Bull. Natl. Mus. Nat. Sci., Ser. B, **34**(4), pp. 175–181, December 22, 2008

Foliar Flavonoids from Two *Begonia* Species in Japan

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Abstract Flavonoid compounds in the leaves of two Japanese *Begonia* species, *B. formosana* and *B. fenicis*, were surveyed. The compounds were isolated by various chromatographic techniques and identified by UV spectra, LC-MS, acid hydrolysis, and direct TLC and HPLC comparisons with authentic samples. Four *C*-glycosylflavones, isoorientin, orientin, vicenin-2 and apigenin 6-*C*-pentoside-8-*C*-hexoside (or 6-*C*-hexoside-8-*C*-pentoside), and two flavonol *O*-glycosides, quercetin 3-*O*-rutinoside and kaempferol 3-*O*-gentiobioside, were detected in *B. formosana*. On the other hand, only one flavonol *O*-glycoside, quercetin 3-*O*-rutinoside was found in *B. fenicis*. From the results obtained in this survey, the flavonoid diversity of the genus *Begonia* including ca. 900 species was presumed.

Key words: Begoniaceae, *Begonia fenicis*, *Begonia formosana*, *C*-glycosylflavones, flavonoids, flavonol *O*-glycosides.

Introduction

The genus *Begonia* consists of ca. 900 species and is distributed to warm temperate from tropical zone, especially North America (Mabberley, 1997). They were hybridized among some species, and many cultivars were made and grown as ornamentals. Only two wild species, *Begonia formosana* (Hayata) Masam. and *B. fenicis* Merr. are distributed in Japan (Hatusima and Amano, 1994), and listed as endangered plants by the Ministry of Environment, Japan (Environment Agency of Japan, 2000).

Flavonoid components have been reported in some *Begonia* species. In many cases, the anthocyanins in the leaves and flowers have been surveyed. Cyanidin 3-*O*-glucoside, 3-*O*-sambubioside, 3-*O*-sophoroside, 3-*O*-glucosylrutinoside, 3-*O*-xylosylrutinoside were detected in *Begonia coccinea* Hook., *B. metallica* W. G. Smith, *B. pearcei* Hook. f., *B. haageana* Regel et Schmidt var. *frostii*, *B. glaucophylla* Hook. f., *B. manicata* Brongn., *B. argenteo-guttata* Hort. ex L.H. Bailey, *B. rex* Putz., *B.* ×*richmondensis*, *B. fuchsioides* Hook. var. *rosea*, *B. martiana* Link et Otto, *B.* cv. "Woodruff's Scarlet", *B.* cv. "Courbeille de Feu" and *B.* cv. "President Carnot" (Harborne and Hall, 1964). Of their anthocyanins, cyanidin 3-*O*-xylosylrutinoside was completely identified as cyanidin 3-*O*-(2″-xylosyl-6″-rhamnosylglucoside) which were present as a major pigment in the leaves of 18 *Begonia* species, e.g., *B. angularis* Raddi, *B. dietrichiana* Irmsch. and *B. sanguinea* Raddi (Langhammer and Grandet, 1974). Cyanidin 3-*O*-gentiobioside has been detected in 51 *Begonia* species such as *B. bunchii* Hort. ex L. H. Bailey, *B. manicata* Brongn. and *B. metachroa* Fotsch. (Bopp, 1957). Pelargonidin 3-*O*-glucoside and peonidin 5-*O*-glucoside were found in the flowers of *B. suther-*

landii Hook. f. (Harborne and Hall, 1964) and the leaves of *B. pavonina* Rindl. (Gould *et al.*, 1995), respectively. Partially characterized cyanidin glycosides were reported from the flowers of *B. heracleifolia* Cham. et Schlecht., *B. senperflorens* Link et Otto, *B. socotrana* Hook. f. and *B. froebelii* A. DC. (Forsyth and Simmonds, 1954). Six acylated anthocyanins were isolated from the flowers of various *Begonia* cultivars and identified as cyanidin 3-*O*-(2″-xylosyl-6″-*trans*-caffeoylglucoside), 3-*O*-(2″-xylosyl-6″-*cis*-caffeoylglucoside), 3-*O*-(2″-xylosyl-6″-*trans*-*p*-coumaroylglucoside), 3-*O*-(2″-xylosyl-6″-*cis*-*p*-coumaroylglucoside), 3-*O*-(2″-glucosyl-6″-*trans*-*p*-coumaroylglucoside) and 3-*O*-(2″-glucosyl-6″-*cis*-*p*-coumaroylglucoside) (Chirol and Jay, 1995).

Flavan 3-ols and proanthocyanidins, (+)-catechin and procyanidin B_1, and (−)-epicatechin and procyanidin B_2 were isolated from the leaves of *B. glabra* Aublet and *B. fagifolia* Fischer (Ensemeyer *et al.*, 1980; Ensemeyer and Langhammer, 1984). Flavones, luteolin and its 7-*O*-glycoside, and 5,7,4′-trihydroxyflavone 6-*O*-glucoside were reported from *B. erythrophylla* Neum. (Vereskovskii *et al.*, 1988a) and *B. evansiana* Andr. (Zhang *et al.*, 1997), respectively. However, this flavonoid may be 5,7,4′-trihydroxyflavone 6-*C*-glucoside, i.e., isovitexin, and misidentified by Zhang *et al.* (1997), because hydroxyl group is not present at 6-position of 5,7,4′-trihydroxyflavone. In *B. erythrophylla*, four *C*-glycosylflavones, orientin, isoorientin, vitexin and isovitexin were accompanied with flavone *O*-glycoside (Vereskovskii *et al.*, 1988b). Flavonols and their glycosides were reported from almost species which have been surveyed for flavonoids. Quercetin and its 3-*O*-rutinoside (rutin), 3-*O*-rhamnoside (quercitrin), 3-*O*-glucoside (isoquercitrin) and 3-*O*-xyloside (reynoutrin), kaempferol 3-*O*-glucoside (astragalin), isokaempferide (kaempferol 3-methyl ether) and its 7-*O*-glucoside, quercetin 3-methyl ether and its 7-*O*-glycoside, pachypodol (quercetin 3,7,3′-trimethyl ether), and ternatin (gossypetin 3,7,8,3′-tetramethyl ether) were isolated from the leaves and/or flowers of *B.* ×*lucerna*, *B. metallica*, *B. haageana* var. *rosea*, *B. glaucophylla*, *B.* ×*argenteo-guttata*, *B. rex*, *B.* ×*richmondensis*, *B. pearcei*, *B. manicata* and *B. martiana* (Harborne and Hall, 1964), *B. erythrophylla* (Vereskovskii *et al.*, 1988a), 30 *Begonia* species (Bopp, 1957), *B. fagifolia* (Esemeyer and Langhammer, 1984), and *B. glabra* Ruiz ex Klotzsch. (Esemeyer and Langhammer, 1982). However, flavonoids in *B. formosana* and *B. fenicis*, which were used as plant materials in this experiment, have not been surveyed. In this paper, the isolation and identification of the foliar flavonoids in their species are described.

Materials and Methods

Plant materials

Two *Begonia* species, *B. formosana* (Hayata) Masam. (Fig. 1) and *B. fenicis* Merr. (Fig. 2) were used as plant materials. *Begonia formosana* and *B. fenicis* were collected in south slope of Mt. Banna, Ishigaki Is., Okinawa, Japan (GK305), and Utsun River, Iriomote Is., Okinawa, Japan (GK1335), respectively. Voucher specimens have been depositerd in the National Museum of Nature and Science, Japan (TNS).

Extraction and isolation of flavonoids

Fresh leaves of *B. formosana* (6.4 g) and *B. fenicis* (95.3 g) were extracted with MeOH, respectively. The concentrated extracts were 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 (Pharmacia) column chromatography using solvent system, 70% MeOH.

High performance liquid chromatography (HPLC)

Qualitative 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

Fig. 1. *Begonia formosana* (Hayata) Masam.

was 190–700 nm and eluent was MeCN/H_2O/H_3PO_4 (20 : 80 : 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. Ltd.), at a flow-rate of 0.2 ml min^{-1}, eluent: MeCN/H_2O/HCOOH (15 : 80 : 5), and ESI^+ 4.5 kV, ESI^- 3.5 kV, 250°C.

Identification of flavonoids

The isolated flavonoids were identified by UV spectra according to Mabry *et al.* (1970), LC-MS, acid hydrolysis and characterization of hy-

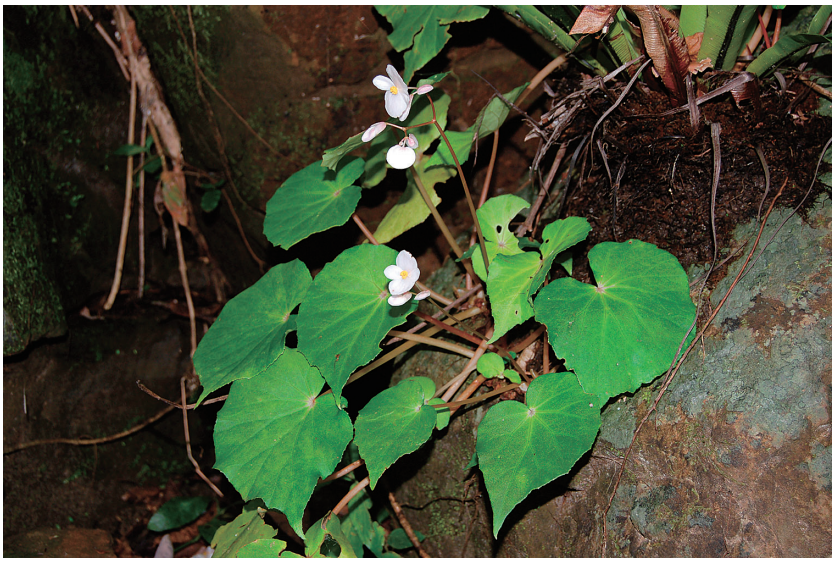

Fig. 2. *Begonia fenicis* Merr.

drolysates, and direct TLC and HPLC comparisons with authentic samples. TLC, HPLC, UV, LC-MS and acid hydrolysis data were as follows.

Quercetin 3-*O*-rutinoside (rutin, **1**). TLC: Rf 0.47 (BAW), 0.53 (BEW), 0.46 (15% HOAc); UV — dark purple, UV/NH_3 — yellow. HPLC: *t*R (min) 7.35. UV: λmax (nm) MeOH 257, 265sh, 358; +NaOMe 273, 326, 411 (inc.); +$AlCl_3$ 274, 432; +$AlCl_3$/HCl 267, 300, 363, 396sh; +NaOAc 273, 326, 400; +NaOAc/H_3BO_3 262, 380. LC-MS: *m/z* 611 $[M+H]^+$, 609 $[M-H]^-$ (quercetin + each 1 mol glucose and rhamnose); *m/z* 465 $[M-146+H]^+$ (quercetin + 1 mol glucose); *m/z* 303 $[M-308+H]^+$ (quercetin). Acid hydrolysis (in 12% HCl, 100°C, 30 min): quercetin, glucose and rhamnose.

Kaempferol 3-*O*-gentiobioside (**2**). TLC: Rf 0.41 (BAW), 0.56 (BEW), 0.48 (15%HOAc); UV — dark purple, UV/NH_3 — dark greenish yellow. HPLC: *t*R (min) 7.48. UV: λmax (nm) MeOH 266, 297sh, 351; +NaOMe 275, 325, 399 (inc.); +$AlCl_3$ 274, 305, 355, 395; +$AlCl_3$/HCl 275, 303, 349, 393; +NaOAc 275, 312, 391; +NaOAc/H_3BO_3 267, 297sh, 355. LC-MS: *m/z* 611 $[M+H]^+$, 609 $[M-H]^-$ (kaempferol + 2 mol glucose). Acid hydrolysis: kaempferol and glucose.

Luteolin 6-*C*-glucoside (isoorientin, **3**). TLC: Rf 0.43 (BAW), 0.52 (BEW), 0.24 (15% HOAc); UV — dark purple, UV/NH_3 — yellow. HPLC: *t*R (min) 5.34. UV: λmax (nm) MeOH 257, 270, 349; +NaOMe 270, 327sh, 411 (inc.); +$AlCl_3$ 275, 422; +$AlCl_3$/HCl 263sh, 277, 296sh, 360, 384sh; +NaOAc 275, 327sh, 400; +NaOAc/H_3BO_3 267, 379, 427sh. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (luteolin + 1 mol glucose). Acid hydrolysis: unhydrolyzable.

Luteolin 8-*C*-glucoside (orientin, **4**). TLC: Rf 0.22 (BAW), 0.29 (BEW), 0.09 (15% HOAc); UV — dark purple, UV/NH_3 — yellow. HPLC: *t*R (min) 6.19. UV: λmax (nm) MeOH 257, 268, 293sh, 349; +NaOMe 271, 327sh, 407 (inc.); +$AlCl_3$ 274, 422; +$AlCl_3$/HCl 265, 276, 297, 358, 385sh; +NaOAc 276, 327sh, 396; +NaOAc/H_3BO_3 266, 377, 427sh. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (luteolin + 1 mol glucose). Acid hydrolysis: unhydrolyzable.

Apigenin 6,8-di-*C*-glucoside (vicenin-2, **5**). TLC: Rf 0.22 (BAW), 0.32 (BEW), 0.39 (15% HOAc); UV — dark purple, UV/NH_3 — dark greenish yellow. HPLC: *t*R (min) 4.12. UV: λmax (nm) MeOH 273, 332; +NaOMe 283, 334, 400 (inc.); +$AlCl_3$ 280, 306, 353, 385sh; +$AlCl_3$/HCl 280, 304, 348, 383sh; +NaOAc 282, 397; +NaOAc/H_3BO_3 277sh, 285, 321, 355, 411. LC-MS: *m/z* 593 $[M-H]^-$ (apigenin + 2 mol glucose). Acid hydrolysis: unhydrolyzable.

Apigenin 6-*C*-pentoside-8-*C*-hexoside (or 6-*C*-hexoside-8-*C*-pentoside, **6**). TLC: Rf 0.26 (BAW), 0.32 (BEW), 0.32 (15%HOAc); UV — dark purple, UV/NH_3 — dark greenish yellow. HPLC: *t*R (min) 5.10. UV: λmax (nm) MeOH 274, 334; +NaOMe 283, 333, 401 (inc.); +$AlCl_3$ 227, 306, 356, 383sh; +$AlCl_3$/HCl 275, 305, 353, 383sh; +NaOAc 283, 396; +NaOAc/H_3BO_3 276, 285, 321, 352, 413. LC-MS: *m/z* 565 $[M+H]^+$, *m/z* 563 $[M-H]^-$ (apigenin + each 1 mol hexose and pentose). Acid hydrolysis: unhydrolyzable.

Results and Discussion

Six flavonoids were isolated from the leaves of *B. formosana* (Table 1). On the other hand, only one flavonoid (**1**) was found in *B. fenicis*. Flavonoid **1**, which is present in both species, was obtained as pale yellow powder. Quercetin, glucose and rhamnose were liberated by acid hydrolysis. UV spectral survey in addition to various shift reagents showed the presence of free 5-, 7-, 3′- and 4′-hydroxyl and a substituted 3-hydroxyl groups. The attachment of each 1 mol glucose and rhamnose to quercetin was shown by LC-MS. Finally, flavonoid **1** was identified as quercetin 3-*O*-rutinoside (Fig. 3) by direct TLC and HPLC comparison with authentic rutin from the aerial parts of *Osyris alba* L. (Santalaceae) (Iwashina *et al.*, 2008a). Quercetin 3-*O*-rutinoside has been found in *Begonia* species (Bopp, 1957; Harborne and Hall, 1964; Vereskovskii *et al.*, 1988a).

Table 1. Distribution of flavonoids in the leaves of *Begonia formosana* and *B. fenicis*

Species	Flavonoids					
	1	**2**	**3**	**4**	**5**	**6**
B. formosana	+	+	++	++	+	+
B. fenicis	++					

1=quercetin 3-*O*-rutinoside, 2=kaempferol 3-*O*-gentiobioside, 3=isoorientin, 4=orientin, 5=vicenin-2 and 6=apigenin 6-*C*-pentoside-8-*C*-hexoside or 6-*C*-hexoside-8-*C*-pentoside.

Flavonoid **2** was obtained as a minor compound from *B. formosana*. UV spectral survey according to Mabry *et al.* (1970) showed the presence of free 5-, 7- and 4′-hydroxyl and a substituted 3-hydroxyl groups. Since kaempferol and glucose were produced by acid hydrolysis, it is clear that glucose is attached to 3-position of kaempferol. LC-MS was appeared the molecular ion peak, *m*/*z* 611 $[M+H]^+$, showing the attachment of 2 mol glucose to kaempferol. Thus, flavonoid **2** was characterized as kaempferol 3-*O*-diglucoside and finally identified as kaempferol 3-*O*-glucosyl-(1→6)-glucoside, i.e. kaempferol 3-*O*-gentiobioside (Fig. 4) by direct TLC and HPLC comparison with authentic sample from the fronds of *Asplenium incisum* Thunb. (Aspleniaceae) (Iwashina *et al.*, 2000). Kaempferol 3-O-gentiobioside was isolated from *Begonia* species for the first time.

Flavonoids **3** and **4** were obtained as pale yellow powders from *B. formosana*. UV spectral properties of them were those of 5,7,3′,4′-tetrahydroxyflavones. Since they were not hydrolyzable by hot acid treatment, it is clear that their flavonoids are C-glycosylflavones. Molecular ion peak, *m*/*z* 449 $[M+H]^+$ appeared by LC-MS, showing the attachment of 1 mol hexose to 5,7,3′,4′-tetrahydroxyflavone. Finally, flavonoids **3** and **4** were identified as luteolin 6-*C*-glucoside (Fig. 5) and luteolin 8-*C*-glucoside (Fig. 6) by direct TLC and HPLC comparisons with authentic isoorientin and orientin from the leaves of *Acer palmatum* Thunb. (Aceraceae) (Iwashina and Murai, 2008). Isoorientin and orientin have been reported from the leaves of *B. erythrophylla* (Vereskovskii *et al.*, 1988b).

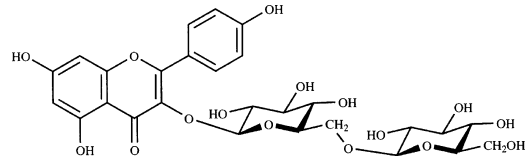

Fig. 4. Kaempferol 3-*O*-gentiobioside (**2**).

Flavonoids **5** and **6** were also *C*-glycosylflavones which were shown by hot acid treatment. It was shown by LC-MS survey that the former is apigenin di-*C*-hexoside. Finally, flavonoid **5** was identified as apigenin 6,8-di-*C*-glucoside (vicenin-2, Fig. 7) by direct TLC and HPLC comparison with authentic sample from the fronds of *Asplenium normale* D. Don (Aspleniaceae) (Iwashina *et al.*, 2008b). Vicenin-2 was isolated from *Begonia* species for the first time. It was shown by LC-MS that flavonoid **6** is apigenin attached each 1 mol pentose and hexose. The attachment of their sugars to both 6- and 8-

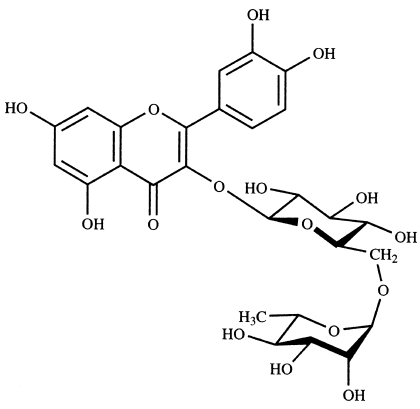

Fig. 3. Quercetin 3-*O*-rutinoside (**1**).

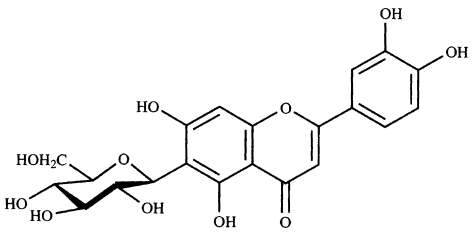

Fig. 5. Isoorientin (**3**).

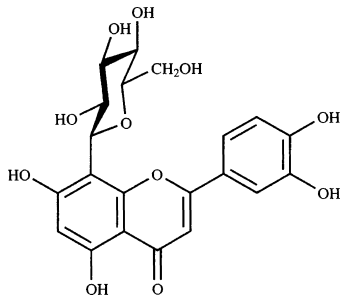

Fig. 6. Orientin (**4**).

positions was determined by UV spectral survey according to Mabry *et al.* (1970). However, complete identification of the glycoside was not performed, since NMR spectra was not measured for meager amount of the isolated compound. Thus, flavonoid **6** was partially characterized as apigenin 6-*C*-pentoside-8-*C*-hexoside or 6-*C*-hexoside-8-*C*-pentoside such as schaftoside and isoschaftoside (Iwashina *et al.*, 2008a), which have not been found in *Begonia* species.

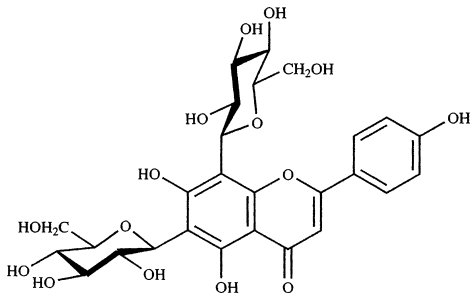

Fig. 7. Vicenin-2 (**5**).

Only two species, *B. formosana* and *B. fenicis* are distributed in Japan. However, their flavonoid composition was remarkably different to each other, i.e., flavonol only in *B. fenicis* and flavonols and *C*-glycosylflavones in *B. formosana*. Flavonoids have fragmentarily been surveyed among some *Begonia* species in spite of the big genus including ca. 900 species. However, five flavonoid classes, i.e. anthocyanins, flavones, C-glycosylflavones, flavonols, and flavans and proanthocyanidins have been reported (Bopp 1957; Chirol and Jay, 1995; Ensemeyer *et al.*, 1980; Ensemeyer and Langhammer, 1982, 1984; Forsyth and Simmonds, 1954; Gould *et al.*, 1995; Harborne and Hall, 1964; Langhammer and grandet, 1974; Vereskovskii *et al.*, 1987a, 1987b; Zhang *et al.*, 1997). From the results obtained in this survey and also references, flavonoid diversity of the *Begonia* was presumed and we are now in progress for flavonoid survey of the genus.

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