筑波実験植物園研報　15：1-18, 1996.
Ann. Tsukuba Bot. Gard.

Detection and Distribution of Chrysanthemin and Idaein in Autumn Leaves of Plants by High Performance Liquid Chromatography

by

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岩科　司*: 高速液体クロマトグラフィーによる紅葉のクリサンテミンおよびイデインの検出とその分布

Chrysanthemin (cyanidin 3-*O*-glucoside) is common anthocyanin in higher plants (Timberlake and Bridle 1975; Yoshitama and Ishikura 1988) and have been reported to be a major pigment in autumn leaves (Hattori und Hayashi 1937; Hayashi und Abe 1955). On the other hand, idaein (cyanidin 3-*O*-glucoside), which have been isolated from the fruits of *Vaccinium vitis-idaea* L. (Willstätter und Mallison 1915) and the pericarps of *Fatsia japonica* Decaisne et Planchon (Hayashi 1939), have been sometimes reported in plants (Timberlake and Bridle 1975; Yoshitama and Ishikura 1988). In autumn leaves, idaein have been reported from *Cotinus coggygria* Scop. (Anacardiaceae) (Tanchev and Timberlake 1969), *Agrimonia pilosa* Ledeb. (Rosaceae), *Acalypha australis* L., *Euphorbia sendaica* Makino (Euphorbiaceae), *Shortia soldanelloides* Makino var. *magna* Makino, *S. uniflora* (Maxim.) Maxim. (Diapensiaceae), and seven Polygonaceous plants, *Fagopyrum esculentum* Moench, *Polygonum longisetum* De. Bruyn, *P. nipponense* (Nakai) Makino, *P. thunbergii* Sieb. et Zucc., *P. hydropiper* L., *P. nodosum* Pers. and *P. blumei* Meisn. (Kato 1982). However, the chemical characters, especially their paper and thin-layer chromatographic properties, are very similar and hardly distinguished between chrysanthemin and idaein.

Recently, high performance liquid chromatographic (HPLC) techniques developped for isolation of flavonoids including anthocyanins (Williams *et al.* 1978; Hostettmann and Hostettmann 1982; Hayashi *et al.* 1989). Thus, flavonoid monoglycosides attaching similar hexoses, glucose, galactose, glucuronic acid and rarely allose have been easily distinguished by HPLC (Iwashina and Ootani 1990; Iwashina and Hatta 1992).

In this experiment, I investigated the distribution of chrysanthemin, idaein and other anthocyanins in autumn leaves of 63 species (21 families) by HPLC.

Materials and Methods

Plant materials

The autumn leaves of 63 species (21 families) (see Table 1) which were used as plant materials were collected in Tsukuba Botanical Garden, National Science Museum, Tsukuba-shi, Ibaraki Pref.

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HPLC separation of anthocyanins

HPLC separation was performed with JASCO HPLC systems including a 880-51 2-line degasser and Syringe loading sample injector 25 model 7125 (Rheodyne Inc.). Multi channel UV-visible detector Multi-330 coupled with a computer was used recording chromatograms and UV-visible spectra. Tosoh TSKgel ODS 80TM column (I. D. 150mm × 4.6mm) was used.

Coloured autumn leaves were extracted with 0.2% MeOH-HCl, filtrated and evaporated *in vacuo.* Residue was dissolved in MeOH, filtrated through Toyopak ODS M (Tosoh) and Maisyoridisc H-13-5 0.45 μm (Tosoh) pre-cartridge, and eluted with H_3PO_4-AcOH-CH_3CN-H_2O (3: 8: 6: 83). Detection was at 350-700 nm and flow-rate was 1.0 ml/min.

Chrysanthemin and idaein were identified by HPLC comparisons with authentic samples (chrysanthemin: from autumn leaves of three *Acer* species, Hattori und Hayashi 1937; and idaein: from the pericarps of *Fatsia japonica*, Hayashi 1939).

Acid hydrolysis of crude extracts and identification of anthocyanidins

Crude extracts were hydrolyzed with 12% aq. HCl in directly on fire, for 3 min. After cooling, anthocyanidins were extracted with isoAmOH and identified by direct paper-chromatographic comparisons (solvent system; AcOH/conc. HCl/H_2O = 30: 3: 10) with authentic pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (see Table 1).

Results and Discussion

The presence of chrysanthemin, idaein and other anthocyanins in autumn leaves of 63 plants were shown in Table 1. It was shown by PC characterization of acid hydrolysates that cyanidin glycosides were major pigments in all species. In many cases, chrysanthemin and idaein were co-presence in autumn leaves (Table 1, 2 and Fig. 1-12).

Gramineae, Fagaceae and Berberidaceae

Four species, i.e., *Miscanthus sinensis* Anderss. (Gramineae), *Quercus crispula* Blume, *Q. aliena* Blume (Fagaceae), *Nandina domestica* Thunb. and *Berberis thunbergii* DC., were surveyed for anthocyanins by HPLC (Fig. 1). In all cases, chrysanthemin was detected as a major pigment (85.3-100% of total anthocyanins). Though idaein was found in *M. sinensis* and *B. thunbergii*, it was very small amount (0.1-0.2%). Other anthocyanins which may be cyanidin glycosides were also present in small amount (1.0-14.7%). In *Q. crispula*, chrysanthemin alone was found.

The anthocyanins in autumn leaves of *N. domestica* and *Q. crispula* have been characterized as cyanidin 3-*O*-monohexoside (Hayashi und Abe 1955) and that of *B. thunbergii* as chrysanthemin (Murrell and Wolf 1969). In this experiment, all of them were determined to be cyanidin 3-*O*-glucoside.

Saxifragaceae

Deutzia scabra Thunb. and *Hydrangea macrophylla* (Thunb. ex Murray) Ser. f. *normalis* (Wilson) Hara were surveyed for anthocyanins in autumn leaves (Table 1 and Fig. 2). Their major pigment was chrysanthemin with a small amount of idaein (4.3-8.1%). However, HPLC patterns of their anthocyanins in autumn leaves were different (Fig. 2). Foliar anthocyanins of *H. macrophylla* f. *normalis* mainly consist of chrysanthemin (93.3%), while those of *D. scabra* were composed of chrysanthemin and other two anthocyanins, which may be cyanidin glycosides, since cyanidin alone was produced by total acid hydrolysis.

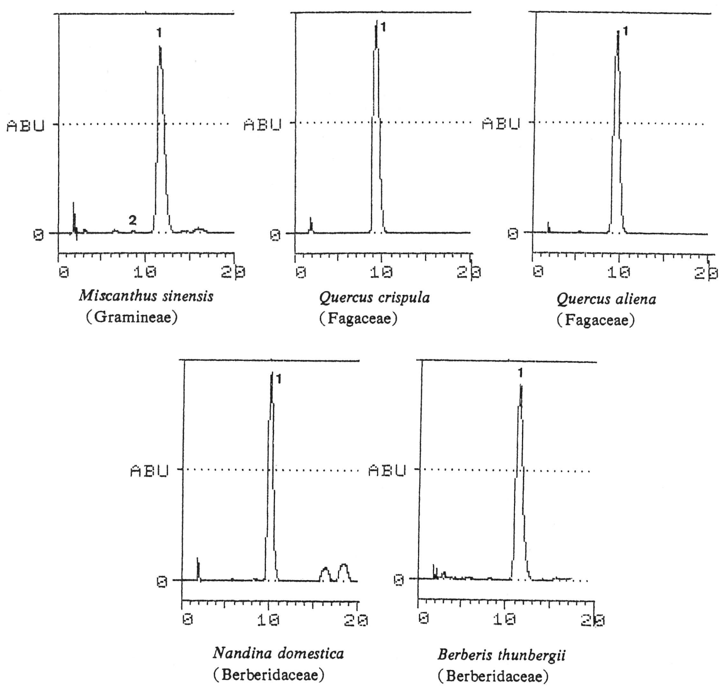


Fig. 1. HPLC patterns of anthocyanins in autumn leaves (Gramineae, Fagaceae and Berberidaceae). Column: Tosoh TSKgel ODS 80TM (I.D. 150mm × 4.6 mm), Eluent: $H_3PO_4/AcOH/CH_3CN/H_2O$ (3: 8: 6: 83), Detection: 520 nm, and Flow-rate: 1.0 ml/min.
1= chrysanthemin, **2**= idaein, and other peaks = other anthocyanins.

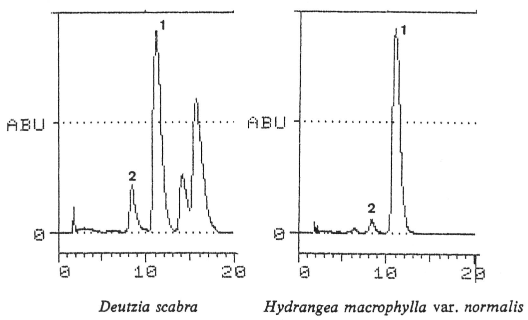


Fig. 2. HPLC patterns of anthocyanins in autumn leaves (Saxifragaceae).

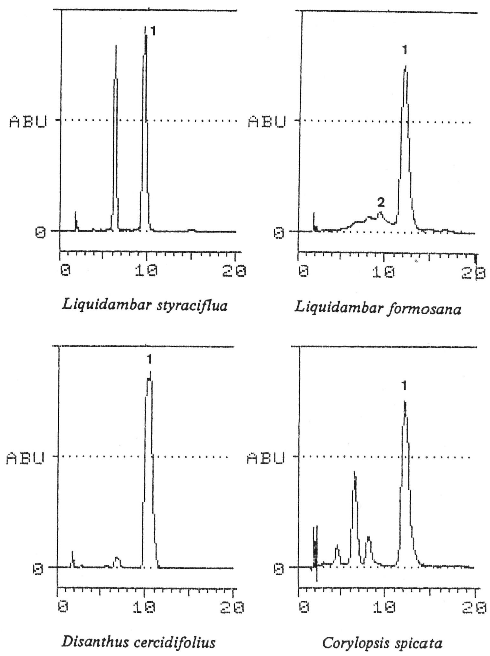


Fig. 3. HPLC patterns of anthocyanins in autumn leaves (Hamamelidaceae).

Table 1. Distribution of chrysanthemin, idaein and other anthocyanins in autumn leaves of plants

Species	chrysanthemin %	idaein %	other anthocyanins %	hydrolysates of crude extr.	references
Gramineae					
Miscanthus sinensis (ススキ)	98.9	0.1	1.0(2)	Cy	
Fagaceae					
Quercus aliena (ナラガシワ)	99.0	0	1.0(2)	Cy	
Q. crispula (ミズナラ)	100	0	0	Cy	Cy3Hex[1)]
Berberidaceae					
Berberis thunbergii (メギ)	98.2	0.2	1.6(2)	Cy	Cy3Gl[2)]
Nandina domestica (ナンテン)	85.3	0	14.7(2)	Cy, Pn(t)	Cy3Hex[1)]
Saxifragaceae					
Deutzia scabra (マルバウツギ)	44.8	8.1	47.1(2)	Cy	
Hydrangea macrophylla f. *normalis* (ガクアジサイ)	93.3	4.3	2.4(2)	Cy, Dp(t)	Cy3Hex[1)]
Hamamelidaceae					
Corylopsis spicata (トサミズキ)	63.6	0	36.4(3)	Cy	
Disanthus cercidifolius (マルバノキ)	95.4	0	4.6(3)	Cy, Pn(t), Dp (t)	
Liquidambar formosana (フウ)	79.8	9.9	10.3(2)	Cy	
L. styraciflua (モミジバフウ)	60.0	0	40.0(1)	Cy, Pn(t)	
Rosaceae					
Cotoneaster horizontalis (ベニシタン)	0	99.0	1.0(3)	Cy	
Prunus buergeriana (イヌザクラ)	97.1	0	2.9(3)	Cy, Pn (t)	
P. tomentosa (ユスラウメ)	99.1	0	0.9(2)	Cy	
Rhaphiolepis indica var. *umbellata* (シャリンバイ)	0.7	99.3	0	Cy	
Rubus crataegifolius (クマイチゴ)	98.5	0	1.5(3)	Cy, Dp(t)	
Sorbus commixta (ナナカマド)	1.2	98.2	0.6(1)	Cy, Pn(t)	
Spiraea japonica (シモツケ)	0.4	91.8	7.8(3)	Cy	Cy3Gl[1)] CyGy[1)]
Stephanandra incisa (コゴメウツギ)	0	91.0	9.0(4)	Cy, Pn(t)	

-(continued)-

Table 1. -(continued)-

Species	chrysanthemin %	idaein %	other anthocyanins %	hydrolysates of crude extr.	references
Euphorbiaceae					
Sapium japonicum (シラキ)	98.7	0	1.3(2)	Cy	
Coriariaceae					
Coriaria japonica (ドクウツギ)	64.7	29.5	5.8(5)	Cy	
Anacardiaceae					
Cotinus coggygria (ハグマノキ)	8.6	78.6	12.8(5)	Cy	Cy3Gal[3], Dp3Gal[3], Pt3Gl[3], Dp7Gl[3], Cy3Gl7R[3]
Rhus succedanea (ハゼノキ)	0.8	84.3	13.9(2)	Cy, Pn(t)	Cy3Gl[4], Pn3Gl[4]
Celastraceae					
Euonymus alatus f. *striatus* (コマユミ)	86.9	0	13.1(2)	Cy, Pn(t), Dp(t)	Cy3Gl[4]
Staphyleaceae					
Euscaphis japonica (ゴンズイ)	97.2	0	2.8(2)	Cy	
Staphylea bumalda (ミツバウツギ)	96.3	0	3.7(3)	Cy	
Aceraceae					
Acer cissifolium (ミツデカエデ)	96.7	0	3.3(2)	Cy, Pn(t)	Cy3Gl[5], Cy35Gl[5], Cy3galGl[5], Cy3galRGl[5]
A. ginnala (カラコギカエデ)	94.6	3.3	2.1(2)	Cy	Cy3Gl[5], Cy35Gl[5], Cy3galGl[5]
A. japonicum (ハウチワカエデ)	97.1	0	2.9(2)	Cy, Pn(t)	Cy3Gl[1][5], Cy35Gl[5]
A. palmatum (イロハモミジ)	97.3	0	2.7(4)	Cy, Pn(t)	Cy3Gl[4][5], Cy35Gl[5], Cy3galGl[5]
A. pycnanthum (ハナノキ)	93.6	0	6.4(4)	Cy	Cy3Gl[5], Cy3galGl[5], Cy3galRGl[5]
Sapindaceae					
Koelreuteria paniculata (モクゲンジ)	97.5	0	2.5(2)	Cy, Pn(t)	
Sabiaceae					
Meliosma myriantha (アワブキ)	87.9	12.1	0	Cy	
Theaceae					
Stuartia pseudo-camellia (ナツツバキ)	0	99.2	0.8(2)	Cy	

-(continued)-

Table 1. -(continued)-

Species	chrysanthemin %	idaein %	other anthocyanins %	hydrolysates of crude extr.	references
Stachyuraceae					
Stachyurus praecox (キブシ)	99.7	0	0.3(1)	Cy	
Lythraceae					
Lagerstroemia indica (サルスベリ)	90.4	0.8	8.8(4)	Cy	Cy3Gl[6]
Cornaceae					
Cornus brachypoda (クマノミズキ)	2.2	95.3	2.5(2)	Cy	
C. controversa (ミズキ)	3.1	94.9	2.0(2)	Cy	Cy3Hex[1]
C. florida (ハナミズキ)	45.4	49.8	4.8(2)	Cy, Pn(t), Dp(t)	
C. kousa (ヤマボウシ)	90.8	9.2	0	Cy, Pn(t)	Cy3Hex[1]
C. officinalis (サンシュユ)	0	36.0	64.0(4)	Cy, Pn	
Clethraceae					
Clethra barvinervis (リョウブ)	10.7	7.7	81.6(4)	Cy, Pn(t)	Cy3HexPen[1], CyGly[1]
Ericaceae					
Elliottia paniculata (ホツツジ)	0	100	0	Cy	
Enkianthus campanulatus var. *rubicundus* (ベニサラサドウダン)	2.0	81.0	17.0(1)	Cy, Dp(t)	Cy3Hex[1], CyGly[1]
E. perulatus (ドウダンツツジ)	1.6	85.3	13.1(1)	Cy	Cy3Hex[1], CyGly[1]
Lyonia ovalifolia var. *elliptica* (ネジキ)	2.6	87.5	9.9(1)	Cy	
Rhododendron dilatatum (ミツバツツジ)	96.9	2.4	0.7(2)	Cy, Pn(t)	
R. japonicum (レンゲツツジ)	1.6	27.4	71.0(1)	Cy	Cy3Hex[1], CyGly[1]
R. macrosepalum (モチツツジ)	1.4	36.3	62.3(1)	Cy, Dp(t)	
R. obtusum var. *kaempferi* (ヤマツツジ)	5.8	28.1	66.1(2)	Cy	Cy3Gl[1], Dp3Hex[1]
R. quinquefolium (シロヤシオ)	93.4	4.3	2.3(1)	Cy, Pn(t), Dp(t)	Cy3Gl[1]
R. reticulatum (コバノミツバツツジ)	71.3	0.8	27.9(1)	Cy, Pn(t), Dp(t)	

-(continued)-

Table 1. -(continued)-

Species	chrysanthemin %	idaein %	other anthocyanins %	hydrolysates of crude extr.	references
R. wadanum (トウゴクミツバツツジ)	97.5	2.3	0.2(1)	Cy, Pn(t),	Cy3Hex[1]
Vaccinium corymbosum (ブルーベリー)	33.0	36.1	30.9(2)	Cy, Pn(t) Dp(t)	Cy3Gl[7], Dp3Gl[7], Pt3Gl[7], Mv3Gl[7],
V. oxycoccus (ツルコケモモ)	1.4	50.5	48.1(2)	Cy, Dp(t)	
Oleaceae					
Forsythia suspensa (レンギョウ)	44.1	2.0	53.9(4)	Cy, Pn(t)	
F. viridissima (シナレンギョウ)	50.7	19.2	30.1(6)	Cy	
Ligustrum obtusifolium (イボタノキ)	73.7	0	26.3(2)	Cy	Cy3Gl[1], CyGly[1]
Caprifoliaceae					
Viburnum dilatatum (ガマズミ)	97.6	2.1	0.3(1)	Cy, Pn(t)	Cy3Hex[1]
V. odorattissimum var. *awabuki* (サンゴジュ)	4.4	92.5	3.1(2)	Cy, Pn(t)	
V. wrightii (ミヤマガマズミ)	99.1	0.4	0.5(1)	Cy, Pn(t)	Cy3Hex[1]
Weigela decora (ニシキウツギ)	99.2	0.7	0.1(1)	Cy, Pn(t)	
W. floribunda (ヤブウツギ)	17.3	80.7	2.0(3)	Cy	

Cy = cyanidin, Pn = peonidin, Dp = delphinidin, Cy3Hex = cyanidin 3-hexoside, Cy3Gl = cyanidin 3-glucoside (chrysanthemin), Cy3Gal = cyanidin 3-galactoside (idaein), Dp3Gal = delphinidin 3-galactoside, Cy3Gl7R = cyanidin 3-glucoside-7-rhamnoside, Pt3Gl = petunidin 3-glucoside, Dp7Gl = delphinidin 7-glucoside, Pn3Gl = peonidin 3-glucoside, Gy35Gl = Cyanidin 3, 5-diglucoside (cyanin), Cy3galGl = cyanidin 3-galloylglucoside, Cy3galRGl = cyanidin 3-galloylrutinoside, Dp3Hex = delphinidin 3-hexoside, Cy3HexPen = cyanidin 3-hexosylpentoside, CyGly = cyanidin glycoside, Dp3Gl = delphinidin 3-glucoside and Mv3Gl = malvidin 3-glucoside.

( ) = numbers of other anthocyanins.

% = each peak area (Abs•sec)/total peak areas of anthocyanins.

[1] Hayashi und Abe (1955), [2] Murrell and Wolf (1969), [3] Tanchev and Timberlake (1969), [4] Ishikura (1972), [5] Ji *et al.* (1992), [6] Kato (1982) and [7] Hrazdina (1982).

In spite of major pigment in autumn leaves of *H. macrophylla* f. *normalis* is chrysanthemin, its leaf colour represents purple which may be coloured by co-pigmentation and/or chelation such as its flower colour (Takeda *et al.* 1985).

Foliar anthocyanin of *H. macrophylla* f. *normalis* have been reported to be cyanidin 3-*O*-*monohexoside* (*Hayashi und Abe 1955*).

Hamamelidaceae

Four Hamamelidaceae plants, *Liquidambar styraciflua* L., *L. formosana* Hance, *Disanthus cercidifolius* Maxim. and *Corylopsis spicata* Sieb. et Zucc., were surveyed for foliar anthocyanins (Fig. 4). Major pigment of all species was chrysanthemin (Table 1). Idaein was detected in *L. formosana* as a minor pigment, but not other three species. In *L. styraciflua*, another cyanidin glycoside was detected with chrysanthemin as a major pigment.

Rosaceae

Eight species, *Prunus tomentosa* Thunb. ex Murray, *P. buergeriana* Miq., *Rubus crataegifolius* Bunge, *Cotoneaster horizontalis* Decne., *Stephanandra incisa* (Thunb.) Zabel, *Sorbus commixta* Hedl.,

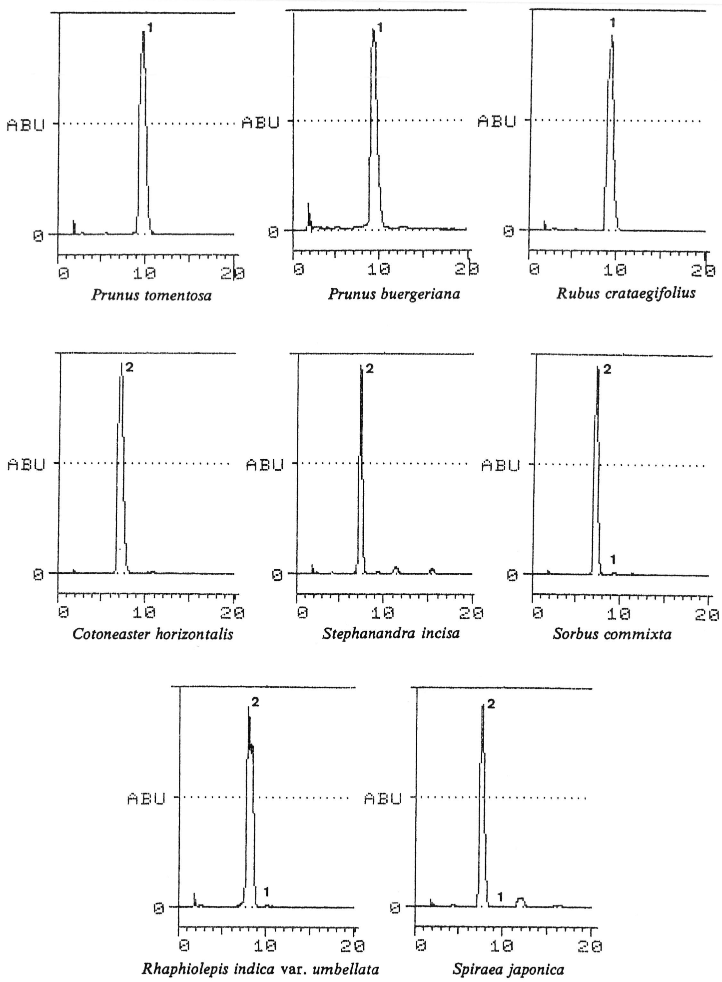


Fig. 4. HPLC patterns of anthocyanins in autumn leaves (Rosaceae).

Rhaphiolepis indica (L.) Lindl. ex Ker var. *umbellata* (Thunb. ex Murray) Ohashi and *Spiraea japonicum* L. fil., were surveyed for anthocyanins in autumn leaves (Fig. 4). They could divided into two types due to difference of major antocyanin, i.e., **A**) chrysanthemin: two *Prunus* species and *Rubus crataegifolius*, and **B**) idaein with a trace of chrysanthemin: *Cotoneaster horizontalis*, *Stephanandra incisa*, *Sorbus commixta*, *Rhaphiolepis indica* var. *umbellata* and *Spiraea japonica*.

Of Rosaceae species which were used in this experiment, foliar anthocyanins of *S. japonica* have only been characterized as chrysanthemin with a trace of cyanidin-monoglycoside (Hayashi und Abe 1955). However, idaein was detected from its autumn leaves as a major pigment in this survey by HPLC. Fourteen Rosaceae species have been investigated for anthocyanins in autumn leaves, and their major pigments have been characterized as chrysanthemin or cyanidin 3-*O*-monohexoside (Hayashi und Abe 1955; Ishikura 1972; Kato 1982). The presence of cyanidin 3-*O*-galactoside in autumn leaves of Rosaceous plants was reported in this paper for the first time.

Euphorbiaceae, Coriariaceae and Anacardiaceae

Sapium japonicum (Sieb. et Zucc.) Pax et K. Hoffm. (Euphorbiaceae), *Coriaria japonica* A. Gray (Coriariaceae), *Rhus succedanea* L. and *Cotinus coggygria* Scop. (Anacardiaceae) were surveyed for anthocyanins in autumn leaves (Table 1 and Fig. 5). Major anthocyanin of *Sapium japonicum* was cyanidin 3-*O*-glucoside (98.7% of total anthocyanins). That of *Coriaria japonica* was also chrysanthemin (64.7%) but it accompanied with idaein (29.5%). Two Anacardiaceae plants, *Rhus succedanea* and *Cotinus coggygria* contained idaein as a major pigment in their autumn leaves with a trace of chrysanthemin (Table 1). The presence of idaein in the leaves of *C. coggygria* have been reported by Tanchev and Timberlake (1969). On the other hand, chrysanthemin have been found with a small amount of peonidin 3-*O*-glucoside in autumn leaves of *R. succedanea* (Ishikura 1972).

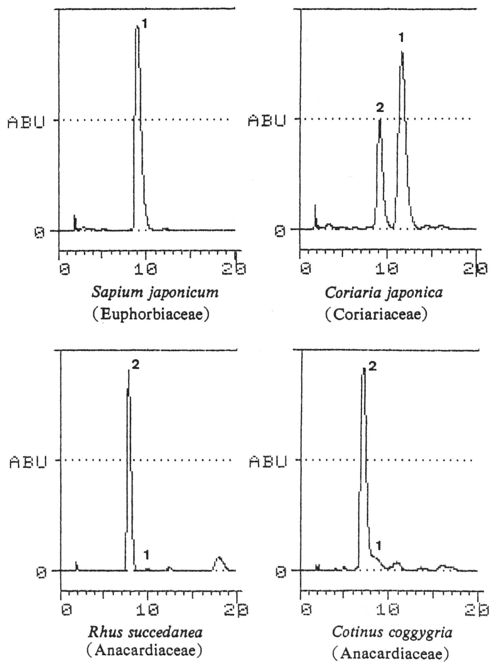


Fig. 5. HPLC patterns of anthocyanins in autumn leaves (Euphorbiaceae, Coriariaceae and Anacardiaceae).

Celastraceae and Staphyleaceae

Major anthocyanins in autumn leaves of *Euonymus alatus* (Thunb.) Sieb. f. *striatus* (Thunb.) Makino (Celastraceae), *Euscaphis japonica* (Thunb.) Kanitz and *Staphylea bumalda*(Thunb.) DC. (Staphyleaceae) were identified as chrysanthemin (Table 1 and Fig. 6). Idaein was not detected in their species by HPLC analysis. The presence of chrysanthemin in *E. alatus* f. *striatus* have been reported by Ishikura (1972). The anthocyanins in autumn leaves of Staphyleaceae plants were characterized in this experiment for the first time.

Aceraceae

Five Aceraceae species, *Acer cissifolium* (Sieb. et Zucc.), *A. ginnala* Maxim., *A. japonicum* Thunb., *A. palmatum* Thunb. and *A. pycnanthum* K. Koch were surveyed for anthocyanins in autumn leaves (Fig. 7). Since many *Acer* species are the representative woody plants of which leaves are

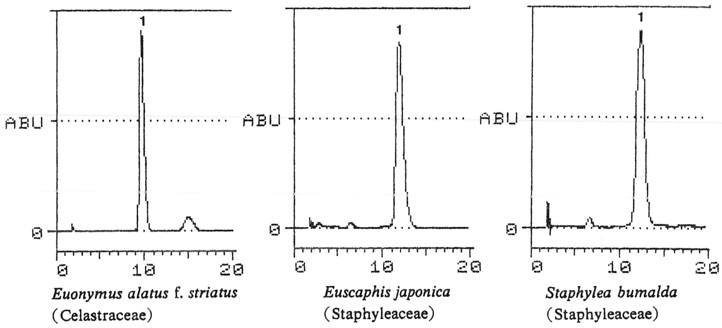


Fig. 6. HPLC patterns of anthocyanins in autumn leaves (Celastraceae and Staphyleaceae).

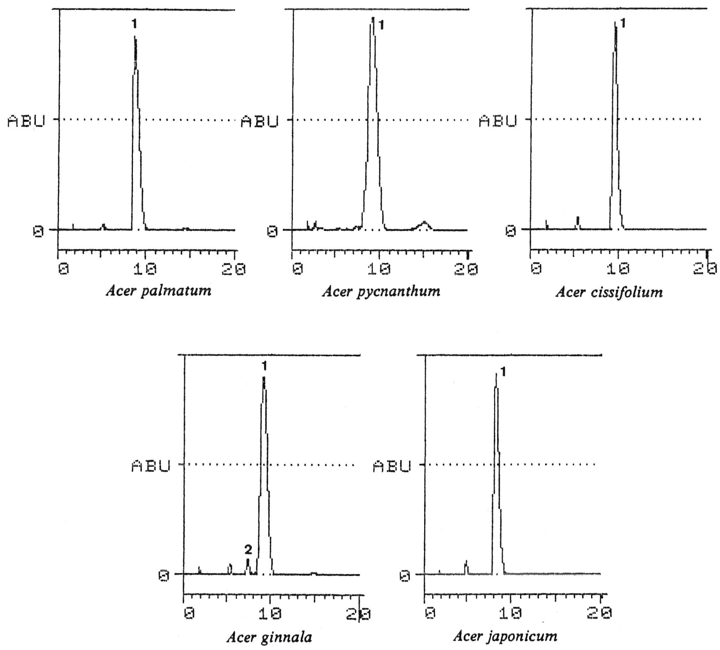


Fig. 7. HPLC patterns of anthocyanins in autumn leaves (Aceraceae).

coloured to red in autumn, the anthocyanins in autumn leaves of many species have been surveyed in earlier times. Thus, chrysanthemin have been detected as a major pigment in autumn leaves (Hattori und Hayashi 1937; Hayashi und Abe 1955; Ishikura 1972; Delendick 1990). Recently, 119 taxa of Aceraceae were surveyed for anthocyanins in autumn and spring leaves, and chrysanthemin was isolated from almost *Acer* species with small amount of cyanidin 3,5-di-*O*-glucoside, cyanidin 3-*O*-galloylglucoside, cyanidin 3-*O*-galloylrutinoside, cyanidin 3-*O*-rutinoside or delphinidin 3-*O*-glucoside (Ji *et al.* 1992). In also this experiment, chrysanthemin was detected as a major anthocyanin (93.6–97.3% of total anthocyanins) (Table 1). Idaein which have not been reported in Aceraceae was found in *A. ginnala* as a minor anthocyanin but not in other four species.

Sapindaceae, Sabiaceae, Theaceae, Stachyuraceae, Lythraceae and Clethraceae

The autumn leaves of *Koelreuteria paniculata* Laxm. (Sapindaceae), *Meliosma myriantha* Sieb. et Zucc. (Sabiaceae), *Stuartia pseudo-camellia* Maxim. (Theaceae), *Stachyurus praecox* Sieb. et Zucc. (Stachyuraceae), *Lagerstroemia indica* L. (Lythraceae) and *Clethra barvinervis* Sieb. et Zucc. (Clethraceae) were analyzed for anthocyanins by HPLC (Fig. 8). Major pigment of *K. paniculata* and *S. praecox* was chrysanthemin, while that of *S. pseudo-camellia* was idaein (Table 1). Chrysanthemin was also detected as a major anthocyanin in *M. myriantha*, but it was accompanied with a small amount of idaein. Another cyanidin glycoside which is more hydrophilic rather than cyanidin 3-*O*-glucoside and 3-*O*-galactoside was present in *C. barvinervis* as a major pigment, but chrysanthemin and idaein were as minor anthocyanins (Table 1 and Fig. 8).

It has been reported by Hayashi und Abe (1955) that major anthocyanin in autumn leaves of *Clethra barvinervis* was not chrysanthemin.

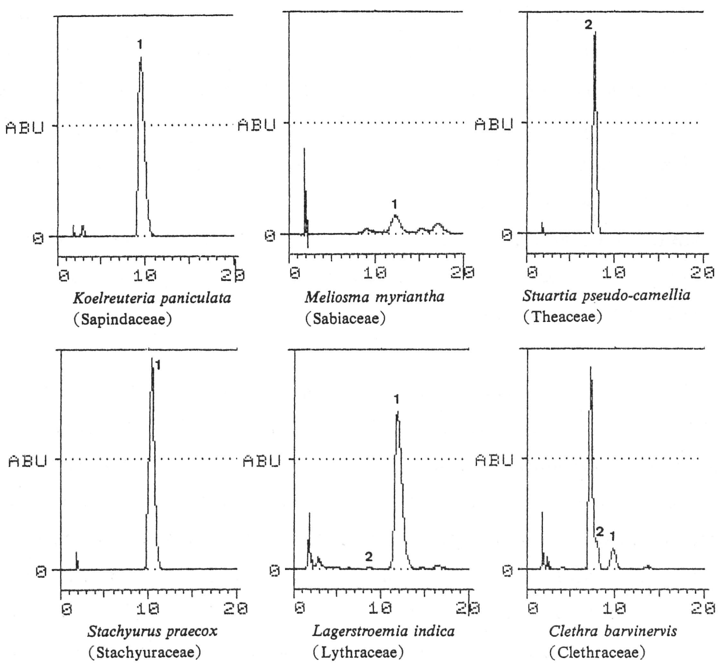


Fig. 8. HPLC patterns of anthocyanins in autumn leaves (Sapindaceae, Sabiaceae, Theaceae, Stachyuraceae, Lythraceae and Clethraceae).

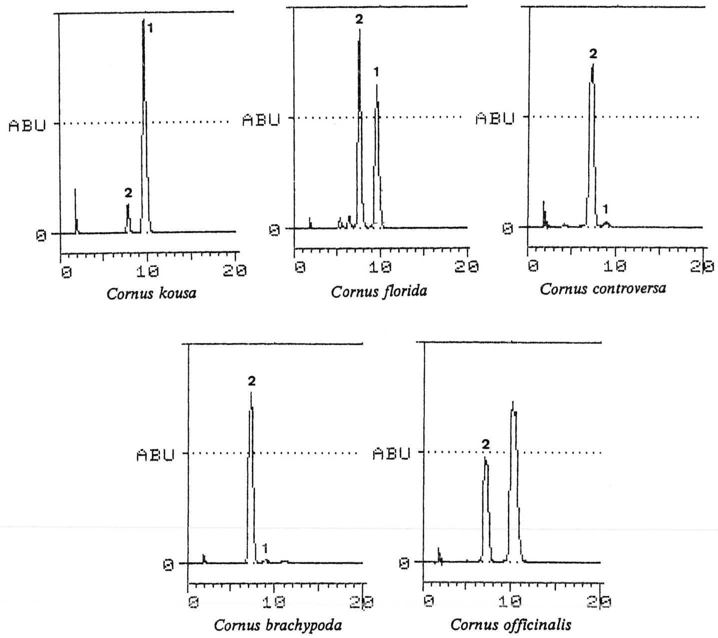


Fig. 9. HPLC patterns of anthocyanins in autumn leaves (Cornaceae).

Cornaceae

Five *Cornus* species, *C. brachypoda* C. A. Meyer, *C. controversa* Hemsl., *C. florida* L., *C. kousa* Buerg. ex Hance and *C. officinalis* Sieb. et Zucc. were analyzed for anthocyanins in autumn leaves by HPLC. In four species except *C. officinalis*, chrysanthemin co-existed with idaein (Fig. 9). Major anthocyanins of *C. officinalis* were idaein and another one which may be peonidin glycoside. Quantitatively, *C. kousa* produced chrysanthemin (90.8%) as a major anthocyanin. In contrast, *C. controversa* and *C. brachypoda* were mainly occurred idaein (each 94.9% and 95.3%). *C. florida* equivalently produced their two anthocyanins.

Foliar anthocyanin of *C. kousa* and *C. controversa* have been characterized as cyanidin 3-*O*-monohexoside (Hayashi und Abe 1955). It was shown in this survey that the anthocyanins were cyanidin 3-*O*-glucoside and 3-*O*-galactoside.

Ericaceae

Seven *Rhododendron* species, two *Enkianthus* species, two *Vaccinium* species, *Elliottia paniculata* (Sieb. et Zucc.) Benth. et Hook. and *Lyonia ovalifolia* (Wall.) Drude var. *elliptica* (Sieb et Zucc.) Hand.-Mazz. were surveyed for anthocyanins in autumn leaves (Table 1). Chrysanthemin and idaein were detected in all species except *Elliottia paniculata* which only produced the former anthocyanin. However, idaein was more quantitatively predominant than chrysanthemin in *Enkianthus perulatus* (Miq.) Schneider, *E. campanulatus* (Miq.) Nicholson var. *rubicundus* (Matsumura et Nakai) Makino and *Lyonia ovalifolia* var. *elliptica* (Fig. 10). *Vaccinium oxycoccus* L. and *V. corymbosum* L. were also similar situation with the species described above, but another cyanidin glycoside co-existed with idaein as a major anthocyanin.

Rhododendron species were divided into two groups due to the anthocyanin patterns, i.e., **A)**

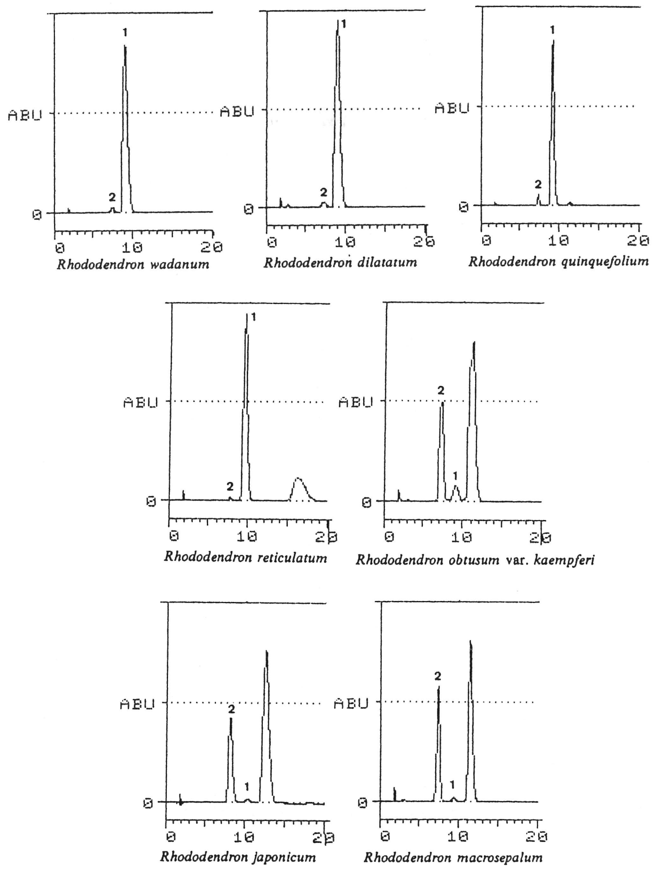


Fig. 10a. HPLC patterns of anthocyanins in autumn leaves (Ericaceae-*Rhododendron*).

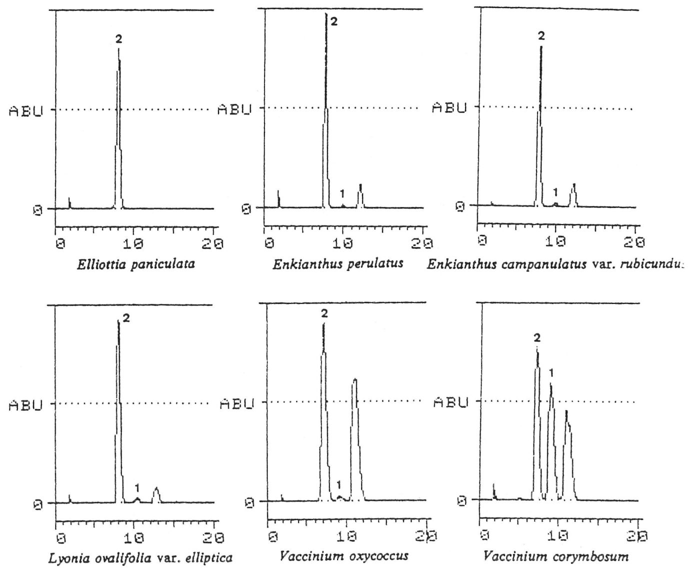


Fig. 10b. HPLC patterns of anthocyanins in autumn leaves (Ericaceae-other genera).

predominant presence of chrysanthemin (71.3–97.5% of total anthocyanins) with a trace of idaein: *R. dilatatum* Miq., *R. quinquefolium* Bisset et Moore, *R. reticulatum* D. Don and *R. wadanum* Makino; **B**) predominant presence of idaein and another cyanidin glycoside with a trace of chrysanthemin: *R. obtusum* (Lindl.) Planchon var. *kaempferi* (Planchon) Wilson, *R. japonicum* (A. Gray) Suringar and *R. macrosepalum* Maxim. (Table 1 and Fig. 10). It is noteworthy that all the species in former anthocyanin type belong to sub-genus Sciadorhodion (Yamazaki 1989).

Of Ericaceae plants investigated in this experiment, major anthocyanin of *Enkianthus perulatus, E. campanulatus* var. *rubicundus, Rhododendron japonicum* and *R. wadanum* have been characterized as cyanidin 3-*O*-monohexoside with a small amount of cyanidin glycoside (Hayashi und Abe 1955). Now, their 3-*O*-hexosides were determined as to be 3-*O*-galactoside, except *R. japonicum* and *R. wadanum* (3-*O*-glucoside).

Though a major anthocyanin of *R. obtusum* var. *kaempferi* have been identified as chrysanthemin

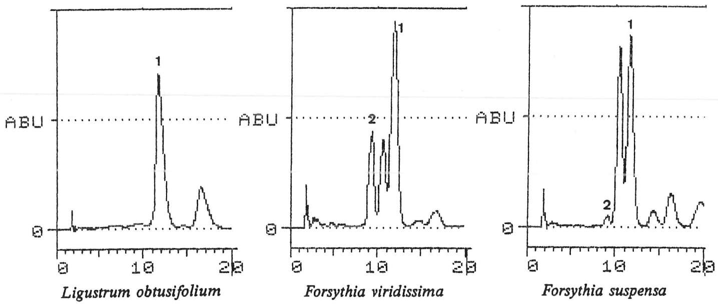


Fig. 11. HPLC patterns of anthocyanins in autumn leaves (Oleaceae).

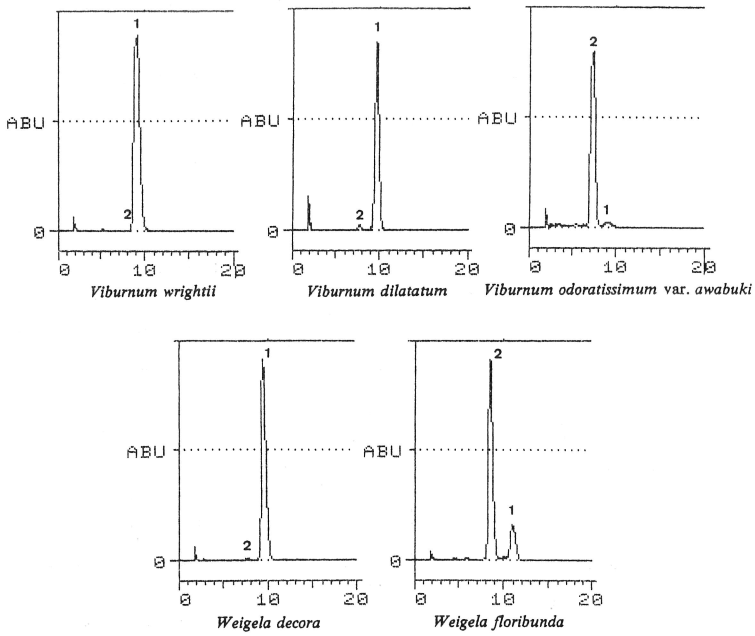


Fig. 12. HPLC patterns of anthocyanins in autumn leaves (Caprifoliaceae).

with a trace of delphinidin 3-*O*-monohexoside (Hayashi und Abe 1955), idaein and another cyanidin glycoside were detected in this survey.

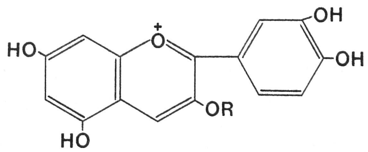
Oleaceae

Two *Forsythia* species, *F. suspensa* (Thunb.) Vahl and *F. viridissima* Lindl. and *Ligustrum obtusifolium* Sieb. et Zucc. were analyzed for foliar anthocyanins by HPLC. Though it was shown that

Table 2. Distribution of anthocyanin types in autumn leaves of plants

major chrysanthemin without idaein*	*Quercus aliena, Quercus crispula, Nandina domestica, Colylopsis spicata, Disanthus cercidifolius, Liquidambar styraciflua, Prunus buergeriana, Prunus tomentosa, Rubus crataegifolius, Sapium japonicum, Euonymus alatus* f. *striatus, Euscaphis japonica, Staphylea bumalda, Acer cissifolium, Acer japonicum, Acer palmatum, Acer pycnanthum, Koelreuteria paniculata, Stachyurus praecox, Ligustrum obtusifolium*
major chrysanthemin with a trace of idaein*	*Miscanthus sinensis, Berberis thunbergii, Deutzia scabra, Hydrangea macrophylla* f. *normalis, Liquidambar formosana, Coriaria japonica, Acer ginnala, Meliosma myriantha, Lagerstroemia indica, Cornus kousa, Rhododendron dilatatum, Rhododendron quinquefolium, Rhododendron reticulatum, Rhododendron wadanum, Forsythia suspensa, Forsythia viridissima, Viburnum dilatatum, Viburnum wrightii, Weigela decora*
major chrysanthemin and idaein*	*Cornus florida, Vaccinium corymbosum*
major idaein without chrysanthemin*	*Cotoneaster horizontalis, Stephanandra incisa, Stuartia pseudo-camellia, Elliottia paniculata*
major idaein with a trace of chrysanthemin*	*Rhaphiolepis indica* var. *umbellata, Sorbus commixta, Spiraea japonica, Cotinus coggygria, Rhus succedanea, Cornus brachypoda, Cornus controversa, Enkianthus campanulatus* var. *rubicundus, Enkianthus perulatus, Lyonia ovalifolia* var. *elliptica, Vaccinium oxycoccus, Viburnum odorattissimum* var. *awabuki, Weigela floribunda*
major other anthocyanins with chrysanthemin and/or idaein	*Cornus officinalis, Clethra barvinervis, Rhododendron japonicum, Rhododendron macrosepalum, Rhododendron obtusum* var. *kaempferi*

*with or without small amount of other anthocyanin(s).



R = glucosyl: chrysanthemin

R = galactosyl: idaein

Fig. 13. Chemical structures of chrysanthemin and idaein.

chrysanthemin was a major anthocyanin, their HPLC patterns were appearently different among three species (Fig. 11). Idaein was found in two *Forsythia* species as a minor pigment.

It has been reported that a major anthocyanin in autumn leaves of *L. obtusifolium* was chrysanthemin (Hayashi und Abe 1955).

Caprifoliaceae

Three *Viburnum* and two *Weigela* species were surveyed for anthocyanins in autumn leaves. Both chrysanthemin and idaein were detected in all species by HPLC (Fig. 12). However, the amount of chrysanthemin is more predominant than that of idaein in *Viburnum dilatatum* Thunb., *V. wrightii* Miq. and *Weigela decora* (Nakai) Nakai. In contrast, idaein is predominant in *V. odoratissimum* Ker-Gawler var. *awabuki* (K. Koch) Zabel and *W. floribunda* (Sieb. et Zucc.) K. Koch (Table 1).

The anthocyanin of *V. dilatatum* have been reported as to be cyanidin 3-*O*-monohexoside (Hayashi und Abe 1955).

It was confirmed by HPLC analysis that a major anthocyanin in autumn leaves of many plants, e.g., *Quercus aliena*, *Q. crispula*, *Prunus tomentosa*, *Rubus crataegifolius*, *Stachyurus praecox*, *Acer* spp., *Weigela decora* etc, was chrysanthemin, as described by Hayashi und Abe (1955) and Ji *et al.* (1992). On the other hand, idaein of which chemical characters were very similar with those of chrysanthemin was also detected in many species (43 of 63 spp, Table 1 and 2). Though idaein co-existed with chrysanthemin whether it was major or minor, it was found in four species, *Cotoneaster horizontalis*, *Stephanandra incisa*, *Stuartia pseudo-camellia* and *Elliottia paniculata* as a major pigment without chrysanthemin (Table 2).

In Rosaceae, Anacardiaceae, Theaceae, Cornaceae and Ericaceae, the major anthocyanin was replaced to idaein from chrysanthemin.

Idaein have been reported from some Polygonaceous species, *Fagopyrum esculentum*, *Polygonum longisetum*, *P. nipponense*, *P. thunbergii*, *P. hydropiper*, *P. nodosum* and *P. blumei* as a major anthocyanin in autumn leaves (Kato 1982), but it hardly found in other plants.

It is presumed that idaein was miss-identified as or hidden by chrysanthemin due to the similarities of PC and TLC properties between chrysanthemin and idaein.

Acknowledgement

The author wish to express my hearty thanks to the late Dr. Kôzô Hayashi for his special courtesy to give adequate advices during the study.

Summary

Chrysanthemin (cyanidin 3-*O*-glucoside), idaein (cyanidin 3-*O*-galactoside) and other anthocyanins in autumn leaves of sixty-three species (21 families) were surveyed by high performance liquid chromatography. Though chrysanthemin was a major pigment as described by Hayashi und Abe (1955), it was accompanied with idaein and other anthocyanins in almost cases. In some species, i.e., *Cotoneaster horizontalis*, *Rhaphiolepis indica* var. *umbellata*, *Stephanandra incisa*, *Rhus succedanea*, *Stuartia pseudo-camellia*, *Elliottia paniculata*, *Enkianthus campanulatus* var. *rubicundus*, *E. perulatus*, *Lyonia ovalifolia* var. *elliptica*, *Vaccinium oxycoccus*, *Viburnum odoratissimum* and

Weigela floribunda, idaein was appeared as a major pigment with or without a small volume of chrysanthemin. Thus, it was shown that idaein was a predominant anthocyanin with chrysanthemin in autumn leaves of some plants. It is presumed that idaein have been miss-identified as or hidden by chrysanthemin, because PC and TLC properties of cyanidin 3-*O*-galactoside was very similar to those of cyanidin 3-*O*-glucoside.

摘　　要

21科63種の植物の紅葉に含まれているクリサンテミン（cyanidin 3-*O*-glucoside），イデイン（cyanidin 3-*O*-galactoside）およびその他のアントシアニン色素が高速液体クロマトグラフィーによって検出された。

以前に Hayashi und Abe（1955）によって指摘されたように，ナラガシワ，ミズナラ，イヌザクラ，クマイチゴ，コマユミ，数種のカエデ科植物などの紅葉に含まれる主要アントシアニンの多くはクリサンテミンであることが判明したが，一方，ペーパーおよび薄層クロマトグラフィーでは識別が困難であるイデインもまた多くの植物で主要，あるいは微量の如何にかかわらず，クリサンテミンと共存していることが判明した。さらに，ベニシタン，コゴメウツギ，ナツツバキおよびホツツジではクリサンテミンはまったく検出されず，主要アントシアニンはイデインのみであった。

これまで，紅葉からのイデインの検出はタデ科など（Kato 1982）極めて限られていたが，実際にはイデインはクリサンテミンとともに多くの植物の紅葉に含まれているのではないかと推定された。

References

Delendick, T. J., 1990. A survey of foliar flavonoids in the Aceraceae. Memories of the New York Botanical Garden, Vol. 54. The New York Botanical Garden, New York.

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

Hayashi, K., 1939. Studien über Anthocyane, V. Über die Farbstoffe der Beeren von *Fatsia japonica*. Acta Phytochim. **11**: 91–108.

—— und Y. Abe, 1955. Studien über Anthocyane, XXVII. Papierchromatographische Übersicht der Anthocyane in Pflanzenreich (II). Farbstoffe des roten Herbstlaubes. Bot. Mag. Tokyo **68**: 299–307.

——, S. Ootani and T. Iwashina, 1989. Comparative analysis of the flavonoid and related compounds occurring in three varieties of *Iris setosa* in the flora of Japan. Sci. Rep. Res. Inst. Evolut. Biol. **6**: 30–60 (in Japanese with English summary).

Hostettman, K. and M. Hostettmann, 1982. Isolation of techniques for flavonoids. *In* Harborne, J. B. and T. J. Mabry (eds.), The Flavonoids: Advances in Research. Chapman and Hall, London, p. 1–18.

Hrazdina, G., 1982. Anthocyanins. *In* Harborne, J. B. and T. J. Mabry (eds.), The Flavonoids: Advances in Research. Chapman and Hall, London, p. 135–188.

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

Iwashina, T. and S. Ootani, 1990. Three flavonol allosides from *Glaucidium palmatum*. Phytochemistry **29**: 3639–3641.

—— and H. Hatta, 1992. The flavonoid glycosides in the leaves of *Cornus* species II. The flavonoids of *C. canadensis* and *C. suecica*. Ann. Tsukuba Bot. Gard. **11**: 23–34.

Ji, S.-B., M. Yokoi, N. Saito and L.-S. Mao, 1992. Distribution of anthocyanins in Aceraceae leaves. Biochem. Syst. Ecol. **20**: 771–781.

Kato, N., 1982. Souhon-Syokubutsu-no-Kouyou-ni-okeru-Antosianin-no-Teisei-to-sono-Bunpu（草本植物の紅葉におけるアントシアニンの定性とその分布）. Niigata-ken Seibutsu-Kyouiku Kenkyu Kaishi（新潟県生物教育研究会誌）**17**: 1–6 (In Japanese).

——, 1988. Toudaigusa-ka-Syokubutsu-ni-okeru-Antosianin-Sikiso-no-Soshikigakuteki-Sonzai-Bui-oyobi-Sosei（ト

ウダイグサ科植物におけるアントシアニン色素の組織学的存在部位および組成). Niigata-ken Seibutsu-Kyouiku Kenkyu Kaishi (新潟県生物教育研究会誌) **23**: 1–8 (in Japanese).

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

Takeda, K., M. Kariuda and H. Itoi, 1985. Blueing of sepal colour of *Hydrangea macrophylla*. Phytochemistry **24**: 2251–2254.

Tanchev, S. S. and C. F. Timberlake, 1969. Anthocyanins in leaves of *Cotinus coggygria*. Phytochemistry **8**: 2367–2369.

Timberlake, C. F. and P. Bridle, 1975. The Anthocyanins. *In* Harborne, J. B., T. J. Mabry and H. Mabry (eds.), The Flavonoids. Chapman and Hall, London, p. 214–266.

Williams, M., G. Hrazdina, M. M. Wilkinson, J. G. Sweeny and G. A. Iacobucci, 1978. High-pressure liquid chromatographic separation of 3-glucosides, 3,5-diglucosides, 3-(6-*O*-P-coumaryl) glucosides and 3-(6-*O*-P-coumarylglucoside)-5-glucosides of anthocyanins. J. Chromatog. **155**: 389–398.

Willstätter, R. und H. Mallison, 1915. Über den Farbstoff der Preiselbeere. Liebigs Ann. Chem. **408**: 15–41.

Yamazaki, T., 1989. Ericaceae. *In* Satake, Y., H. Hara, S. Watari and T. Tominari (eds.), Wild Flowers of Japan. Woody Plants II. Heibonsha Ltd., Tokyo. p. 127–145 (in Japanese).

Yoshitama, K. and N. Ishikura, 1988. Distribution of anthocyanin pigments in plants native to Japan. *In* Hayashi, K. (ed.), Plant Pigments — An Introduction to Research and Experiments —. Yokendo Ltd., Tokyo. p. 481–511.