Flavone O- and C-Glycosides from *Pothos chinensis* (Araceae)

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Abstract

Nine flavonoid glycosides were isolated from the aerial parts of *Pothos chinensis*. Of their flavonoids, eight ones were identified as vitexin, vitexin 7-O-glucoside, isovitexin 7-O-glucoside, scoparin 7-O-glucoside, isoscoparin 7-O-glucoside, schaftoside, isoschaftoside and chrysoseryl 7-O-rhamnosylglucoside. Another flavonoid was characterized as vitexin 7-O-diglucoside or vitexin 7,X"-di-O-glucoside. The flavonoids of *P. chinensis* were reported in this paper for the first time.

Key words: Araceae, flavone glycosides, flavonoids, *Pothos chinensis*.

Introduction

The genus *Pothos* belongs to the family Araceae and consists of ca. 50 species (Mabberley, 1997). In Taiwan and Japan, *P. chinensis* (Raf.) Merr. is only growing. Though the species is abundant at medium elevations through Taiwan (Huang, 2000), it is endemic to Daito Islands in Japan (Hatusima and Amano, 1994) and nominated as endangered plant by the Ministry of Environment, Japan (Environment Agency of Japan, 2000).

Flavonoids of the family Araceae have roughly been investigated by Williams et al. (1981). They were surveyed the leaf flavonoids of 144 species from 58 genera and showed that flavone C-glycosides are the most characteristic flavonoid constituents of the family. In inflorescence, fruits, leaf or petiole of 59 representative species, they were detected cyanidin 3-O-rutinoside as a common anthocyanin, together with pelargonidin 3-O-rutinoside and cyanidin 3-O-glucoside (Williams et al., 1981). Eight C-glycosylflavones and a flavone O-glycoside were isolated from the leaves of cultivated taro (*Colocasia esculenta* (L.) Schott) and identified as orientin, isoorientin, isovitexin, vicenin-2, orientin 7-O-glucoside, isovitexin 4′-O-glucoside, vitexin X"-O-glucoside, and luteolin 7-O-glucoside (Iwashina et al., 1999). The anthocyanins, cyanidin and pelargonidin glycosides were found in two *Anthurium* species, and cyanidin and delphinidin glycosides in three *Philodendron* species (Forsyth and Simmonds, 1954), cyanidin 3,5-di-O-glucoside in the spathe of *Arisaema serratum* Schott. f. thunbergii Makino (Ueno et al., 1969), pelargonidin 3-O-glucoside, cyanidin 3-O-rhamnoside and cyanidin 3-O-glucoside in the peeled corms of taro (Chan Jr. et al., 1977), and cyanidin and peonidin glycosides in the spathe of *Symphoricarpus foetidus* Nott. var. *latissimus* (Makino) Hara (Yoshitama et al., 1980). Common flavones and C-glycosylflavones, apigenin, luteolin, vitexin, isovitexin, orientin, isoorientin and isovitexin 7-O-glucoside were isolated from the leaves of *Synandrospadix vermitoxicus* (Grisebach) Engler which is a monotypic species and grows in Argentina and Bolivia (Sosa et al., 1978). Vitexin 2″-O-glucoside and its sinapoyl derivative were reported from *Cryptocoryne* spp. as their chemo-
taxonomic marker (Franke et al., 2006). The former compound was also found in *Lasia spinosa* (L.) Thw. native to Vietnam, together with vitexin and a flavonol glycoside, isorhamnetin 3-O-rutinoside (Hong Van et al., 2006). Another flavonol glycoside, kaempferol 3-O-sophoroside-7-O-rhamnoside was isolated from the leaves of *Gymnostachys anceps* R. Br. (Williams et al., 1971). However, flavonoid compounds in *Pothos* species have not been reported until now. In this paper, flavonoid glycosides in the aerial parts of *Pothos chinensis* are described.

**Materials and Methods**

**Plant materials**

*Pothos chinensis* (Raf.) Merr. (Fig. 1) was collected in hiking way to Shihfen Waterfall, Pingchi Hsiang, Taipei, Taiwan, during 5 November, 2006.

**Extraction and isolation of flavonoids**

Fresh aerial parts (35.4 g) of *P. chinensis* were extracted with MeOH. The concentrated extracts were applied to preparative paper chromatography using solvent systems: BAW (n-BuOH/HOAc/H₂O = 4:1:5, upper phase), 15% HOAc and then BEW (n-BuOH/EtOH/H₂O = 4:1:2.2). The isolated flavonoids were purified by Sephadex LH-20 column chromatography using solvent system: 70% MeOH.

**High performance liquid chromatography (HPLC)**

HPLC was performed with Shimadzu HPLC systems using a PEGASIL ODS column (I.D. 6.0×150 mm; Senshu Scientific Co. Ltd.), at a flow-rate of 1.0 ml min⁻¹. Detection was 190–700 nm and eluent was MeCN/H₂O/H₃PO₄ (18 : 82 : 0.2).

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

LC-MS was measured using a PEGASIL ODS column (I.D. 2.0×150 mm, Senshu Scientific Co.)
UV—dark purple, UV/NH₃—dark yellow. Rf 0.09 (BAW), 0.10 (BEW), 0.20 (15% HOAc); 333;
332;
UV—dark purple, UV/NH₃—dark greenish yellow. HPLC: Rt 10.90 min. UV: λₘₐₓ (nm) MeOH 270, 331; +NaOMe 279, 328, 393 (inc.); +AlCl₃ 276, 305, 354, 384sh; +AlCl₃/HCl 276, 303, 349, 383sh; +NaOAc 279, 311, 389; +NaOAc/H₃BO₃ 272, 344. LC-MS: m/z 433 [M+H]^+, 431 [M–H]– (apigenin+1 mol hexose).

Vitexin (1). TLC: Rf 0.45 (BAW), 0.48 (BEW), 0.16 (15% HOAc); UV—dark purple, UV/NH₃—dark yellow. HPLC: Rt 6.81 min. UV: λₘₐₓ (nm) MeOH 272, 332; +NaOMe 275, 384 (inc.); +AlCl₃ 275, 303, 349, 377sh; +AlCl₃/HCl 276, 302, 343, 375sh; +NaOAc 257sh, 269, 392; +NaOAc/H₃BO₃ 270, 342. LC-MS: m/z 595 [M+H]^+, 593 [M–H]– (apigenin+2 mol hexose).

Isoschaftoside (6). TLC: Rf 0.22 (BAW), 0.18 (BEW), 0.51 (15% HOAc); UV—dark purple, UV/NH₃—dark greenish yellow. HPLC: Rt 6.63 min. UV: λₘₐₓ (nm) MeOH 273, 333; +NaOMe 283, 331, 400 (inc.); +AlCl₃ 278, 306, 354, 386sh; +AlCl₃/HCl 278, 304, 349, 386sh; +NaOAc 282, 398; +NaOAc/H₃BO₃ 276sh, 285, 322, 348sh. LC-MS: m/z 565 [M+H]^+, 563 [M–H]– (apigenin+each 1 mol hexose and pentose).

Chrysoeriol 7-O-rhamnosylglucoside (8). TLC: Rf 0.44 (BAW), 0.43 (BEW), 0.08 (15% HOAc); UV—dark purple, UV/NH₃—yellow. UV: 7.18 min. UV: λₘₐₓ (nm) MeOH 255sh, 272, 342; +NaOMe 281, 398 (inc.); +AlCl₃ 264sh, 277, 301, 358; +AlCl₃/HCl 260sh, 278, 301, 354; +NaOAc 271, 280sh, 406; +NaOAc/H₃BO₃ 274, 285sh, 351. LC-MS: m/z 625 [M+H]^+, 623 [M–H]– (chrysoeriol+2 mol hexose).

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MeOH 269, 332; +NaOMe 275, 384 (inc.); +AlCl₃ 275, 303, 349, 377sh; +AlCl₃/HCl 276, 302, 343, 375sh; +NaOAc 257sh, 269, 392; +NaOAc/H₃BO₃ 270, 342. LC-MS: m/z 757 [M+H]⁺ (apigenin+3 mol hexose), 595 [M–162+H]⁺ (apigenin+2 mol hexose).

Results and Discussion

Nine flavonoids were isolated from the aerial parts of Pothos chinensis from Taiwan. Of their compounds, flavonoids 2 (3 mg) and 3 (7 mg) were obtained as pale yellow powder.

UV spectral properties of flavonoid 1 showed the compound is flavone glycoside having free 5-, 7- and 4’-hydroxyl groups (Mabry et al., 1970). By hot acid treatment (in 12% HCl, 100°C, 30 min) of the original compound, another flavonoid was produced by Wessely-Moser rearrangement, showing the original flavonoid is 6- or 8-C-glycosylflavone (Markham, 1982). Attachment of 1 mol hexose to 5,7,4’-trihydroxyflavone was shown by the appearance of a molecular ion peak, m/z 433 [M+H]⁺ on LC-MS. Finally, flavonoid 1 was identified as 5,7,4’-trihydroxyflavone 8-C-glucoside, i.e., vitexin (Fig. 2) by direct TLC and HPLC comparison with authentic sample from the flowers of Iris ensata Thunb. (Iridaceae) (Iwashina et al., 1996).

UV spectral survey of flavonoids 2 and 3 showed that they are 7-substituted apigenin (Mabry et al., 1970). Since vitexin, isovitexin and glucose were liberated by acid hydrolysis of them, it was indicated that the original glycosides are 7-O-glucoside of vitexin and isovitexin, respectively. Of their glycosides, Rf values and retention times of flavonoid 3 agreed with those of authentic saponarin from the flowers of Strongylodon macrobotrys A. Gray (Leguminosae) (Iwashina et al., 1984). Thus, flavonoid 3 was identified as isovitexin 7-O-glucoside (Fig. 3). On the other hand, flavonoid 2 was considered as vitexin 7-O-glucoside, i.e., isosaponarin (Fig. 3).

Since the molecular ion peak, m/z 625 [M+H]⁺ appeared on LC-MS of flavonoids 4 and 5, their compounds were presumed as trihydroxy-methoxyflavone dihexosides. Their UV spectral survey of them showed the presence of free 5- and 4’-hydroxyl and substituted 7- and 3’-hydroxyl groups. Each one product was liberated by acid hydrolysis of them, together with glucose. Their hydrolysates were proved to be 3’-substituted 5,7,4’-trihydroxyflavones by UV spectral survey, showing the attachment of glucose to 7-hydroxyl group and the presence of methoxyl group. Thus, flavonoids 4 and 5 were estimated as scoparin (chrysoeriol 8-C-glucoside) and isoscoparin (chrysoeriol 6-C-glucoside) 7-O-glucosides, respectively (Fig. 5 and 6).

Flavonoids 6 and 7 were also shown to be C-glycosylflavones by hot acid treatment, and to be 5,7,4’-trihydroxyflavones by UV spectral survey.
The molecular ion peaks, \( m/z \) 565 \([\text{M}+\text{H}]^+\) appeared on LC-MS of their compounds, showing the attachment of each 1 mol hexose and pentose to apigenin nucleus. Finally, they were identified as apigenin 6-C-glucoside-8-C-arabinoside (6) (Fig. 7) and apigenin 6-C-arabinoside-8-C-glucoside (7) (Fig. 8) by direct TLC and HPLC comparisons with authentic schaftoside and isoschaftoside from the aerial parts of Osyris alba L. (Santalaceae) (Iwashina et al., 2008).

Acid hydrolysis of flavonoid 8 liberated chrysoeriol (5,7,4’-trihydroxy-3’-methoxyflavone), rhamnose and glucose. Since the presence of free 5- and 4’-hydroxyl groups was shown by UV spectra, it was proved that the sugars are attached to 7-position of chrysoeriol. A molecular ion peak, \( m/z \) 609 \([\text{M}+\text{H}]^+\) appeared on LC-MS, showing the attachment of each 1 mol glucose and rhamnose to chrysoeriol. In addition, a fragment ion peak, \( m/z \) 463 \([\text{M}−146+\text{H}]^+\) appeared, showing that the original glycoside is 7-O-rhamnosylglucoside but not 7-O-glucosylrhamnoside. Thus, flavonoid 8 was characterized as chrysoeriol 7-O-rhamnosylglucoside.

It was proved by UV spectra that flavonoid 9 was also 7-substituted C-glycosylapigenin. Vitexin and glucose were liberated by acid hydrolysis. Moreover, the presence of an additional glucose was proved by LC-MS, i.e., the appearance of a molecular ion peak, \( m/z \) 757 \([\text{M}+\text{H}]^+\), showing the attachment of 3 mol glucose to apigenin. However, the attached position of an additional glucose could not be determined. From the results described above, flavonoid 9 was presumed as vitexin 7-O-diglucoside or 7,\( X''\)-di-O-glucoside.

C-Glycosylflavones have been known as characteristic flavonoids in the family Araceae (Williams et al., 1981). In this survey, C-glycosylflavones and their O-glycosides were isolated from the aerial parts of Pothos chinensis as major flavonoids. Of their compounds, relatively rare C-glycosylflavones, schaftoside and isoschaftoside, have been reported from other Araceous species, Anthurium bellum and Philodendron eichleri (Williams et al., 1981). In spite of Pothos is relatively large genus including 50 species, their flavonoid information has only been reported from Pothos scandens L. (Williams et al., 1981). However, no flavonoid was found in this species. It was shown for the first time that
flavonoid character of the genus was C-glyco-
sylflavones as well as almost other Araceae gen-
era.

References