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Flavone and Flavonol Glycosides from the Leaves of *Triumfetta procumbens* in Ryukyu Islands

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Abstract Four flavonoids were isolated from the leaves of *Triumfetta procumbens* in Ryukyu Islands for the first time. They were identified as apigenin 7-*O*-glucuronide, luteolin 7-*O*-glucuronide, schaftoside and kaempferol 3-*O*-(*p*-coumaroylglucoside) by UV, LC-MS, acid hydrolysis, and direct TLC and HPLC comparisons with authentic samples. From the species belonging to the family Tiliaceae *sensu stricto* including the genus *Triumfetta*, some flavonols and flavones have been found together with a few dihydroflavonols and proanthocyanidins. However, three flavonoid *O*-glycosides and *C*-glycoside except for kaempferol 3-*O*-(*p*-coumaroylglucoside), which were isolated in this experiment, were reported from the family Tiliaceae s.s. for the first time.

Key words: apigenin and luteolin 7-glucuronides, flavonoids, kaempferol 3-(*p*-coumaroylglucoside), schaftoside, Tiliaceae s.s., *Triumfetta*.

Introduction

The genus *Triumfetta* belongs to the family Tiliaceae, which was recently included in the family Malvaceae together with Bombacaceae and Sterculiaceae by APG. The genus consists of ca. 40–150 species and is distributed from tropical to temperate zones (Satake, 1982; Yamazaki, 1989). In Japan, four species, i.e. *Triumfetta japonica* Makino, *T. bartramia* L., *T. procumbens* G. Forst. and *T. repens* (Blume) Merr. et Rolfe, are growing in Honshu to Ryukyu (Noshiro, 1999). *Triumfetta procumbens* is low spreading evergreen shrubs and native to Ryukyu Islands in Japan, and Indian Ocean, Malaysia, Polynesia and Micronesia (Noshiro, 1999; Hatusima and Amano, 1994).

A few flavonoids have been reported from the species of the genus *Triumfetta*, i.e., triumboidin and sorbifolin from the leaves of *T. rhamboidea* Jacq. (Srinivasan and Subramanian, 1981). Though they were characterized as scutellarein 7-*O*-rhamnosylarabinoside and scutellarein 7-*O*-rhamnoside, respectively (Srinivasan and Subramanian, 1981), chemical structure of triumboidin was later revised as scutellarein 6-*O*-*β*-D-xyloside-7-*O*-*α*-L-rhamnopyranoside by ^{1}H and ^{13}C NMR and FAB-MS methods (Nair *et al.*, 1986).

Some flavonoids have been reported from other Tiliaceae species. The genus *Tilia* has much surveyed for flavonoid compounds, i.e., acacetin 7-*O*-rutinoside (linarin), quercetin 3-*O*-rhamnoside (quercitrin) and quercetin 3-*O*-glucoside (isoquercitrin) from *T. cordata* Mill., *T. spaethii* Spaeth and *T.* × *vulgaris* Hayne, diosmetin 7-*O*-rutinoside (diosmin) from *T. dasystyla* Stev. and *T.* × *euchlora* Koch (Hegnauer, 1973), acacetin 7-*O*-glucoside (tilianin) and quercetin 3-*O*-rutinoside (rutin) from *T. japonica* (Miq.) Simonkai (Nakaoki *et al.*, 1960), quercetin 3-*O*-glucoside-7-*O*-rhamnoside (vincetoxicoside = petiolaroside) from *T. miqueliana* Maxim., *T.* × *moltkei* Spaeth and *T. petiolaris* Hook.f.,

apigenin 7-*O*-neohesperidoside (rhoifolin) from *T. mongolica* Maxim., kaempferol 3,7-di-*O*-rhamnoside (kaempferitrin) and tiliroside from *T. platyphyllos* Scop. together with leucocyanidins (Hegnauer, 1973). Tiliroside has been isolated from the flowers of *T. argentea* Desf. (= *T. tomentosa* Moench.) (Hörhammer *et al.*, 1959) and completely identified as kaempferol 3-*O*-(6″-*p*-coumaroylglucoside) by Harborne (1964). In *T. argentea*, vincetoxicoside, kaempferol 3-*O*-glucoside-7-*O*-rhamnoside, kaempferitrin, isoquercitrin, quercitrin, kaempferol 3-*O*-glucoside (astragalin), kaempferol 3-*O*-rhamnoside (afzelin) and quercetin rhamnosylxyloside were isolated together with tiliroside (Hegnauer, 1973). Two dihydroflavonols, pinobanksin and aromadendrin were isolated from *T. platyphyllos* (Harborne and Baxter, 1999).

In the genus *Corchorus*, quercetin was found in *C. aestuans* L. (Hegnauer, 1973) and kaempferol 3-*O*-arabinofuranoside (juglanin) in *C. olitorius* L. (common name: molukhyia) (Harborne and Baxter, 1999). Two anthocyanins, peonidin 3-*O*-xylosylglucoside and petunidin 3-*O*-xylosylglucoside were reported from the young leaves of *Elaeocarpus mastersii* King (Lowry, 1970). However, *Triumfetta procumbens* have never been surveyed for flavonoids.

In this paper, we describe the isolation and identification of flavonoid compounds in the leaves of *Triumfetta procumbens*.

Materials and Methods

Plant materials

Triumfetta procumbens G. Forst. which was used as plant materials was collected in Miyako-jima Island, Okinawa Prefecture in 14 May 2008 (GK10304). Voucher specimens were deposited in the herbarium of National Museum of Nature and Science, Japan (TNS).

Extraction and isolation

Fresh leaves (70.2 g) were extracted with MeOH. The concentrated extract was applied to preparative paper chromatography using solvent systems, BAW (*n*-BuOH/HOAc/H_2O = 4 : 1 : 5, upper phase), 15% HOAc and then BEW (*n*-BuOH/EtOH/H_2O = 4 : 1 : 2.2). The isolated flavonoids were purified by Sephadex LH-20 column chromatography using solvent system, 70% MeOH. Four flavonoids (**1**–**4**) were obtained as pure solutions.

Qualitative 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 and eluent was MeCN/H_2O/H_3PO_4 (20 : 80 : 0.2).

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 0.1 ml min^{-1}, ESI^+ 4.5 kV and ESI^- 3.5 kV, 250°C. Eluent was MeCN/H_2O/HCOOH (20 : 75 : 5).

Acid hydrolysis

Acid hydrolysis was performed in 12% HCl, 100°C, 30 min. After shaking with diethyl ether, 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 flavonoids

Flavonoids were identified by UV spectral survey according to Mabry *et al.* (1970), LC-MS, acid hydrolysis, and direct TLC and HPLC comparisons with authentic samples. Following authentic standards were used, i.e., apigenin 7-*O*-glucuronide from the aerial parts of *Aeginetia indica* L. (Orobanchaceae) (Iwashina, 2010), luteolin 7-*O*-glucuronide from the leaves

of *Vitex rotundifolia* L.fil. (Verbenaceae) (Iwashina *et al.*, 2011) and schaftoside from the aerial parts of *Osyris alba* L. (Santalaceae) (Iwashina *et al.*, 2008). TLC, HPLC, UV and LC-MS data of the isolated flavonoids were as follows.

Apigenin 7-*O*-glucuronide (**1**). TLC (Rf): 0.55 (BAW), 0.41 (BEW) and 0.16 (15%HOAc); color (365 nm): UV—dark purple, UV/NH_3—dark greenish yellow. HPLC (*t*R): 7.33 min. UV λ_{max} (nm): MeOH 268, 332; + NaOMe 274, 379 (inc.); + $AlCl_3$ 266sh, 275, 298, 348, 375sh; + $AlCl_3$/HCl 266sh, 275, 299, 342, 374sh; + NaOAc 255sh, 267, 389; + NaOAc/H_3BO_3 267, 338. LC-MS: *m/z* 447 $[M+H]^+$, 445 $[M-H]^-$ (molecular ion peaks, apigenin + 1 mol glucuronic acid), *m/z* 271 $[M-176+H]^+$, 269 $[M-176-H]^-$ (fragment ion peaks, apigenin).

Luteolin 7-*O*-glucuronide (**2**). TLC (Rf): 0.36 (BAW), 0.21 (BEW) and 0.07 (15%HOAc); color (365 nm): UV—dark purple, UV/NH_3—dark yellow. HPLC (*t*R): 5.38 min. UV λ_{max} (nm): MeOH 256, 266sh, 349; + NaOMe 267, 393 (inc.); + $AlCl_3$ 273, 425; + $AlCl_3$/HCl 265, 273sh, 294sh, 359, 384sh; + NaOAc 261, 404; + NaOAc/H_3BO_3 259, 373. LC-MS: *m/z* 463 $[M+H]^+$, 461 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucuronic acid), *m/z* 287 $[M-176+H]^+$, 285 $[M-176-H]^-$ (fragment ion peaks, luteolin).

Schaftoside (apigenin 6-*C*-glucoside-8-*C*-arabinoside, **3**). TLC (Rf): 0.29 (BAW), 0.23 (BEW) and 0.31 (15%HOAc); color (365 nm): UV—dark purple, UV/NH_3—dark greenish yellow. HPLC (*t*R): 4.12 min. UV λ_{max} (nm): MeOH 274, 331; + NaOMe 276, 283, 330, 399 (inc.); + $AlCl_3$ 266, 275, 305, 354, 382sh; + $AlCl_3$/HCl 265, 276, 305, 353, 382sh; + NaOAc 276, 284, 409; + NaOAc/H_3BO_3 276, 284, 322, 350sh, 409. LC-MS: *m/z* 565 $[M+H]^+$, 563 $[M-H]^-$ (molecular ion peaks, apigenin + each 1 mol glucose and arabinose).

Kaempferol 3-*O*-(*p*-coumaroylglucoside) (tiliroside?, **4**). TLC (Rf): 0.92 (BAW), 0.95 (BEW) and 0.21 (15%HOAc); color (365 nm): UV—dark purple, UV/NH_3—dark greenish yellow. HPLC (*t*R): 20.98 min. UV λ_{max} (nm): MeOH 266, 297sh, 315, 353sh; + NaOMe 275, 367 (inc.); + $AlCl_3$ 275, 306, 320sh, 346sh, 388; + $AlCl_3$/HCl 276, 305, 318sh, 349sh, 387sh; + NaOAc 275, 312, 374; + NaOAc/H_3BO_3 267, 299sh, 317, 359sh. LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (molecular ion peaks, kaempferol + each 1 mol glucose and *p*-coumaric acid), *m/z* 287 $[M-324+H]^+$ (fragment ion peak, kaempferol).

Results and Discussion

Four flavonoids (**1**–**4**) were isolated from the leaves of *Triumfetta procumbens*.

It was shown by UV spectral properties that **1** is 5,4′-dihydroxy-7-substituted flavone. Apigenin and glucuronic acid were liberated by acid hydrolysis of **1**. Attachment of 1 mol glucuronic acid to apigenin was shown by LC-MS, i.e., appearance of a molecular ion peak, *m/z* 447 $[M+H]^+$. Finally, **1** was identified as apigenin 7-*O*-glucuronide (Fig. 1) by direct TLC and HPLC comparison with authentic sample.

Flavonoid **2** produced luteolin and glucuronic acid by acid hydrolysis. Since UV spectral properties of **2** were those of 7-substituted luteolin, it was shown that glucuronic acid is attached to 7-position of luteolin. Attachment of 1 mol glucuronic acid was indicated by LC-MS, i.e., appearance of a molecular ion peak, *m/z* 463 $[M+H]^+$. TLC and HPLC properties of **2** agreed with those of authentic luteolin 7-*O*-glucuronide. Thus, **2** was elucidated as luteolin 7-*O*-glucuronide (Fig. 2).

It was shown by UV spectra that **3** is a flavone having free 5-, 7- and 4′-hydroxyl groups. Moreover, since **3** is not hydrolysable by hot acid treatment, it is *C*-glycosylflavone. Attachment of

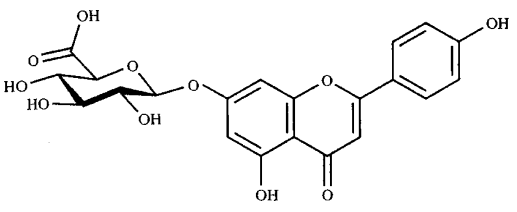

Fig. 1. Apigenin 7-*O*-glucuronide (**1**).

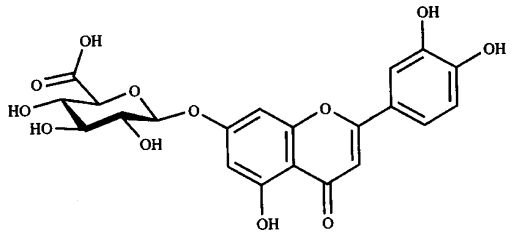

Fig. 2. Luteolin 7-*O*-glucuronide (**2**).

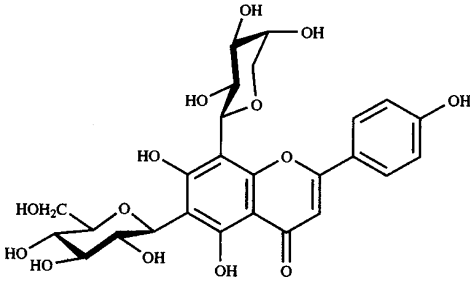

Fig. 3. Schaftoside (apigenin 6-*C*-glucoside-8-*C*-arabinoside, **3**)

each 1 mol hexose and pentose to apigenin was proved by LC-MS, i.e., appearance of a molecular ion peak, *m/z* 565 $[M+H]^+$. Finally, **3** was identified as apigenin 6-*C*-glucoside-8-*C*-arabinoside by direct TLC and HPLC comparison with authentic schaftoside (Fig. 3).

Absorption maxima of **4** were 266 (Band II) and 315 (Band I) nm and it seemed to be flavone glycoside. However, flavonol, kaempferol, was liberated by acid hydrolysis, together with glucose and also *p*-coumaric acid. Since UV spectral survey of the original glycoside showed the presence of free 5-, 7- and 4′-hydroxyl groups of kaempferol, both glucose and *p*-coumaric acid are on 3-position of kaempferol. LC-MS survey of **4** showed the attachment of each 1 mol glucose and *p*-coumaric acid. Thus, **4** was characterized as kaempferol 3-*O*-(*p*-coumaroylglucoside) (Fig. 4).

As flavonoid characters of the family Tiliaceae s. s., flavonols, kaempferol, quercetin and myricetin, flavones, apigenin and luteolin, *C*-glycosylflavonoids and proanthocyanidins have been reported (Giannasi, 1988). In this survey, the flavonoids of *Triumfetta procumbens* were reported

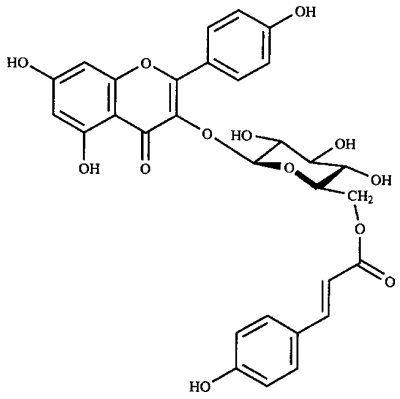

Fig. 4. Kaempferol 3-*O*-(*p*-coumaroylglucoside) (**4**).

for the first time. Of four flavonoid glycosides isolated, **1** and **2** were flavone glucuronides, and **3** and **4** were *C*-glycosylflavone and acylated flavonol glycoside, respectively. The flavonoids in *Triumfetta* species have been reported from *T. rhomboidea* and two flavone glycosides, scutellarein 7-*O*-rhamnoside and scutellarein 6-*O*-xyloside-7-*O*-rhamnoside, were detected (Srinivasan and Subramanian, 1981; Nair *et al.*, 1986). Flavone glucuronides were found in the family Tiliaceae s. s. for the first time and are new chemotaxonomic character of the genus *Triumfetta*, together with *C*-glycosylflavone. Kaempferol 3-*O*-(*p*-coumaroylglucoside) has been reported from two *Tilia* species, *T. argentea* and *T. platyphyllos* (Hegnauer, 1973; Hörhammer *et al.*, 1959). It was named as tiliroside and completely identified as kaempferol 3-*O*-(6″-*p*-coumaroylglucoside) by Harborne (1964). Though it could not be determined that kaempferol 3-*O*-(*p*-coumaroylglucoside), which was isolated in this survey, is the same with tiliroside, acylated flavone glycoside was also found in the genus *Triumfetta* for the first time.

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