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Flavonoid Glycosides from the Fern, *Schizaea* (Schizaeaceae) in South Pacific Region, and their Distribution Pattern

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Abstracts Eight *Schizaea* species, *S. balansae*, *S. bifida*, *S. dichotoma*, *S. fistulosa*, *S. intermedia*, *S. laevigata*, *S. melanesica* and *S. tenuis*, were chemotaxonomically surveyed for flavonoid glycosides. Seventeen flavonoids, e.g. 3-*O*-glucosides and 3-*O*-rutinosides of kaempferol and quercetin, isorhamnetin 3-*O*-glucoside, luteolin 7-*O*-glucoside and apigenin 6,8-di-*C*-glucoside, were isolated and identified from the fronds of the species. Nine acylated flavonols such as kaempferol 3-*O*-(feruloylglucoside) and 3-*O*-(*p*-coumaroylglucoside), and quercerin 3-*O*-(*p*-coumaroylglucoside) were basically present in all species. On the other hand, 3-*O*-rutinosides of kaempferol and quercetin were absent in four species, *S. intermedia*, *S. laevigata*, *S. melanesica* and *S. tenuis*.

Key words: acylated flavonoids, chemotaxonomy, flavonoids, flavonols, *Schizaea*.

Introduction

The genus *Schizaea* (Schizaeaceae) consists of ca. 30 species, of which nine are growing in the South Pacific region (Sahashi, 2008). The species are native to tropical and subtropical zones. A few flavonoids have been reported from *Schizaea* species, i.e. kaempferol and quercetin 3-*O*-glucosides and 3-*O*-rhamnosylglucosides from *S. bifida* (Wallace and Markham, 1978), kaempferol, quercetin, procyanidin and prodelphinidin from *S. pectinata* Sw. (Cooper-Driver, 1977). In *S. dichotoma* (Fig. 1), though it was reported that leucoanthocyanins are present, their isolation and characterization were not performed (Cambie *et al.*, 1961).

In this paper, we chemotaxonomically describe the flavonoid properties and their distribution patterns in eight species of the genus *Schizaea* in the South Pacific region.

Materials and Methods

General

UV spectra were recorded on a Shimadzu MPS-2000 Multi purpose recording spectrophotometer (Shimadzu Inc., Japan) according to Mabry *et al.* (1970). LC-MS were measured on a Shimadzu LC-MS systems using a Inertsil ODS-4 column [I.D. 2.1 × 100 mm (GL Sciences Inc., Japan)], at a flow-rate of 0.1 ml min^{-1} eluting with MeCN/H_2O/HCOOH (30 : 65 : 5) (solv. I) for acylated flavonoids and (18 : 77 : 5) (solv. II) for non-acylated flavonoids, ESI^+ 4.5 kV and ESI^- 3.5 kV, 250°C. HPLC survey of the isolated flavonoids and their aglycones, which were obtained by acid hydrolysis, and crude extracts was performed with a Shimadzu HPLC systems using a 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 wavelength was 350 nm and eluents were used MeCN/H_2O/H_3PO_4 (20 : 80 : 0.2) for glycosides and (35 : 65 : 0.2) for aglycones.

Fig. 1. *Schizaea dichotoma*. Grande Terre Is., New Caledonia.

Acid hydrolysis of the flavonoid glycosides was performed in 12% aq.HCl, 100°C, 30 min. After shaken with diethyl ether, aglycones migrated to organic layer and glycosidic sugars remained in aqueous layer. Aglycones were identified by HPLC comparisons with authentic samples, and glycosidic sugars were characterized by paper chromatographic comparisons with authentic samples using solvent systems: BBPW (*n*-BuOH/benzene/pyridine/H_2O = 5 : 1 : 3 : 3) and BTPW (*n*-BuOH/toluene/pyridine/H_2O = 5 : 1 : 3 : 3).

The solvent systems of qualitative TLC (Merck, Germany) and preparative paper chromatography (PC) (Advantec, Japan) are as follows; BAW (*n*-BuOH/HOAc/H_2O = 4 : 1 : 5, upper phase), 15% HOAc and BEW (*n*-BuOH/EtOH/H_2O = 4 : 1 : 2.2).

Plant materials

Eight *Schizaea* species were used as plant materials. Collection sites and dates of the species are as follows.

S. balansae E.Fourn.: Mts. Koghis, Grande Terre Is., New Caledonia. 3 Aug. 1999 (TNS9531048).

S. bifida Willd.: Mts. Koghis, Grande Terre Is., New Caledonia. 8 Aug. 1994 (TNS9520603).

S. dichotoma (L.) J.Sm.: Mts. Koghis, Grande Terre Is., New Caledonia. 11 Dec. 1996 (Iwashina 3285 and 3275); Mt. Dzumac, Grande Terre Is., New Caledonia. 5 Nov. 1997 (Matsumoto 499); Rivière Bleue, Grande Terre Is., New Caledonia. 2 Nov. 1997 (Matsumoto 386 and 405); Mt. Nakobalevu, Viti Levu Is., Fiji. 11 Nov. 1997 (Sugimura 4236); Mt. Bernier, Efate Is., Vanuatu. 28 Oct. 1997 (Matsumoto 373) (Matsumoto *et al.*, 1998; Hashimoto *et al.*, 1998).

S. fistulosa Labill.: Mts. Koghis, Grande Terre Is., New Caledonia. 4 Nov. 1997 (Matsumoto 451) (Hashimoto *et al.*, 1998).

S. intermedia Mett.: Rivière Bleue, Grande Terre Is., New Caledonia. 11 Dec. 1996 (Iwashina 3282) (Hashimoto *et al.*, 1998).

S. laevigata Mett.: Mouirange, Grande Terre Is., New Caledonia. 5 Aug. 1994 (TNS9521384).

S. melanesica Selling: Yate, Grande Terre Is., New Caledonia. 5 Aug. 1994 (TNS9527915).

S. tenuis E.Fourn.: Yate Lake and Pass, Grande Terre Is., New Caledonia. 24 Dec. 2005 (TNS9527245).

Almost all plant materials were collected during the botanical expedition to Vanuatu and adjacent countries in 1996 and 1997. Voucher specimens were deposited in the Herbarium of National Museum of Nature and Science, Japan (TNS).

Extraction and separation

Dried fronds of *S. dichotoma* (95.3 g), *S. fistulosa* (4.9 g) and *S. intermedia* (38.9 g) were extracted with MeOH. After concentration, crude extracts were applied to prep. PC using solvent systems, BAW, 15% HOAc and then BEW. The obtained flavonoids were purified by Sephadex LH-20 column chromatography using solvent system, 70% MeOH. Dried fronds of *S. balansae*, *S. bifida*, *S. laevigata*, *S. melanesica* and *S. tenuis* were extracted with MeOH for qualitative HPLC.

Authentic samples

The flavonoid compounds, which were used as authentic samples, are as follows. Kaempferol 3-*O*-glucoside from the fronds of *Cyrtomium falcatum* (L.f.) C.Presl. (Dryopteridaceae) (Iwashina *et al.*, 2006); kaempferol 3-*O*-rutinoside from the aerial parts of *Osyris alba* L. (Santalaceae) (Iwashina *et al.*, 2008); quercetin 3-*O*-glucoside from the leaves of *Phytolacca americana* L. (Phytolaccaceae) (Iwashina and Kitajima, 2009); quercetin 3-*O*-rutinoside from the leaves of *Saruma henryi* Oliver (Aristolochiaceae) (Iwashina *et al.*, 2002); quercetin 3-*O*-sophoroside from the leaves of *Asarum yakusimense* Masam. (Aristolochiaceae) (Iwashina *et al.*, 2005); apigenin 6,8-di-*C*-glucoside from the fronds of *Asplenium normale* D.Don (Aspleniaceae) (Iwashina *et al.*, 2010); luteolin 7-*O*-glucoside from the leaves of *Schmalhausenia nidulans* Petrak (Asteraceae) (Iwashina and Kadota, 1999); isorhamnetin 3-*O*-glucoside from the leaves of *Asarum asperum* F.Maek. (Aristolochiaceae) (Iwashina *et al.*, 2005); kaempferol 3-*O*-gentiobioside from the flowers of *Glycine max* (L.) Merr. (Leguminosae) (Iwashina *et al.*, 2007).

Identification

The flavonoids were identified by UV spectroscopy, LC-MS, characterization of acid hydrolysates, and direct HPLC and TLC comparisons with authentic samples. TLC, HPLC, UV, acid hydrolysis and LC-MS data of the isolated flavonoids are as follows.

Kaempferol 3-*O*-glucoside (astragalin, **1**). TLC: Rf 0.81 (BAW), 0.83 (BEW), 0.31 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark greenish yellow. HPLC: t_R 12.14 min (solv. II). UV λmax (nm): MeOH 266, 349; + NaOMe 275, 323, 397 (inc.); + $AlCl_3$ 274, 304, 351, 393; + $AlCl_3$/HCl 275, 302, 347, 388; + NaOAc 275, 313, 389; + NaOAc/H_3BO_3 266, 356. Acid hydrolysis: kaempferol and glucose. LC-MS: *m*/*z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, kaempferol + 1 mol glucose); *m*/*z* 287 $[M-162+H]^+$ (fragment ion peak, kaempferol).

Kaempferol 3-*O*-rutinoside (nicotiflorin, **2**). TLC: Rf 0.66 (BAW), 0.68 (BEW), 0.49 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark greenish yellow. HPLC: t_R 10.66 min (solv. II). UV λmax (nm): MeOH 266, 344; + NaOMe 276, 324, 394 (inc.); + $AlCl_3$ 274, 304, 354, 386sh; + $AlCl_3$/HCl 274, 304, 347, 387sh; + NaOAc 275, 314, 390; + NaOAc/H_3BO_3 266, 353. Acid hydrolysis: kaempferol, glucose and rhamnose. LC-MS: *m*/*z* 593 $[M-H]^-$ (molecular ion peak, kaempferol + each 1 mol of glucose and rhamnose); *m*/*z* 449 $[M-146+H]^+$ (fragment ion peak, kaempferol + 1 mol glucose); *m*/*z* 287 $[M-308+H]^+$ (fragment ion peak, kaempferol).

Quercetin 3-*O*-glucoside (isoquercitrin, **3**). TLC: Rf 0.63 (BAW), 0.66 (BEW), 0.23 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark yellow. HPLC: t_R 8.39 min (solv. II). UV λmax (nm): MeOH 257, 266sh, 358; + NaOMe 274, 328, 410 (inc.); + $AlCl_3$ 275, 432; + $AlCl_3$/HCl 268, 299, 360, 396; + NaOAc 273, 326, 397; + NaOAc/H_3BO_3 262, 378. Acid hydrolysis: quercetin and glucose. LC-MS: *m*/*z* 465 $[M+H]^+$, 463 $[M-H]^-$ (molecular ion peaks, quercetin + 1 mol glucose); *m*/*z* 303 $[M-162+H]^+$ (fragment ion peak, quercetin).

Quercetin 3-*O*-rutinoside (rutin, **4**). TLC: Rf 0.46 (BAW), 0.49 (BEW), 0.43 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark yellow. HPLC: t_R 7.32 min (solv. II). UV λmax (nm): MeOH 257, 265sh, 358; + NaOMe 273, 323, 409 (inc.); + $AlCl_3$ 275, 431; + $AlCl_3$/HCl 269, 299sh, 359, 395; + NaOAc 273, 326, 401; + NaOAc/H_3BO_3 262, 376. Acid hydrolysis: quercetin, glucose and rhamnose. LC-MS: *m*/*z* 611 $[M+H]^+$, 609 $[M-H]^-$ (molecular ion peaks, quercetin + each 1 mol of glucose and rhamnose); *m*/*z* 465 $[M-146+H]^+$ (fragment ion peak, quercetin + 1 mol glucose); *m*/*z* 303 $[M-308+H]^+$ (fragment ion peak, quercetin).

Quercetin 3-*O*-sophoroside (baimaside, **5**). TLC: Rf 0.27 (BAW), 0.34 (BEW), 0.44 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark yellow. HPLC: t_R 5.19 min (solv.

II). Acid hydrolysis: quercetin and glucose. LC-MS: *m/z* 627 $[M+H]^+$, 625 $[M-H]^-$ (molecular ion peaks, quercetin + 2 mol glucose); *m/z* 465 $[M-162+H]^+$, 463 $[M-162-H]^-$ (fragment ion peaks, quercetin + 1 mol glucose); *m/z* 303 $[M-324+H]^+$ (fragment ion peak, quercetin).

Apigenin 6,8-di-*C*-glucoside (vicenin-2, **6**). TLC: Rf 0.18 (BAW), 0.21 (BEW), 0.36 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark greenish yellow. HPLC: t_R 4.23 min (solv. II). UV λmax (nm): MeOH 273, 332; + NaOMe 283, 333, 401 (inc.); + $AlCl_3$ 280, 304, 350, 380sh; + $AlCl_3$/HCl 280, 304, 345, 378sh; + NaOAc 282, 335, 393; + NaOAc/H_3BO_3 284, 318, 350sh, 410sh. Acid hydrolysis: unhydrolyzable. LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (molecular ion peaks, apigenin + 2 mol glucose).

Luteolin 7-*O*-glucoside (glucoluteolin, **7**). TLC: Rf 0.42 (BAW), 0.35 (BEW), 0.04 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–yellow. HPLC: t_R 8.63 min (solv. II). UV λmax (nm): MeOH 255, 266sh, 349; + NaOMe 269, 395 (inc.); + $AlCl_3$ 273, 425; + $AlCl_3$/HCl 263, 273sh, 295, 359, 386sh; + NaOAc 260, 404; + NaOAc/H_3BO_3 259, 374. Acid hydrolysis: luteolin and glucose. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucose).

Kaempferol 3-*O*-(feruloylglucoside) (**9** and **10**). TLC: Rf 0.98 (BAW), 0.91 (BEW), 0.15 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark greenish yellow. HPLC: t_R 8.05 min (**9**) and 8.83 min (**10**) (solv. I). UV λmax (nm): MeOH 266, 327; + NaOMe 275, 381 (inc.); + $AlCl_3$ 275, 306, 332, 394sh; + $AlCl_3$/HCl 275, 303, 330, 394sh; + NaOAc 275, 319, 379; + NaOAc/H_3BO_3 267, 328. Acid hydrolysis: kaempferol, glucose and ferulic acid, and kaempferol 3-*O*-glucoside (intermediate). LC-MS: *m/z* 625 $[M+H]^+$, 623 $[M-H]^-$ (molecular ion peaks, kaempferol + each 1 mol of glucose and ferulic acid); *m/z* 287 $[M-338+H]^+$ (fragment ion peak, kaempferol).

Kaempferol 3-*O*-(*p*-coumaroylglucoside) (**11** and **12**). TLC: Rf 0.95 (BAW), 0.95 (BEW), 0.11 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark greenish yellow. HPLC: t_R 13.98 min (**11**) and 15.14 min (**12**) (solv. I). UV λmax (nm): MeOH 267, 297sh, 313; + NaOMe 276, 368 (inc.); + $AlCl_3$ 276, 307, 322sh, 392; + $AlCl_3$/HCl 277, 305, 322sh, 392sh; + NaOAc 275, 314, 377; + NaOAc/H_3BO_3 268, 317. Acid hydrolysis: kaempferol, glucose and *p*-coumaric acid, and kaempferol 3-*O*-glucoside (intermediate). LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (molecular ion peaks, kaempferol + each 1 mol of glucose and *p*-coumaric acid); *m/z* 287 $[M-308+H]^+$ (fragment ion peak, kaempferol).

Kaempferol 3-*O*-(*p*-coumaroyl-feruloylglucoside) (**13** and **14**). TLC: Rf 0.95 (BAW), 0.95 (BEW), 0.03 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark greenish yellow. HPLC: t_R 25.46 min (solv. I). UV λmax (nm): MeOH 268, 298sh, 317; + NaOMe 275, 370 (inc.); + $AlCl_3$ 277, 307, 318sh, 396; + $AlCl_3$/HCl 278, 307, 318, 395sh; + NaOAc 275, 315, 372; + NaOAc/H_3BO_3 268, 319. Acid hydrolysis: kaempferol, glucose, *p*-coumaric acid and ferulic acid, and kaempferol 3-*O*-glucoside (intermediate). LC-MS: *m/z* 771 $[M+H]^+$, 769 $[M-H]^-$ (molecular ion peaks, kaempferol + each 1 mol of glucose, *p*-coumaric acid and ferulic acid); *m/z* 287 $[M-484+H]^+$ (fragment ion peak, kaempferol).

Kaempferol 3-*O*-(feruloylgentiobioside) (**15**). TLC: Rf 0.95 (BAW), 0.95 (BEW), 0.03 (15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark greenish yellow. HPLC: t_R 25.46 min (solv. I). UV λmax (nm): MeOH 268, 298sh, 317; + NaOMe 275, 370 (inc.); + $AlCl_3$ 277, 307, 318sh, 396; + $AlCl_3$/HCl 278, 307, 318, 395sh; + NaOAc 275, 315, 372; + NaOAc/H_3BO_3 268, 319. Acid hydrolysis: kaempferol, glucose and ferulic acid, and kaempferol 3-*O*-gentiobioside (intermediate). LC-MS: *m/z* 787 $[M+H]^+$, 785 $[M-H]^-$ (molecular ion peaks, kaempferol + 2 mol glucose and 1 mol ferulic acid).

Quercetin 3-*O*-(*p*-coumaroylglucoside) (**16** and **17**). TLC: Rf 0.95 (BAW), 0.95 (BEW), 0.11

(15%HOAc); Color UV (365 nm)–dark purple, UV/NH_3–dark greenish yellow. HPLC: t_R 9.80 min (**16**) and 10.51 min (**17**) (solv. I). Acid hydrolysis: quercetin, glucose and *p*-coumaric acid, and quercetin 3-*O*-glucoside (intermediate). LC-MS: *m/z* 611 $[M+H]^+$, 609 $[M-H]^-$ (molecular ion peaks, quercetin + each 1 mol of glucose and *p*-coumaric acid); *m/z* 303 $[M-308+H]^+$ (fragment ion peak, quercetin).

Results and Discussion

Identification of flavonoids

Seventeen flavonoids were found in *Schizaea dichotoma*, *S. fistulosa* and *S. intermedia*. Of their compounds, nine were acylated flavonol glycosides. On the other hand, each one flavone *O*-glycoside and *C*-glycoside, and six non-acylated flavonol glycosides were also characterized.

Flavonoid **1** was isolated from all three *Schizaea* species. Kaempferol and glucose were produced by acid hydrolysis. The attachment of 1 mol glucose to 3-position of kaempferol was shown by UV spectral survey according to Mabry *et al.* (1970) and LC-MS. Finally, **1** was identified as kaempferol 3-*O*-glucoside (astragalin) by TLC and HPLC comparison with authentic sample. Astragalin has already been reported from *Schizaea bifida* (Wallace and Markham, 1978).

Flavonoid **2** was liberated kaempferol, glucose and rhamnose by acid hydrolysis. The attachment of each 1 mol glucose and rhamnose to kaempferol was proved by occurrence of molecular ion peak, *m/z* 593 $[M-H]^-$ on LC-MS. Since their glycosidic sugars were determined to be attached to 3-position of kaempferol by UV spectra, **2** was characterized as kaempferol 3-*O*-rhamnosylglucoside. Finally, **2** was identified as kaempferol 3-*O*-rhamnosyl-(1→6)-glucoside, i.e. 3-*O*-rutinoside (nicotiflorin), by TLC and HPLC comparison with authentic sample. Though kaempferol 3-*O*-rhamnosylglucoside has been found in *Schizaea bifida* (Wallace and Markham, 1978), it was determined as to be kaempferol 3-*O*-rutinoside by the present survey.

By the similar procedures, **3** and **4** were identified as quercetin 3-*O*-glucoside (isoquercitrin) and 3-*O*-rutinoside (rutin), respectively. Rutin has been reported from *S. bifida* (Wallace and Markham, 1978). Though quercetin 3-*O*-rhamnosylglucoside has also been isolated from *S. bifida* (Wallace and Markham, 1978), this compound was completely identified as quercetin 3-*O*-rutinoside in the present survey for the first time.

Flavonoid **5** was isolated from *S. intermedia*. Quercetin and glucose were liberated by acid hydrolysis of **5**. The attachment of 2 mol glucose to 3-position of quercetin was shown by UV spectra and LC-MS. Thus, **5** was characterized as quercetin 3-*O*-diglucoside. Finally, this compound was identified as quercetin 3-*O*-glucosyl-(1→2)-glucoside, i.e. 3-*O*-sophoroside, by direct TLC and HPLC comparison with authentic sample. Quercetin 3-*O*-sophoroside was reported from *Schizaea* species for the first time.

UV spectral properties of **6** showed that this compound is the glycoside having free hydroxyl groups to 5-, 7- and 4′-positions of flavone. Moreover, since **6** was unhydrolyzable by hot acid treatment, it was proved that the compound is *C*-glycosylflavonoid. The attachment of 2 mol hexose to trihydroxyflavone was shown by LC-MS. Flavonoid **6** was finally identified as apigenin 6,8-di-*C*-glucoside, i.e. vicenin-2, by TLC and HPLC comparison with authentic sample. *C*-Glycosylflavone was reported from *Schizaea* species for the first time.

It was shown by UV spectral survey that **7** is 7-substituted and 5-, 3′- and 4′-hydroxylated flavone. Luteolin and glucose were produced by acid hydrolysis. Finally, **7** was identified as luteolin 7-*O*-glucoside by direct TLC and HPLC comparison with authentic sample. Flavone *O*-glycoside was also found in *Schizaea* species for the first time.

Though flavonoid **8** was cannot be isolated from the plant materials, retention time and UV spectral properties of its peak on the HPLC agreed with those of authentic isorhamnetin 3-*O*-glucoside.

Flavonoids **9** and **10**, **11** and **12**, **13** and **14**,

Table 1. Distribution of flavonoids in eight *Schizaea* species in South Pacific region

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
S. dichotoma	+	+	+	+			t		t	t	t	t	t	t	+	t	t
S. fistulosa	+	+	+	+	t	t	t	t	t	t	t	t	t	+	t	t	t
S. balansae	+	+	+	+				t	t	t	t		t	+	t		
S. bifida	+	+	+	+		t			t	t	t	t	+	+	t	t	t
S. laevigata	+		+		t	t	t	+	t	t	t	t	t	+	t	t	t
S. melanesica	+		+		t	t		t	t	t	t	t	+	+	t	t	t
S. tenuis	t		+		t	t		+	t	t	t		t	+	t		
S. intermedia	+		+		+	t			t	t	t	t	t	+	t	t	t

1 = kaempferol 3-*O*-glucoside, **2** = kaempferol 3-*O*-rutinoside, **3** = quercetin 3-*O*-glucoside, **4** = quercetin 3-*O*-rutinoside, **5** = quercetin 3-*O*-sophoroside, **6** = apigenin 6,8-di-*C*-glucoside, **7** = luteolin 7-*O*-glucoside, **8** = isorhamnetin 3-*O*-glucoside, **9** and **10** = kaempferol 3-*O*-(feruloylglucosides), **11** and **12** = kaempferol 3-*O*-(*p*-coumaroylglucosides), **13** and **14** = kaempferol 3-*O*-(*p*-coumaroyl-feruloylglucosides), **15** = kaempferol 3-*O*-(feruloylgentiobioside), and **16** and **17** = quercetin 3-*O*-(*p*-coumaroylglucosides). + = presence, t = trace.

and **16** and **17** were isolated from *S. dichotoma*, *S. intermedia* and *S. fistulosa* as the mixtures, respectively. By their acid hydrolysis, ferulic acid (**9** and **10**, and **13** and **14**) and/or *p*-coumaric acid (**11**–**14**, and **16** and **17**) were liberated together with kaempferol (**9**–**14**) or quercetin (**16** and **17**), and glucose. UV spectral properties of **9**–**14**, and **16** and **17** showed that they are kaempferol 3-*O*-glycosides and quercetin 3-*O*-glycosides, respectively. Thus, **9** and **10**, **11** and **12**, **13** and **14**, and **16** and **17** were characterized as kaempferol 3-*O*-(feruloylglucosides), 3-*O*-(*p*-coumaroylglucosides) and 3-*O*-(*p*-coumaroyl-feruloylglucosides), and quercetin 3-*O*-(*p*-coumaroylglucosides), respectively. They may be *cis*- and *trans*-form mixtures of the same acylated glycosides in each other. Flavonoid **15** liberated kaempferol, glucose and ferulic acid by acid hydrolysis. Since the attachment of acylated gentiobioside to 3-position of kaempferol was shown by UV spectral survey, **15** was characterized as kaempferol 3-*O*-(feruloylgentiobioside). Acylated flavonoids were reported from *Schizaea* species for the first time.

Distribution of the flavonoids in eight Schizaea *species*

In this survey, six non-acylated and nine acylated flavonols, and each one *C*-glycosylflavone and flavone *O*-glycoside were found in *Schizaea* species (Table 1). In *S. dichotoma*, though the individials of some populations including New Caledonia, Fiji and Vanuatu were surveyed for flavonoids, their distribution patterns were essentially the same in each other. Of their compounds, acyalted flavonols (**9**–**17**) were found in all species as minor compounds in almost cases, except for the absence of kaempferol 3-*O*-(*p*-coumaroylglucoside) (**12**) in *S. balansae* and *S. tenuis* (Table 1). Kaempferol and quercetin 3-*O*-glucosides (**1** and **3**) were detected from all species. However, though kaempferol and quercetin 3-*O*-rutinosides (**2** and **4**), which attach rhamnose, were found in *S. dichotoma*, *S. fistulosa*, *S. balansae* and *S. bifida*, they were absent in *S. intermedia*, *S. laevigata*, *S. melanesica* and *S. tenuis*. Quercetin 3-*O*-sophoroside (**5**) and *C*-glycosylflavone, vicenin-2 (**6**), were detected from *S. intermedia*, *S. fistulosa*, *S. laevigata*, *S. melanesica* and *S. tenuis* as minor compounds. Vicenin-2 was also found in *S. bifida*.

As the flavonoids of *Schizaea* species, flavonols, kaempferol and quercetin glycosides have been reporterd from *S. bifida* and *S. pectinata* (Wallace and Markham, 1978; Cooper-Driver, 1977). The genus *Schizaea* is considered as comparatively 'primitive' fern (Wallace and Markham, 1978), together with *Stromatopteris*, *Gleichenia*, *Cardiomanes* and *Hymenophyllum*, and they have been reported to accumulate only flavonols, kaempferol and quercetin glycosides (Markham, 1988). However, in this survey, it was proved that flavonoid characters of *Schizaea* species are acylated and non-acylated flavonol glycosides.

On the other hand, flavone *O*- and *C*-glycosides were also isolated from a few species.

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