Bull. Natl. Mus. Nat. Sci., Ser. B, 41(1), pp. 33–44, February 20, 2015

Anthocyanins and Other Flavonoids from *Amorphophallus titanum* Having Largest Inflorescence in Plant Kingdom, and Other Two Species

Tsukasa Iwashina[1,*], Ayumi Uehara[1,2], Junichi Kitajima[3] and Tomohisa Yukawa[1]

[1] Department of Botany, National Museum of Nature and Science,
Amakubo 4–1–1, Tsukuba, Ibaraki 305–0005, Japan
[2] Department of Chemistry, Hiyoshi Campus, Keio University,
Hiyoshi 4–1–1, Kohoku-ku, Yokohama, Kanagawa 223–8521, Japan
[3] Laboratory of Pharmacognosy, Showa Pharmaceutical University,
Higashi-tamagawagakuen 3, Machida, Tokyo 194–8543, Japan
* E-mail: iwashina@kahaku.go.jp

(Received 8 November 2014; accepted 24 December 2014)

Abstracts *Amorphophallus titanum* has a largest inflorescence of the plant kingdom. In this survey, five anthocyanins and 14 other flavonoids were isolated from the spathe and spadix appendix of *A. titanum*. Anthocyanins were identified as cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, peonidin 3-*O*-glucoside, peonidin 3-*O*-rutinoside and pelargonidin 3-*O*-rhamnosylglucoside. On the other hand, other flavonoids were characterized as vitexin, vitexin 2″-*O*-glucoside, isovitexin, isovitexin 2″-*O*-glucoside, orientin, isoorientin, vicenin-2, lucenin-2, schaftoside, isoschaftoside, isovitexin X″-*O*-rhamnoside, isoscoparin X″-*O*-glucoside (*C*-glycosylflavones), chrysoeriol 7-*O*-glucoside (flavone) and kaempferol 3-*O*-robinobioside (flavonol). Anthocyanins and other flavonoids in the inflorescences of other two *Amorphophallus* species, *A. rivieri* and *A. paeoniifolius* were also isolated and identified.

Key words: *Amorphophallus paeoniifolius*, *Amorphophallus rivieri*, *Amorphophallus titanum*, Anthocyanins, Flavonols, *C*-Glycosylflavones.

Introduction

The genus *Amorphophallus* is mainly distributed in the tropical zone of the Old World and consists of 80–90 species (Ohashi, 1982). Of their species, *A. titanum* is endemic to Sumatra Island and has the largest inflorescence, ca. 3 m high and 1 m wide, of the plant kingdom. Its flowering in the botanical gardens is very rare. In 2012 and 2014, the flowers of the species came out at the Tsukuba Botanical Garden, National Museum of Nature and Science, Japan. The spathe and spadix appendix of the species are dark purple and their anthocyanins have been reported to be cyanidin 3-*O*-glucoside and 3-*O*-rutinoside, and peonidin and pelargonidin 3-*O*-(*p*-coumaloylglucosides) (Gallori *et al.*, 2004).

Amorphophallus paeoniifolius grows in Sri Lanka, the Philippines, Malaysia, Indonesia, and other Southeast Asia countries. Flavonol, quercetin, has been isolated from the corm of the species (Sharstry *et al.*, 2010). *Amorphophallus rivieri* is native to Indochina and southern China and cultivated in Japan for edible. However, polyphenols including flavonoids are not reported until now.

As the flavonoids of other *Amorphophallus* species, anthocyanin, cyanidin 3-*O*-rutinoside has been found in the spathe of *A. abyssinicus* (A.Rich.) N.E.Brown and *A. stuhlmannii* (Engl.) Engl. & Gehrm. (Williams *et al.*, 1981). A rare flavonol, 3,5-diacetyltambulin (7,8,4′-trimethoxy-3,5-diacetoxyflavone), has been isolated from the

tuberous roots of *A. campanulatus* Blume ex Decne (= *A. paeoniifolius*) (Khan *et al.*, 2008). Unknwon *C*-glycosylflavone was detected from the leaves of *A. stuhlmannii* (Williams *et al.*, 1981). In this paper, we describe the isolation and identification of the anthocyanins, flavone, flavonols and *C*-glycosylflavones from the inflorescences of three *Amorphophallus* species, *A. titanum*, *A. paeoniifolius* and *A. rivieri*.

Materials and Methods

General

UV spectra were recorded on a Shimadzu MPS-2000 Multipurpose recording spectrophotometer (Shimadzu, Japan) according to Mabry *et al.* (1970). LC-MS were measured on Shimadzu LC-MS systems using Inertsil ODS-4 column [I.D. 2.1 × 100 mm (GL Sciences, Japan)], at a flow-rate of 0.1 ml min^{-1}, eluting with MeCN/H_2O/HCOOH (5 : 90 : 5) for anthocyanins and (12 : 83 : 5 or 15 : 80 : 5) for other flavonoids, ESI^+ 4.5 kV and ESI^- 3.5 kV, 250°C. HPLC survey of the isolated compounds and crude extracts was performed with a Shimadzu HPLC systems using Inertsil ODS-4 column (I.D. 6.0 × 150 mm) at a flow-rate of 1.0 ml min^{-1}, eluting with MeCN/HOAc/H_2O/H_3PO_4 (10 : 8 : 79 : 3) (Solv. I) for anthocyanins, 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}, eluting with MeCN/H_2O/H_3PO_4 (15 : 85 : 0.2) (Solv. II) and (18 : 82 : 0.2) (Solv. III) for other flavonoids. Detection wave length was 530 nm for anthocyanins and 350 nm for other flavonoids. Acid hydrolyses of the anthocyanins and other flavonoids were performed in 12% HCl, 100°C, 30 min. After reaction, isoamyl alcohol (anthocyanins) or diethyl ether (other flavonoids) was added in the solution. Aglycones migrated to organic layer, and glycosidic sugars and *C*-glycosylflavones remained in aqueous layer. Aglycones and *C*-glycosylflavones were identified by HPLC comparisons with authentic samples, and sugars were compared with authentic sugars by paper chromatography or TLC 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 (PPC) are as follows; BAW (*n*-BuOH/HOAc/H_2O = 4 : 1 : 5, upper phase), BEW (*n*-BuOH/EtOH/H_2O = 4 : 1 : 2.2) and 15% HOAc. NMR spectra were measured in pyridine-d_5 at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR).

Plant materials

Three *Amorphophallus* species, *A. titanum* (Becc.) Becc. ex Arcangeli (TBG157495), *A. paeoniifolius* (Dennst.) Nicolson (TBG84663) and *A. rivieri* Durieu (= *A. konjac* K.Koch) (TBG157597) (Fig. 1) were used as plant materials. They are cultivated in the Tsukuba Botanical Garden, National Museum of Nature and Science, Tsukuba, Japan.

Extraction and separation

Fresh spathe (454 g) and spadix appendix (945 g) of *A. titanum*, and fresh inflorescences (138 g and 300 g) of *A. paeoniifolius* and *A. rivieri* were extracted with MeOH : HCOOH (92 : 8). After filtration, the concentrated extracts were applied to PPC using solvent systems, BAW and 15% HOAc. Other flavonoids were further separated with solvent system, BEW. The isolated anthocyanins and other flavonoids were purified by Sephadex LH-20 column chromatography using solvent systems, MeOH/H_2O/HCOOH (70 : 25 : 5) and 70% MeOH, respectively.

Identification

The isolated compounds were identified by UV spectroscopy, LC-MS, characterization of acid hydrolysates, ^{1}H and ^{13}C NMR, and TLC and HPLC comparisons with authentic samples. TLC, HPLC, LC-MS and NMR data of the isolated compounds are as follow.

Cyanidin 3-*O*-glucoside (chrysanthemin, **1**). HPLC: *t*R (min) 8.22 (solv. I). UV: λmax (nm) 0.01%MeOH-HCl 282, 529; + $AlCl_3$ 556; E_{440}/E_{max} 28%. LC-MS: *m/z* 449 $[M]^+$ (molecular ion

Fig. 1. Three *Amorphophallus* species used as plant materials.

peak, cyanidin + 1 mol glucose), *m/z* 287 $[M-162]^+$ (fragment ion peak, cyanidin).

Cyanidin 3-*O*-rutinoside (keracyanin, **2**). HPLC: *t*R (min) 9.22 (solv. I). UV: λmax (nm) 0.01%MeOH-HCl 280, 527; + $AlCl_3$ 546; E_{440}/E_{max} 29%. LC-MS: *m/z* 595 $[M]^+$ (molecular ion peak, cyanidin + each 1 mol rhamnose and glucose), *m/z* 287 $[M-308]^+$ (fragment ion peak, cyanidin).

Peonidin 3-*O*-glucoside (oxycoccicyanin, **3**). HPLC: *t*R (min) 16.81 (solv. I). LC-MS: *m/z* 463 $[M]^+$ (molecular ion peak, peonidin + 1 mol glucose), *m/z* 301 $[M-162]^+$ (fragment ion peak, peonidin).

Peonidin 3-*O*-rutinoside (**4**). HPLC: *t*R (min) 17.82 (solv. I). UV: λmax (nm) 0.01%MeOH-HCl 280, 529; + $AlCl_3$ 528; E_{440}/E_{max} 30%. LC-MS: *m/z* 609 $[M]^+$ (molecular ion peak, peonidin + each 1 mol rhamnose and glucose), *m/z* 301 $[M-308]^+$ (fragment ion peak, peonidin).

Pelargonidin 3-*O*-rhamnosylglucoside (**5**). HPLC: *t*R (min) 13.86 (solv. I). UV: λmax (nm) 0.01%MeOH-HCl 277, 512; + $AlCl_3$ 512. LC-MS: *m/z* 579 $[M]^+$ (molecular ion peak, pelargonidin + each 1 mol rhamnose and glucose), *m/z* 271 $[M-308]^+$ (fragment ion peak, pelargonidin).

Cyanidin 3-*O*-diglucoside (**6**). HPLC: *t*R (min) 6.18 (solv. I). UV: λmax (nm) 0.01%MeOH-HCl 280, 527; + $AlCl_3$ 546; E_{440}/E_{max} 25%. LC-MS: *m/z* 611 $[M]^+$ (molecular ion peak, cyanidin + 2 mol glucose), *m/z* 287 $[M-324]^+$ (fragment ion peak, cyanidin).

Pelargonidin 3-*O*-glucoside (callistephin, **7**). HPLC: *t*R (min) 11.72 (solv. I). UV: λmax (nm) 0.01%MeOH-HCl 272, 512; + $AlCl_3$ 513; E_{440}/E_{max} 42%. LC-MS: *m/z* 433 $[M]^+$ (molecular ion peak, pelargonidin + 1 mol glucose).

Vitexin (**8**). TLC: Rf 0.48 (BAW), 0.44 (BEW), 0.16 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 6.11 (solv. II). UV: λmax (nm) MeOH 270, 329; + NaOMe 278, 326, 394 (inc.); + $AlCl_3$ 275, 304, 346, 377sh; + $AlCl_3$/HCl 274, 303, 344, 376sh; + NaOAc 279, 310, 390; + NaOAc/H_3BO_3 271, 344. LC-MS: *m/z* 431 $[M-H]^-$ (molecular ion peak, apigenin + 1 mol glucose).

Vitexin 2″-*O*-glucoside (**9**). TLC: Rf 0.36 (BAW), 0.40 (BEW), 0.59 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 6.37 (solv. II). UV: λmax (nm) MeOH 270, 334; + NaOMe 280,

330, 397 (inc.); +$AlCl_3$ 277, 303, 349, 383; +$AlCl_3$/HCl 278, 302, 343, 382sh; +NaOAc 280, 312, 392; +NaOAc/H_3BO_3 271, 345. LC-MS: *m/z* 593 $[M-H]^-$ (molecular ion peak, apigenin + 2 mol glucose).

Isovitexin (**10**). TLC: Rf 0.71 (BAW), 0.68 (BEW), 0.42 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 8.02 (solv. II). UV: λmax (nm) MeOH 271, 334; +NaOMe 279, 327, 399 (inc.); +$AlCl_3$ 278, 303, 350, 377sh; +$AlCl_3$/HCl 279, 303, 346, 376sh; +NaOAc 278, 313, 394; +NaOAc/H_3BO_3 272, 347. LC-MS: *m/z* 433 $[M+H]^+$, 431 $[M-H]^-$ (molecular ion peaks, apigenin + 1 mol glucose).

Isovitexin 2″-*O*-glucoside (**11**). TLC: Rf 0.45 (BAW), 0.51 (BEW), 0.69 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 6.52 (solv. II). UV: λmax (nm) MeOH 270, 334; +NaOMe 280, 330, 397 (inc.); +$AlCl_3$ 277, 303, 349, 383; +$AlCl_3$/HCl 278, 302, 343, 382sh; +NaOAc 280, 312, 392; +NaOAc/H_3BO_3 271, 345. LC-MS: *m/z* 593 $[M-H]^-$ (molecular ion peak, apigenin + 2 mol glucose).

Orientin (**12**). TLC: Rf 0.31 (BAW), 0.27 (BEW), 0.11 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark yellow. HPLC: *t*R (min) 5.27 (solv. II). UV: λmax (nm) MeOH 257, 269, 349; +NaOMe 270, 327sh, 407 (inc.); +$AlCl_3$ 274, 422; +$AlCl_3$/HCl 263sh, 276, 296, 358, 385sh; +NaOAc 272, 276, 327sh, 400; +NaOAc/H_3BO_3 266, 376, 427sh. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucose).

Isoorientin (**13**). TLC: Rf 0.47 (BAW), 0.45 (BEW), 0.28 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark yellow. HPLC: *t*R (min) 6.01 (solv. II). UV: λmax (nm) MeOH 258, 270, 350; +NaOMe 270, 337sh, 411 (inc.); +$AlCl_3$ 276, 422; +$AlCl_3$/HCl 262sh, 278, 296sh, 357, 384sh; +NaOAc 270, 402; +NaOAc/H_3BO_3 267, 378, 426sh. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucose).

Vicenin-2 (**14**). TLC: Rf 0.17 (BAW), 0.19 (BEW), 0.40 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 4.73 (solv. II). UV: λmax (nm) MeOH 272, 334; +NaOMe 282, 335, 399 (inc.); +$AlCl_3$ 279, 303, 353, 384sh; +$AlCl_3$/HCl 279, 303, 347, 383sh; +NaOAc 282, 398; +NaOAc/H_3BO_3 278, 283sh, 323, 349, 410sh. LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (molecular ion peaks, apigenin + 2 mol glucose).

Lucenin-2 (**15**). TLC: Rf 0.09 (BAW), 0.09 (BEW), 0.31 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark yellow. HPLC: *t*R (min) 4.14 (solv. II). UV: λmax (nm) MeOH 255sh, 272, 344; +NaOMe 268, 280, 340sh, 411 (inc.); +$AlCl_3$ 279, 402; +$AlCl_3$/HCl 260sh, 279, 296sh, 356, 385sh; +NaOAc 268sh, 281, 327sh, 404; +NaOAc/H_3BO_3 275, 285sh, 361, 422. LC-MS: *m/z* 611 $[M+H]^+$, 609 $[M-H]^-$ (molecular ion peaks, luteolin + 2 mol glucose).

Schaftoside (**16**). TLC: Rf 0.21 (BAW), 0.20 (BEW), 0.40 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 5.63 (solv. II). UV: λmax (nm) MeOH 272, 333; +NaOMe 283, 334, 399 (inc.); +$AlCl_3$ 279, 305, 350, 384sh; +$AlCl_3$/HCl 279, 303, 345, 384sh; +NaOAc 282, 314sh, 397; +NaOAc/H_3BO_3 276sh, 284, 322, 350sh, 410. LC-MS: *m/z* 565 $[M+H]^+$, 563 $[M-H]^-$ (molecular ion peaks, apigenin + each 1 mol arabinose and glucose).

Isoschaftoside (**17**). TLC: Rf 0.32 (BAW), 0.35 (BEW), 0.40 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 5.63 (solv. II). UV: λmax (nm) MeOH 272, 333; +NaOMe 283, 334, 399 (inc.); +$AlCl_3$ 279, 305, 350, 384sh; +$AlCl_3$/HCl 279, 303, 345, 384sh; +NaOAc 282, 314sh, 397; +NaOAc/H_3BO_3 276sh, 284, 322, 350sh, 410. LC-MS: *m/z* 565 $[M+H]^+$, 563 $[M-H]^-$ (molecular ion peaks, apigenin + each 1 mol arabinose and glucose).

Isovitexin X″-*O*-rhamnoside (**18**). TLC: Rf 0.53 (BAW), 0.53 (BEW), 0.75 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 6.75 (solv. II). UV: λmax (nm) MeOH 270, 329; +NaOMe 280,

331, 393 (inc.); + $AlCl_3$ 273, 304, 349, 377sh; + $AlCl_3$/HCl 278, 303, 339, 380sh; + NaOAc 280, 313, 389; + NaOAc/H_3BO_3 270, 347. LC-MS: *m/z* 579 $[M+H]^+$, 577 $[M-H]^-$ (molecular ion peaks, apigenin + each 1 mol rhamnose and glucose).

Isoscoparin X″-*O*-glucoside (**19**). TLC: Rf 0.14 (BAW), 0.12 (BEW), 0.36 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 5.25 (solv. II). UV: λmax (nm) MeOH 273, 345; + NaOMe 282, 341sh, 411 (inc.); + $AlCl_3$ 262sh, 277, 300sh, 360, 387sh; + $AlCl_3$/HCl 257sh, 277, 298sh, 358, 387sh; + NaOAc 282, 337sh, 409; + NaOAc/H_3BO_3 276, 285sh, 344, 412. LC-MS: *m/z* 625 $[M+H]^+$ (molecular ion peak, chrysoeriol + 2 mol glucose), *m/z* 461 $[M-162-H]^-$ (fragment ion peak, chrysoeriol + 1 mol glucose).

Chrysoeriol 7-*O*-glucoside (**20**). TLC: Rf 0.48 (BAW), 0.41 (BEW), 0.09 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 yellow. HPLC: *t*R (min) 16.16 (solv. II). UV: λmax (nm) MeOH 252, 269, 342; + NaOMe 264, 276sh, 391 (inc.); + $AlCl_3$ 262sh, 274, 296, 358, 385sh; + $AlCl_3$/HCl 259sh, 276, 297, 354, 385sh; + NaOAc 258, 265sh, 404; + NaOAc/H_3BO_3 269, 348. LC-MS: *m/z* 463 $[M+H]^+$, 461 $[M-H]^-$ (molecular ion peaks, chrysoeriol + 1 mol glucose).

Kaempferol 3-*O*-robinobioside (**21**). TLC: Rf 0.69 (BAW), 0.63 (BEW), 0.75 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 9.33 (solv. II). UV: λmax (nm) MeOH 267, 343; + NaOMe 276, 327, 400 (inc.); + $AlCl_3$ 275, 303, 352, 384; + $AlCl_3$/HCl 276, 302, 347, 387sh; + NaOAc 275, 316, 394; + NaOAc/H_3BO_3 267, 353. LC-MS: *m/z* 593 $[M-H]^-$ (molecular ion peak, kaempferol + each 1 mol rhamnose and galactose), *m/z* 287 $[M-308+H]^+$ (fragment ion peak, kaempferol).

Quercetin 3-*O*-glucoside (isoquercitrin, **22**). TLC: Rf 0.65 (BAW), 0.65 (BEW), 0.31 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 yellow. HPLC: *t*R (min) 7.33 (solv. II). UV: λmax (nm) MeOH 257, 265sh, 358; + NaOMe 274, 331, 407 (inc.); + $AlCl_3$ 274, 431; + $AlCl_3$/HCl 268, 300, 359, 394sh; + NaOAc 273, 327, 395; + NaOAc/H_3BO_3 261, 380. LC-MS: *m/z* 463 $[M-H]^-$ (molecular ion peak, quercetin + 1 mol glucose), *m/z* 302 $[M-162+H]^+$ (fragment ion peak, quercetin).

Vitexin 2″-*β*-*O*-xylopyranoside (**23**). TLC: Rf 0.47 (BAW), 0.55 (BEW), 0.73 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 6.22 (solv. II). UV: λmax (nm) MeOH 270, 330; + NaOMe 279, 331, 394 (inc.); + $AlCl_3$ 276, 304, 348, 384sh; + $AlCl_3$/HCl 277, 302, 343, 383sh; + NaOAc 279, 311, 390; + NaOAc/H_3BO_3 271, 345. LC-MS: *m/z* 565 $[M+H]^+$, 563 $[M-H]^-$ (molecular ion peaks, apigenin + each 1 mol xylose and glucose). ^{1}H NMR (600 MHz, pyridine-d_5): *δ* 13.85 (1H, s, 5-OH), 8.25 (2H, d, *J* = 6.7 Hz, H-2′,6′), 7.24 (2H, d, *J* = 6.7 Hz, H-3′,5′), 6.82 (1H, s, H-3), 6.74 (1H, s, H-6), 5.84 (1H, d, *J* = 9.2 Hz, glucosyl H-1), 5.01 (1H, brs, xylosyl H-1), 4.95 (1H, m, glucosyl H-2), 4.53 (1H, m, glucosyl H-4), 4.52 (1H, m, glucosyl H-6a), 4.42 (1H, m, glucosyl H-6b), 4.41 (1H, m, glucosyl H-3), 4.08 (1H, m, glucosyl H-5), 3.83 (1H, m, xylosyl H-3), 3.82 (1H, m, xylosyl H-5a), 3.82 (1H, m, xylosyl H-2), 3.76 (1H, m, xylosyl H-4), 3.18 (1H, m, xylosyl H-5b). ^{13}C NMR (150 MHz, pyridine-d_5): (apigenin) *δ* 165.1 (C-2), 103.5 (C-3), 183.2 (C-4), 162.5 (C-5), 99.4 (C-6), 164.7 (C-7), 105.4 (C-8), 158.3 (C-9), 105.4 (C-10), 123.2 (C-1′), 129.5 (C-2′,6′), 117.0 (C-3′,5′), 162.6 (C-4′); (*C*-glucose) *δ* 73.5 (C-1), 82.0 (C-2), 80.6 (C-3), 72.2 (C-4), 83.5 (C-5), 62.9 (C-6); (xylose) *δ* 106.7 (C-1), 75.0 (C-2), 77.4 (C-3), 70.8 (C-4), 66.5 (C-5).

Kaempferol 3-*O*-glucoside (astragalin, **24**). TLC: Rf 0.81 (BAW), 0.77 (BEW), 0.39 (15%HOAc); color UV (365 nm) dark purple, UV/NH_3 dark greenish yellow. HPLC: *t*R (min) 8.34 (solv. III). UV: λmax (nm) MeOH 266, 349; + NaOMe 275, 325, 398 (inc.); + $AlCl_3$ 274, 303, 351, 391; + $AlCl_3$/HCl 274, 302, 348, 389; + NaOAc 275, 313, 392; + NaOAc/H_3BO_3 267, 353. LC-MS: *m/z* 447 $[M-H]^-$ (molecular ion

peak, kaempferol + 1 mol glucose), *m/z* 287 $[M - 162 + H]^+$ (fragment ion peak, kaempferol).

Authentic samples

Authentic samples, which were used in this survey, and their origin were as follows: cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, peonidin 3-*O*-glucoside, peonidin 3-*O*-rutinoside and pelargonidin 3-*O*-glucoside from Extra Synthese, France, vitexin from the fronds of *Adiantum venustum* Don (Parkeriaceae) (Iwashina *et al.*, 1995), isovitexin from the flowers of *Iris ensata* Thunb. (Iridaceae) (Iwashina *et al.*, 1996), isovitexin 2″-*O*-glucoside and vitexin 2″-*O*-glucoside from the fronds of *Cyrtomium falcatum* (L.f.) C. Presl (Dryopteridaceae) (Iwashina *et al.*, 2006), orientin from the leaves of *Begonia formosana* (Hayata) Masam. (Begoniaceae) (Iwashina *et al.*, 2008a), isoorientin from the leaves of *Vitex rotundifolia* L.fil. (Verbenaceae) (Iwashina *et al.*, 2011), vicenin-2 and lucenin-2 from the fronds of *Asplenium normale* D.Don (Aspleniaceae) (Iwashina *et al.*, 2010), schaftoside and isoschaftoside from the aerial parts of *Osyris alba* L. (Santalaceae) (Iwashina *et al.*, 2008b), kaempferol 3-*O*-robinobioside from the aerial parts of *Cassytha filiformis* L. (Lauraceae) (Murai *et al.*, 2008), kaempferol 3-*O*-glucoside from the flowers of *Glycine max* (L.) Merr. (Leguminosae) (Iwashina *et al.*, 2007), and quercetin 3-*O*-glucoside from the leaves of *Calystegia sordanella* (L.) Roem. et Schult. (Convolvulaceae) (Murai *et al.*, 2014).

Results and Discussion

Anthocyanins from Amorphophallus titanum, A. rivieri *and* A. paeoniifolius

Five anthocyanins (**1**–**5**) were isolated from the spathe and spadix appendix of *Amorphophallus titanum* and the inflorescence of *A. rivieri*. Their anthocyanin composition was the same with each other. On the other hand, three anthocyanins (**1**, **6** and **7**) were found in the inflorescence of *A. paeoniifolius* (Fig. 2).

Acid hydrolysis of **1** and **2** produced cyanidin as anthocyanidin. And also, glucose, and glucose and rhamnose were detected as glycosidic sugars, respectively. As the results, they were identified as cyanidin 3-*O*-glucoside (chrysanthemin, **1**, Fig. 3) and cyanidin 3-*O*-rutinoside (keracyanin, **2**, Fig. 3) by HPLC comparisons with authentic samples. Similarly, **3** and **4** were identified as peonidin 3-*O*-glucoside (oxycoccicyanin, Fig. 3) and peonidin 3-*O*-rutinoside (Fig. 3). Anthocyanin **5** was obtained as a minor compo-

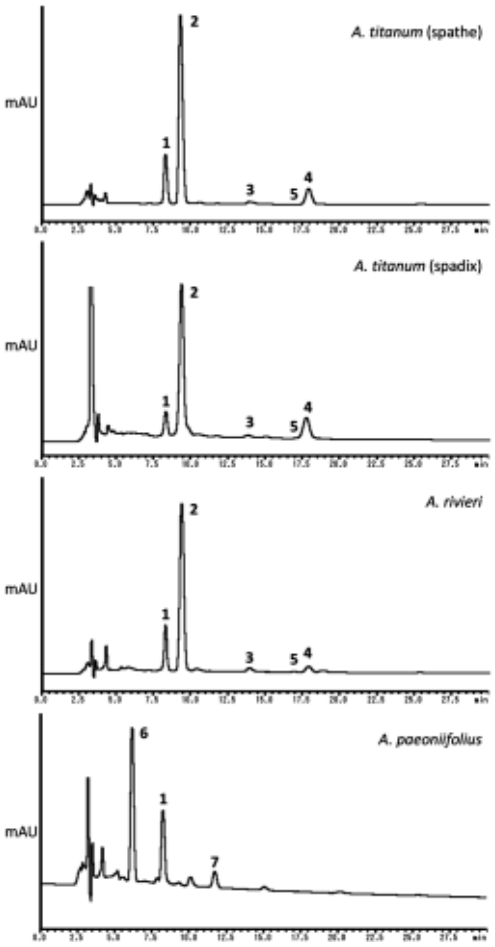
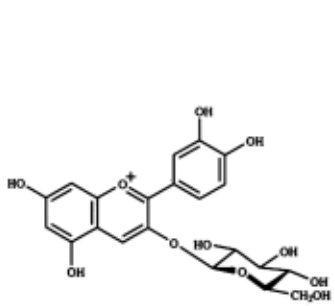
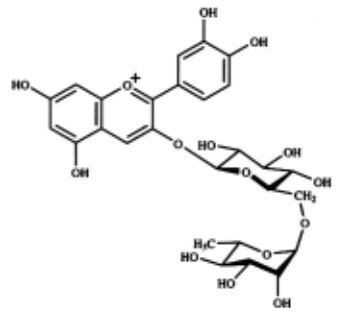


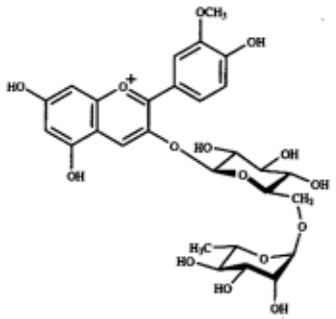
Fig. 2. HPLC patterns of the anthocyanins in the inflorescences of three *Amorphophallus* species. **1**: cyanidin 3-*O*-glucoside, **2**: cyanidin 3-*O*-rutinoside, **3**: peonidin 3-*O*-glucoside, **4**: peonidin 3-*O*-rutinoside, **5**: pelargonidin 3-*O*-rhamnosylglucoside, **6**: cyanidin 3-*O*-diglucoside and **7**: palergonidin 3-*O*-glucoside.



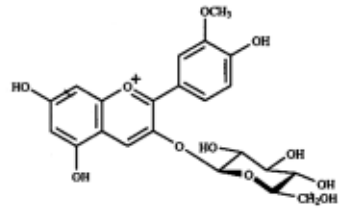
Cyanidin 3-*O*-glucoside (**1**)



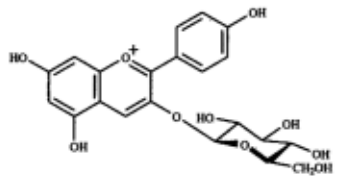
Cyanidin 3-*O*-rutinoside (**2**)



Peonidin 3-*O*-rutinoside (**4**)



Peonidin 3-*O*-glucoside (**3**)



Pelargonidin 3-*O*-glucoside (**7**)

Fig. 3. Anthocyanins isolated from the inflorescences of three *Amorphophallus* species.

nent from *A. titanum* and *A. rivieri*. Pelargonidin, glucose and rhamnose were liberated by acid hydrolysis. Attachment of each 1 mol glucose and rhamnose to pelargonidin was shown by appearance of molecular ion peak, *m/z* 579 $[M]^+$ of LC-MS. Thus, **5** was characterized as pelargonidin 3-*O*-rhamnosylglucoside. Anthocyanins **6** and **7** were isolated from *A. paeoniifolius*. They produced cyanidin and pelargonidin by acid hydrolysis, respectively. Attachment of 2 mol glucose to 3-position of cyanidin was shown by LC-MS survey, i.e. appearance of molecular ion peak, *m/z* 611 $[M]^+$, and UV spectral survey. From the results described above, **6** was characterized as cyanidin 3-*O*-diglucoside. On the other hand, **7** was identified as pelargonidin 3-*O*-glucoside (callistephin) (Fig. 3) by HPLC comparison with authentic sample.

In this survey, five anthocyanins, cyanidin 3-*O*-glucoside (**1**), cyanidin 3-*O*-rutinoside (**2**), peonidin 3-*O*-glucoside (**3**), peonidin 3-*O*-rutinoside (**4**) and pelargonidin 3-*O*-rhamnosylglucoside (perhaps 3-*O*-rutinoside, **5**), were isolated and characterized from *A. titanum* and *A. rivieri*.

Though the spathe and spadix appendix of *A. titanum* were separately extracted, their anthocyanin composition was qualitatively the same. On the other hand, three anthocyanins, cyanidin 3-*O*-glucoside (**1**), cyanidin 3-*O*-diglucoside (**6**) and pelargonidin 3-*O*-glucoside (**7**), were isolated from the inflorescence of *A. paeoniifolius*. *A. titanum* and *A. paeoniifolius*, and *A. rivieri* are native to tropical Asia, and temperate zones of Asia, respectively. However, anthocyanin pattern was the same between *A. titanum* and *A. rivieri*, but not *A. paeoniifolius*. Anthocyanins in the inflorescence of *A. titanum* have been surveyed, and 3-*O*-glucoside and 3-*O*-rutinoside of cyanidin, and peonidin and pelargonidin 3-*O*-(*p*-coumaroylglucosides) were reported (Gallori *et al.*, 2004). Of their anthocyanins, though the former two were found in this survey, the latter two acylated anthocyanins were not detected. Instead, three anthocyanins, peonidin 3-*O*-glucoside (**3**), peonidin 3-*O*-rutinoside (**4**) and pelargonidin 3-*O*-rhamnosylglucoside (**5**) were newly isolated. The anthocyanins in the inflorescences of *A. rivieri* and *A. paeoniifolius* were reported for the first time.

Flavonoids in the spathe and spadix appendix of Amorphophallus titanum

Fourteen flavonoids were isolated from the spathe and spadix appendix of *A. titanum*. UV spectral properties of flavonoids **8** and **10** showed that they are flavones having 5-, 7- and 4′-hydroxyl groups (Mabry *et al.*, 1970). Since they were unhydrolyzable by hot acid treatment, it was shown that they are *C*-glycosylflavones. Attachment of 1 mol hexose to apigenin was cleared by LC-MS, i.e. appearance of molecular ion peaks, *m/z* 433 $[M+H]^+$ and 431 $[M-H]^-$. Finally, they were identified as vitexin (5,7,4′-trihydroxyflavone 8-*C*-glucoside, **8**) and isovitexin (5,7,4′-trihydroxyflavone 6-*C*-glucoside, **10**) (Fig. 4) by TLC and HPLC comparisons with authentic samples. UV spectral properties of **9** and **11** were also the same with those of **8** and **10**, showing that they are 5,7,4′-trihydroxyflavone derivatives. Vitexin and isovitexin were liberated by acid hydrolysis of **9** and **11**, respectively. Attachment of additional 1 mol glucose to vitexin and isovitexin was proved by LC-MS, i.e. appearance of molecular ion peak, *m/z* 593 $[M-H]^-$. Finally, **9** and **11** were identified as vitexin 2″-*O*-glucoside and isovitexin 2″-*O*-glucoside (Fig. 5) by TLC and HPLC comparisons with authentic standards. It was shown by UV, hot acid treatment and LC-MS that **12** and **13** are 5,7,3′,4′-tetrahydroxylated flavone *C*-monohexosides. Thus, they were identified as orientin (5,7,3′,4′-tetrahydroxyflavone 8-*C*-glucoside, **12**) and isoorientin (5,7,3′,4′-tetrahydroxyflavone 6-*C*-glucoside, **13**) (Fig. 6) by TLC and HPLC comparisons with authentic samples. Flavonoid **14** was unhydrolyzable by hot acid treatment, showing that it is *C*-glycosylflavone. Attachment of 2 mol hexose to 5,7,4′-trihydroxyflavone was shown by LC-MS, i.e. appearance of molecular ion peaks, *m/z* 595 $[M+H]^+$ and 593 $[M-H]^-$. Finally, **14** was identified as vicenin-2 (5,7,4′-trihydroxyflavone 6,8-di-*C*-glucoside) (Fig. 7) by TLC and HPLC comparison with authentic sample. Similarly, **15** was estimated as lucenin-2 (5,7,3′,4′-tetrahydroxyflavone 6,8-di-*C*-glucoside) (Fig. 7). UV spectral survey and hot acid treatment of **16** and **17** showed that they are *C*-glycosylflavones having free 5-, 7- and 4′-hydroxyl

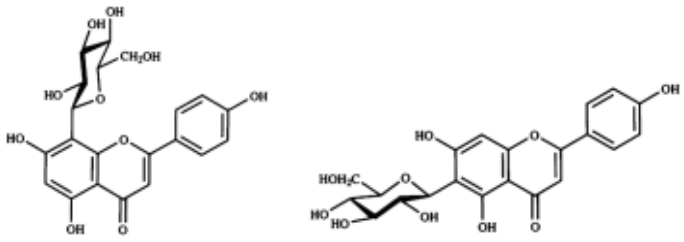


Fig. 4. Chemical structures of vitexin (left, **8**) and isovitexin (right, **10**).

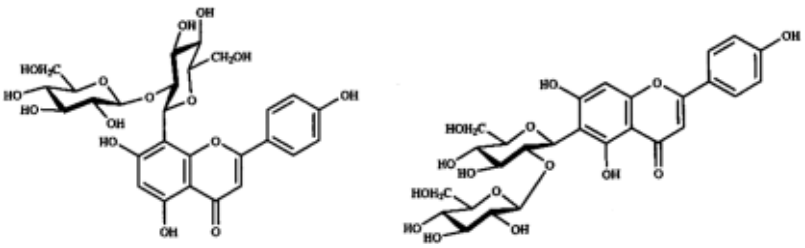


Fig. 5. Chemical structures of vitexin 2″-*O*-glucoside (left, **9**) and isovitexin 2″-*O*-glucoside (right, **11**).

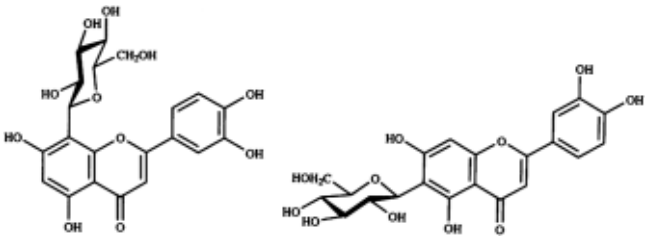


Fig. 6. Chemical structures of orientin (left, **12**) and isoorientin (right, **13**).

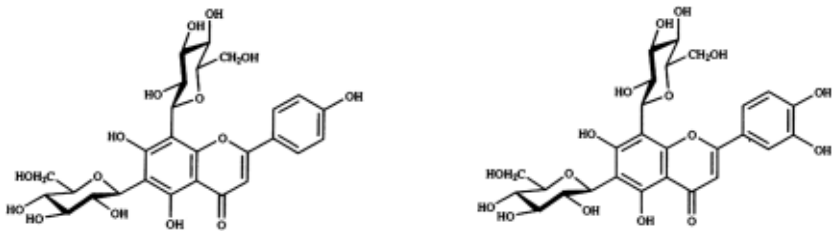


Fig. 7. Chemical structures of vicenin-2 (left, **14**) and lucenin-2 (right, **15**).

groups. By LC-MS survey of their compounds, molecular ion peaks, *m/z* 565 $[M+H]^+$ and 563 $[M-H]^-$, appeared on the chromatograms, showing that they are apigenin monopentosylhexoside. It was cleared that they are schaftoside (apigenin 6-*C*-glucoside-8-*C*-arabinoside, **16**) and isoschaftoside (apigenin 6-*C*-arabinoside-8-*C*-glucoside, **17**) by TLC and HPLC comparisons with authentic samples (Fig. 8). Flavonoid **18** produced isovitexin and rhamnose by acid hydrolysis. Since UV spectral properties of original compound were essentially the same with those of isovitexin, rhamnosyl moiety was considered to be attached to 6-*C*-glucosyl group of isovitexin. From the results described above, **18** was characterized as isovitexin X″-*O*-rhamnoside. However, interglycosidic linkage could not be determined. To flavonoid **19** is trihydroxy-monomethoxyflavone dihexoside proved by LC-MS, i.e. appearance of molecular ion peak, *m/z* 625 $[M+H]^+$. Isoscoparin (5,7,4′-trihydroxy-3′-methoxyflavone 6-*C*-glucoside) and glucose were liberated by acid hydrolysis of **19**. Since UV spectral properties of **19** were the same with those of isoscoparin, it was shown that *O*-glucose is attached to 6-*C*-glucosyl group. Thus, **19** was characterized as isoscoparin X″-*O*-glucoside. UV spectral survey in addition of various shift reagents showed that **20** is 7,3′-substituted luteolin (Mabry *et al.*, 1970). Chrysoeriol (5,7,4′-trihydroxy-3′-methoxyflavone) and glucose were produced by acid hydrolysis. Since molecular ion peaks, *m/z* 463 $[M+H]^+$ and 461 $[M-H]^-$, appeared on the chromatogram of LC-MS, it was shown that

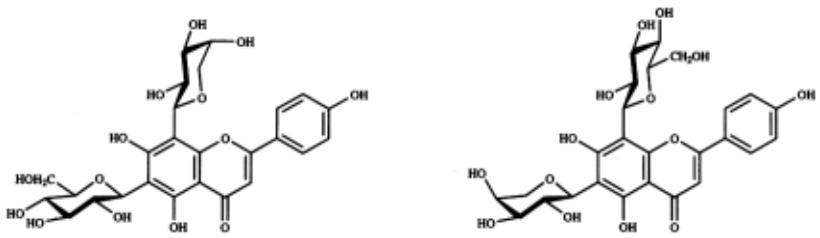


Fig. 8. Chemical structures of schaftoside (left, **16**) and isoschaftoside (right, **17**).

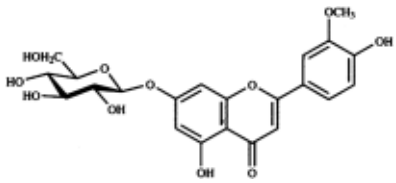


Fig. 9. Chemical structure of chrysoeriol 7-*O*-glucoside (**20**).

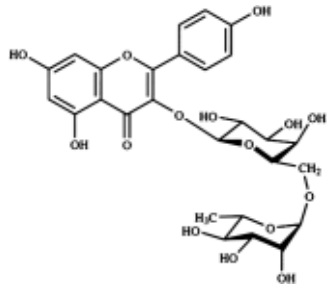


Fig. 10. Chemical structure of kaempferol 3-*O*-robinobioside (**21**).

1 mol glucose is attached to chrysoeriol. Thus, **20** was identified as chrysoeriol 7-*O*-glucoside (Fig. 9). Flavonoid **21** was only one flavonol from the inflorescence of *A. titanum*. Kaempferol, rhamnose and galactose were liberated by acid hydrolysis of **21**. UV spectral properties of the compound showed that this is 3-substituted kaempferol. Molecular ion peak, *m/z* 593 $[M-H]^-$, appeared on the chromatogram of LC-MS, showing the attachment of each 1 mol rhamnose and galactose to kaempferol. Finally, **21** was identified as kaempferol 3-*O*-robinobioside [= 3-*O*-rhamnosyl-(1→6)-galactoside] (Fig. 10) by TLC and HPLC comparison with authentic sample. Though the presence of other flavonoids was noticed by HPLC analysis of the crude extract of *A. titanum*, they could not be isolated for small amounts.

Flavonoids in the inflorescences of A. rivieri *and* A. paeoniifolius

Five flavonoids were isolated from the inflorescence of *A. rivieri*. On the other hand, seven flavonoids were identified from *A. paeoniifolius*. Of the flavonoids in *A. rivieri*, three were identified as isovitexin (**10**), orientin (**12**) and isoorientin (**13**). Flavonoid **22** liberated quercetin and glucose by acid hydrolysis. Since UV spectral properties of original compound showed that **22** is 3-substituted quercetin, it was proved that glucose is attached to 3-position of quercetin. Finally, **22** was identified as quercetin 3-*O*-glucoside (isoquercitrin) (Fig. 11) by TLC and HPLC comparison with authentic sample. Flavonoid **23** was obtained as pale yellow powder (yield, ca. 30 mg). Vitexin and xylose were found as acid hydrolysates of **23**. Attachment of 1 mol xylose to 8-*C*-glucosyl group of vitexin was shown by *m/z* 565 $[M+H]^+$ and 563 $[M-H]^-$ of LC-MS and UV spectral properties. In ^{1}H NMR, six aromatic proton signals, δ 6.82, 6.74, 8.25 and 7.24 derived from H-3, H-6, H-2′,6′ and H-3′,5′, and two anomeric proton signals, δ 5.84 (d, J = 9.2 Hz) and 5.01 (brs) derived from glucose and xylose, appeared. The interglycosidic linkage was determined as xylosyl-(1→2)-glucose from HMBC correlation between xylosyl anomeric proton at δ 5.01 and C-2 signal of 8-*C*-glucose at δ 82.0. Thus, **23** was identified as apigenin 8-*C*-β-xylopyranosyl-(1→2)-glucopyranoside (vitexin 2″-*O*-xyloside) (Fig. 12).

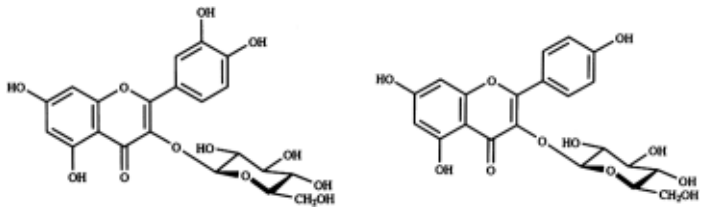


Fig. 11. Chemical structures of quercetin 3-*O*-glucoside (left, **22**) and kaempferol 3-*O*-glucoside (light, **24**).

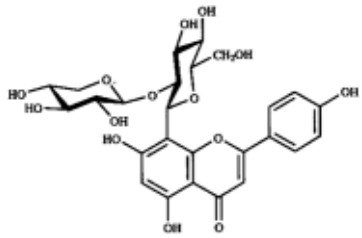


Fig. 12. Chemical structure of vitexin 2″-*O*-xyloside (**23**).

Of seven flavonoids isolated from the inflorescence of *A. paeoniifolius*, six were identified as vitexin (**8**), isovitexin (**10**), orientin (**12**), vicenin-2 (**14**), schaftoside (**16**) and quercetin 3-*O*-glucoside (**22**). Acid hydrolysis of another flavonoid **24** liberated kaempferol and glucose. Attachment of 1 mol glucose to 3-position of kaempferol was determined by LC-MS and UV spectra. Finally, **24** was identified as kaempferol 3-*O*-glucoside (astragalin) (Fig. 11) by TLC and HPLC comparison with authentic sample. In *A. paeoniifolius* (= *A. campanulatus*), quercetin and a rare flavonol, diacetyltambulin, have been isolated from the corm and tuberous roots (Sharstry *et al.*, 2010; Khan *et al.*, 2008). However, they were not found in this survey.

Distribution of anthocyanins and other flavonoids in the inflorescences of three Amorphophallus *species*

The anthocyanins and other flavonoids isolated from three *Amorphophallus* species are shown in Table 1. In *A. titanum*, flavonoids were separately extracted from the spathe and spadix appendix. Though anthocyanin composition of their organs was qualitatively the same, other flavonoid composition was different. In the spadix appendix, all flavonoids which were found in *A. titanum* in this survey were present. However, two flavonoids only, vitexin (**8**) and orientin (**12**), were isolated from the spathe. This may be means the difference of the function of the flavonoids between the spathe and spadix appendix of *A. titanum*. Among the three *Amorphophallus* species used as plant materials, their flavonoid pattern was different to each other. However, their major flavonoids are *C*-glycosylflavones, together with minor flavonols. Anthocyanins and other flavonoids have been surveyed in many species of the family Araceae, and it has been reported that major anthocyanins are cyanidin 3-*O*-rutinoside and 3-*O*-glucoside (Williams *et al.*, 1981). Furthermore, *C*-glycosylflavones were found in many Araceous species with minor flavonols, kaempferol and quercetin glycosides (Williams *et al.*, 1981). In *A. titanum*, though chrysoeriol 7-*O*-glucoside (**20**) was found as only one flavone *O*-glycoside, another flavone *O*-glycoside, luteolin 7-*O*-glucoside, has been reported from the leaves of *Colocasia esculenta* (Iwashina *et al.*, 1999). Thus, the anthocyanin and other flavonoid characters of three *Amorphophallus* species, *A. titanum*, *A. rivieri* and *A. paeoniifolius* show the same patterns with those of many Araceous species.

References

Gallori, S., Giaccherini, C., Bilia, A. R., Mulinacci, N. and Vincieri, F. F. 2004. Identification of anthocyanins in *Amorphophallus titanum* (Becc.) Becc. Chromatographia 59: 647–651.

Iwashina, T., Matsumoto, S. and Nakaike, T. 1995. Flavonoid characters of five *Adiantum* species in Pakistan.

Table 1. Anthocyanins and other flavonoids isolated from the inflorescences of three *Amorphophallus* species

A. titanum (spathe)
Anthocyanins: cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, peonidin 3-*O*-glucoside, peonidin 3-*O*-rutinoside, pelargonidin 3-*O*-rhamnosylglucoside
***C*-Glycosylflavones**: vitexin, orientin
A. titanum (spadix appendix)
Anthocyanins: cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, peonidin 3-*O*-glucoside, peonidin 3-*O*-rutinoside, pelargonidin 3-*O*-rhamnosylglucoside
***C*-Glycosylflavones**: vitexin, vitexin 2″-*O*-glucoside, isovitexin, isovitexin 2″-*O*-glucoside, orientin, isoorientin, vicenin-2, lucenin-2, schaftoside, isoschaftoside, isovitexin X″-*O*-rhamnoside, isoscoparin X″-*O*-glucoside
Flavone: chrysoeriol 7-*O*-glucoside
Flavonol: kaempferol 3-*O*-robinobioside
A. rivieri (inflorescence)
Anthocyanins: cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, peonidin 3-*O*-glucoside, peonidin 3-*O*-rutinoside, pelargonidin 3-*O*-rhamnosylglucoside
***C*-Glycosylflavones**: isovitexin, orientin, isoorientin, vitexin 2″-*O*-xyloside
Flavonol: quercetin 3-*O*-glucoside
A. paeoniifolius (inflorescence)
Anthocyanins: cyanidin 3-*O*-glucoside, cyanidin 3-*O*-diglucoside, pelargonidin 3-*O*-glucoside
***C*-Glycosylflavones**: vitexin, isovitexin, orientin, vicenin-2, schaftoside
Flavonols: kaempferol 3-*O*-glucoside, quercetin 3-*O*-glucoside

In: Watanabe, M. and Hagiwara, H. (eds.), Cryptogams of the Himalayas, Vol. 3. Nepal and Pakistan. Department of Botany, National Science Museum, Tsukuba. pp. 179–191.

Iwashina, T., Kamenosono, K. and Yabuya, T. 1996. Isolation and identification of flavonoid and related compounds as co-pigments from the flowers of *Iris ensata*. Journal of Japanese Botany 71: 281–287.

Iwashina, T., Konishi, T., Takayama, A., Fukada, M. and Ootani, S. 1999. Isolation and identification of the flavonoids in the leaves of taro. Annals of the Tsukuba Botanical Garden 18: 71–74.

Iwashina, T., Kitajima, J. and Matsumoto, S. 2006. Flavonoids in the species of *Cyrtomium* (Dryopteridaceae) and related genera. Biochemical Systematics and Ecology 34: 14–24.

Iwashina, T., Githiri, S. M., Benitez, E. R., Takemura, T., Kitajima, J. and Takahashi, R. 2007. Analysis of flavonoids in flower petals of soybean near-isogenic lines for flower and pubescence color genes. Journal of Heredity 98: 250–257.

Iwashina, T., Saito, Y., Peng, C.-I, Yokota, M. and Kokubugata, G. 2008a. Foliar flavonoids from two *Begonia* species in Japan. Bulletin of the National Museum of Nature and Science, Series B 34: 175–181.

Iwashina, T., López-Saez, J. A. and Kitajima, J. 2008b. Flavonoids from *Osyris alba*. Biochemical Systematics and Ecology 36: 146–147.

Iwashina, T., Matsumoto, S., Kitajima, J., Nakamura, T., Kokubugata, G., Suleiman, M. and Said, I. M. 2010. Apigenin di- and trirhamnoside from *Asplenium normale* in Malaysia. Natural Product Communications 5: 39–42.

Iwashina, T., Setoguchi, H. and Kitajima, J. 2011. Flavonoids from the leaves of *Vitex rotundifolia* (Verbenaceae), and their qualitative and quantitative comparison between coastal and inland population. Bulletin of the National Museum of Nature and Science, Series B 37: 87–94.

Khan, A., Rahman, M. and Islam, M. S. 2008. Antibacterial, antifungal and cytotoxic activities of 3,5-diacetyltambulin isolated from *Amorphophallus campanulatus* Blume ex Decne. Daru 16: 239–244.

Mabry, T. J., Markham, K. R. and Thomas, M. B. 1970. The Systematic Identification of Flavonoids. Springer, Berlin.

Murai, Y., Kokubugata, G., Yokota, M., Kitajima, J. and Iwashina, T. 2008. Flavonoids and anthocyanins from six *Cassytha* taxa (Lauraceae) as taxonomic markers. Biochemical Systematics and Ecology 36: 745–748.

Murai, Y., Setoguchi, H., Ono, E. and Iwashina, T. 2014. Flavonoids and their qualitative variation in *Calystegia soldanella* and related species (Convolvulaceae). Natural Product Communications, in press.

Ohashi, H. 1982. Araceae. In: Satake, Y., Ohwi, J., Kitamura, S., Watari, S. and Tominari, T. (eds.), Wild Flowers of Japan, pp. 127–139. Heibonsha, Tokyo.

Sharstry, R. A., Biradar, S. M., Mahadevan, K. M. and Habbu, P. V. 2010. Isolation and characterization of secondary metabolite from *Amorphophallus paeoniifolius* for hepatoprotective activity. Research Journal of Pharmaceutical, Biological and Chemical Sciences 1: 429–437.

Williams, C. A., Harborne, J. B. and Mayo, S. J. 1981. Anthocyanin pigments and leaf flavonoids in the family Araceae. Phytochemistry 20: 217–234.