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Flavonoids in the Leaves and Flowers of *Myoporum bontioides* Native to Northernmost Region in the Myoporaceae

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Abstract Ten flavonoids, one anthocyanin and two organic acids were isolated from the leaves and flowers of *Myoporum bontioides*, which is native to northernmost region in the family Myoporaceae. Each one anthocyanin and organic acid, and two flavonoids from the flowers were cyanidin 3,5-di-*O*-glucoside, acteoside and apigenin 7-*O*-glucuronide and luteolin 7-*O*-glucuronide. On the other hand, fourteen foliar flavonoids were characterized as apigenin 7-*O*-glucuronide, luteolin 7-*O*-glucoside, luteolin 7-*O*-glucuronide, chrysoeriol 7-*O*-glucoside, chrysoeriol 7-*O*-glucuronide, selagin 7-*O*-glucoside, selagin 7-*O*-glucuronide, tricin 7-*O*-glucuronide, quercetin 3-methyl ether, chrysoeriol, isokaempferide, apigenin, luteolin, quercetin 3,4′-dimethyl ether. Two organic acids were identified as chlorogenic acid and acteoside.

Key words: flavones, flavonoids, Myoporaceae, *Myoporum bontioides*, northernmost species.

Introduction

The genus *Myoporum* is consists of ca. 30 species and mainly distributed in Australia, and a few species in New Zealand, New Guinea, Pacific islands and East Asia (Yamazaki, 1989). Two species, *M. bontioides* (Sieb. et Zucc.) A. Gray and *M. boninense* Koidz., were native to Japan as northernmost species in the family. Both species were nominated as endangered plants by the Ministry of Environment, Japan (Environment Agency of Japan, 2000).

Flavonoids in the family Myoporaceae has been reported from *Eremophila* and *Myoporum* species. Harborne and Williams (1971) have surveyed the flavonoids in the leaves of six Myoporaceous species, and detected quercetin and kaempferol from *Eremophila oppositifolia* R. Br. and *E. sturtii* R. Br., unidentified flavone from *E. latifolia* F. Muell., luteolin from *Myoporum deserti* A. Cunn. and *M. acuminatum* R. Br., and kaempferol from *M. serratum* R. Br. Flavonoids have been isolated from *Eremophila ramosissima* Lehm. and *E. alternifolia* R. Br. and identified as a dihydroflavonol, pinobanksin and a flavonol, galangin (Jefferies *et al*., 1962). 5-Hydroxy-3,6,7,3′,4′,5′-hexamethoxyflavone and 5,3′,5′-trihydroxy-3,6,7,4′-tetramethoxyflavone were reported from another *Eremophila* species, *E. fraseri* F. Muell. (Jefferies *et al*., 1962).

In the genus *Myoporum*, eleven flavone aglycones and glycosides, apigenin and its 7-*O*-rutinoside, chrysoeriol and its 7-*O*-gentiobioside and 7-*O*-rutinoside, luteolin and its 7-*O*-glucoside, 7-*O*-gentiobioside and 7-*O*-rutinoside, tricin and its 7-*O*-glucuronide, and a flavanone, eriodictyol 7-*O*-rutinoside have been reported from the leaves of *M. tenuifolium* G. Forster (Tomas *et al*., 1985). Recently, a flavanone, pinocembrin and two flavonols, galangin and ermanin have been isolated from the leaves of *Myoporum bontioides* as bioactivity compounds to *Pletella xylostella* (L.) (Gu *et al*., 2004). Futhermore, four flavone glycosides, 7-*O*-glucosides of apigenin, luteolin, chrysoeriol and tricin, were isolated from the same species, together with some iridoids, aceto-

genin and monoterpene (Kanemoto *et al*., 2008).

In this paper, isolation and identification of the flavonoids from the leaves and flowers of northernmost species, *M. bontioides*, in the Myoporaceae are described.

Materials and Methods

Plant materials

Myoporum bontiodes (Sieb. et Zucc.) A. Gray (Fig. 1) used as plant materials in this experiment was collected in Uchiumi, Amami-Ohshima Is. Kagoshima Pref. Japan. Voucher specimen was deposited to the herbarium of National Museum of Nature and Science, Japan (TNS).

Extraction and isolation of anthocyanin and other flavonoids in flowers

Fresh flowers (6.5 g) were extracted with 8% HCOOH in MeOH. The concentrated extracts were applied to paper chromatography (PPC) using solvent systems: BAW (*n*-BuOH/HOAc/H_2O=4 : 1 : 5, upper phase) and then 15% HOAc. One anthocyanin band and a few flavonoid mixture bands were obtained and eluted with 8% HCOOH in MeOH (anthocyanin) or MeOH (other flavonoids). Anthocyanin was purified by Sephadex LH-20 column chromatography using solvent system: MeOH/H_2O/HCOOH (70 : 25 : 5). On the other hand, other flavonoid mixtures were applied to PPC using solvent system: BEW (*n*-BuOH/EtOH/H_2O=4 : 1 : 2.2) and then purified by Sephadex LH-20 column chromatography using solvent system: 70% MeOH.

Extraction and isolation of flavonoids in leaves

Fresh leaves (383 g) were extracted with MeOH. After concentration, flavonoid compounds were isolated from the extracts by PPC using solvent systems: BAW, 15% HOAc and then BEW. The crude flavonoids were furthermore purified by Sephadex LH-20 column chromatography using solvent system: 70% MeOH.

Fig. 1. *Myoporum bontioides* (Sieb. et Zucc.) A. Gray.

High-performance liquid chromatography (HPLC)

HPLC was performed with Shimadzu HPLC systems using a Senshu Pak 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 530 nm (anthocyanin) or 350 nm (other flavonoids) and the eluents were MeCN/HOAc/H_2O/H_3PO_4 (6 : 8 : 83 : 3) for anthocyanin and MeCN/H_2O/H_3PO_4 (20 : 80 : 0.2) for other flavonoids.

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. The eluents were MeCN/H_2O/HCOOH (12 : 83 : 5) for anthocyanin and MeCN/H_2O/HCOOH (18 : 77 : 5, glycosides and organic acids) or (30 : 65: 5, aglycones).

Identification of anthocyanin and other compounds

Anthocyanin, other flavonoids and organic acids were identified by UV spectral survey, acid hydrolysis (in 12% HCl, 100°C, 3 min, for anthocyanin, 30 min for other flavonoids), LC-MS and direct TLC and HPLC comparisons with authentic samples. TLC, UV spectral and LC-MS data of their compounds are as follows.

Cyanidin 3,5-di-*O*-glucoside (cyanin, A1).

TLC: Rf 0.13 (BAW), 0.08 (1% HCl); visible—red purple. UV: λ_{max} (nm) 0.1% MeOH–HCl 277, 524; E_{440}/E_{max} 22.3%. LC-MS: *m/z* 611 $[M]^+$ (cyanidin+2 mol glucose).

Apigenin 7-*O*-glucuronide (F1). TLC: Rf 0.67 (BAW), 0.45 (BEW), 0.13 (15% HOAc); UV—dark purple; UV/NH_3—dark greenish yellow. UV: λ_{max} (nm) MeOH 268, 332; +NaOMe 273, 379 (inc.); +$AlCl_3$ 275, 299, 347, 378; +$AlCl_3$/HCl 276, 298, 340, 376; +NaOAc 256, 266, 389; +NaOAc/H_3BO_3 268, 340. LC-MS: *m/z* 447 $[M+H]^+$, *m/z* 445 $[M-H]^-$(apigenin+1 mol glucuronic acid), *m/z* 271 $[M-176+H]^+$, *m/z* 269 $[M-176-H]^-$ (apigenin).

Luteolin 7-*O*-glucuronide (F2). TLC: Rf 0.35 (BAW), 0.33 (BEW), 0.05 (15% HOAc); UV—dark purple; UV/NH_3—dark yellow. UV: λ_{max} (nm) MeOH 254, 267, 347; +NaOMe 266, 391 (inc.); +$AlCl_3$ 273, 415; +$AlCl_3$/HCl 265, 273sh, 358, 381; +NaOAc 259, 406; +NaOAc/H_3BO_3 258, 363. LC-MS: *m/z* 463 $[M+H]^+$, *m/z* 461 $[M-H]^-$ (luteolin+1 mol glucuronic acid), *m/z* 287 $[M-176+H]^+$, *m/z* 285 $[M-176-H]^-$ (luteolin).

Luteolin 7-*O*-glucoside (F3). TLC: Rf 0.47 (BAW), 0.45 (BEW), 0.05 (15% HOAc); UV—dark purple; UV/NH_3—dark yellow. UV: λ_{max} (nm) MeOH 255, 265sh, 350; +NaOMe 268, 393 (inc.); +$AlCl_3$ 273, 426; +$AlCl_3$/HCl 266, 273sh, 359, 384sh; +NaOAc 260, 403; +NaOAc/H_3BO_3 259, 373. LC-MS: *m/z* 449 $[M+H]^+$, *m/z* 447 $[M-H]^-$ (luteolin+1 mol glucose), *m/z* 287 $[M-162+H]^+$ (luteolin).

Mixture of tricin 7-*O*-glucuronide (F4) and selagin 7-*O*-glucuronide (F5). TLC: Rf 0.37 (BAW), 0.24 (BEW), 0.03 (15% HOAc); UV—dark purple; UV/NH_3—dark yellow. UV: λ_{max} (nm) MeOH 247, 268sh, 351; +NaOMe 247sh, 261, 399 (inc.); +$AlCl_3$ 272, 303sh, 364sh, 403; +$AlCl_3$/HCl 257sh, 275, 300, 363, 385sh; +NaOAc 258, 422; +NaOAc/H_3BO_3 267sh, 360, 385sh. LC-MS: *m/z* 493 $[M+H]^+$, *m/z* 491 $[M-H]^-$ (selagin+1 mol glucuronic acid), *m/z* 317 $[M-176+H]^+$, *m/z* 315 $[M-176-H]^-$ (selagin), *m/z* 507 $[M+H]^+$, *m/z* 505 $[M-H]^-$ (tricin+1 mol glucuronic acid), *m/z* 331 $[M-176+H]^+$, *m/z* 329 $[M-176-H]^-$ (tricin).

Selagin 7-*O*-glucoside (F6). TLC: Rf 0.39 (BAW), 0.33 (BEW), 0.04 (15% HOAc); UV—dark purple; UV/NH_3—dark yellow. UV: λ_{max} (nm) MeOH 247, 266sh, 353; +NaOMe 265, 401 (inc.); +$AlCl_3$ 271, 433; +$AlCl_3$/HCl 266sh, 275, 302, 363, 387; +NaOAc 257, 421; +NaOAc/H_3BO_3 256, 379. LC-MS: *m/z* 479 $[M+H]^+$, *m/z* 477 $[M-H]^-$ (selagin+1 mol glucose), *m/z* 317 $[M-162+H]^+$, *m/z* 315 $[M-162-H]^-$ (selagin).

Chrysoeriol 7-*O*-glucuronide (F7). TLC: Rf 0.53 (BAW), 0.33 (BEW), 0.07 (15% HOAc); UV—dark purple; UV/NH_3—dark yellow. UV: λ_{max} (nm) MeOH 251, 268, 345; +NaOMe 245sh, 263, 389 (inc.); +$AlCl_3$ 265, 272, 295sh, 365, 383; +$AlCl_3$/HCl 262, 273, 295sh, 356, 383sh; +NaOAc 259, 408; +NaOAc/H_3BO_3 250sh, 266, 349. LC-MS: *m/z* 477 $[M+H]^+$, *m/z* 475 $[M-H]^-$ (chrysoeriol+1 mol glucuronic acid), *m/z* 301 $[M-176+H]^+$, *m/z* 299 $[M-176-H]^-$ (chrysoeriol).

Chrysoeriol 7-*O*-glucoside (F8). LC-MS: *m/z* 463 $[M+H]^+$, *m/z* 461 $[M-H]^-$ (chrysoeriol+1 mol glucose).

Quercetin 3-methyl ether (F9). λ_{max} (nm) MeOH 257, 264sh, 358; +NaOMe 272, 328, 403 (inc.); +$AlCl_3$ 275,435; +$AlCl_3$/HCl 267, 298, 360, 395sh; +NaOAc 272, 326, 395; +NaOAc/H_3BO_3 261, 297sh, 376. LC-MS: *m/z* 317 $[M+H]^+$, *m/z* 315 $[M-H]^-$ (quercetin 3-methyl ether).

Isokaempferide (F10). LC-MS: *m/z* 301 $[M+H]^+$, *m/z* 299 $[M-H]^-$ (isokaempferide).

Chrysoeriol (F11). LC-MS: *m/z* 301 $[M+H]^+$, *m/z* 299 $[M-H]^-$ (chrysoeriol).

Apigenin (F12). LC-MS: *m/z* 271 $[M+H]^+$, *m/z* 269 $[M-H]^-$ (apigenin).

Luteolin (F13). UV: λ_{max} (nm) MeOH 257sh, 265, 365; +NaOMe 265sh, 273, 328sh, 399 (inc.); +$AlCl_3$ 267sh, 272,420; +$AlCl_3$/HCl 265, 273sh, 296sh, 357, 387sh; +NaOAc 267, 273sh, 393; +NaOAc/H_3BO_3 266, 375. LC-MS: *m/z* 287 $[M+H]^+$, *m/z* 285 $[M-H]^-$ (luteolin).

Quercetin 3,4′-dimethyl ether (F14). UV: λ_{max} (nm) MeOH 268, 287, 350; +NaOMe 274, 325,

384 (dec.); +$AlCl_3$ 275, 310, 367, 395sh; +$AlCl_3$/HCl 276, 304, 359, 393sh; +NaOAc 275, 326, 378; +NaOAc/H_3BO_3 268, 287, 349. LC-MS: *m/z* 331 $[M+H]^+$, *m/z* 329 $[M-H]^-$ (quercetin 3,4′-dimethyl ether).

Chlorogenic acid (O1). LC-MS: *m/z* 355 $[M+H]^+$, *m/z* 353 $[M-H]^-$ (chlorogenic acid).

Acteoside (O2). UV: λ_{max} (nm) MeOH 246sh, 292, 331; +NaOMe 259, 382 (inc.); +$AlCl_3$ 261, 302, 363; +$AlCl_3$/HCl 244sh, 290sh, 331; +NaOAc 252sh, 291, 354sh, 372; +NaOAc/H_3BO_3 256, 296, 354. LC-MS: *m/z* 623 $[M-H]^-$ (acteoside).

Results and Discussion

In HPLC survey of crude extracts from the flowers and leaves of *Myoporum bontioides*, some flavonoid peaks appeared on the chromatograms (Figs. 2 and 3).

Anthocyanin A1 was isolated from the red spotted pale reddish purple flowers of this species. Cyanidin and glucose were liberated by

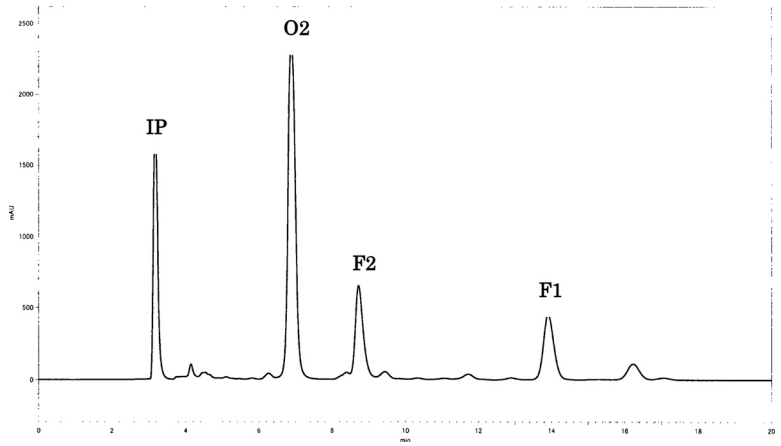

Fig. 2. HPLC chromatogram of MeOH extracts from the flowers of *Myoporum bontioides*.
F1=apigenin 7-glucuronide, F2=luteolin 7-glucuronide, O2=acteoside and IP=injection peak.

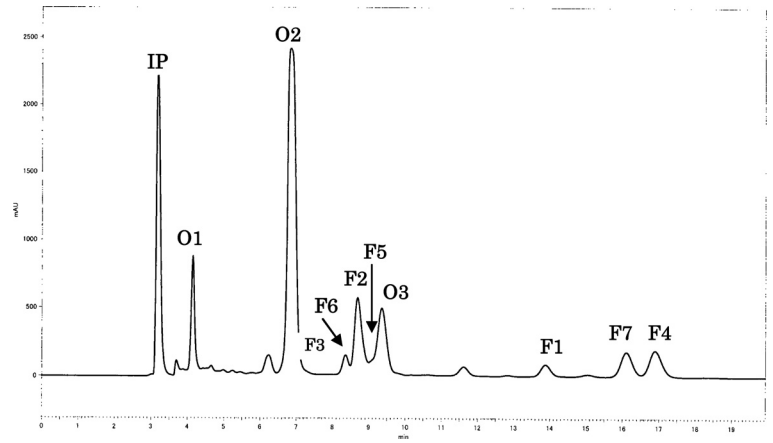

Fig. 3. HPLC chromatogram of MeOH extracts from the leaves of *Myoporum bontioides*. F1=apigenin 7-glucuronide, F2=luteolin 7-glucuronide, F3=luteolin 7-glucoside, F4=tricin 7-glucuronide, F5=selagin 7-glucuronide, F6=selagin 7-glucoside, F7=chrysoeriol 7-glucuronide, O1=chlorogenic acid, O2=acteoside, O3=unidentified organic acid and IP=injection peak.

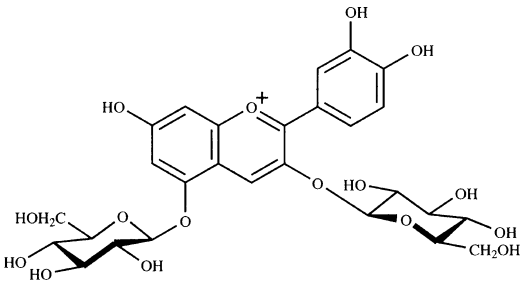

Fig. 4. Cyanidin 3,5-di-*O*-glucoside (cyanin, A1).

acid hydrolysis of this anthocyanin. E_{440}/E_{max} value of UV spectra of the original glycoside was 22.3%, showing that A1 is 3,5-substituted anthocyanin. The attachment of 2 mol glucose to cyanidin was shown by LC-MS survey, i.e. the occurrence of the molecular ion peak, *m/z* 611 $[M]^+$. Finally, anthocyanin A1 was identified as cyanidin 3,5-di-*O*-glucoside (Fig. 4) by direct TLC and HPLC comparison with authentic deacylated succinylcyanin from the blue flowers of *Centaurea cyanus* L. (Takeda *et al*., 2005). Though it was shown in this experiment by HPLC survey that two anthocyanins are contained in the flowers, another one was not isolated. It was clear for the first time that the flower color of *M. bontioides* is due to anthocyanins, cyanidin 3,5-di-*O*-glucoside and unknown anthocyanin.

Flavonoid F1 was obtained from the flowers and leaves. UV spectral properties of this compound were those of 7-substituted apigenin (Mabry *et al*., 1970). Apigenin and glucuronic acid were liberated by acid hydrolysis of the original glycoside. The attachment of 1 mol glucuronic acid to apigenin was shown by LC-MS survey, i.e. the occurrence of the molecular ion peak, *m/z* 447 $[M+H]^+$ and a fragment ion peak, *m/z* 271 $[M-176+H]^+$. It was shown by direct TLC and HPLC comparison with authentic specimen from the leaves of *Uncarina grandidieri* (Baill.) Stapf. (Pedaliaceae) (Yamazaki *et al*., 2007) that compound F1 is apigenin 7-*O*-glucuronide (Fig. 5). Though apigenin 7-*O*-glucoside has been reported from the leaves of this species (Kanemoto *et al*., 2008), the presence of

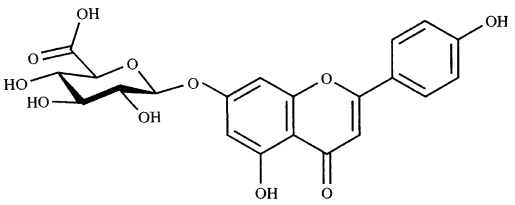

Fig. 5. Apigenin 7-*O*-glucuronide (F1).

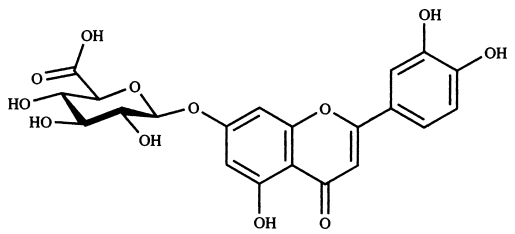

Fig. 6. Luteolin 7-*O*-glucuronide (F2).

7-*O*-glucuronide in the leaves and flowers was shown for the first time.

Flavonoid F2 was also isolated from the flowers and leaves as major compound. Luteolin and glucuronic acid were produced by acid hydrolysis. The attachment of 1 mol glucuronic acid to 7-hydroxyl group of luteolin was shown by UV spectral survey and the occurrence of molecular ion peak, *m/z* 463 $[M+H]^+$ and a fragment ion peak, *m/z* 287 $[M-176+H]^+$ on LC-MS. Finally, F2 was identified as luteolin 7-*O*-glucuronide (Fig. 6) by TLC and HPLC comparison with authentic sample from *U. grandidieri* (Yamazaki *et al*., 2007). Luteolin 7-*O*-glucuronide was reported from this species for the first time.

UV spectral properties of flavonoid F3 were essentially the same with those of luteolin 7-*O*-glucuronide. However, glucose but not glucuronic acid was liberated by acid hydrolysis as a glycosidic sugar. Molecuar ion peak, *m/z* 449 $[M+H]^+$ and a fragment ion peak, 287 $[M-162+H]^+$ appeared on the chromatogram of LC-MS, showing that 1 mol glucose is attached to luteolin. Flavonoid F3 was finally identified as luteolin 7-*O*-glucoside (Fig. 7) by direct TLC and HPLC comparison with authentic sample from the leaves of *Helwingia japonica* (Thunb.) F.G. Dietrich (Cornaceae) (Iwashina *et al*., 1997). Luteolin 7-*O*-glucoside has already been detected

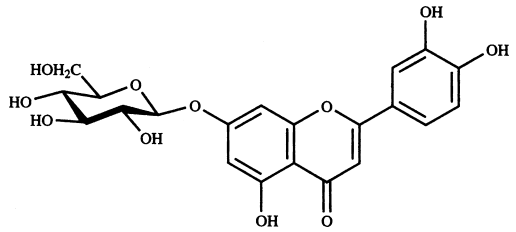

Fig. 7. Luteolin 7-*O*-glucoside (F3)

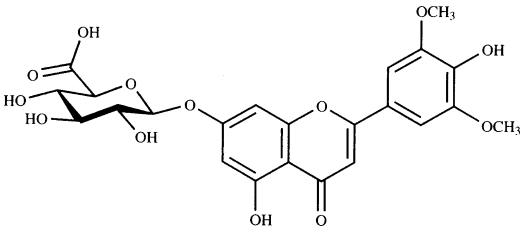

Fig. 8. Tricin 7-O-glucuronide (F4).

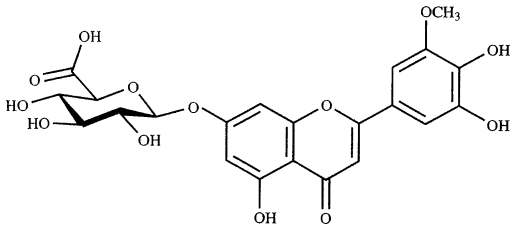

Fig. 9. Selagin 7-*O*-glucuronide (F5).

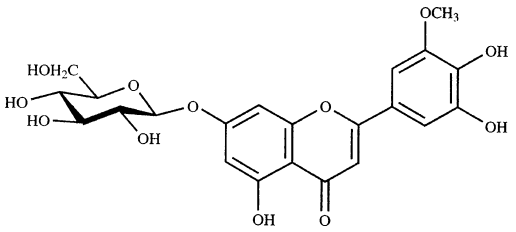

Fig. 10. Selagin 7-*O*-glucoside (F6).

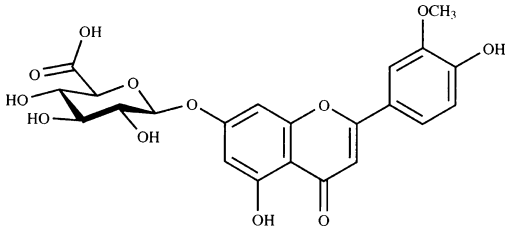

Fig. 11. Chrysoeriol 7-*O*-glucuronide (F7).

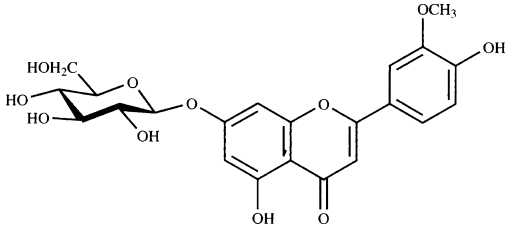

Fig. 12. Chrysoeriol 7-*O*-glucoside (F8).

from this species (Kanemoto *et al*., 2008).

Flavonoids F4 and F5 were obtained as a mixture. Their aglycones were determined to be trihydroxy-dimethoxyflavone and tetrahydroxy-monomethoxyflavone by LC-MS survey, respectively. Glucuronic acid was obtained as a glycosidic sugar by acid hydrolysis of the mixture. LC-MS survey of the mixture showed the attachment of each 1 mol glucuronic acid to the respective aglycones. Thus, it was presumed that the mixture consists of tricin 7-*O*-glucuronide (F4, Fig. 8) and selagin 7-*O*-glucuronide (F5, Fig. 9). Their compounds never found in this species, until now.

Flavonoid F6 liberated tetrahydroxy-monomethoxyflavone, which was shown to be the same aglycone with that of flavonoid F5, and glucose by acid hydrolysis. UV spectral and LC-MS survey of the original glycoside indicated the attachment of 1 mol glucose to 7-hydroxyl group of the aglycone. From the results described above, F6 was characterized as selagin 7-*O*-glucoside (Fig. 10), which was reported from this species for the first time.

Flavonoids F7 and F8 were shown to be chrysoeriol 7-*O*-glycosides by UV spectroscopy and LC-MS. Acid hydrolysis of F7 produced chrysoeriol and glucuronic acid. Since the molecular ion peak, *m/z* 477 $[M+H]^+$ and a fragment ion peak, *m/z* 301 $[M-176+H]^+$ appeared by LC-MS survey of F7, it was shown that 1 mol glucuronic acid is attached to chrysoeriol. Thus, flavonoid F7 was identified as chrysoeriol 7-*O*-glucuronide (Fig. 11). On the other hand, another compound F8 was characterized as chrysoeriol 7-*O*-glucoside (Fig. 12) by LC-MS. Though chrysoeriol 7-*O*-glucoside has been reported from this species, chrysoeriol 7-*O*-glucuronide

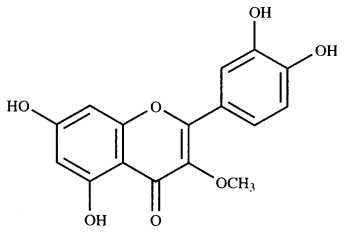

Fig. 13. Quercetin 3-methyl ether (F9).

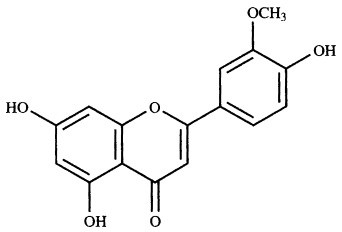

Fig. 15. Chrysoeriol (F11).

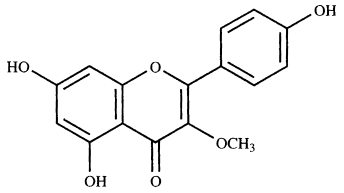

Fig. 14. Isokaempferide (F10).

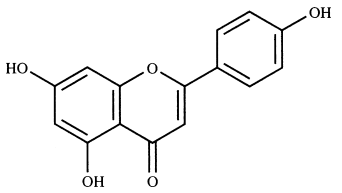

Fig. 16. Apigenin (F12).

was first isolated.

It was presumed by HPLC survey that flavonoid F9 is a free flavonoid. Its UV spectral properties showed that the compound is 3-substituted quercetin. LC-MS survey of F9 indicated the presence of monomethyl ether in flavonol nucleus. Finally, the component was identified as a free flavonol, quercetin 3-methyl ether (Fig. 13) by HPLC comparison with authentic sample from the flowers of *Neochilenia* spp. (Cactaceae) (Iwashina *et al*., 1984).

The appearance of molecular ion peaks, *m/z* 301 $[M+H]^+$ (trihydroxy-monomethoxyflavone) (F10 and F11) and *m/z* 271 $[M+H]^+$ (trihydroxyflavone) (F12), of flavonoids F10, F11 and F12 on LC-MS showed that they correspond to isokaempferide (kaempferol 3-methyl ether, Fig. 14), chrysoeriol (Fig. 15) and apigenin (Fig. 16), respectively. Moreover, the retention times of F11 and F12 agreed with those of authentic chrysoeriol from *Disporum kawakamii* Hayata (Liliaceae) (Saito *et al*., 2009) and apigenin from *Glycine max* (L.) Merr. (Leguminosae) (Iwashina *et al*., 2006). Though apigenin and chrysoeriol glycosides have been isolated from this plant, they were reported as free states in this paper for the first time.

Flavonoids F13 and F14 were also free flavonoids. UV spectral properties of their compounds were those of the flavone having free 5-, 7-, 3′- and 4′-hydroxyl groups, and flavonol having free 5-, 7- and 3′-hydroxyl and substituted 3- and 4′-hydroxyl groups, respectively. Molecular ion peaks, *m/z* 287 $[M+H]^+$ and *m/z* 331 $[M+H]^+$ appeared by LC-MS survey of them, showing that they are tetrahydroxyflavone and trihydroxy-dimethoxyflavone, respectively. Thus, flavonoids F13 and F14 were characterized as luteolin (Fig. 17) and quercetin 3,4′-dimethyl ether (Fig. 18). Retention time of F13 agreed with that of authentic luteolin from the pubescence of *Glycine max* (Iwashina *et al*., 2006) by HPLC survey.

Two caffeic acid derivatives (O1 and O2) were isolated from the leaves together with flavonoids. Of their compounds, O1 was identified as chlorogenic acid (Fig. 19) by LC-MS survey and direct HPLC comparison with authentic sample from the seeds of *Coffea arabica* L. (Rubiaceae) (Hayashi, unpublished data). Another O2 was identified as a phenylethanoid, acteoside (Fig. 20) by UV spectral properties, molecular ion peak, *m/z* 623 $[M-H]^-$, of LC-MS, and HPLC comparison with authentic sample from the leaves of *Plantago asiatica* L. (Plantaginaceae) (Murai *et al*., 2009).

In this experiment, each one anthocyanin and caffeoyl derivative, and two flavone glycosides

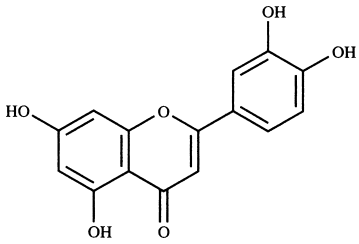

Fig. 17. Luteolin (F13).

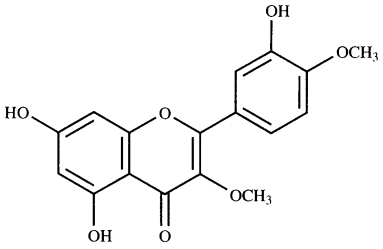

Fig. 18. Quercetin 3,4′-dimethyl ether (F14).

were isolated from the flowers. On the other hand, three flavone and two flavonol aglycones, and eight flavone glycosides and two caffeoyl derivatives were detected from the leaves. Of their flavonoids, five flavonoid aglycones may be derived from external exudates on the leaves. Four flavone glycosides, 7-*O*-glucosides of apigenin, luteolin, chrysoeriol and tricin have been reported from this species which was collected in a coastal area of Yaeyama-gun, Okinawa Pref., Japan (Kanemoto *et al*., 2008). Though we detected luteolin and chrysoeriol 7-*O*-glucosides in this survey, other two glucosides could not be found in the plants which were collected in Amami-ohsima Is., Kagoshima Pref., Japan. Instead, 7-*O*-glucuronides of apigenin, luteolin, chrysoeriol, tricin and selagin were isolated and identified, together with selagin 7-*O*-glucoside. It was shown for the first time that the difference of their flavonoid composition occurs between two islands.

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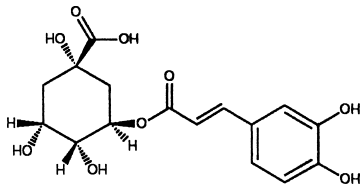

Fig. 19. Chlorogenic acid (O1).

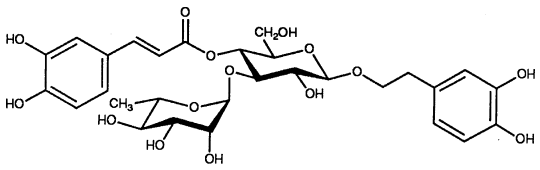

Fig. 20. Acteoside (O2).

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