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Quantitative Variation of Anthocyanins and Other Flavonoids in Autumn Leaves of *Acer palmatum*

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Abstract Anthocyanins and other flavonoids in autumn leaves of *Acer palmatum* were qualitatively and quantitatively surveyed. Cyanidin 3-*O*-glucoside (chrysanthemin) was found in autumn leaves of *Acer palmatum* as a major pigment, together with a minor cyanidin glycoside. Six *C*-glycosylflavones were also isolated as other flavonoids and characterized as isoorientin, orientin, isovitexin, vitexin, and acylated isoorientin and orientin. The anthocyanin content increased with the progress of coloration of the leaves and decrease of chlorophyll and carotenoid contents. On the other hand, *C*-glycosylflavones gradually decreased. Thus, it was shown that the anthocyanins and *C*-glycosylflavones were independently synthesized to each other in autumn leaves, in spite of the same biosynthetic pathway, i.e., shikimate pathway.

Key words : *Acer palmatum*, anthocyanins, autumn leaves, chrysanthemin, *C*-glycosylflavones.

Introduction

The genus *Acer* is famous for the beautiful coloring of the leaves in autumn. It has been reported by Hattori and Hayashi (1937) for the first time that anthocyanin, cyanidin 3-*O*-glucoside, i.e. chrysanthemin, is a major pigment in autumn leaves of *Acer circumlobatum* Maxim. (=*A. japonicum* Thunb.) and *A. ornatum* Carr. var. *matsumurae* (Koidz.) Koidz. (=*A. amoenum* Carr. var. *matsumurae* (Koidz.) Ogawa). Later, Ji *et al.* (1992a) have surveyed the autumn colored and spring sprouted leaves of 119 *Acer* taxa by HPLC, and showed that the major anthocyanin is cyanidin 3-*O*-glucoside as the cases of the autumn colored leaves, together with cyanidin 3-*O*-rutinoside, 3-*O*-galloylglucoside and 3,5-di-*O*-glucoside and 3-*O*-galloylrutinoside as minor pigments. Moreover, chrysanthemin has been proved to be major anthocyanin in autumn leaves of the various plant species (Hayashi and Abe, 1955; Murrell and Wolf, 1969; Ishikura, 1972; Yoshitama and Ishikura, 1988; Iwashina, 1996). On the other hand, related cyanidin 3-*O*-galactoside, i.e. idaein, was found in some plants, e.g. *Coteneaster horizontalis* Decne., *Rhaphiolepis indica* (L.) Lindle. ex Ker. var. *umbellata* (Thunb. ex Murray) Ohashi (Rosaceae), *Stuartia pseudocamellia* Maxim. (Theaceae), *Elliottia paniculata* (Sieb. & Zucc.) Benth. & Hook. (Ericaceae) and so on, as major anthocyanin (Iwashina, 1996).

Twenty-six *Acer* species are native to Japan (Shimizu, 1989). Of their species, *A. palmatum* Thunb. is most popular one and has most beautiful colored leaves in autumn, and many cultivars have been grown as ornamentals. Two anthocyanins, cyanidin 3-*O*-glucoside and 3-*O*-rutinoside, were isolated from the colored leaves of the species, together with leucoanthocyanidins (Ishikura, 1972). Two acylated anthocyanins, cyanidin 3-*O*-[2″-*O*-(galloyl)-*β*-D-glucoside] and 3-*O*-[2″-*O*-(galloyl)-6″-*O*-(L-rhamnosyl)-*β*-D-glucoside], were also isolated from the red spring leaves (Ji *et al.*, 1992b). Though the former pigment was also observed in autumn red leaves, major anthocyanin is cyanidin 3-*O*-glucoside as well as other *Acer* species (Iwashina, 1996).

Four *C*-glycosylflavones, vitexin, isovitexin,

orientin and isoorientin, have been found in green leaves of *A. palmatum* as other flavonoid compounds (Aritomi, 1962, 1963). A flavonol, kaempferol was also detected from the leaves, together with ellagic acid (Bate-Smith, 1978).

The mechanism of autumn leaf coloration has been reviewed by some authors (e.g. Hayashi, 1968; Takeda, 1983; Lee, 2002). However, qualitative and quantitative variation of other flavonoids, e.g. flavones, flavonols, flavanones or dihydroflavonols, which are synthesized on the same biosynthetic pathway, i.e. shikimate pathway, was hardly surveyed, in relation with autumn coloration of the leaves.

In this paper, we describe quantitative variation of anthocyanin, other flavonoids, and total chlorophylls and carotenoids along with the progress of autumn leaves coloration of *Acer palmatum*.

Materials and Methods

Plant Materials

Acer palmatum Thunb., which was used in this study (acc. No. TBG50382), is growing in the Tsukuba Botanical Garden, National Museum of Nature and Science, Tsukuba, Japan.

Isolation of flavonoids

Fresh leaves (12.5 g) of *A. palmatum* were collected in 26 Oct. 2006 and extracted with MeOH. The concentrated extracts were applied to 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 using solvent system: 70% MeOH. Of their flavonoids, four ones, F1 (ca. 50 mg), F3 (ca. 5 mg), F4 (ca. 10 mg) and F5 (trace amount), were obtained as pale yellow powders.

Quantitative HPLC analysis of anthocyanins and other flavonoids

Fresh leaves (each 0.2 g) of *A. palmatum* (each 5 samples) were collected in 26 Oct., 2 Nov., 13 Nov., 18 Nov., 22 Nov, 25 Nov. and 29 Nov., 2006, along with the progress of autumn coloration, and were extracted with 0.1% HCl in MeOH (3 ml) for anthocyanins and MeOH (3 ml) for other flavonoids. After filtration with Maisyori-disc H-13-5 (Tosoh), the extracts were analysed by HPLC using Pegasil ODS (I.D. 6.0×150 mm, Senshu Scientific Co. Ltd.), at flow-rate: 1.0 ml min^{-1}, detection: 190–700 nm, and eluent: MeCN/HOAc/H_2O/H_3PO_4 (6 : 8 : 83 : 3) for anthocyanins and MeCN/H_2O/H_3PO_4 (22 : 78 : 0.2) (Sol. I) and (15 : 85 : 0.2) (Sol. II) for other flavonoids. Relative anthocyanin and other flavonoid contents were determined from the peak area of each compounds on the HPLC chromatograms.

Quantitative visible spectral analysis of total chlorophyll and carotenoid contents

The crude MeOH extracts, which were obtained for quantitative HPLC analysis (see above), were directly measured visible spectra (400–700 nm) by Shimadzu Multipurpose Spectrophotometer MPS-2000. Relative chlorophyll and carotenoid contents were determined from the absorbance of λmax 663 nm (chlorophylls) or that of λmax 446 nm (carotenoids).

Liquid chromatograph-mass spectra (LC-MS)

LC-MS were measured using a Pegasil-ODS (I.D. 2.0×150 mm, Senshu Scientific Co. Ltd.), at a flow-rate of 0.2 ml min^{-1}, eluting with HCOOH/MeCN/H_2O (5 : 15 : 80 or 22 : 5 : 73), injection: 10 μl, ESI^+ 4.5 kV, ESI^- 3.5 kV, 250°C.

Identification of anthocyanin and other flavonoids

Major anthocyanin from the leaves of *A. palmatum* was identified as cyanidin 3-*O*-glucoside by direct HPLC comparison with authentic chrysanthemin from *Acer japonicum* (Hattori and Hayashi, 1937). Six flavonoid glycosides were identified by UV spectroscopy according to Mabry *et al.* (1970), LC-MS, characterization of acid hydrolysates in 12% HCl, 100°C, 30 min,

Fig. 1. Color variation of the leaves of *Acer palmatum* with the progress of autumn coloration.

and direct TLC and HPLC comparisons with authentic samples. TLC, UV, acid hydrolysis and LC-MS data of the isolated flavonoids were as follows.

Luteolin 6-*C*-glucoside (isoorientin, F1). TLC: Rf 0.39 (BAW), 0.47 (BEW), 0.23 (15% HOAc); UV — dark purple, UV/NH_3 — yellow. UV: λmax (nm) MeOH 257 sh, 270, 347; +NaOMe 275, 330 sh, 406 (inc.); +$AlCl_3$ 276, 422; +$AlCl_3$/HCl 262 sh, 278, 297, 358, 384 sh; +NaOAc 268 sh, 277, 330 sh, 399; +NaOAc/H_3BO_3 268, 373. Acid hydrolysis: unhydrolyzable. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (luteolin+1 mol glucose).

Apigenin 6-*C*-glucoside (isovitexin, F2). TLC: Rf 0.63 (BAW), 0.74 (BEW), 0.35 (15% HOAc); UV — dark purple, UV/NH_3 — dark yellow. UV: λmax (nm) MeOH 271, 334; +NaOMe 278, 331, 398 (inc.); +$AlCl_3$ 278, 303, 354, 383 sh; +$AlCl_3$/HCl 278, 302, 347, 378 sh; +NaOAc 278, 311, 393; +NaOAc/H_3BO_3 273, 345. Acid hydrolysis: unhydrolyzable. LC-MS: *m/z* 433 $[M+H]^+$, 431 $[M-H]^-$ (apigenin+1 mol glucose).

Luteolin 8-*C*-glucoside (orientin, F3). TLC: Rf 0.24 (BAW), 0.27 (BEW), 0.08 (15% HOAc); UV — dark purple, UV/NH_3 — bright yellow. UV: λmax (nm) MeOH 256, 267 sh, 349; +NaOMe 267 sh, 277, 328 sh, 407 (inc.); +$AlCl_3$ 271, 422; +$AlCl_3$/HCl 259, 297, 359, 386 sh; +NaOAc 268 sh, 278, 326 sh, 397; +NaOAc/H_3BO_3 266, 373. Acid hydrolysis: unhydrolyzable. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (luteolin+1 mol glucose).

Apigenin 8-*C*-glucoside (vitexin, F4). TLC: Rf 0.39 (BAW), 0.50 (BEW), 0.13 (15% HOAc); UV — dark purple, UV/NH_3 — dark yellow. UV: λmax (nm) MeOH 270, 337; +NaOMe 279, 329, 395 (inc.); +$AlCl_3$ 276, 304, 353, 384 sh; +$AlCl_3$/HCl 277, 302, 350, 380 sh; +NaOAc 279, 312, 392; +NaOAc/H_3BO_3 271, 348. Acid hydrolysis: unhydrolyzable. LC-MS: *m/z* 433 $[M+H]^+$, 431 $[M-H]^-$ (apigenin+1 mol glucose).

Acylated orientin (F5). TLC: Rf 0.37 (BAW), 0.49 (BEW), 0.09 (15% HOAc); UV — dark purple, UV/NH_3 — yellow. UV: λmax (nm) MeOH 270, 287 sh, 347; +NaOMe 278, 320 sh, 407 (inc.); +$AlCl_3$ 273, 302, 366, 426; +$AlCl_3$/HCl 268, 296 sh, 359, 393 sh; +NaOAc 277, 324, 394; +NaOAc/H_3BO_3 271, 296 sh, 375. Acid hydrolysis: orientin (major) and isoorientin (minor). LC-MS: *m/z* 601 $[M+H]^+$, 599 $[M-H]^-$ (luteolin+each 1mol glucose and unknown compound having molecular weight 152).

Acylated isoorientin (F6). TLC: Rf 0.49 (BAW), 0.61 (BEW), 0.06 (15% HOAc); UV — dark purple, UV/NH_3 — yellow. UV: λmax (nm) MeOH 257 sh, 271, 350; +NaOMe 277, 339, 415 (inc.); +$AlCl_3$ 277, 300 sh, 365 sh, 422; +$AlCl_3$/HCl 264 sh, 276, 296 sh, 361, 387 sh; +NaOAc 276, 322, 401; +NaOAc/H_3BO_3 271, 300 sh, 378. Acid hydrolysis: isoorientin (major) and orientin (minor). LC-MS: *m/z* 601 $[M+H]^+$, 599 $[M-H]^-$ (luteolin+each 1 mol glucose and unknown compound having molecular weight 152).

Results and Discussion

Anthocyanins and other flavonoids in autumn leaves of A. palmatum

In this survey, two anthocyanin peaks (A1 and A2) appeared on the HPLC chromatograms (Fig. 2). Of their anthocyanins, A1 was the major pigment and identified as cyanidin 3-*O*-glucoside (Fig. 3), as reported by Iwashina (1996). Though minor A2 was not isolated, it may be cyanidin 3-*O*-galloylglucoside, which has been isolated from this plant by Ji *et al.* (1992b). Cyanidin 3-*O*-glucoside was contained in 99.3% of total anthocyanin in the leaves of *A. palmatum* collected in 22 Nov. 2006 and recorded highest anthocyanin content (Table 1). On the other hand, seven flavonoid peaks appeared on the HPLC chromatograms (Fig. 4). Of their flavonoids, six ones were isolated and four major ones were characterized as *C*-glycosylflavones, isoorientin (F1), isovitexin (F2), orientin (F3) and vitexin (F4) (Fig. 3), which have already been reported from this species (Aritomi, 1962, 1963). Two minor flavonoids (F5 and F6) were characterized as ori-

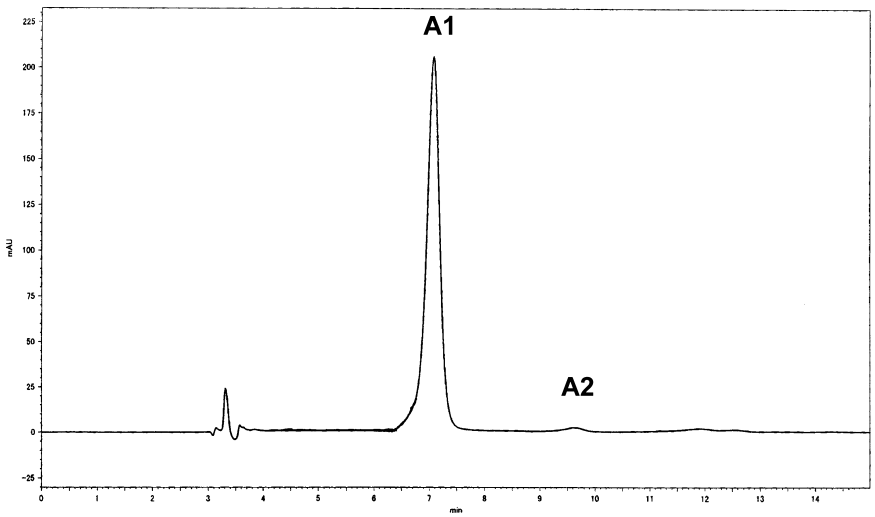
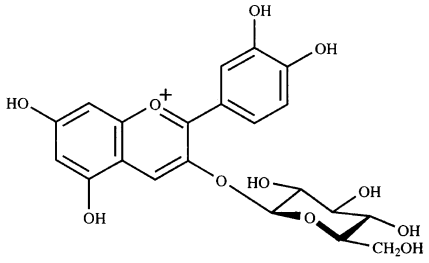


Fig. 2. HPLC chromatogram of the anthocyanins in autumn leaves of *Acer palmatum* (22 Nov. 2006). Flow-rate: 1.0 ml min^{-1}; Detection: 530 nm; and Eluent: MeCN/HOAc/H_2O/H_3PO_4 (6 : 8 : 83 : 3). A1=Cyanidin 3-*O*-glucoside and A2=Cyanidin glycoside.

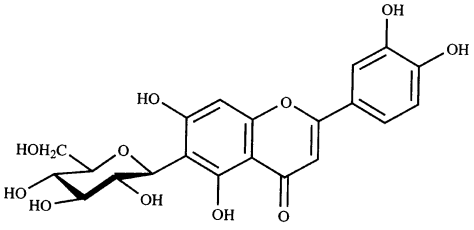
Table 1. Quantitative variations of anthocyanins, and total chlorophylls and carotenoids in *Acer palmatum* leaves.

Date	Total chlorophylls	Total carotenoids	Anthocyanins		
			A1	A2	Total anthocyanins
26 Oct.	1.23[1]	2.40[1]	138,849[2] 100.0%	—	138,849
	(1.05)	(1.12)	(0.24)		(0.19)
2 Nov.	1.17	2.15	589,996 79.1%[3]	156,065 20.9%	746,061
	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
13 Nov.	0.73	1.68	2,738,880 95.4%	132,830[2] 4.6%	2,871,710
	(0.62)	(0.78)	(4.64)	(0.85)	(3.85)
18 Nov.	0.61	1.46	3,240,981 99.0%	32,933 1.0%	3,273,914
	(0.52)	(0.68)	(5.49)	(0.21)	(4.39)
22 Nov.	0.08	0.99	5,396,960 99.3%	39,829 0.7%	5,436,789
	(0.07)	(0.46)	(9.15)	(0.26)	(7.29)
25 Nov.	0.03	0.79	4,739,052 99.6%	19,886 0.4%	4,758,938
	(0.03)	(0.37)	(8.03)	(0.13)	(6.38)
29 Nov.	0.02	0.67	3,944,404 99.5%	19,541 0.5%	3,963,945
	(0.02)	(0.31)	(6.69)	(0.13)	(5.31)

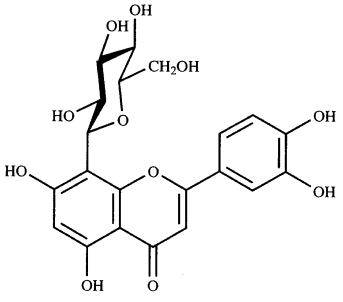
Each fresh leaves (0.2 g) was extracted with 0.1% MeOH–HCl (3 ml) for anthocyanins or MeOH (3 ml) for chlorophylls and carotenoids; measurement was at 530 nm (anthocyanins), 663 nm (chlorophylls) or 446 nm (carotenoids).
[1] Absorbance at 663 nm (chlorophylls) or 446 nm (carotenoids).
[2] Peak area at 530 nm.
( )=Relative amounts of chlorophylls, carotenoids and anthocyanins as absorbance or peak area of the samples collected in 2 Nov, 2006 is 1.00.
[3] Each anthocyanin percentage.
A1=Cyanidin 3-*O*-glucoside, A2=cyanidin glycoside.



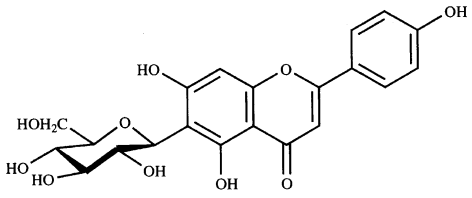
Cyanidin 3-*O*-glucoside (Chrysanthemin, A1)



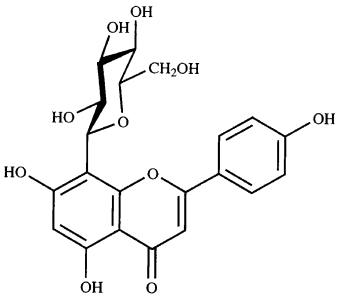
Isoorientin (F1)



Orientin (F3)



Isovitexin (F2)



Vitexin (F4)

Fig. 3. Chemical structures of the anthocyanin and *C*-glycosylflavones isolated from the leaves of *Acer palmatum*.

entin and isoorientin, which were acylated by an unknown substance having molecular weight 152, such as monohydroxymethyl benzoic acid, by LC-MS survey.

Anthocyanin and other flavonoid composition in the leaves was qualitatively the same during all sampling period.

Quantitative variation of anthocyanin and other flavonoids with the progress of autumn leaf coloration

The variation of total anthocyanins, *C*-glycosylflavones, chlorophylls and carotenoids was shown in Tables 1–3, and Figs. 5 and 6. Anthocyanin content increased with the progress of autumn leaf coloration, and reached to maximum amount (7.29 times as compared with that of the

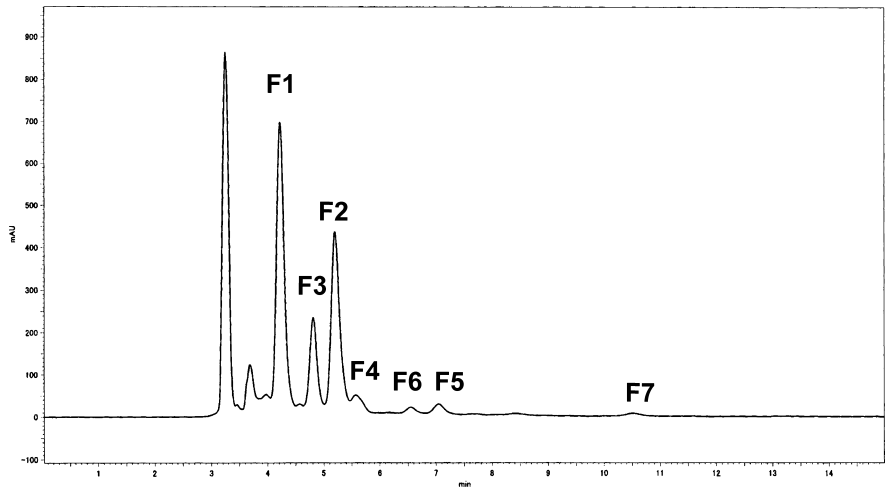


Fig. 4. HPLC chromatogram of *C*-glycosylflavones in autumn leaves of *Acer palmatum* (22 Nov. 2006). Flow-rate: 1.0 ml min^{-1}; Detection: 350 nm; and Eluent: $MeCN/H_2O/H_3PO_4$ (22 : 78 : 0.2). F1=Isoorientin, F2=Isovitexin, F3=Orientin, F4=Vitexin, F5=Acyalted orientin, F6=Acylated isoorientin and F7=Unknown flavonoid.

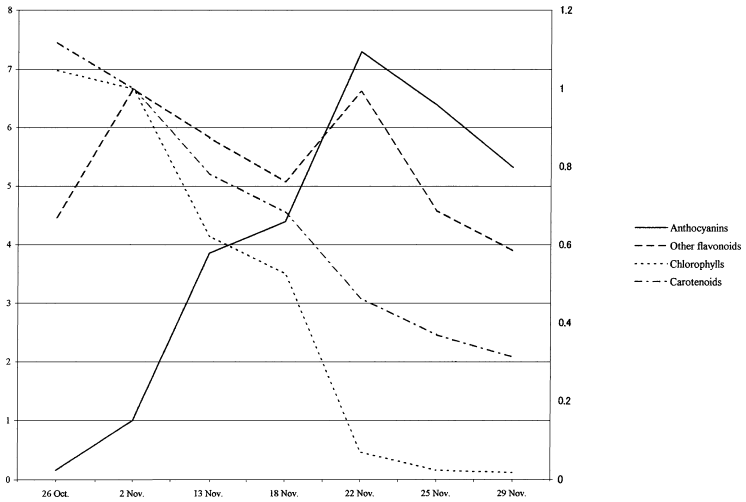


Fig. 5. Qualitative variations of total anthocyanins, other flavonoids, chlorophylls and carotenoids in autumn leaves of *Acer palmatum*. Left and right axes show the relative level of anthocyanin and other flavonoid contents.

leaves in 2 Nov. 2006) in 22 Nov. 2006 (Table 1 and Fig. 5). Thereafter, anthocyanin content gradually decreased. Total chlorophyll and carotenoid contents were also decreased. Especially, they were rapidly decreased in 22 Nov. with increase of anthocyanin. On the other hand, total *C*-glycosylflavone content gradually decreased, though it temporarily increased in 22 Nov. with increase of anthocyanin content. However, they ephemerally increased in the samples collected in 22 Nov. 2006, in which anthocyanin content maximally increased. Relative amount of each *C*-glycosylflavones did not basically varied during the collection period. Seasonal variation of anthocyanins and other flavonoids in autumn leaves has been investigated on aquatic plant,

Table 2. Quantitative variations of other flavonoids in *Acer palmatum* leaves.

Date	Other flavonoids							
	F1	F2	F3	F4	F5	F6	F7	Total
26 Oct.	5,054,538[1]	3,001,959	1,351,310	635,166	495,615	291,233	163,644	10,993,465
	46.0%[2]	27.3%	12.3%	5.8%	4.5%	2.6%	1.5%	
	(0.72)	(0.48)	(0.54)	(13.00)	(1.86)	(1.76)	(1.39)	(0.67)
2 Nov.	7,041,040	6,281,028	2,521,542	48,864	267,045	165,558	117,521	16,442,598
	42.8%	38.2%	15.3%	0.3%	1.6%	1.0%	0.7%	
	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
13 Nov.	6,263,892	4,408,140	2,094,037	664,984	385,203	280,592	153,494	14,250,342
	44.0%	30.9%	14.7%	4.7%	2.7%	2.0%	1.1%	
	(0.89)	(0.70)	(0.83)	(13.61)	(1.44)	(1.69)	(1.31)	(0.87)
18 Nov.	5,542,055	3,681,992	1,745,989	686,907	402,756	285,190	159,959	12,504,848
	44.3%	29.4%	14.0%	5.5%	3.2%	2.3%	1.3%	
	(0.79)	(0.59)	(0.69)	(14.06)	(1.51)	(1.72)	(1.36)	(0.76)
22 Nov.	7,077,709	4,823,225	2,255,561	855,694	591,201	446,507	252,923	16,302,820
	43.4%	29.6%	13.8%	5.2%	3.6%	2.7%	1.6%	
	(1.01)	(0.77)	(0.89)	(17.51)	(2.21)	(2.70)	(2.15)	(0.99)
25 Nov.	5,209,621	3,221,106	1,348,229	613,585	439,452	298,747	147,724	11,278,464
	46.2%	28.6%	12.0%	5.4%	3.9%	2.6%	1.3%	
	(0.74)	(0.51)	(0.53)	(12.56)	(1.64)	(1.80)	(1.26)	(0.69)
29 Nov.	4,163,022	2,712,609	1,312,825	632,278	370,586	263,971	144,561	9.599,852
	43.4%	28.3%	13.7%	6.6%	3.9%	2.7%	1.5%	
	(0.59)	(0.43)	(0.52)	(12.94)	(1.38)	(1.59)	(1.23)	(0.58)

Each fresh leaves (0.2 g) was extracted with MeOH (3 ml); measurement was at 350 nm.

[1] Peak area at 350 nm.

[2] Each flavonoid percentage.

( )=Relative amounts of other flavonoids as peak area of the samples collected in 2 Nov, 2006 is 1.00.

F1=Isoorientin, F2=Isovitexin, F3=Orientin, F4=Vitexin, F5=Acylated orientin, F6=Acylated isoorientin and F7=Unknown flavonoids.

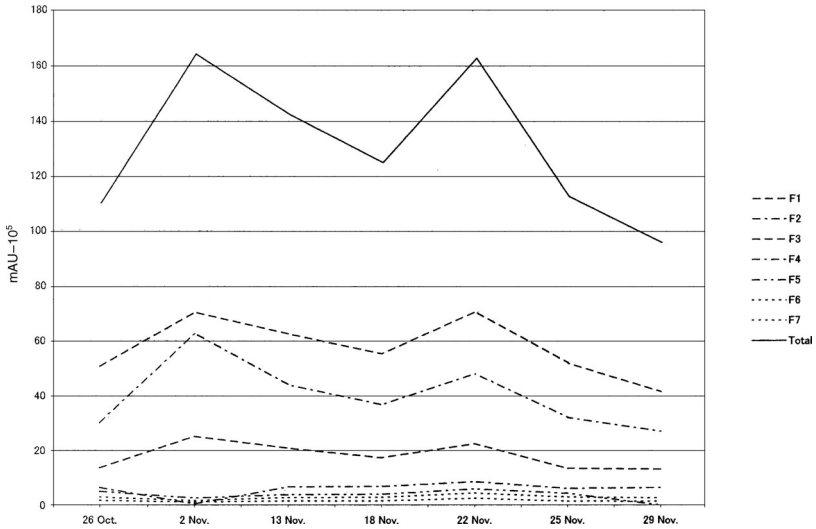


Fig. 6. Qualitative variations of each *C*-glycosylflavones in autumn leaves of *Acer palmatum*. F1=Isoorientin, F2=Isovitexin, F3=Orientin, F4=Vitexin, F5=Acylated orientin, F6=Acylated isoorientin and F7=Unknown flavonoid.

Egeria densa Planch. (Momose, 1998). The leaves of the species are commonly green, but the detached ones easily induces an rare anthocyanin, cyanidin 5-methyl ether 3-*O*-glucoside, i.e. erodenin, in sucrose solution under light (Momose *et al.*, 1977; Momose, 1996). Flavone *O*-glycoside, luteolin 7-*O*-diglucuronide, accompanied with erodenin and two luteolin derivatives in colored leaves as other flavonoids (Momose, 1998). This compound was remained stable during incubation of detached leaves, but two luteolin derivatives and some unknown compounds including caffeic acid derivatives were newly synthesized with coloration.

Major aspects of the overall biosynthetic pathway to major groups of the flavonoids have fully been described, and the main enzymes have been demonstrated so far in cell-free extracts (Stafford, 1990). In the case of *Egeria densa* leaves coloration, flavone *O*-glycosides were accompanied with increase of the anthocyanin (Momose, 1998). On the other hand, *C*-glycosylflavones were synthesized in the case of *Acer palmatum*. Though *O*-glycosylation of the flavones was clearly proved to occur in the terminal of flavonoid biosynthesis, that of *C*-glycosylation has hardly been reported (Stafford, 1990). Kerschr and Franz (1987) have reported that a *C*-glucosyltransferase from *Fagopyrum esculentum* Moench seedlings catalyzed the transfer of glucose from UDP-glucose or 2-hydroxyflavanones, e.g. 2,5,7,4′-tetrahydroxyflavanone and 2,5,7-trihydroxyflavanone were appropriate substrates. Moreover, naringenin, chalcononaringenin, apigenin and chrysin can not act as glucosyl acceptors in *C*-glycosylflavonoid biosynthesis. This demonstrates that *C*-glucosylation occurs after oxidation of flavanone. If such the case is the fact, *C*-glycosylation early occurs in flavonoid biosynthesis. In contrast, anthocyanins are synthesized in later step. As the results, it was presumed that seasonal variation between anthocyanins and *C*-glycosylflavones does not correlate each other in the case of autumn leaves coloration of *A. palmatum*. Among the plant species varied the leaves to red from green in autumn, some species, e.g. *Euonymus alatus* (Thunb.) Sieb. and *Parthenocissus tricuspidata* (Sieb. & Zucc.) Planch., are occurred flavonols such as quercetin and kaempferol together with anthocyanin, cyanidin 3-*O*-glucoside (Ishikura, 1977). The biosynthetic step of the anthocyanins and

flavonols are comparatively near than that of *C*-glycosylflavones. The seasonal variation between anthocyanins and flavonols should be surveyed as plant materials which synthesize flavonols but not *C*-glycosylflavones in relation with autumn leaves coloration.

References

Aritomi, M. 1962. Studies on the chemical constituents in leaves of *Acer palmatum* Thunberg. *Yakugaku Zasshi* **82**: 1329–1331 (in Japanese with English summary).

Aritomi, M. 1963. Chemical constituents in Aceraceous plants. I. Flavonoid constituents in the leaves of *Acer palmatum* Thunberg. *Yakugaku Zasshi* **83**: 737–740.

Bate-Smith, E.C. 1978. Systematic aspects of the astringent tannins of *Acer* species. *Phytochemistry* **17**: 1945–1948.

Hattori, S. and Hayashi, K. 1937. Studien über Anthocyane. II. Über die Farbstoffe aus den roten Herbstblättern von einigen *Acer*-Arten. *Acta Phytochimica* **10**: 129–138.

Hayashi, K. and Abe, Y. 1955. Studien über Anthocyane, XXVII. Papierchromatographische übersicht der Anthocyane in Pflanzenreich (II). Farbstoffe des roten Herbstlaubes. *The Botanical Magazine*, *Tokyo* **68**: 299–307.

Hayashi, K. 1968. Phylogeny of the autumn leaves. *In*: Ohwi, J., Aritaki, T. and Nakamura, T. (eds.), *Momiji-to-Kaede*. pp. 246–253. Seibundo-Shinkosha, Tokyo (in Japanese).

Ishikura, N. 1972. Anthocyanins and other phenolics in autumn leaves. *Phytochemistry* **11**: 2555–2558.

Iwashina, T. 1996. Detection and distribution of chrysanthemin and idaein in autumn leaves of plants by high performance liquid chromatography. *Annals of the Tsukuba Botanical Garden* (15): 1–18.

Ji, S.-B., Yokoi, M., Saito, N. and Mao, L.-S. 1992a. Distribution of anthocyanins in Aceraceous leaves. *Biochemical Systematics and Ecology* **20**: 771–781.

Ji, S.-B., Saito, N., Yokoi, M., Shigihara, A. and Honda, T. 1992b. Galloylcyanidin glycosides from *Acer*. *Phytochemistry* **31**: 655–657.

Kerscher, F. and Franz, G. 1987. Biosynthesis of vitexin and isovitexin: enzymatic synthesis of the *C*-glucosylflavones vitexin and isovitexin with an enzyme preparation from *Fagopyrum esculentum* M. seedlings. *Zeitschrift für Naturforschung* **42c**: 519–524.

Lee, D.W. 2002. Anthocyanins in autumn leaf senescence. *In*: Gould, K.S. and Lee, D.W. (eds.), *Advances in Botanical Research Incorporating Advances in Plant Pathology. Anthocyanins in Leaves*. pp. 147–165. Academic Press, Amsterdam.

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

Momose, T., Abe, K. and Yoshitama, K. 1977. 5-*O*-Methylcyanidin 3-glucoside from leaves of *Egeria densa*. *Phytochemistry* **16**: 1321.

Momose, T. 1996. Study of autumnal coloration in *Egeria densa* 1. Induction factors of autumnal coloration. *The Journal of Biology Teacher's Association of Tokyo* (32): 1–6 (in Japanese with English summary).

Momose, T. 1998. Study of autumnal coloration in *Egeria densa* 3. Qualitative and quantitative variation of flavonoid compounds in the leaves of *E. densa* with its autumnal coloration. *The Journal of Biology Teacher's Association of Tokyo* (34): 11–16 (in Japanese with English summary).

Murrell Jr., J.T. and Wolf, F.T. 1969. The anthocyanin of the autumn leaves of Japanese barberry. *Bulletin of the Torrey Botanical Club* **96**: 594–596.

Shimizu, T. 1989. Aceraceae. *In*: Satake, Y., Hara, H., Watari, S. and Tomimori, T. (eds.), *Wild Flowers of Japan. Woody Plants II*. pp. 7–18. Heibonsya, Tokyo.

Stafford, H.A. 1990. *Flavonoid Metabolism*. CRC Press, Boca Raton.

Takeda, K. 1983. The mechanism of autumn leaves. *The Nature and Plants*, **17**(11): 2–6 (in Japanese).

Yoshitama, K. and Ishikura, N. 1988. Distribution of anthocyanin pigments in plants native to Japan. *In*: Hayashi, K. (ed.), *Plant Pigments — An Introcuction to Research and Experiments —*. pp. 481–551. Yokendo, Tokyo (In Japanese).