Flavonoids from the Flowers and Aerial Parts of
Torenia concolor var. formosana

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Abstract Two flavones and three anthocyanins were isolated from the violet-blue flowers of Torenia concolor var. formosana. They were characterized as apigenin and luteolin 7-O-glucuronides, and malvidin and peonidin glycosides and malvidin 3,5-di-O-glucoside, respectively, by UV, acid hydrolysis, LC-MS and TLC and HPLC comparisons with authentic samples. On the other hand, C-glycosylflavone was isolated from the aerial parts, together with minor two unknown flavonoids, and identified as apigenin 6,8-di-C-arabinoside by UV, LC-MS, and TLC and HPLC comparison with authentic sample. Flavonoids were reported from this species for the first time. Flavonoid pattern was the same among the some populations of Japan and Taiwan.

Key words: anthocyanins, apigenin 6,8-di-C-arabinoside, C-glycosylflavone, flavones, flavonoids, Torenia concolor var. formosana.

Introduction

The genus Torenia consists of ca. 30–40 species and is distributed in mainly tropical and subtropical zones of East Asia (Mabberley, 1987; Yamazaki, 1981). In Japan, two taxa, Torenia glabra Osbeck and T. concolor Lindl. var. formosana Yamazaki, are growing, and some cultivars of T. fournieri Linden ex Fourn. are cultivated as ornamentals (Yamazaki, 1981). Of their taxa, T. concolor var. formosana is native to Amami-Ohshima Is. and Miyako Is., Japan, and Taiwan (Hatusima and Amano, 1994), and is designated to endangered plant in Japan.

The flavonoids in Torenia species have hardly been reported. The anthocyanins, 3,5-di-O-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin were isolated from the flowers of T. fournieri cultivars and wild type (Aida et al., 2000). Flavones, apigenin 7-O-glucuronide, luteolin 7-O-glucoside and luteolin 7-O-glucuronide, were also found as copigment substances in the flowers of the same species (Aida et al., 2000). The anthocyanin, malvidin 3-O-glucoside-5-O-(6″-p-coumaroylglucoside) was isolated from the flowers of Torenia hybrida ‘Summerwave Blue’ together with minor peonidin 3-O-glucoside-5-O-(6″-p-coumaroylglucoside) (Suzuki et al., 2000; Fukusaki et al., 2004). Though the presence of the anthocyanins was showed in the flowers of Torenia baillonii Godfroy ex André, their identification was not performed (Lang and Potrykus, 1971).

As a series of flavonoid survey of endangered plants in Ryukyus, we have studied the flavonoids in Cassytha spp. (Lauraceae) (Murai et al., 2008), Begonia spp. (Begoniaceae) (Iwashina et al., 2008), Pothos chinensis (Raf.) Merr. (Ara-ceae) (Iwashina et al., 2010), Myoporum bontiodes (Sieb. et Zucc.) A. Gray (Myoporaceae) (Iwashina and Kokubugata, 2010) and Triumfetta procumbens G. Forst. (Malvaceae) (Iwashina and Kokubugata, 2012).
In this paper, we describe the isolation and characterization of the flavonoids including the anthocyanins of the flowers and aerial parts of *Torenia concolor* var. *formosana* in Japan and Taiwan.

**Materials and Methods**

**Plant materials**

*Torenia concolor* Lindl. var. *formosana* Yamazaki was used as plant material. Collection sites and dates of the plants are as follows: Honohoshi, Amami-Ohshima Is., Kagoshima Pref., Japan, 12 Nov. 2006 (GK4512); Tatsugo, Amami-Ohshima Is., Kagoshima Pref., Japan, 6 Feb. 2009; Fan Papshan, Taiwan, 12 Nov. 2006 (GK8820); Lienhua-yu, Taiwan, 12 Nov. 2006 (GK8821); Tungyen-shan, Taiwan, 6 Nov. 2006; Bitou Cape, Taiwan, 31 Oct. 2011. Voucher specimens are deposited in the Herbarium of National Museum of Nature and Science, Japan (TNS).

**Extraction and separation**

Fresh aerial parts (168.4 g) and violet-blue flowers (0.9 g) of *T. concolor* var. *formosana* were extracted with MeOH and HCOOH/MeOH (8:92), respectively. The concentrated extracts were applied to preparative paper chromatography using solvent systems, BAW (n-BuOH/HOAc/H₂O=4:1:5, upper phase) and 15% HOAc. Flavone mixtures were, moreover, chromatographed using solvent system, BEW (n-BuOH/EtOH/H₂O=4:1:2:2). The isolated compounds were purified by Sephadex LH-20 column chromatography using solvent systems, 70% MeOH (flavones) and MeOH/H₂O/HCOOH (65:30:5) (anthocyanins).

**High performance liquid chromatography (HPLC)**

HPLC was performed with Shimadzu HPLC systems using Senshu pak PEGASIL ODS column (I.D. 6.0 × 150 mm, Senshu Scientific Co. Ltd., Japan) at a flow-rate of 1.0 ml min⁻¹. Detection was 350 nm (flavones) and 530 nm (anthocyanins). Eluents were MeCN/H₂O/H₃PO₄ (15:85:0.2) (flavones) and MeCN/HOAc/H₂O/H₃PO₄ (6:8:83:3) (anthocyanins).

**Liquid chromatograph-mass spectra (LC-MS)**

LC-MS was performed with Shimadzu LC-MS systems using Senshu pak PEGASIL ODS column (I.D. 2.0 × 150 mm, Senshu Scientific Co. Ltd.) at a flow-rate of 0.2 ml min⁻¹, ESI⁺ 4.5 kV and ESI⁻ 3.5 kV, 250°C. Eluents were MeCN/H₂O/HCOOH (10:85:5) (flavones) and (8:5:82 or 20:5:75) (anthocyanins).

**Acid hydrolysis**

Acid hydrolysis was performed in 12% aq. HCl, 100°C, 30 min. After shaking with diethyl ether (flavones) and isoamyl alcohol (anthocyanins), aglycones migrated to the organic layer, and sugars and C-glycosylflavone were left in aqueous layer.

**Thin layer chromatography (TLC)**

TLC was performed with Cellulose F plastic plate (Merck, Germany) using solvent systems, BAW, BEW and 15% HOAc.

**Identification of flavones and anthocyanins**

Flavones were identified by UV spectral survey according to Mabry et al. (1970), LC-MS, characterization of acid hydrolysates, and TLC and HPLC comparisons with authentic samples. Anthocyanins were characterized by UV and LC-MS, and HPLC comparison with authentic sample. Following authentic samples were used:
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malvidin 3,5-di-<i>O</i>-glucoside from the flowers of *Gladiolus* cultivars (Takemura et al., 2008), apigenin 7-<i>O</i>-glucuronide from the flowers of *Aeginetia indica* L. (Orobanchaceae) (Iwashina, 2010), luteolin 7-<i>O</i>-glucuronide from the leaves of *Uncarina grandiflora* (Baill.) Stapf. (Pedaliaceae) (Yamazaki et al., 2007) and apigenin 6,8-di-C-arabinoside from the leaves of *Ajuga decumbens* Thunb. (Lamiaceae) (Inomata et al., 2013). TLC, HPLC, UV and LC-MS data of the isolated compounds are as follows.

Apigenin 7-<i>O</i>-glucuronide (1). TLC: Rf 0.59 (BAW), 0.45 (BEW), 0.18 (15%HOAc); color UV (365 nm)—dark purple, UV/NH₃—dark greenish yellow. HPLC: t<sub>R</sub> (min) 11.38. UV: λ<sub>max</sub> (nm) MeOH 268, 333; +NaOMe 274, 299, 348, 375sh; +AlCl₃/ HCl 275, 298, 340, 375sh; +NaOAc 257, 266, 390; +NaOAc/H₃BO₃ 268, 340. LC-MS: m/z 447 [M + H]<sup>+</sup>, 445 [M − H]<sup>−</sup> (molecular ion peaks, apigenin + 1 mol glucuronic acid), m/z 269 [M − 176 − H]<sup>−</sup> (fragment ion peak, apigenin).

Luteolin 7-<i>O</i>-glucuronide (2). TLC: Rf 0.36 (BAW), 0.27 (BEW), 0.11 (15%HOAc); color UV (365 nm)—dark purple, UV/NH₃—dark yellow. HPLC: t<sub>R</sub> (min) 7.86. UV: λ<sub>max</sub> (nm) MeOH 255, 266sh, 348; +NaOMe 264, 392 (inc.); +AlCl₃ 273, 342; +AlCl₃/HCl 264, 273sh, 296, 359, 384sh; +NaOAc 259, 358sh, 401; +NaOAc/H₃BO₃ 259, 373. LC-MS: m/z 463 [M + H]<sup>+</sup>, 461 [M − H]<sup>−</sup> (molecular ion peaks, luteolin + 1 mol glucuronic acid), m/z 287 [M − 176 + H]<sup>+</sup> (fragment ion peak, luteolin).

Apigenin 6,8-di-C-arabinoside (3). TLC: Rf 0.31 (BAW), 0.28 (BEW), 0.35 (15%HOAc); color UV (365 nm)—dark purple, UV/NH₃—dark greenish yellow. HPLC: t<sub>R</sub> (min) 7.21. UV: λ<sub>max</sub> (nm) MeOH 273, 332; +NaOMe 283, 333, 401 (inc.); +AlCl₃ 280, 305, 350, 385sh; +AlCl₃/HCl 280, 303, 346, 384sh; +NaOAc 282, 398; +NaOAc/H₂BO₃ 285, 319, 361, 417. LC-MS: m/z 535 [M + H]<sup>+</sup>, 533 [M − H]<sup>−</sup> (molecular ion peaks, apigenin + 2 mol pentose).

Malvidin glycoside (A1). HPLC: t<sub>R</sub> (min) 7.69. LC-MS: m/z 801 [M]<sup>+</sup> (molecular ion peak, malvidin + 1 mol rhamnose or <i>p</i>-coumaric acid, and 2 mol glucose), m/z 655 [M − 146]<sup>+</sup> (fragment ion peak, malvidin + 2 mol glucose).

Peonidin glycoside (A2). HPLC: t<sub>R</sub> (min) 6.34. LC-MS: m/z 771 [M]<sup>+</sup> (molecular ion peak, peonidin + 1 mol rhamnose or <i>p</i>-coumaric acid, and 2 mol glucose).

Malvidin 3,5-di-<i>O</i>-glucoside (A3). HPLC: t<sub>R</sub> (min) 8.84. LC-MS: m/z 655 [M]<sup>+</sup> (molecular ion peak, malvidin + 2 mol glucose).

**Results and Discussion**

Flavones and anthocyanins in the flowers

Two flavones 1 and 2 were isolated from the flowers. Flavonoid 1 liberated apigenin and glucuronic acid, which were characterized by HPLC (aglycone) and PC (sugar) comparisons with authentic samples, by acid hydrolysis. The
attachment of 1 mol glucuronic acid to 7-position of apigenin was proved by UV spectral data and LC-MS survey. Finally, 1 was identified as apigenin 7-O-glucuronic acid by TLC and HPLC comparison with authentic specimen. Apigenin 7-O-glucuronic acid has been reported from the flowers of *Torenia fournieri* cultivars and wild type (Aida et al., 2000). It was shown by UV spectral survey according to Mabry et al. (1970) that 2 is 5,3′,4′-tri-hydroxy-7-substituted flavone. Luteolin and glucuronic acid were produced by acid hydrolysis. The attachment of 1 mol glucuronic acid to luteolin was determined by LC-MS. Thus, 2 was identified as luteolin 7-O-glucuronic acid by direct TLC and HPLC comparison with authentic sample. Luteolin 7-O-glucuronic acid was also reported from the flowers of garden torenia (Aida et al., 2000).

Three anthocyanins A1–A3 were isolated from the flowers. Molecular weights, 801, 771 and 655 were assigned to A1, A2 and A3 by LC-MS, showing that they are malvidin monorhamnosyl-dihexoside or p-coumaroyl-dihexoside, peonidin monorhamnosyl-dihexoside or p-coumaroyl-dihexoside, and malvidin dihexoside, respectively. Malvidin 3-O-glucoside-5-O-(6″-p-coumaroylglucoside) and peonidin 3-O-glucoside-5-O-(6″-p-coumaroylglucoside), which are corresponding to their molecular weight, have been reported from the flowers of *Torenia hybrida* ‘Summerwave Blue’ (Suzuki et al., 2000; Fukusaki et al., 2004). However, their retention times of HPLC in literature (Suzuki et al., 2000; Fukusaki et al., 2004) are faster compared with those of A1 and A2, presuming that they are not acylated. Though A3 was identified as malvidin 3,5-di-O-glucoside by HPLC comparison with authentic sample, A1 and A2 could not be identified for minute amount of each plant material. Malvidin 3,5-di-O-glucoside has been found in the flowers of *T. fournieri* (Aida et al., 2000).

**C-Glycosylflavone from the aerial parts**

Only one flavonoid 3 was isolated from the aerial parts of *T. concolor* var. *formosana*. UV spectral properties of 3 showed the presence of free 5-, 7- and 4′-hydroxyl groups in flavone nucleus. Since 3 was unhydrolyzable by hot acid treatment, it was proved that this is C-glycosylflavone. LC-MS survey showed the attachment of 2 mol pentose to apigenin. Finally, 3 was identified as apigenin 6,8-di-C-arabinoside by direct TLC and HPLC comparison with authentic sample. C-Glycosylflavone was reported from *Torenia* species for the first time. Two very minor flavonoids were found in the aerial parts by HPLC survey, together with 3. However, they could not be isolated for minute amount. Though the flavonoids in the flowers has been reported from *Torenia* species (Aida et al., 2000; Suzuki et al., 2000; Fukusaki et al., 2004), those of the aerial parts were found by this experiment for the first time.

In this survey, both the flowers and aerial parts were used as plant materials. However, their flavonoid composition was completely different with each other, i.e. flavone O-glycosides and anthocyanins in flowers and C-glycosylflavone in aerial parts. It may be due to the difference of their function of flavonoids in each plant parts. Though the plants of two Japanese and three Taiwanese populations were surveyed for flavonoids, their patterns were essentially the same with each other.

**References**


Iwashina, T., Saito, Y., Peng, C.-I, Yokota, M. and


