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Polyphenols in *Iris setosa* var. *canadensis* and Their Chemotaxonomic Comparisons with Three Japanese Varieties

by

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岩科　司*・大谷俊二**：カナダヒオウギアヤメのポリフェノール成分の定性並びに日本産ヒオウギアヤメ3変種との化学分類学的比較

Iris setosa Pallas (Iridaceae) is widely distributed in mainly subarctic zone of Northern Hemisphere, such as northeastern Asia, the Aleutian Islands, Alaska, central and northern Honshu and Hokkaido (Satake 1982). In Japan, its two varieties, i. e., *I. setosa* var. *nasuensis* Hara endemic to Nasu regions, Tochigi Pref. and var. *hondoensis* Honda endemic to Kirigamine, Nagano Pref., are also exist (Hara and Kurosawa 1963). Apart from them, another variety, *I. setosa* var. *canadensis* M. Foster (=*I. hookeri* Penny) is recognized in Canada (Kitamura *et al.* 1976).

In compliance with the interest shown by the late Emperor Showa in the origin of *I. setosa* var. *nasuensis*, we have carried out the chemotaxonomical study of three Japanese varieties with the flavonoid and related compounds in order to clarify the affinity (Hayashi *et al.* 1989). As a result, it has been shown that three classes of compounds are present in the flowers and leaves of *Iris setosa* group; namely 10 anthocyanins, 23 *C*-glycosylflavones and their *O*-glycosides, and 7 *C*-glycosylxanthones. Among them, anthocyanins and xanthones were almost commom to all three varieties, whereas an appreciable difference was obserbed in *C*-glycosylflavones. Thus, there was a good reason to reconfirm three varieties within a group of *Iris setosa* occurring in Japanese flora. However, the flavonoid characterization in *I. setosa* var. *canadensis* was not performed owing to absence of plant materials.

In this paper, we describe the flavonoid and related compound charactes of *I. setosa* var. *canadensis*, and discussed the phytochemical relationship among Canadian variety and three Japanese ones, var. *setosa*, var. *hondoensis* and var. *nasuensis*.

Materials and Methods

Plant materials

Iris setosa var. *canadensis* M. Foster was collected at following three locarities in Canada; 1) Point Riche, North-western New Foundland, 2) La Grande Bergeronne, Lower St. Laurence, Quebec and 3) Les Escoumins, Golf of St. Laurence, Quebec, by Mr. Tony Huber and brought to us via Mr. Hiroshi Shimizu. Live plants which are not flowering are growing at Kirigamine-Gakuen in

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Kirigamine, Nagano Pref. by Mr. Takeshi Takeuchi.

Isolation of the flavonoid and related compounds

Fresh leaves were extracted with methanol. Concentrated extracts were applied to preparative paper chromatography (PPC) as described by Hayashi *et al.* (1989). Isolated compounds were finally purified by Sephadex LH-20 column (solvent system: 70% MeOH).

High performance liquid chromatography (HPLC)

Extracts and isolated compounds were filtered through Toyopak ODS M and then Maisyoridisc H-13-5, 0.45 μm, and injected onto a Tosoh TSKgel ODS-80TM column (15 × 0.46cm) using MeCN/H_2O/H_3PO_4 (22:78:0.2) with flow-rate of 1ml/min.

Identification of compounds

The flavonoid and related compounds were identified by paper chromatographic (solvent systems: BAW=n-BuOH/AcOH/H_2O (4:1:5, upper phase), BEW=n-BuOH/AcOH/H_2O (4:1:2.2), 15%AcOH and 5%AcOH) and HPLC comparisons with authentic specimens, UV spectra, and characterization of acid hydrolysates as described by Hayashi *et al.* (1989) and Mabry *et al.* (1970).

Isoorientin (**1**). PC Rf: 0.49 (BAW), 0.51 (BEW), 0.38 (15%AcOH), 0.24 (5%AcOH); UV: dark purple, UV/NH_3: yellow. HPLC Rt min: 3.18. UV λ_{max}^{MeOH} nm: 256, 267sh, 350; +NaOMe: 270, 337sh, 408 (inc.); +$AlCl_3$: 272, 422; +$AlCl_3$/HCl: 266, 275sh, 295sh, 359, 382sh; +NaOAc: 272, 397; +NaOAc/H_3BO_3 : 264, 376. Acid hydrolysis (12% aq. HCl, 100℃, 30 min.): unhydrolyzable; Wessely-Moser rearrangement: positive (orientin).

Orientin (**2**). PC Rf: 0.27 (BAW), 0.28 (BEW), 0.18 (15%AcOH), 0.08 (5%AcOH); UV: dark purple, UV/NH_3 : dark yellow. HPLC Rt min: 3.61. UV λ_{max}^{MeOH} nm: 257, 351. Acid hydrolysis: unhydrolyzable; Wessely-Moser rearrangement: positive (isoorientin).

Vicenin-2 (**3**). PC Rf: 0.24 (BAW), 0.19 (BEW), 0.52 (15%AcOH), 0.40 (5%AcOH); UV: dark purple, UV/NH_3: dark yellow. HPLC Rt min: 2.79. UV λ_{max}^{MeOH} nm: 273, 332. Acid hydrolysis: unhydrolyzable; Wessely-Moser rearrangement: negative.

Mangiferin (**4**). PC Rf: 0.47 (BAW), 0.54 (BEW), 0.45 (15%AcOH), 0.33 (5%AcOH); UV: dark orange, UV/NH_3: bright yellow. HPLC Rt min: 2.48. UV λ_{max}^{MeOH} nm: 241, 257, 317, 368; +NaOMe: 273, 303sh, 344sh, 392 (inc.); +$AlCl_3$: 236, 270, 287sh, 356, 418; +$AlCl_3$/HCl: 232, 266, 279sh, 338, 403; +NaOAc: 270, 303, 386; +NaOAc/H_3BO_3: 263, 326, 361, 414sh. Acid hydrolysis: unhydrolyzable; Wessely-Moser rearrangement: positive (isomangiferin).

Isomangiferin (**5**). PC Rf: 0.39 (BAW), 0.43 (BEW), 0.49 (15%AcOH), 0.37 (5%AcOH); UV: dark orange, UV/NH_3: bright yellow. HPLC Rt min: 2.74. UV λ_{max}^{MeOH} nm: 241, 257, 313, 365; +NaOMe: 273, 302sh, 344sh, 392 (inc.); +$AlCl_3$: 235, 268, 287sh, 356, 418; +$AlCl_3$/HCl: 234, 265, 278sh, 336, 404; +NaOAc: 271, 304, 385; +NaOAc/H_3BO_3: 262, 320, 358, 414sh. Acid hydrolysis: unhydrolyzable; Wessely-Moser rearrangement: positive (mangiferin).

Luteolin 6, 8-di-*C*-glycoside (**6**). PC Rf: 0.18 (BAW), 0.12 (BEW), 0.42 (15%AcOH), 0.28 (5%AcOH); UV: dark purple, UV/NH_3: yellow. HPLC Rt min: 2.33. UV λ_{max}^{MeOH} nm: 255sh, 270sh, 348. Acid hydrolysis: unhydrolyzable; Wessely-Moser rearrangement: negative.

Luteolin 6, 8-di-*C*-glycoside (**7**). PC Rf: 0.13 (BAW), 0.09 (BEW), 0.36 (15%AcOH), 0.24

(5%AcOH); UV: dark purple, UV/NH_3: yellow. HPLC Rt min: 2.33. UV λ_{max}^{MeOH} nm: 259sh, 272, 348; +NaOMe: 272, 338sh, 413 (inc.); +$AlCl_3$: 278, 426; +$AlCl_3$/HCl: 264sh, 278, 296sh, 361, 384sh; +NaOAc: 270sh, 282, 404; +NaOAc/H_3BO_3: 267, 382, 428sh. Acid hydrolysis: unhydrolyzable; Wessely-Moser rearrangement: negative.

Xanthone *C*-glycoside (**8**). PC Rf: 0.39 (BAW), 0.43 (BEW), 0.49 (15%AcOH), 0.37 (5%AcOH); UV: dark orange, UV/NH_3: yellow. HPLC Rt min: 2.16. UV λ_{max}^{MeOH} nm: 253, 283, 326; +NaOMe: 264, 295sh, 376 (inc.); +$AlCl_3$: 245, 269, 291, 386; +$AlCl_3$/HCl: 262, 283sh, 352; +NaOAc: 264sh, 290sh, 375; +NaOAc/H_3BO_3: 259, 285sh, 345, 385sh. Acid hydrolysis: unhydrolyzable; Wessely-Moser rearrangement: negative?.

Xanthone *O*-galactoside (**9**). PC Rf: 0.42 (BAW), 0.48 (BEW), 0.28 (15%AcOH), 0.14 (5%AcOH); UV: dark orange, UV/NH_3: yellow. HPLC Rt min: 5.26. UV λ_{max}^{MeOH} nm: 251, 278sh, 319; +NaOMe: 247, 260sh, 311sh, 366 (inc.); +$AlCl_3$: 233, 266, 286sh, 390; +$AlCl_3$/HCl: 232, 263, 280sh, 342; +NaOAc: 265sh, 277sh, 371; +NaOAc/H_3BO_3: 254sh, 281sh, 343, 373sh. Acid hydrolysis: unknown xanthone aglycone and galactose.

Aglycone of **9**. PC Rf: 0.84 (BAW), 0.88 (BEW), 0.07 (15%AcOH); UV: dark orange, UV/NH_3: bright yellow. UV λ_{max}^{MeOH} nm: 250, 282, 321.

Results

Five flavonoids and four xanthones appeared on two-dimensional paper chromatography (2D-PC) and HPLC (Fig. 1).

Isoorientin (**1**). UV spectra of flavonoid **1** showed the presence of free 5-, 7-, 3′- and 4′-hydroxyl groups. By hot acid treatment, the glycoside was unhydrolyzable, but another spot which was produced by Wessely-Moser rearrangement (Markham 1982) appeared with original one on the chromatogram, showing to be 6- or 8-mono-*C*-glycosylflavone. Finally, flavonoid **1** was identified as luteolin 6-*C*-glucoside (isoorientin) by PC and HPLC comparisons with authentic specimen (Fig. 2).

Orientin (**2**). The spots of isoorientin, which was identified by PC and HPLC, and original glycoside appeared on the chromatogram after hot acid treatment. Accordingly, flavonoid **2** was regarded as luteolin 8-*C*-glucoside (orientin) (Fig. 2).

Vicenin-2 (**3**). UV spectra of **3** was presumed that the flavonoid was apigenin type. Original glycoside was unhydrolyzable nor isomerized by hot acid treatment, showing that the flavonoid was 6, 8-di-*C*-glycoside. Flavonoid **3** was identified as apigenin 6, 8-di-*C*-glucoside (vicenin-2) by PC and HPLC comparisons with authentic specimen (Fig. 2).

Mangiferin (**4**). Color (dark orange change to bright yellow after exposure to fuming ammonia) under UV light and UV spectral properties of **4** were presumed that the component was xanthone. Original glycoside strongly resisted to acid hydrolysis. Finally, **4** was identified as 1, 3, 6, 7-tetrahydroxyxanthone 2-*C*-glucoside (mangiferin) by PC and HPLC comparisons with authentic specimen (Fig. 2).

Isomangiferin (**5**). Component **5** had similar color reactions and UV spectral properties with those of mangiferin. PC and HPLC data of the original glycoside agreed with those of isomer which was produced by hot acid treatment of mangiferin. Thus, **5** was regarded as 1, 3, 6, 7-tetrahydoxyxanthone 4-*C*-glucoside (isomangiferin) (Fig. 2).

Luteolin 6, 8-di-*C*-glycosides (**6** and **7**). Both compounds **6** and **7** could not be hydrolyzed nor

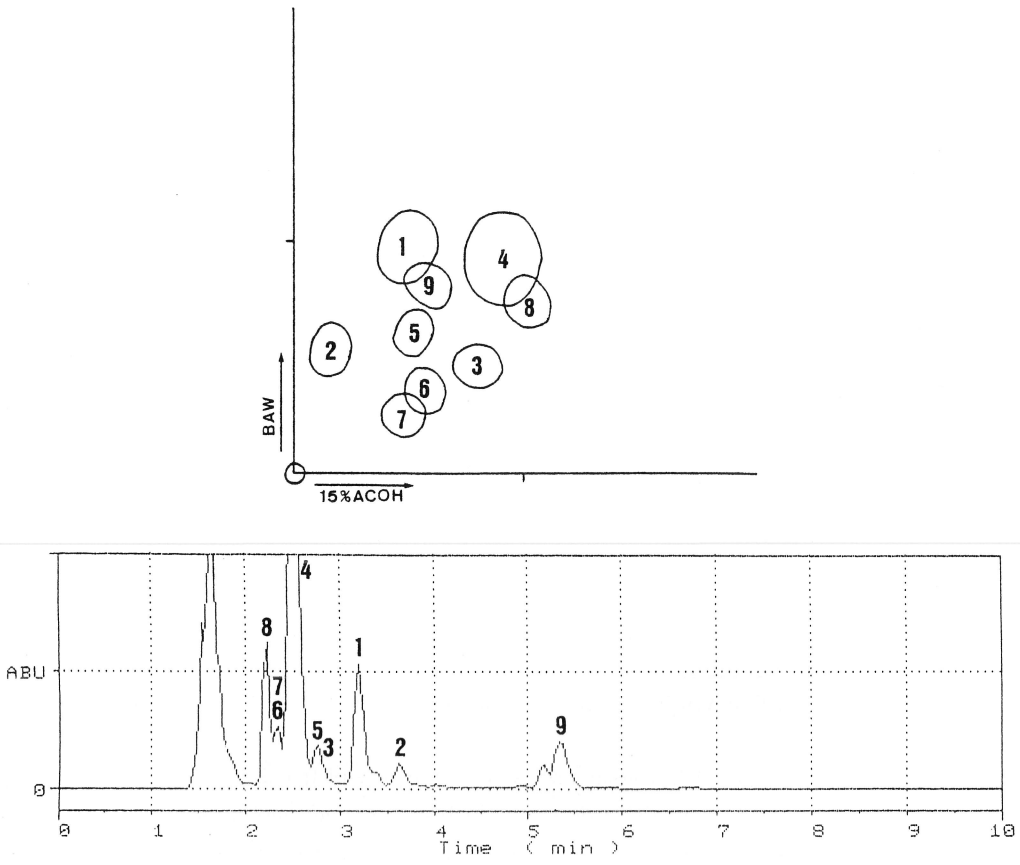
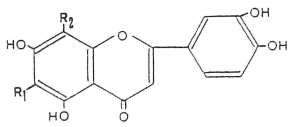


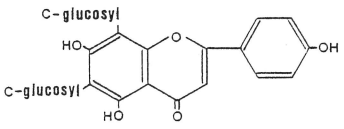
Fig. 1. 2D-PC and HPLC separation of phenolic compounds in the leaves of *Iris setosa* var. *canadensis*. HPLC: Eluents=MeCN/H_2O/H_3PO_4 (22:78:0.2), Flow-rate=1.0ml/min, Injection=10 μl and Detection=345nm.
1=luteolin 6-*C*-glucoside (isoorientin), **2**=luteolin 8-*C*-glucoside (orientin), **3**=apigenin 6, 8-di-*C*-glucoside (vicenin-2), **4**=1, 3, 6, 7-tetrahydroxyxanthone 2-*C*-glucoside (mangiferin), **5**=1, 3, 6, 7-tetrahydroxyxanthone 4-*C*-glucoside (isomangiferin), **6** and **7**=luteolin 6, 8-di-*C*-glycosides, **8**=xanthone *C*-glycoside and **9**=xanthone *O*-galactoside.

isomerized by hot acid treatment, showing to be 6, 8-di-*C*-glycosylflavones. UV spectra of **7** showed the presence of free 5, 7, 3′, 4′-tetraOH groups. Though *C*-glycosyl groups which were attached to 6-and 8-positions were not determined, flavonoids **6** and **7** were characterized as luteolin 6, 8-di-*C*-glycosides (either of two compounds is probabry lucenin-2, i. e., luteolin 6, 8-di-*C*-glucoside) by UV and PC data.

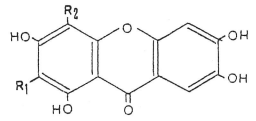
Xanthone *C*-glycoside (**8**) and *O*-galactoside (**9**). Color reaction under UV light and UV spectral properties of their compounds showed that they were xanthones (see Materials and Methods). By hot acid treatment, xanthone **9** liberated an unidenfied aglycone and galactose, whereas **8** was unhydrolyzable. From the results described above, their compounds were characterized as xanthone *C*-glycoside (**8**) and xanthone *O*-galactoside (**9**).



R_1=*C*-glucosyl, R_2=H: isoorientin (**1**)
R_1=H, R_2=*C*-glucosyl: orientin (**2**)
R_1=R_2=C-glycosyl: luteolin 6, 8-di-*C*-glycosides (**6** and **7**)



vicenin-2 (**3**)



R_1=*C*-glucosyl, R_2=H: mangiferin (**4**)
R_1=H, R_2=*C*-glucosyl: isomangiferin (**5**)

Fig. 2. Chemical structures of phenolic compounds in the leaves of *Iris setosa* var. *canadensis*.

Discussion

We have isolated and identified many *C*-glycosylflavones and *C*-glycosylxanthones, and their *O*-glycosides from three Japanese *Iris setosa* varieties, var. *setosa*, var. *hondoensis* and var. *nasuensis* (Hayashi *et al.* 1989). Common *C*-glycosylflavones such as vicenin-2, vitexin, isovitexin, orientin and isoorientin, and *C*-glycosylxanthone such as mangiferin were present in the leaves of all three varieties, whereas appreciable difference among their varieties was observed in *O*-glycosylated and *O*-methylated *C*-glycosylflavones. Namely, *I. setosa* var. *setosa* and other two Japanese varieties could be distinguished by the presence of isovitexin 4′-*O*-glucoside (isosaponarin) in latter varieties. Moreover, var. *nasuensis* was differentiated with var. *hondoensis* by the presence of *O*-methylated *C*-glycosylflavones, isovitexin 7-methyl ether (swertisin), vitexin 7-methyl ether (isoswertisin), isoorientin 7-methyl ether (swertiajaponin), orientin 7-methyl ether (isoswertiajaponin) and so on in former.

In *I. setosa* var. *canadensis*, mangiferin, isoorientin and orientin were present as major components, but isovitexin and vitexin which were common in three Japanese varieties were not found. Moreover, 4′-*O*-glycosylated and *O*-methylated *C*-glycosylflavones were also absence in the leaves (Table 1). Such the facts show that origin of *I. setosa* var. *canadensis* native to Canada is phytochemically different from that of var. *hondoensis* and var. *nasuensis*. Cytologically, Canadian variety may be homoploid (plant materials were cultivated from seeds), whereas two Japanese ones were aneuploids (2n=ca. 54) (Serizawa and Kondo 1992). Since flavonoid pattern of *I. setosa* var. *canadensis* was also distinguished from var. *setosa*, *I. setosa* var. *canadensis* may be regarded as an independent species, *Iris hookeri* Penny.

Table 1. Comparison of phenolic compounds in the leaves among four *Iris setosa* varieties

Compounds	*setosa*	*hondoensis*	*nasuensis*	*canadensis*
C-glycosylflavones				
apigenin				
6, 8-di-*C*-glycosides	+	+	+	+
luteolin				
mono-*C*-glycosides	+	+	+	+
apigenin				
mono-*C*-glycosides	+	+	+	−
X″-*O*-glycosides	+	+	+	−
4′-*O*-glycosides	−	+	+	−
O-methylated flavones	−	+	+	−
xanthone				
C-glycosylxanthones	+	+	+	+
O-glycosylxanthone	−	−	−	+

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Summary

Five flavonoids and four xanthones in the leaves of *Iris setosa* var. *canadensis* M. Foster were isolated by paper and column chromatography and identified as isoorientin (**1**), orientin (**2**), vicenin-2 (**3**), luteolin 6, 8-di-*C*-glycosides (**6** and **7**), mangiferin (**4**), isomangiferin (**5**), xanthone *C*-glycoside (**8**) and xanthone *O*-galactoside (**9**) based on PC and HPLC comparisons with authentic specimens, UV spectra, and characterization of acid hydrolysates. Phenolic characters of the variety was compared with those of three Japanese *I. setosa* varieties, var. *setosa*, var. *hondoensis* and var. *nasuensis* of which flavonoid and related compounds have already been reported (Hayashi *et al.* 1989). Chemicals of var. *canadensis* were clearly different from those of other varieties by the absence of isovitexin and vitexin, and presence of xanthone *O*-glycoside in former variety. As a result, *Iris setosa* var. *canadensis* may be regarded as an independent species, *Iris hookeri* Penny rather than a variety in *I. setosa*.

摘　　要

カナダヒオウギアヤメ（*Iris setosa* var. *canadensis* M. Foster）の葉から 5 種類のフラボノイドと 4 種類のキサントンが分離され，それぞれ isoorientin（**1**），orientin（**2**），vicenin-2（**3**），2 種類の luteolin 6, 8-di-*C*-glycoside（**6** および **7**），mangiferin（**4**），isomangiferin（**5**），xanthone *C*-glycoside（**8**）および xanthone *O*-galactoside（**9**）と同定された。これらのフェノール成分のパターンがすでに報告のある日本産のヒオウギアヤメの 3 変種，すなわちヒオウギアヤメ（var. *setosa* Pallas），キリガミネヒオウギアヤメ（var. *hondoensis* Honda）およびナスヒオウギアヤメ（var. *nasuensis* Hara）のものと比較された。その結果，カナダヒオウギアヤメでは4′-*O*-グリコシル化フラボンやメトキシル化フラボンばかりでなく，他の変種に共通に存在する一般的な *C*-グリコシルフラボンの isovitexin や vitexin さえも欠失しており，逆に他の変種では見いだされなかったキサントンの *O*-配糖体が存在することでキリガミネヒオウギアヤメやナスヒオウギアヤメはもとより母種のヒオウギアヤメとも異なっていることが判明し，化学分類学的にはヒオウギアヤメの変種よりもむしろ独立した種，*Iris hookeri* Penny とみなすのが妥当と考えられた。

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