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Isolation and Identification of the Flavonoids in the Leaves of Taro

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岩科　司*・小西達夫*・高山明子**・深田未央子**・大谷俊二**：
タロイモの葉に含まれるフラボノイド化合物の分離と同定

Taro, i.e., *Colocasia esculenta* (Araceae) is widespreadly cultivated in the tropical and warm temperate zones of the world, and considered to be the most important traditional crop (Konishi *et al.*, 1994). The species may be originally native to the tropical zone of Asia and its cultivation was more extended to the world with the migration of the peaple. As the results, many cultivars which are distinguished by colors and shapes of the petioles and corms etc, were formed. Rhizomes are utilized in almost cultivars, but the leaves are not used as foods.

As a series of the research of useful substances contained the unused parts of vegetables and crops, we report the isolation and identification of the flavonoids in the leaves of *Colocasia esculenta.*

Materials and Methods

Plant materials

Colocasia esculenta (L.) Schott has been cultivated in Tsukuba Botanical Garden, National Science Museum, Tsukuba, Ibaraki Pref. Japan.

Isolation of flavonoids

The fresh leaves (ca. 500 g) were extracted with MeOH. The extracts were filtered, concentrated to a small volume and shaken with petroleum ether for the removal of chlorophylls. Aqueous residues were completely evaporated to dryness, dissolved to a small volume of MeOH and applied to preparative paper chromatography (PPC) using solvent systems: BAW (n-BuOH/HOAc/H_2O = 4 : 1 : 5, upper phase), 15% HOAc and BEW (n-BuOH/EtOH/H_2O = 4 : 1 : 2.2). The isolated flavonoids were finally purified by Sephadex LH-20 column (solvent system: 70% MeOH).

High performance liquid chromatography (HPLC)

HPLC separation of the flavonoids was performed with TSKgel ODS-80TM column (I.D. 4.6 ×150 mm), at flow-rate: 1.0 ml/min, detection: 190-350 nm and eluent: MeCN/H_2O/H_3PO_4 (22 : 78 : 0.2).

Identification of flavonoids

The flavonoids were identified by UV spectral analysis according to Mabry *et al.* (1970), characterization of the hydrolysates which were liberated by acid hydrolysis (in 12% aq.HCl, 100℃, 30

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min), and PC and HPLC comparisons with authentic specimens. PC, HPLC and UV spectral data were as follows.

Orientin (**1**). PC: Rf 0.34 (BAW), 0.44 (BEW), 0.33 (15%HOAc), 0.15 (5%HOAc); UV - dark purple, UV/NH_3 - yellow. HPLC: retention times (Rt) 3.94 min. UV λ max (nm): MeOH 257, 269, 348; +NaOMe 269, 327sh, 406 (inc.); + $AlCl_3$ 275, 422; +$AlCl_3$/HCl 276, 297, 356, 384; +NaOAc 267sh, 277, 396; +NaOAc/H_3BO_3 264, 374.

Isoorientin (**2**). PC: Rf 0.54 (BAW), 0.63 (BEW), 0.34 (15%HOAc), 0.14 (5%HOAc); UV - dark purple, UV/NH_3 - bright yellow. HPLC: Rt 3.48 min. UV λ max (nm): MeOH 258, 270, 348; +NaOMe 270, 328sh, 407 (inc.); +$AlCl_3$ 276, 422; +$AlCl_3$/HCl 278, 295sh, 358, 384; +NaOAc 276, 398; +NaOAc/H_3BO_3 266, 375.

Isovitexin (**3**). PC: Rf 0.71 (BAW), 0.79 (BEW), 0.45 (15%HOAc), 0.23 (5%HOAc); UV - dark purple, UV/NH_3 - dark greenish yellow. HPLC: Rt 5.45 min. UV λ max (nm): MeOH 271, 334; +NaOMe 279, 330, 398 (inc.); +$AlCl_3$ 279, 304, 352, 379sh; +$AlCl_3$/HCl 279, 302, 345, 378; +NaOAc 278, 306, 392; +NaOAc/H_3BO_3 272, 343.

Vicenin-2 (**4**). PC: Rf 0.25 (BAW), 0.29 (BEW), 0.26 (15%HOAc), 0.10 (5%HOAc); UV - dark purple, UV/NH_3 - dark yellow. HPLC: Rt 3.24 min. UV λ max (nm): MeOH 273, 332; +NaOMe 282, 333, 400 (inc.); +$AlCl_3$ 280, 305, 352, 384sh; +$AlCl_3$/HCl 280, 304, 346, 384sh; +NaOAc 282, 395; +NaOAc/H_3BO_3 276sh, 283, 321.

Orientin 7-*O*-glucoside (**5**). PC: Rf 0.50 (BAW), 0.39 (BEW), 0.23 (15%HOAc); UV - dark purple, UV/NH_3 - greenish yellow. HPLC: Rt 2.26 min. UV λ max (nm): MeOH 257, 270sh, 349; +NaOMe 270, 399 (inc.); +$AlCl_3$ 274, 422; +$AlCl_3$/HCl 274, 297sh, 358, 384sh; +NaOAc 267, 399; +NaOAc/H_3BO_3 262, 372.

Isovitexin 4′-*O*-glucoside (**6**). PC: Rf 0.55 (BAW), 0.37 (BEW), 0.81 (15%HOAc); UV and UV/NH_3 - dark purple. HPLC: Rt 2.26 min. UV λ max (nm): MeOH 272, 325; +NaOMe 279, 382 (dec.); +$AlCl_3$ 279, 302, 348, 384sh; +$AlCl_3$/HCl 281, 300, 337, 384sh; +NaOAc 279, 379; +NaOAc/H_3BO_3 272, 317.

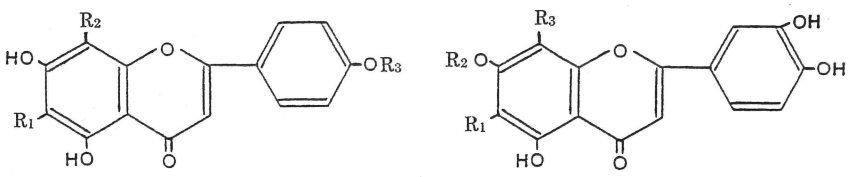
Vitexin X″-*O*-glucoside (**7**). PC: Rf 0.55 (BAW), 0.65 (BEW), 0.60 (15%HOAc), 0.49 (5%HOAc); UV - dark purple, UV/NH_3 - dark greenish yellow. UV λ max (nm): MeOH 271, 329; +NaOMe 280, 332, 394 (inc.); +$AlCl_3$ 278, 304, 349, 385sh; +$AlCl_3$/HCl 278, 303, 344, 386sh; +NaOAc 280, 304, 389; +NaOAc/H_3BO_3 273, 323.

Luteolin 7-*O*-glucoside (**8**). PC: Rf 0.25 (BAW), 0.29 (BEW), 0.26 (15%HOAc), 0.10 (5%HOAc); UV - dark purple, UV/NH_3 - yellow. HPLC: Rt 5.44 min. UV λ max (nm): MeOH 254, 266sh, 348; +NaOMe 266, 391 (inc.); +$AlCl_3$ 273, 426; +$AlCl_3$/HCl 273, 294sh, 360, 385; +NaOAc 260, 406; +NaOAc/H_3BO_3 259, 373.

Results

Eight flavonoid glycosides were isolated from the leaves of Taro.

UV spectra of flavonoids **1** and **2** showed the presence of free 5-, 7-, 3′- and 4′-hydroxyl groups (Mabry *et al.*, 1970). The glycosides could not be hydrolyzed by hot acid treatment, showing that the compounds are *C*-glycosides but not *O*-glycosides. Finally, flavonoids **1** and **2** were identified as orientin and isoorientin (Fig. 1) by direct PC and HPLC comparisons with authentic specimens, respectively.



R_1 = *C*-glucosyl, R_2 = R_3 = H: isovitexin (**3**)
R_1 = R_2 = *C*-glucosyl, R_3 = H: vicenin-2 (**4**)
R_1 = *C*-glucosyl, R_2 = H, R_3 = glucosyl: isovitexin 4′-*O*-glucoside (**6**)
R_1 = R_3 = H, R_2 = *C*-glucoglucosyl: vitexin X″-*O*-glucoside (**7**)

R1 = R_2 = H, R_3 = *C*-glucosyl: orientin (**1**)
R_1 = *C*-glucosyl, R_2 = R_3 = H: isoorientin (**2**)
R_1 = H, R_2 = glucosyl, R_3 = *C*-glucsyl: orientin 7-*O*-glucoside (**5**)
R_1 = R_3 = H, R_2 = glucosyl: luteolin 7-*O*-glucoside (**8**)

Fig 1. Chemical Structures of the flavonoids isolated from the leaves of Taro.

Flavonoid **3** could not be also hydrolyzed by hot HCl. UV spectral properties of **3** agreed with that of 5,7,4′-trihydroxyflavone *C*-glycoside, and PC and HPLC data coincided with those of authentic isovitexin.

UV spectra of flavonoid **4** also showed that the compound is 5,7,4′-trihydroxyflavone *C*-glycoside. By hot acid treatment, **4** was not hydrolyzable nor occurred a corresponding isomer which is produced by Wessely-Moser rearrangement, showing that the compound is 6,8-di-*C*-glycoside. The glycoside **4** was identified as vicenin-2 (apigenin 6,8-di-*C*-glucoside, Fig. 1) by direct PC and HPLC comparison with authentic specimen. Though **4** was accomapined with the trace of three other apigenin 6,8-di-*C*-glycosides, they could not be completely characterized.

Flavonoid **5** liberated orientin and glucose, which were characterized by direct PC comparisons with authenic specimens, by acid hydrolysis. Since UV spectra of the original glycoside showed the presence of free 5-, 3′- and 4′-hydroxyl and a substituted 7-hydroxyl groups, it is clear that the glucose moiety is attached to 7-hydroxyl group. Moreover, PC data, especially Rfs of aqueous solvent systems: 15% HOAc and 5 % HOAc, of **5** was presumed that glucosyl group on the flavonoid nucleus is 1 mol. Thus, flavonoid **5** was identified as orientin 7-*O*-glucoside (Fig. 1).

Acid hydrolysis of **6** liberated isovitexin and glucose which were characterized by direct PC comparisons with authentic specimens. Since UV spectra of original glycoside showed the presence of free 5- and 7-hydroxyl and a substituted 4′-hydroxyl groups, glucose must be on 4′-hydroxyl group. Thus, flavonoid **6** was considered as isovitexin 4′-*O*-glucoside (Fig. 1).

UV spectra of **7** showed the presence of free 5-, 7- and 4′-hydroxyl groups and the absence of substituted hydroxyl group. However, vitexin and glucose were produced by acid hydrolysis. As a result, *O*-glucosyl group was considered to be on 8-*C*-glucosyl group. From the results described above, flavonoid **7** was identified as vitexin X″-*O*-glucoside (Fig. 1).

Acid hydrolysis of flavonoid **8** liberated luteolin and glucose. Since the presence of free 5-, 3′- and 4′-hydroxyl groups was shown by UV spectra of original glycoside, a glucosyl moiety was considered to be attached to 7-position. Finally, flavonoid **8** was identified as luteolin 7-*O*-glucoside (Fig. 1) by direct PC and HPLC comparison with authentic specimen.

Discussion

Seven *C*-glycosylflavones and their *O*-glycosides, and one flavone *O*-glycoside were detected in this experiment. The flavonoids of Taro have been surveyed by a few authors (Chan Jr. *et al.*, 1977; Williams *et al.*, 1981). Thus, three anthocyanins, i.e., pelargonidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rhamnosylglucoside have been isolated from the corms of *Colocasia esculenta* (L.) Schott var. *lehua maoli* (Chan Jr. *et al.*, 1977). The presence of *C*-glycosylflavones, quercetin glycoside and procyanidin in the leaves of Taro have been reported by Williams *et al.* (1981). However, the exact identification of each flavonoid has not been performed. In this survey, it was elucidated that Taro's major *C*-glycosylflavones were orientin (**1**), isoorientin (**2**), isovitexin (**3**), vicenin-2 (**4**), orientin 7-*O*-glucoside (**5**), isovitexin 4′-*O*-glucoside (**6**) and vitexin X″-*O*-glucoside (**7**). The presence of a flavone *O*-glycoside, i.e., luteolin 7-*O*-glucoside (**8**), which have not been reported by Williams *et al.* (1981), was also clear, but flavonol, quercetin glycoside could not be detected in this experiment. Though several minor flavonoids, probabry *C*-glycosylflavones and their *O*-glycosides, were noticed by preparatory two-dimensional paper chromatography, those characterization is now in progress.

Summary

The flavonoids in the leaves of Taro, *Colocasia esculenta* (L.) Schott (Araceae) were surveyed for the research of the useful substances contained the unused vegetable parts. Eight were isolated and identified as orientin (**1**), isoorientin (**2**), isovitexin (**3**), vicenin-2 (**4**), orientin 7-*O*-glucoside (**5**), isovitexin 4′-*O*-glucoside (**6**), vitexin X″-*O*-glucoside (**7**) and luteolin 7-*O*-glucoside (**8**) by UV spectra, characterization of hydrolysates, and PC and HPLC comparisons with authentic specimens.

摘　要

野菜などの未利用部分に含まれる有用物質の探索を行う目的で，今回タロイモ（*Colocasia esculenta* (L.) Schott）の葉に含まれるフラボノイド成分が分析された。

8種類のフラボノイドが分離され，これらは紫外・可視吸収スペクトル，酸加水分解物や熱塩酸処理による反応物の定性および基準標品とのペーパークロマトグラフィーおよび高速液体クロマトグラフィーによる比較によって orientin (**1**), isoorientin (**2**), isovitexin (**3**), vicenin-2 (**4**), orientin 7-*O*-glucoside (**5**), isovitexin 4′-*O*-glucoside (**6**), vitexin X″-*O*-glucoside (**7**) および luteolin 7-*O*-glucoside (**8**) と同定された。

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