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Flavonoid Variation in Fronds of *Cyrtomium falcatum* Complex

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岩科　司[*]・松本　定[*]：オニヤブソテツ（広義）に含まれるフラボノイドの変異

The genus *Cyrtomium* consists of ca. 15 species and is mainly distributed from the Himalayas to Japan (Iwatsuki 1992). Ten species, *Cyrtomium hookerianum* (C. Presl) C. Chr., *C. balansae* (Christ) C. Chr., *C. caryotideum* (Wall. ex Hook. & Grev.) C. Presl, *C. falcatum sensu lato, C. fortunei* J. Sm., *C. macrophyllum* (Makino) Tagawa, *C. atropunctatum* Sa. Kurata, *C. microindusium* Sa. Kurata *sensu* S. Matsumoto *in shed.*, *C. tukusicola* Tagawa and *C. laetevirens* (Hiyama) Nakaike, are native to Japan (Nakaike 1992). Of their species, *C. falcatum* are widely distributed from Japan, Korea, China, Vietnam, Taiwan, India to Hawaii, North America, Europe. Matsumoto (2003) divided the species into *C. falcatum* subsp. *falcatum sensu* S. Matsumoto, subsp. *australe S.* Matsumoto *nom. nud.* and subsp. *littorale* S. Matsumoto *nom. nud.* and *C. devexiscapulae* (Koidz.) Ching.

The flavonoids of the genus *Cyrtomium* have been reported from some species. A common flavonol, quercetin 3-*O*-glucoside was found from *C. falcatum sensu lato, C. fortunei, C. caryotideum, C. tukusicola* and *C. macrophyllum* (Kishimoto 1956a). Kaempferol 3-*O*-glucoside was also detected from their species except *C. macrophyllum.* Rare kaempferol 7-*O*-(6″-succinyl)-glucoside was isolated from *C. falcatum sensu lato* and *C. fortunei* (Hiraoka 1978, Hiraoka and Maeda 1979). The common *C*-glycosylflavones, vitexin and orientin were also found from the same taxa (Hiraoka 1978). Two rare *C*-methylflavanone glycosides, cyrtopterin and cyrtomin, were isolated from *C. falcatum sensu lato* (Kishimoto 1956a). Their aglycones were characterized as 5,7,4′-trihydroxy-6,8-di-*C*-methylflavanone (farrerol) and 5,7,3′,4′-tetrahydroxy-6,8-di-*C*-methylflavanone (cyrtominetin) (Kishimoto 1956b, 1956c).

More recently, the flavonoids in nineteen *Cyrtomium* and the related three *Cyrtogonellum* and two *Phanerophlebia* taxa were surveyed and chemotaxonomically discussed (Iwashina *et al.* 2006). In this time, two *C*-methylflavanone glycosides, cyrtopterin and cyrtomin were completely identified as farrerol 7-*O*-β-D-glucopyranoside and cyrtominetin 7-*O*-β-D-glucopyranoside, respectively. Flavonoid composition of *C. falcatum* subsp. *falcatum*, subsp. *australe* and subsp. *littorale*, and *C. devexiscapulae* was also surveyed and pointed out the qualitative difference between *C. falcatum sensu stricto* and *C. devexiscapulae*.

In this paper, flavonoid variation among many individuals and populations of *C. falcatum* subspecies, subsp. *falcatum*, subsp. *australe* and subsp. *littorale*, and *C. devexiscapulae* including *C. falcatum sensu lato* are described.

Materials and Methods

Plant materials

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Fourty-five individuals of *C. devexiscapulae,* 239 of *C. falcatum* subsp. *falcatum*, 94 of *C. falcatum* subsp. *australe*, and 74 of *C. falcatum* subsp. *littorale* were used as plant materials for flavonoid survey. The collection sites, accession numbers and flavonoid composition of plant materials are shown in App. 1. Live plants are grown in Tsukuba Botanical Garden, National Science Museum and voucher specimens were deposited in National Science Museum, Japan (TNS).

Extraction and isolation of flavonoids

Fresh fronds were extracted with MeOH. The flavonoids were isolated by 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 (solvent system: 70% MeOH).

Flavonoid composition

Flavonoid composition was surveyed by two-dimensional paper chromatography (2D-PC) using BAW (1st) and 15%HOAc (2nd) and HPLC using Shim-pack CLC-ODS (I.D. 6.0×150 mm, Shimadzu), at flow-rate: 1.0 ml/min, injection: 10 μl, detection: 190-400 nm, and eluent: MeCN/H_2O/H_3PO_4 (22:78:0.2).

Identification of flavonoids

The isolated flavonoids were identified by UV, ^{1}H and ^{13}C NMR, complete and mild acid hydrolysis (in 12% HCl, 100℃, 30 min, and 1.2% HCl:MeOH = 1:1, 100℃, 5-40 min, respectively), and direct PC and HPLC comparisons with authentic specimens. UV spectral analysis were performed according to Mabry *et al.* (1970). ^{1}H and ^{13}C NMR spectra were performed in pyridine-d_5 at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR). PC, UV, and ^{1}H and ^{13}C NMR data of the isolated flavonoids were as follows.

Quercetin 3-*O*-glucoside (Isoquercitrin, **1**). PC: Rf 0.58 (BAW), 0.57 (BEW), 0.32 (15%HOAc), 0.17 (5%HOAc); Color UV - dark purple, UV/NH_3 - yellow. UV λ max (nm): MeOH 257, 265sh, 296sh, 357; +NaOMe 273, 328, 409 (inc.); +$AlCl_3$ 275, 433; +$AlCl_3$/HCl 269, 299, 361, 399; +NaOAc 273, 326, 394; +NaOAc/H_3BO_3 261, 296, 379.

Kaempferol 3-*O*-glucoside (Astragalin, **2**). PC: Rf 0.72 (BAW), 0.71 (BEW), 0.40 (15%HOAc), 0.24 (5%HOAc); Color UV - dark purple, UV/NH_3 - dark greenish yellow. UV λ max (nm): MeOH 266, 296sh, 349; +NaOMe 275, 325, 399 (inc.); +$AlCl_3$ 274, 304, 352, 395; +$AlCl_3$/HCl 275, 302, 346, 395; +NaOAc 274, 310, 388; +NaOAc/H_3BO_3 267, 296sh, 353.

Kaempferol 3-*O*-glucoside-7-*O*-rhamnoside (**3**). PC: Rf 0.42 (BAW), 0.43 (BEW), 0.63 (15%HOAc), 0.52 (5%HOAc); Color UV - dark purple, UV/NH_3 - dark greenish yellow. UV λ max (nm): MeOH 266, 342; +NaOMe 273, 387 (inc.); +$AlCl_3$ 274, 300, 351, 391; +$AlCl_3$/HCl 276, 299, 343, 394; +NaOAc 267, 376, 394sh; +NaOAc/H_3BO_3 266, 288, 351. ^{1}H NMR (500 MHz, pyridine-d_5): δ 8.45 (2H, *d, J* = 9.2 Hz, H-2′, 6′), 7.26 (2H, *d, J* = 8.9 Hz, H-3′, 5′), 6.94 (1H, *d, J* = 2.1 Hz, H-8), 6.78 (1H, *d, J* = 2.1 Hz, H-6), 6.42 (1H, *d, J* = 8.6 Hz, glucosyl H-1), 6.26 (1H, *d, J* = 1.2 Hz, rhamnosyl H-1), 4.8-4.0 (*m*, sugar protons), 1.66 (3H, *d, J* = 5.8 Hz, rhamnosyl Me). ^{13}C NMR (125 MHz, pyridine-d_5): (kaempferol) δ 156.9 (C-2), 134.8 (C-3), 178.9 (C-4), 162.3 (C-5), 94.8 (C-6), 162.8 (C-7), 106.9 (C-8), 157.8 (C-9), 103.6 (C-10), 123.0 (C-1′), 132.0 (C-2′, C-6′), 116.2 (C-3′, C-5′), 161.9 (C-4′); (glucose) δ 100.4 (C-1), 76.1 (C-2), 78.5 (C-3), 71.5 (C-4), 79.2 (C-5), 62.6 (C-6); (rhamnose) δ 100.1 (C-1), 71.6 (C-2), 72.4 (C-3), 73.6 (C-4), 71.5 (C-5), 18.7 (C-6).

Orientin (**4**). PC: Rf 0.24 (BAW), 0.28 (BEW), 0.14 (15%HOAc), 0.06 (5%HOAc); Color UV - dark

purple, UV/NH_3 - greenish yellow. UV λ max (nm): MeOH 257, 268, 349; +NaOMe 270, 330sh, 406 (inc.); +$AlCl_3$ 274, 424; +$AlCl_3$/HCl 263sh, 276, 297, 358, 385sh; +NaOAc 277, 325sh, 397; +NaOAc/H_3BO_3 265, 375.

Isoorientin (**5**). PC: Rf 0.42 (BAW), 0.43 (BEW), 0.29 (15%HOAc), 0.15 (5%HOAc); Color UV - dark purple, UV/NH_3 - greenish yellow. UV λ max (nm): MeOH 257, 268, 349; +NaOMe 270, 335sh, 409 (inc.); +$AlCl_3$ 275, 425; +$AlCl_3$/HCl 263sh, 277, 296sh, 360, 385sh; +NaOAc 274, 327, 394; +NaOAc/H_3BO_3 264, 376.

Orientin 2″-*O*-glucoside (**6**). PC: Rf 0.20 (BAW), 0.44 (BEW), 0.58 (15%HOAc), 0.49 (5%HOAc); Color UV - dark purple, UV/NH_3 - dark yellow. UV λ max (nm): MeOH 257, 269, 348; +NaOMe 273, 337sh, 409 (inc.); +$AlCl_3$ 274, 422; +$AlCl_3$/HCl 263sh, 276, 298, 358, 387sh; +NaOAc 268sh, 279, 400; +NaOAc/H_3BO_3 264, 375. ^{1}H NMR (500 MHz, pyridine-d_5): δ 8.38 (2H, *d, J* = 9.5 Hz, H-2′, 6′), 7.90 (1H, *d, J* = 7.9 Hz, H-5′), 7.14 (1H, *s*, H-3), 6.74 (1H, *s*, H-6), 6.03 (1H, *d, J* = 10.1 Hz, glucosyl H-1), 5.83 (1H, *d, J* = 10.1 Hz, glucosyl H-1), 4.8-3.9 (*m*, sugar protons). ^{13}C NMR (125 MHz, pyridine-d_5): (luteolin) δ 165.1 (C-2, C-7), 105.3 (C-3), 183.3 (C-4), 162.5 (C-5), 99.2 (C-6), 106.9 (C-8), 155.5 (C-9), 103.6 (C-10), 121.0 (C-1′), 114.9 (C-2′), 147.5 (C-3′), 150.9 (C-4′), 115.6 (C-5′), 120.2 (C-6′); (*O*-glucose) δ 103.6 (C-1), 74.3 (C-2), 76.4 (C-3), 64.7 (C-4), 76.1 (C-5), 62.6 (C-6); (*C*-glucose) δ 71.6 (C-1), 83.5 (C-2), 78.6 (C-3), 71.5 (C-4), 83.4 (C-5), 62.7 (C-6).

Isoorientin 2″-*O*-glucoside (**7**). PC: Rf 0.25 (BAW), 0.37 (BEW), 0.61 (15%HOAc), 0.56 (5%HOAc); Color UV - dark purple, UV/NH_3 - dark yellow. UV λ max (nm): MeOH 257sh, 270, 347; +NaOMe 275, 337sh, 407 (inc.); +$AlCl_3$ 276, 422; +$AlCl_3$/HCl 260sh, 279, 296sh, 357, 385sh; +NaOAc 269sh, 279, 399; +NaOAc/H_3BO_3 267, 375. ^{1}H NMR (500 MHz, pyridine-d_5): δ 8.37 (2H, *d, J* = 8.5 Hz, H-2′, 6′), 7.83 (1H, *dd, J* = 2.1 and 8.2 Hz, H-5′), 7.16 (1H, *s*, H-3), 6.74 (1H, *d, J* = 2.1 Hz, H-8), 6.03 (1H, *d, J* = 9.5 Hz, glucosyl H-1), 5.89 (1H, *d, J* = 9.5 Hz, glucosyl H-1), 4.7-3.9 (*m*, sugar protons). ^{13}C NMR (125 MHz, pyridine-d_5): (luteolin) δ 163.8 (C-2, C-7), 104.0 (C-3), 181.8 (C-4), 156.4 (C-5), 108.7 (C-6), 93.5 (C-8), 160.5 (C-9), 102.7 (C-10), 122.6 (C-1′), 114.6 (C-2′), 146.3 (C-3′), 149.2 (C-4′), 116.8 (C-5′), 119.5(C-6′); (*O*-glucose) δ 104.0 (C-1), 74.4 (C-2), 78.4 (C-3), 70.5 (C-4), 78.1 (C-5), 62.6 (C-6); (*C*-glucose) δ 71.5 (C-1), 83.3 (C-2), 78.5 (C-3), 70.5 (C-4), 80.7 (C-5), 62.6 (C-6).

Vitexin 2″-*O*-glucoside (**8**). PC: Rf 0.35 (BAW), 0.57 (BEW), 0.64 (15%HOAc), 0.54 (5%HOAc); Color UV - dark purple, UV/NH_3 - dark greenish yellow. UV λ max (nm): MeOH 270, 333; +NaOMe 280, 331, 396 (inc.); +$AlCl_3$ 277, 304, 348, 382; +$AlCl_3$/HCl 278, 303, 342, 382sh; +NaOAc 280, 314, 334, 393; +NaOAc/H_3BO_3 271, 345. ^{1}H NMR (500 MHz, pyridine-d_5): δ 8.37 (2H, *d, J* = 8.5 Hz, H-2′, 6′), 7.82 (2H, *d, J* = 8.5 Hz, H-3′, 5′), 7.17 (1H, *s*, H-3), 6.75 (1H, *s*, H-6), 6.03 (1H, *d, J* = 10.1 Hz, glucosyl H-1), 5.83 (1H, *d, J* = 9.5 Hz, glucosyl H-1), 4.8-3.9 (*m*, sugar protons). ^{13}C NMR (125 MHz, pyridine-d_5): (apigenin) δ 164.7 (C-2), 103.6 (C-3), 183.3 (C-4), 162.6 (C-5), 99.3 (C-6), 164.3 (C-7), 106.9 (C-8), 157.9 (C-9), 105.4 (C-10), 122.9 (C-1′), 121.6 (C-2′, C-6′), 116.8 (C-3′, C-5′), 162.7 (C-4′); (*O*-glucose) δ 103.9 (C-1), 73.4 (C-2), 76.1 (C-3, C-5), 71.6 (C-4), 62.6 (C-6); (*C*-glucose) δ 71.8 (C-1), 80.7 (C-2), 78.5 (C-3), 71.6 (C-4), 80.6 (C-5), 62.6 (C-6).

Isovitexin 2″-*O*-glucoside (**9**). PC: Rf 0.35 (BAW), 0.67 (BEW), 0.72 (15%HOAc), 0.64 (5%HOAc); Color UV - dark purple, UV/NH_3 - dark greenish yellow. UV λ max (nm): MeOH 270, 333; +NaOMe 280, 331, 396 (inc.); +$AlCl_3$ 277, 304, 348, 382; +$AlCl_3$/HCl 278, 303, 342, 382sh; +NaOAc 280, 314, 334, 393; +NaOAc/H_3BO_3 271, 345. ^{1}H NMR (500 MHz, pyridine-d_5): δ 8.37 (2H, *d, J* = 8.5 Hz, H-2′, 6′), 7.82 (2H, *d, J* = 8.5 Hz, H-3′, 5′), 7.17 (1H, *s*, H-3), 6.93 (1H, *s*, H-8), 6.03 (1H, *d, J* = 10.1 Hz, glucosyl H-1), 5.83 (1H, *d, J* = 9.5 Hz, glucosyl H-1), 4.8-3.9 (*m*, sugar protons). ^{13}C NMR (125 MHz, pyridine-d_5):

(apigenin) δ 164.7 (C-2, C-7), 103.6 (C-3), 183.3 (C-4), 162.6 (C-5), 111.7 (C-6), 94.5 (C-8), 157.9 (C-9), 105.4 (C-10), 122.9 (C-1′), 129.6 (C-2′, C-6′), 116.9 (C-3′, C-5′), 162.7 (C-4′); (*O*-glucose) δ 103.9 (C-1), 71.8 (C-2, C-5), 73.4 (C-3), 71.6 (C-4), 62.6 (C-6); (*C*-glucose) δ 71.8 (C-1), 83.1 (C-2), 78.5 (C-3), 71.6 (C-4), 80.7 (C-5), 62.6 (C-6).

Vicenin-2 (**10**). PC: Rf 0.23 (BAW), 0.25 (BEW), 0.52 (15%HOAc), 0.37 (5%HOAc); Color UV - dark purple, UV/NH_3 - dark yellow. UV λ max (nm): MeOH 273, 332; +NaOMe 283, 334, 400 (inc.); +$AlCl_3$ 281, 305, 351, 386sh; +$AlCl_3$/HCl 280, 304, 347, 384sh; +NaOAc 282, 315sh, 336, 397; +NaOAc/H_3BO_3 275sh, 285, 321, 348sh, 414sh.

Farrerol 7-*O*-glucoside (**11**). PC: Rf 0.75 (BAW), 0.83 (BEW), 0.54 (15%HOAc), 0.29 (5%HOAc); Color UV - dark purple, UV/NH_3 - greenish dark purple. UV λ max (nm): MeOH 283, 361; +NaOMe 244sh, 287, 392 (inc.); +$AlCl_3$ 287, 312sh, 362; +$AlCl_3$/HCl 285, 311sh, 363; +NaOAc 284, 363; +NaOAc/H_3BO_3 285, 363. ^{1}H NMR (500 MHz, pyridine-d_5): δ 12.59 (1H, *s*, 5-OH), 7.55 (2H, *d*, J = 7.9 Hz, H-2′, 6′), 7.09 (2H, *dd*, J = 1.8 and 9.8 Hz, H-3′, 5′), 5.3-5.5 (Obsc. with H-2 and glucosyl H-1), 4.6-4.0 (*m*, sugar protons), 3.25 (1H, *dd*, J = 10.4 and 17.1 Hz, H-3_{ax}), 2.91 (1H, *dd*, J = 3.4 and 6.7 Hz, H-3_{eq}), 2.65 (3H, *s*, 6 or 8-Me), 2.55 (3H, *s*, 6 or 8-Me). ^{13}C NMR (125 MHz, pyridine-d_5): (farrerol) δ 79.1 (C-2), 43.6 (C-3), 198.6 (C-4), 159.5 (C-5, C-9), 112.1 (C-6), 162.7 (C-7), 111.2 (C-8), 105.9 (C-10), 130.9 (C-1′), 128.6 (C-2′, C-6′), 116.5 (C-3′, C-5′), 158.4 (C-4′), 10.0 (6 or 8-Me), 9.5 (6 or 8-Me); (glucose) δ 105.8 (C-1), 75.8 (C-2), 78.5 (C-3), 71.6 (C-4), 78.8 (C-5), 62.7 (C-6).

Cyrtominetin 7-*O*-glucoside (Cyrtomin, **12**). PC: Rf 0.66 (BAW), 0.72 (BEW), 0.45 (15%HOAc), 0.25 (5%HOAc); Color UV - dark purple, UV/NH_3 - dark purple. UV λ max (nm): MeOH 285, 350; +NaOMe 244sh, 289, 390 (inc.); +$AlCl_3$ 289, 310sh, 357; +$AlCl_3$/HCl 288, 307sh, 360; +NaOAc 286, 350; +NaOAc/H_3BO_3 286, 351. ^{1}H NMR (500 MHz, pyridine-d_5): δ 12.59 (1H, *s*, 5-OH), 7.30 (2H, *d*, J = 8.2 Hz, H-2′, 6′), 7.06 (1H, *d*, J = 1.8 Hz, H-5′), 5.3-5.5 (Obsc. with H-2 and glucosyl H-1), 4.6-4.0 (*m*, sugar protons), 3.25 (1H, *dd*, J = 10.4 and 17.1 Hz, H-3_{ax}), 2.91 (1H, *dd*, J = 3.4 and 6.7 Hz, H-3_{eq}), 2.64 (3H, *s*, 6 or 8-Me), 2.52 (3H, *s*, 6 or 8-Me). ^{13}C NMR (125 MHz, pyridine-d_5): (cyrtominetin) δ 79.3 (C-2), 43.6 (C-3), 198.7 (C-4), 159.5 (C-5, C-9), 112.1 (C-6), 162.8 (C-7), 111.2 (C-8), 105.9 (C-10), 130.9 (C-1′), 115.1(C-2′), 147.6 (C-3′), 147.9 (C-4′), 116.6 (C-5′), 118.3 (C-6′), 10.1 (6 or 8-Me), 9.4 (6 or 8-Me); (glucose) δ 105.8 (C-1), 75.8 (C-2), 78.5 (C-3), 71.6 (C-4), 78.8 (C-5), 62.7 (C-6).

Farrerol (**13**). PC: Rf 0.86 (BAW), 0.88 (BEW), 0.90 (Forestal: HOAc:HCl/H_2O = 30:3:10), 0.14 (15%HOAc); Color UV - dark purple, UV/NH_3 - dark purple. UV λ max (nm): MeOH 294, 348; +NaOMe 243sh, 338 (inc.); +$AlCl_3$ 316, 361, 407sh; +$AlCl_3$/HCl 315, 364sh, 400; +NaOAc 290, 338; +NaOAc/H_3BO_3 296, 348. ^{1}H NMR (500 MHz, pyridine-d_5): δ 13.06 (1H, *s*, 5-OH), 7.60 (1H, *d*, J = 1.8 Hz, H-6′), 7.58 (1H, *d*, J = 2.1 Hz, H-2′), 7.28 (1H, *d*, J = 1.8 Hz, H-5′), 7.25 (1H, *d*, J = 2.1 Hz, H-3′), 5.52 (1H, *dd*, J = 3.1 and 12.5 Hz, H-2), 3.29 (1H, *dd*, J = 12.5 and 17.1 Hz, H-3_{ax}), 2.97 (1H, *dd*, J = 3.2 and 16.9 Hz, H-3_{eq}), 2.44 (3H, *s*, 6 or 8-Me), 2.34 (3H, *s*, 6 or 8-Me). ^{13}C NMR (125 MHz, pyridine-d_5): δ 79.1 (C-2), 43.4 (C-3), 197.2 (C-4), 159.4 (C-5), 104.6 (C-6), 160.1 (C-7), 103.6 (C-8), 164.3 (C-9), 102.9 (C-10), 130.3 (C-1′), 128.6 (C-2′, C-6′), 116.5 (C-3′, C-5′), 158.5 (C-4′), 9.0 (6 or 8-Me), 8.5 (6 or 8-Me).

Cyrtominetin (**14**). PC: Rf 0.86 (BAW), 0.88 (BEW), 0.85 (Forestal), 0.14 (15%HOAc); Color UV - dark purple, UV/NH_3 - dark purple. UV λ max (nm): MeOH 294, 345; +NaOMe 243sh, 337 (inc.); +$AlCl_3$ 315, 360, 405sh; +$AlCl_3$/HCl 315, 370, 405sh; +NaOAc 290, 338; +NaOAc/H_3BO_3 295, 346. ^{1}H NMR (500 MHz, pyridine-d_5): δ 13.01 (1H, *s*, 5-OH), 7.33 (1H, *d*, J = 1.2 Hz, H-6′), 7.30 (1H, *d*, J =1.2 Hz, H-2′), 7.10 (1H, *d*, J = 1.5 Hz, H-5′), 5.52 (1H, *dd*, J = 2.8 and 12.2 Hz, H-2), 3.29 (1H, *dd*, J = 12.4 and 16.9 Hz, H-3_{ax}), 3.02 (1H, *dd*, J = 3.1 and 10.1 Hz, H-3_{eq}), 2.42 (3H, *s*, 6 or 8-Me), 2.30 (3H, *s*, 6 or 8-Me). ^{13}C

NMR (125 MHz, pyridine-d_5): δ 79.1 (C-2), 43.5 (C-3), 197.2 (C-4), 159.4 (C-5), 104.4 (C-6), 160.0 (C-7), 103.7 (C-8), 164.2 (C-9), 102.9 (C-10), 131.2 (C-1′), 115.1 (C-2′), 147.6 (C-3′), 147.8 (C-4′), 116.6 (C-5′), 118.3 (C-6′), 9.0 (6 or 8-Me), 8.4 (6 or 8-Me).

Results and Discussion

Three flavonols (**1** - **3**), seven *C*-glycosylflavones (**4** - **10**) and four *C*-methylflavanones (**11** - **14**) were isolated from *C. falcatum* subsp. *falcatum*, subsp. *australe* and subsp. *littorale,* and *C. devexiscapulae*. Of their flavonoids, characterization of four *C*-methylflavanones have already been described by Iwashina *et al.* (2006). Two flavonols, quercetin 3-*O*-glucoside (**1**) and kaempferol 3-*O*-glucoside (**2**), and three *C*-glycosylflavones, orientin (**4**), isoorientin (**5**) and vicenin-2 (**10**) were identified by UV spectral properties and direct PC and HPLC comparisons with authentic specimens. Another flavonol, kaempferol 3-*O*-glucoside-7-*O*-rhamnoside (**3**), and four *C*-glycosylflavone *O*-glycosides, orientin 2″-*O*-glucoside (**6**), isoorientin 2″-*O*-glucoside (**7**), vitexin 2″-*O*-glucoside (**8**) and isovitexin 2″-*O*-glucoside (**9**) were identified based on UV, ^{1}H and ^{13}C NMR spectral data (see Materials and Methods).

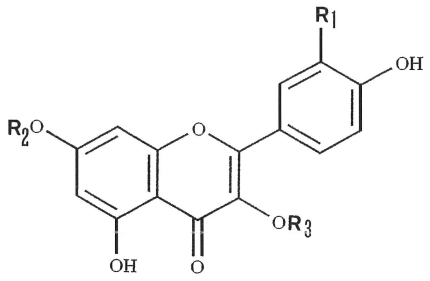
Among three *C. falcatum* subspecies and *C. devexiscapulae,* four *C*-methylflavanones, farrerol and cyrtominetin, and their 7-*O*-glucosides were only detected from *C. devexiscapulae* (Table 1), showing that the presence of flavanones is a chemical character of *C. devexiscapulae* but not *C. falcatum sensu lato.* On the other hand, other flavonols and *C*-glycosylflavones were not detectable except a few individuals, e.g., 417: Oura, Shimoda, Shizuoka Pref.; 437: Fukue Is., Nagasaki Pref. (presence of flavonoid **3**), 444-446: Hou-ji temple, Putuo-shan, Zhoushan Qundao Isls., Zhejiang Prov. China (presence of flavonoid **10**) and so on (App. 1).

Flavonols and *C*-glycosylflavones can commonly detect in three *C. falcatum* subspecies. The appearance of *C*-glycosylflavones was apparently the same among three subspecies, i.e., major 2″-*O*-

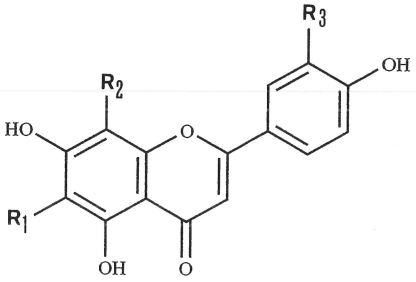
Table 1. Distribution of the flavonoids in *Cyrtomium falcatum* subsp. *falcatum,* subsp. *australe* and subsp. *littorale,* and *C. devexiscapulae* including *C. falcatum sensu lato*

Taxa	Flavonols			*C*-Glycosylflavones							Flavanones			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
C. falcatum subsp.	36	36	168	8	6	153	105	119	118	17	0	0	0	0
falcatum (239)	15%	15%	70%	3%	3%	64%	44%	50%	49%	7%				
C. falcatum subsp.	85	85	78	11	8	64	62	52	51	23	0	0	0	0
australe (94)	90%	90%	83%	12%	9%	68%	66%	55%	54%	24%				
C. falcatum subsp.	74	74	0	7	6	37	38	17	17	0	0	0	0	0
littorale (74)	100%	100%		9%	8%	50%	51%	23%	23%					
C. devexiscapulae (45)	0	0	2	0	0	0	0	0	0	6	45	45	38	38
			4%							13%	100%	100%	90%	90%

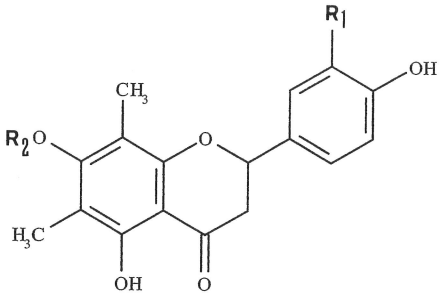
( ) = No. of individuals examined.
1 = Quercetin 3-*O*-glucoside, **2** = Kaempferol 3-*O*-glucoside, **3** = Kaempferol 3-*O*-glucoside-7-*O*-rhamnoside, **4** = Orientin, **5** = Isoorientin, **6** = *O*rientin 2″-*O*-glucoside, **7** = Isoorientin 2″-*O*-glucoside, **8** = Vitexin 2″-*O*-glucoside, **9** = Isovitexin 2″-*O*-glucoside, **10** = Vicenin-2, **11** = Farrerol 7-*O*-glucoside, **12** = Cyrtominetin 7-*O*-glucoside, **13** = Farrerol and **14** = Cyrtominetin.



R_1 = OH, R_2 = H, R_3 = glucosyl: Quercetin 3-*O*-glucoside (Isoquercitrin, **1**)
R_1 = R_2 = H, R_3 = glucosyl: Kaempferol 3-*O*-glucoside (Astragalin, **2**)
R_1 = H, R_2 = rhamnosyl, R_3 = glucosyl: Kaempferol 3-*O*-glucoside-7-*O*-rhamnoside (**3**)



R_1 = H, R_2 = *C*-glucosyl, R_3 = OH: Orientin (**4**)
R_1 = *C*-glucosyl, R_2 = H, R_3 = OH: Isoorientin (**5**)
R_1 = H, R_2 = 2″-*O*-gluco-*C*-glucosyl, R_3 = OH: Orientin 2″-*O*-glucoside (**6**)
R_1 = 2″-*O*-gluco-*C*-glucosyl, R_2 = H, R_3 = OH: Isoorientin 2″-*O*-glucoside (**7**)
R_1 = R_3 = H, R_2 = 2″-*O*-gluco-*C*-glucosyl: Vitexin 2″-*O*-glucoside (**8**)
R_1 = 2″-*O*-gluco-*C*-glucosyl, R_2 = R_3 = H: Isovitexin 2″-*O*-glucoside (**9**)
R_1 = R_2 = *C*-glucosyl, R_3 = H: Vicenin-2 (**10**)



R_1 = H, R_2 = glucosyl: Farrerol 7-*O*-glucoside (**11**)
R_1 = OH, R_2 = glucosyl: Cyrtominetin 7-*O*-glucoside (**12**)
R_1 = R_2 = H: Farrerol (**13**)
R_1 = OH, R_2 = H: Cyrtominetin (**14**)

Fig. 1. Chemical structures of flavonoids isolated from *Cyrtomium falcatum* complex.

glucosides (**6**, **7**, **8** and **9**) and minor orientin (**4**), isoorientin (**5**) and vicenin-2 (**10**). However, major flavonols were different among three subspecies. Major flavonol of subsp. *falcatum* is kaempferol 3-*O*-glucoside-7-*O*-rhamnoside (**3**), but those of subsp. *littorale* are quercetin 3-*O*-glucoside (**1**) and kaempferol 3-*O*-glucoside (**2**). Three flavonols appear in subsp. *australe* as major compounds.

Of the individuals of *C. falcatum sensu lato* used as plant materials in this experiment, flavonols and *C*-glycosylflavones were not found or small amount from some individuals, e.g., 29: Ohse, Minami-Izu, Shizuoka Pref.; 52: Tajiri-hama, Hitachi, Ibaraki Pref.; 128-130: Senkaku-wan, Aikawa, (Sado Is.), Niigata Pref.; 132-133: Nagasaki-hana, Yamakawa, Kagoshima Pref. (App. 1). As the reason, since many individuals are cultivated in the greenhouse, the flavonoid biosynthesis may be controlled. On the other hand, it is noteworthy that the *C*-methylflavanones were abundantly synthesized with or without the cultivation of the plants in the greenhouse.

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Summary

Fourty-two individuals of *Cyrtomium devexiscapulae*, 239 of *C. falcatum* subsp. *falcatum*, 97 of *C. falcatum* subsp. *australe*, 74 of *C. falcatum* subsp. *littorale*, which are included in *C. falcatum sensu lato*, were analysed for flavonoid compounds. Three flavonols, quercetin 3-*O*-glucoside (**1**), kaempferol 3-*O*-glucoside (**2**) and kaempferol 3-*O*-glucoside-7-*O*-rhamnoside (**3**), seven *C*-glycosylflavones, orientin (**4**), isoorientin (**5**), orientin 2″-*O*-glucoside (**6**), isoorientin 2″-*O*-glucoside (**7**), vitexin 2″-*O*-glucoside (**8**), isovitexin 2″-*O*-glucoside (**9**) and vicenin-2 (**10**), and four *C*-methylflavanones, farrerol 7-*O*-glucoside (**11**), cyrtominetin 7-*O*-glucoside (**12**), farrerol (**13**) and cyrtominetin (**14**), were isolated from the fronds. Of their flavonoids, occurrence of four flavanones was restricted to *C. devexiscapulae*, showing that the presence of them is a chemical character of *C. devexiscapulae* alone. The appearance of *C*-glycosylflavones was apparently the same among three *C. falcatum* subspecies. However, occurrence of the flavonols were quantitatively different among three *C. falcatum* subspecies, i.e., major compound of subsp. *falcatum* is **3**, but those of subsp. *littorale* are **1** and **2**. In subsp. *australe*, all three flavonols **1**, **2** and **3** were present as major compounds.

摘　要

広義のオニヤブソテツに属するオニヤブソテツ（狭義）239個体、その亜種であるヒメオニヤブソテツ74個体とムニンオニヤブソテツ94個体、さらに別種とされるナガバヤブソテツ45個体のフラボノイド組成がHPLCなどによって解析された。３種類のフラボノール、Quercetin 3-*O*-glucoside (**1**), Kaempferol 3-*O*-glucoside (**2**)およびKaempferol 3-*O*-glucoside-7-*O*-rhamnoside (**3**)、７種

類の*C*-グリコシルフラボン、Orientin (**4**), Isoorientin (**5**), Orientin 2″-*O*-glucoside (**6**), Isoorientin 2″-*O*-glucoside (**7**), Vitexin 2″-*O*-glucoside (**8**), Isovitexin 2″-*O*-glucoside (**9**)およびVicenin-2 (**10**)、そして4種類の*C*-メチルフラバノン、Farrerol 7-*O*-glucoside (**11**), Cyrtominetin 7-*O*-glucoside (**12**), Farrerol (**13**)およびCyrtominetin (**14**)が分離同定された。これらのフラボノイドのうち、広義のオニヤブソテツに存在するとされていた*C*-メチルフラバノンはナガバヤブソテツからのみ検出され、オニヤブソテツ(広義)の化学的特徴ではなく、ナガバヤブソテツのみの特徴であることが判明した。一方、オニヤブソテツ(狭義)とその2亜種のフラボノイドはフラボノールと*C*-グリコシルフラボンであった。これらのうち、*C*-グリコシルフラボンの出現頻度は3亜種でほとんど変わらなかったが、フラボノールについてはオニヤブソテツ(狭義)でKaempferol 3-*O*-glucoside-7-*O*-rhamnoside (**3**)が主要成分であるのに対して、ヒメオニヤブソテツではQuercetin 3-*O*-glucoside (**1**)とKaempferol 3-*O*-glucoside (**2**)が主要成分であった。さらにムニンオニヤブソテツでは、そのいずれもが主要フラボノイドとして存在した。なお、ナガバヤブソテツからはフラボノールと*C*-グリコシルフラボンはほとんど検出されなかった。

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App. 1. Collection sites, acc. no. and flavonoid composition ( ) of *Cyrtomium falcatum* subsp. *falcatum*, subsp. *australe* and subsp. *littorale*, and *C. devexiscapulae*

Cyrtomium falcatum* subsp. *falcatum

1: Funaura, Taketomi (Iriomote Is.), Okinawa Pref.（沖縄県竹富町船浦）（竹富島）; TBG54814 (**1**,**2**,**3**,**6**,**8**,**9**), 2, 3: Sumiyoshi, Taketomi (Iriomote Is.), Okinawa Pref.（沖縄県竹富町住吉）（竹富島）; SM880330-5, SM880330-6 (**3**), 4, 5: Gyokusen-do, Gushikami, Okinawa Pref.（沖縄県具志頭村玉泉洞）; TBG54774, TBG54773 (**3**,**6**,**8**,**9**), 6-8: White-beach, Katsuren, Okinawa Pref.（沖縄県勝連町ホワイトビーチ）; TBG54777, TBG54775, TBG54776 (**1**,**2**,**3**,**6**,**7**,**8**,**9**), 9: Syurei-no-mon, Naha, Okinawa Pref.（沖縄県那覇市守礼門）; SM880330-1 (**3**,**6**,**8**,**9**), 10-12: Yaku (Yaku Is.), Kagoshima Pref.（鹿児島県屋久町）（屋久島）; SM861011-28, SM861011-29, SM861011-32 (**3**,**6**,**7**,**8**,**9**), 13-15: Kawachi, Kumamoto Pref.（熊本県川内町）; TBG27913, TBG27914, TBG27916 (**3**,**6**,**8**,**9**), 16: Shikimi, Nagasaki, Nagasaki Pref.（長崎県長崎市式見町）(**3**,**6**,**8**,**9**), 17: Hiwasa, Tokushima Pref.（徳島県日和佐町）; TBG53945 (**1**,**2**,**3**,**6**,**7**,**8**,**9**), 18: Kawano, Aki, Kochi Pref.（高知県安芸市河野）; TBG53949 (**3**), 19: Arisaki, Tosa-shimizu, Kochi Pref.（高知県土佐清水市蟻岬）; TBG53962 (**3**), 20: Onigasaki, Kumano, Mie Pref.（三重県熊野市鬼ヶ崎）; SM840210-1 (**6**,**7**,**8**,**9**,**10**), 21-23: Mikura Is., Tokyo（東京都御蔵島）; SM810707, SM810707-12, SM810709-8 (**5**,**6**,**7**), 24: Kaeshi-hama, Kozu Is., Tokyo（東京都神津島返浜）; SM810711-27 (**6**,**8**,**9**), 25-28: Usuki, Miyake Is., Tokyo（東京都三宅島薄木）; SM801010-6, SM801010-7, SM801010-8, SM801010-9 (**1**,**2**,**3**,**6**,**7**,**8**,**9**), 29, 30: Ohse, Minami-Izu, Shizuoka Pref.（静岡県南伊豆町大瀬）; SM760516-26, 760516-27 (**none**), 35-37: Sotoura, Shimoda, Shizuoka Pref.（静岡県下田市外浦）; SM800211-5, SM800211-7, SM800515-8 (**1**,**2**,**3**,**6**,**7**,**8**,**9**,**10**), 38-40: Jogasaki, Ito, Shizuoka Pref.（静岡県伊東市城ヶ崎）; SM800615-3, SM800615-4, SM800615-22 (**1**,**2**,**3**,**6**,**7**,**8**,**9**), 41, 42: Oura-kaigan, Shimoda, Shizuoka Pref.（静岡県下田市大浦海岸）; SM811004-14, SM811004-15 (**3**,**6**,**7**,**8**,**9**), 43: Sotoura-iriguchi, Shimoda, Shizuoka Pref.（静岡県下田市外浦入口）; SM811009-7 (**none**), 44: Suzaki, Shimoda, Shizuoka Pref.（静岡県下田市須崎）; SM811011-20 (**3**,**6**,**7**,**8**,**9**), 45-50: Kisami, Shimoda, Shizuoka Pref.（静岡県下田市吉佐美）; SM820315-10, SM820315-11, SM820315-12, SM820315-13, SM820315-16, SM820315-22 (**1**,**2**,**3**,**6**,**7**,**8**,**9**,**10**), 51: Mochimune, Shizuoka, Shizuoka Pref.（静岡県静岡市用宗）; SM730902-4 (**3**,**6**,**7**,**8**,**9**), 52: Tajirihama, Hitachi, Ibaraki Pref.（茨城県日立市田尻浜）; SM8301120-10 (**none**), 53-57: Ajigaura, Nakaminato, Ibaraki Pref.（茨城県那珂湊市阿字浦）; SM820509-1, SM820509-2, SM820509-3, SM820509-4, SM820509-5 (**3**,**4**,**5**,**6**,**7**,**8**,**9**,**10**), 58: Umiyama, Mikata, Fukui Pref.（福井県三方町海山）; SM870328-1 (**1**,**2**,**3**,**6**,**7**,**8**,**9**,**10**), 59: Sugao, Fukui, Fukui Pref.（福井県福井市菅生）; TBG33273 (**3**,**6**,**7**,**8**,**9**), 31-34, 60: Nanaura-kaigan, Monzen, Ishikawa Pref.（石川県門前町七浦海岸）; SM770502-30, SM770502-31-1, SM770502-31-2, SM770502-31, SM770502-23 (**1**,**2**,**3**,**4**,**5**,**6**,**7**,**8**,**9**,**10**), 61-63: Kinkazan Is., Oshika, Miyagi Pref.（宮城県牡鹿町金華山島）; SM730923-1, SM730923-8, SM730923-9 (**none**), 64, 65: Onoaida, Yaku (Yaku Is.), Kagoshima Pref.（鹿児島県屋久町屋之間）（屋久島）; SM861011-30, SM861011-33 (**3**), 66-70, 72-74: Daio-saki, Daio, Mie Pref.（三重県大王町大王崎）; SM870401-2, SM870401-3, SM870401-4, SM870401-7, SM870401-8, SM870401-3, SM870401-10, SM870401-12 (**3**,**6**,**7**,**8**,**9**), 71: Kakizaki, Shimoda, Shizuoka Pref.（静岡県下田市柿崎）(**3**,**6**,**7**), 75, 76: Borawazawa-kaigan, Hachijo (Hachijo Is.), Tokyo（東京都八丈町洞輪沢海岸）（八丈島）; SM890127-3, SM890127-19 (**1**,**2**,**6**), 77: Borawazawa, Hachijo (Hachijo Is.), Tokyo（東京都八丈町洞輪沢）（八丈島）; SM890127-20 (**1**,**2**,**6**), 78: Sueyoshi, Hachijo (Hachijo Is.), Tokyo（東京都八丈町末吉）（八丈島）; SM890127-17 (**1**,**2**,**6**), 79, 81: Nakanogo, Hachijo (Hachijo Is.), Tokyo（東京都八丈町中之郷）（八丈

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* Plant materials, of which the country names are not recorded, were collected in Japan.