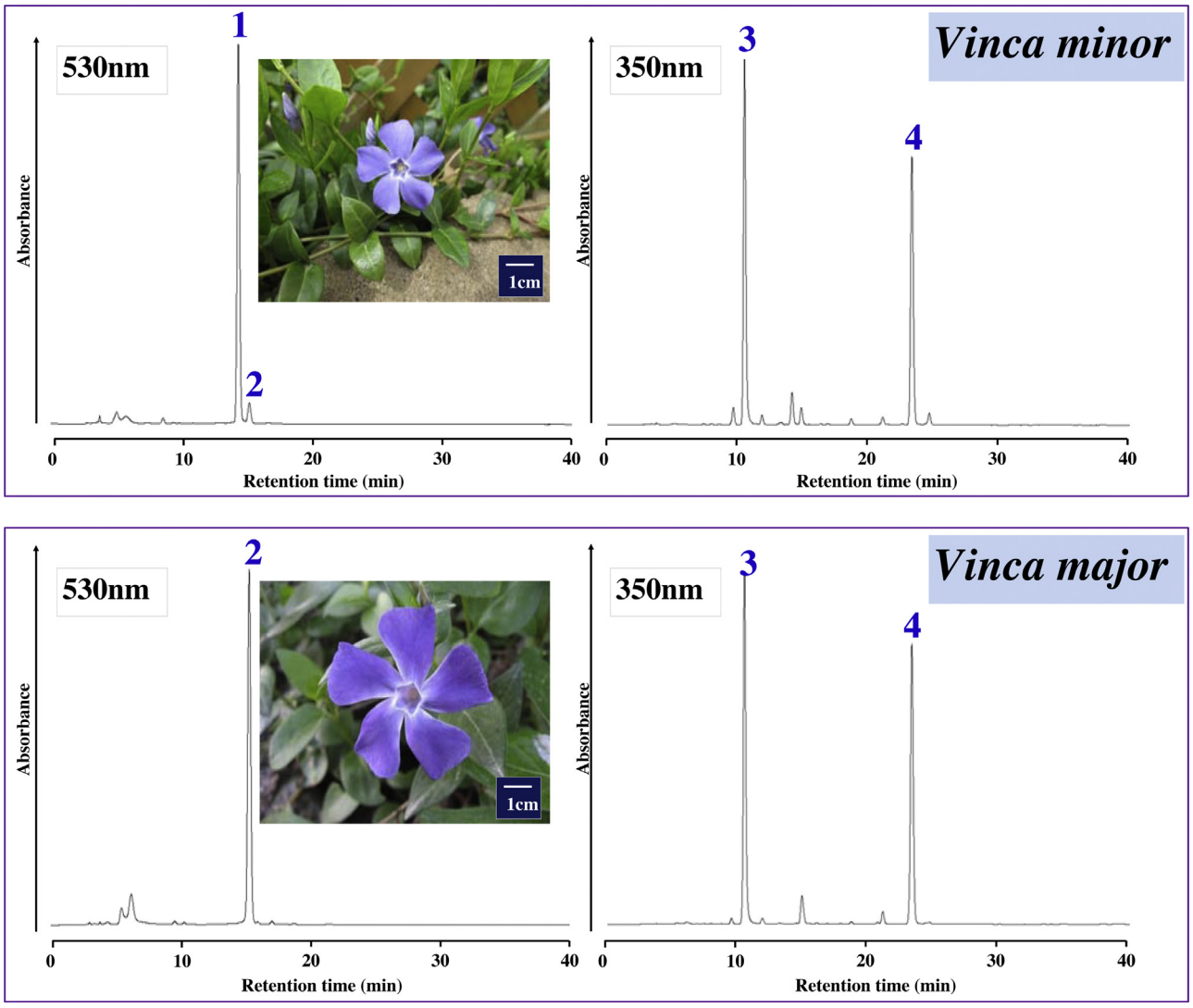

Fig. 1. Comparative HPLC profiles of extracts from violet–blue flowers of *Vinca*.

agrycone, respectively (Harborne, 1984). Moreover, xylose (**1**) (BAW 0.41, EAA 0.34, ETN 0.44, EFW 0.36), galactose (**1**, **2**, and **4**) (BAW 0.32, EAA 0.20, ETN 0.39, EFW 0.20), and rhamnose (**1**, **2**, and **4**) (BAW 0.51, EAA 0.46, ETN 0.64, EFW 0.52) were detected as sugars (Harborne, 1984). These aglycone of flavonoids and sugars were identified by direct comparison with commercial standards (Wako Pure Chemical Industries Co., Ltd., Tokyo, Japan).

The structures of **1**, **2**, and **4** were confirmed based on the analyses of their ^{1}H (400 MHz), ^{13}C (100 MHz) and 2D (COSY, NOESY, ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC) NMR (JNM AL-400, JEOL Ltd., Tokyo, Japan) spectra in CD_3OD–DCl (9:1) and/or DMSO–DCl (9:1) for **1** and **2**, and DMSO-d_6 for **4**, as well as their high resolution fast atom bombardment mass spectra (HR-FABMS) (LMS-700, JEOL Ltd.).

The peak **3** was easily identified to be chlorogenic acid by direct comparison of its HPLC, HR-FAB mass, and ^{1}H NMR data (see Section 4.3.3) with commercial standard (Wako Pure Chemical Industries Co., Ltd., Tokyo, Japan).

2.1. HPLC peak 1

The molecular ion $[M]^+$ of **1** was observed at *m/z* 889 ($C_{38}H_{49}O_{24}$) indicating that **1** was composed of delphinidin with two molecules of rhamnose, one molecule each of galactose and xylose. The elemental components were confirmed by measuring its HR-FABMS, and the mass data are summarized in Section 4.3.1. The structure was elucidated based on the analysis of the NMR spectra (Table 1).

The chemical shifts of 5 aromatic protons of delphinidin moiety with their coupling constants were assigned as shown in Table 1. The chemical shifts of the sugar moieties were observed in the region of δ 5.72–1.15 (DMSO–DCl) and δ 5.80–1.26 (CD_3OD–DCl), where the four anomeric protons exhibited at δ 5.55 (*d*, J = 7.6 Hz, Galactose), δ 4.52 (*brs*, Rhamnose A), δ 5.72 (*brs*, Rhamnose B), and δ 4.56 (*d*, J = 8.0 Hz, Xylose) (DMSO–DCl) and at δ 5.51 (*d*, J = 7.6 Hz, Galactose), δ 4.66 (*brs*, Rhamnose A), δ 5.80 (*d*, J = 1.4 Hz, Rhamnose B), and δ 4.71 (*d*, J = 8.0 Hz, Xylose) (CD_3OD–DCl). Based on the observed coupling constants (Table 1), galactose and xylose were assumed to be in the β-pyranose form and two molecules of rhamnose were assumed to be in the α-pyranose.

Based on results of analyses of their COSY spectra, characteristic mathine proton signal [δ 4.27 (DMSO–DCl) and δ 4.32 (CD_3OD–DCl)] being shifted to lower magnetic field was assigned to H-2 protons of galactose (Fossen and Andersen, 2006). This result indicated that the OH-2 of galactose must be glycosylated with sugar.

NOESY and HMBC spectra (DMSO–DCl) were used to determine the sites of attachment of the sugars and delphinidin moieties

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