

A Chromatographic Survey of Anthocyanins in the Flora of Japan, I

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Abstract

Paper- and thin layer-chromatographic surveys were given to the anthocyanin distribution in the plants, including some conifers and alpine plants, of 106 species belonging to 34 families.

In the present study, cyanidin glycoside was most frequent (65.6%); delphinidin, malvidin, peonidin, pelargonidin and petunidin glycosides were present in descending order. Malvidin glycoside was found in the Polygonaceae and pelargonidin glycoside in the Caprifoliaceae, in the families cyanidin glycoside was mostly detected. In glycosidic pattern, 3-glucoside and 3-rutinoside were frequently found; especially, rutinoside was in the Liliaceae, and arabinoside and galactoside in the Ericaceae.

Introduction

Hitherto, there have been some informations available to an anthocyanin distribution in the flora of Japan. For example, HAYASHI and ABE^{1,2,3)} dealt with anthocyanins of autumn leaves and alpine plants, UENO *et al.*⁴⁾ with those of petals and fruits, YOSHITAMA *et al.*⁵⁾ with sprouting leaves and ISHIKURA^{6,7,8,9)} with the fruits of some plants. Recently, ISHIKURA *et al.*¹⁰⁾ examined in detail glycosidic patterns of anthocyanins of the leguminous plants and discussed the relationship between the glycosidic pattern and the systematics at the level of subfamily and tribe. Notwithstanding these earlier works, it seems to be still far from the perfect to make a comprehensive anthocyanin map of the flora in Japan. Our study was undertaken to accomplish the map, and to disclose some significant correlation between the occurrence of anthocyanins and the systematic grouping of the plant genera, if any. This paper deals with the results of chromatographic analysis of the anthocyanins in some plant organs of 106 species belonging to 34 families, including some conifers and alpine plants.

Materials and Methods

The plant materials were collected from April to November in 1977 to 1978 in the vicinity of Matsumoto, e. g., Utsukushigahara higher plain and Mt. Hachibuse; the region located around Japan Alps. The extraction of the anthocyanins was accomplished by immersing the fresh materials in 0.5% methanolic hydrochloric acid overnight in a refrigerator, and the extract was purified by large scale paper-chromatography with the following solvents: *n*-butanol/acetic acid/water (4 : 1 : 5, v/v) and then acetic acid/HCl/water (15 : 3 : 82, v/v)^{8,11}. The pigment bands on the chromatogram were cut out and eluted with 80% methanol containing 0.1% HCl. After acid hydrolysis of the anthocyanins separated, individual components, *i. e.*, sugars, aglycones and organic acids were identified by standard procedure of paper- and thin layer-chromatography^{5,11}. The identification of original glycosides was made by partial acid hydrolysis¹² and by careful comparison of the R_f-values with those of the authentic samples on TLC-plate with the following solvents: *n*-butanol/acetic acid/water (4 : 1 : 5, v/v), *n*-butanol/2N-HCl (1 : 1, v/v), conc. HCl/water (3 : 97, v/v) and acetic acid/HCl/water (15 : 3 : 82, v/v).

Results and Discussion

From Table 1, it is clear at the level of family that cyanidin glycosides, in which especially 3-monoglucoside (chrysanthemin) is most frequent, are found commonly in the Rosaceae, the Caprifoliaceae, the Compositae and the Polygonaceae. The result is consistent with the findings obtained from other many species of the same families^{13,14}.

As regards aglycone, the first point to be noticed in Table 1 is that malvidin glycoside was detected in *Polygonum senticosum* (Meisn.) Fr. et Sav. in the Polygonaceae. No mention is made about the occurrence of malvidin glycoside in the researches on the family by UENO *et al.*⁴, TIMBERLAKE and BRIDLE¹⁴ and HARBORNE¹³. They showed that cyanidin was an only anthocyanidin in the family. However, noteworthy is the report by ISHIKURA and SUGAHARA⁹ that *P. perfolium* L. belonging to the same section (Echinocaulon) contains malvidin 3,5-diglucoside. Our further survey is in progress on the plants of the section.

The second to be pointed out is that pelargonidin glycoside was found in *Lonicera Morrowii* A. Gray in the Caprifoliaceae: in the family, cyanidin glycoside is mostly found^{4,14} but pelargonidin glycoside has not been reported.

The third is that four different types of aglycones, e. g., cyanidin, delphinidin, petunidin and malvidin, are found in *Abies Veitchii* Lindl. belonging to the Pinaceae. It is known that the plants of the family contain comparatively simple anthocyanidin components¹⁴, however, it was shown now that there is a case also in the Pinaceae

where a plant contains not a few kinds of anthocyanidins.

In glycosidic pattern, 3-glucoside and 3-rutinoside are more frequent among the families examined; especially, rutinoside is in the Liliaceae as shown by ISHIKURA and SUGAHARA⁹⁾, arabinoside and galactoside in the Ericaceae as noted by HARBORNE¹³⁾.

From the results in the survey, we should like to place stress upon the facts that there are some cases where anthocyanin components differ in each organ or in each season. For example, in *Epimedium*, the petal and sepal contain only acylated delphinidin glucoside but the sprouting and autumn leaves and stem contain about equal amount of acylated cyanidin and delphinidin glucosides. Similar fact is also found in *Nandina domestica* and *Cayratia japonica*: in *Nandina*, pelargonidin glycoside was found in the fruit⁸⁾ and delphinidin glycoside in sprouting leaves⁵⁾. This was recently pointed in the chemotaxonomical study on the flavonoids other than anthocyanins by GIANNASI¹⁵⁾. Therefore, an essential requirement is to survey the anthocyanins appearing in every plant parts, e.g., fruit, petal, leaf and stem, at all seasons for more detailed discussion of chemotaxonomy by the anthocyanins. It is necessary to check immediately the number of anthocyanins involved with TLC after the collection of materials, because it is feared that minor components of anthocyanin are lost during the process of purification by the precipitation of lead acetate and/or large scale chromatography.

Table 1. Chromatographically identified anthocyanins of some plants

Plants	Anthocyanins identified	Plant parts	Coloration
Pinaceae			
<i>Pinus pumila</i> Regel*	CyGly	male flower	red
	CyGlu, DpGly, PnGly, CyGly	female flower	purple
	CyGly, PnGly	strobilus	purple
<i>Picea jezoensis</i> var. <i>hondoensis</i> (Mayr) Rehder*	CyGly, DpGly	female flower	reddish orange
<i>Abies Veitchii</i> Lindley*	Cy3Glu, Dp3Glu, Pt-monoGlu, Mv-monoGlu	strobilus	purple
<i>Tsuga diversifolia</i> Masters*	Dp-monoGly, Cy-monoGly	strobilus	purple
Moraceae			
<i>Humulus japonicus</i> Sieb. et Zucc.	CyGly	fruit	dark violet
<i>Broussonetia Kazinoki</i> Sieb.	CyGly	male flower	dark brown
Urticaceae			
<i>Boehmeria tricuspis</i> Makino	Cy3Rt, CyGly, PgGly	flower, stem	red
	Cy3Glu	fruit	red
<i>B. nivea</i> (L.) Gaud. subsp. <i>nivea</i>	CyGyl	young leaf	red

Plants	Anthocyanins identified	Plant parts	Coloration
Polygonaceae			
<i>Polygonum cuspidatum</i> Sieb et Zucc.	Cy3Glu	tepala	red
<i>P. senticosum</i> (Meisn.) Fr. et Sav.	MvGly	sepal, fruit coat	pink
<i>P. filiforme</i> Thunb.	Cy3Glu	sepal	red
<i>Rumex Acetosella</i> L.	Cy3Glu	tepala	pink
<i>R. Acetosa</i> L.	Cy3Glu, CyGlu	tepala	red
<i>R. obtusifolius</i> L.	Cy3Glu	tepala	red
Caryophyllaceae			
<i>Dianthus superbus</i> L. subsp. <i>longicalycinus</i> (Maxim.) Kitam.	CyGlu, CyGly	petal	pink
<i>Silene Armeria</i> L.	CyGly	petal	pink
Berberidaceae			
<i>Epimedium macranthum</i> More. et Decne.	Dp3So5Glu+pC	petal	pink
	Cy-triGlu+pC Dp3So5Glu+pC	sprout, stem	dull red
Aristolochiaceae			
<i>Asarum Sieboldii</i> Miq.	CyGly, PnGly	sepal	dull purple
<i>A. Blumei</i> Duchart.	CyGly	sepal	dark violet
Papaveraceae			
<i>Corydalis lineariloba</i> Sieb. et Zucc.	Cy3Glu, Cy3Rt	petal	violet
<i>C. incisa</i> (Thunb.) Pers.	Cy3Rt, CyGlu CyGly(Glu, Rham)+CA	petal	purple
<i>Dicentra spectabilis</i> (L.) Lemaire	CyGly	petal	pink
Crassulaceae			
<i>Sedum japonicum</i> Sieb.	Cy-monoGlu	stem	pale red
Saxifragaceae			
<i>Ribes japonica</i> Maxim.*	CyGly	fruit coat	red
<i>R. fasciculatum</i> Sieb et Zucc.	CyGly	fruit coat	red
Rosaceae			
<i>Prunus Maximowiczii</i> Rupr.	Cy3Glu, Cy3Rt	petal	red
<i>P. incisa</i> Thunb.	CyGly	fruit coat	dark violet
<i>P. Grayana</i> Maxim.	CyGly	fruit coat	dark red
<i>Rubus crataegifolius</i> Bunge	Cy3Glu	fruit	red
<i>R. parvifolius</i> L.	CyGlu	petal	pink
<i>Rubus pedatum</i> Smith*	Cy3Glu, Cy3Rt	fruit	red
<i>Sorbus sambucifolia</i> (Cham. et Schltdl.) Roem.*	CyGly (Glu, Xy)	fruit	red
<i>Rhaphiolepis umbellata</i> (Thunb.) Makino	CyGly	fruit coat	dark violet
<i>Pyracantha angustifolia</i> Schneid.	Cy3Glu	fruit	red
<i>Malus Sieboldii</i> (Regel) Rehd.	Cy3Glu	fruit coat	red
Leguminosae			
<i>Phaseolus coccineus</i> L.	PgGly	petal	orange
<i>Lathyrus maritimus</i> Biegel	CyGly, MvGly	petal	red purple

Plants	Anthocyanins identified	Plant parts	Coloration
<i>Vicia sativa</i> L.	CyGly	petal	purplish red
<i>V. amoena</i> Fisch.	MvGly	petal	red purple
<i>V. bifolia</i> Nakai	MvGly	petal	purplish red
<i>Desmodium racemosum</i> DC.	MvGly	petal	pink
<i>Trifolium lupinaster</i> L.	CyGly, MvGly, Mv-monoGlu	petal	reddish purple
<i>Robinia Pseudo-acacia</i> L.	Cy-monoGlu, Pn-monoGlu	stem, stipule	purple
Geraniaceae			
<i>Geranium yesoense</i> Fr. et Sav. var. <i>nipponicum</i> Nakai*	Mv3Glu5Glu, MvGly	petal	pink
Euphorbiaceae			
<i>Euphorbia helioscopia</i> L.	CyGlu, DpGlu	stem	red
Daphniphyllaceae			
<i>Daphniphyllum humile</i> Maxim.	Cy3GluXy., CyGlu, Dp3Glu Dp3Rt, DpGluXy, DpGly (Glu, Xy, Rham)	fruit coat	purplish black
Aceraceae			
<i>Acer aidzuense</i> (Franch.) Nakai	CyGlu	samara	red
<i>A. carpinifolium</i> Sieb. et Zucc.	Cy3Glu	bract	red
Lythraceae			
<i>Lagerstroemia indica</i> L.	Cy3Glu, CyGlu, Dp3Glu, DpGly	petal	pink
Umbelliferae			
<i>Ostericum Sieboldi</i> (Miq.) Nakai	CyGly	fruit coat	dark vitole
<i>Torilis japonica</i> (Houtt.) DC.	CyGly	fruit coat	dark violet
Pyrolaceae			
<i>Pyrola incarnata</i> Fisch.*	Cy-monoGlu	petal, anther	red
Ericaceae			
<i>Rhododendron Kaempferi</i> Planch.	Cy3Glu, CyGly Cy-monoAra	petal	reddish orange
<i>R. dilatatum</i> Miq.	MvGly	petal	purplish red
<i>Vaccinium ovalifolium</i> var. <i>membranaceum</i> H. Boiss.*	Cy3Glu, CyGlu, DpGlu, PnGlu, Pt-monoGlu, Mv-monoGlu	fruit	blue violet
<i>Enkianthus perulatus</i> (Miq.) Schneid.	CyGly(Gal, Ara), CyAra	leaf	red
Myrsinaceae			
<i>Ardisia crenata</i> Sims	Cy3Glu	fruit coat	red
Primulaceae			
<i>Lysimachia mauritiana</i> Lam.	CyGly DpGly	fruit coat	red
<i>Primula Sieboldi</i> E. Morren	MvGly	petal	pink
Gentianaceae			
<i>Swertia pseudochinensis</i> Hara	CyGly, DpGly	petal	violet
<i>Gentiana Zollingeri</i> Fawc.	DpGly	petal	blue
Rubiaceae			
<i>Paederia scandens</i> (Lour.) Merr. var. <i>Mairei</i> (Lev.) Hara	Cy3Rt, CyGly (Glu, Rham) CyGlu, Cy3Glu	petal	reddish purple

Plants	Anthocyanins identified	Plant parts	Coloration
Convolvulaceae			
<i>Quamoclit angulata</i> Bojer	PgGly	petal	orange
Verbenaceae			
<i>Caryopteris divaricata</i> (Sieb. et Zucc.) Maxim.	DpGly	petal	blue purple
<i>Vitex rotundifolia</i> L. fil.	DpGly, CyGly	petal	purple
Labiatae			
<i>Lamium album</i> L. var <i>barbatum</i> (Sieb. et Zucc.) Fr. et Sav.	CyGly	petal	pink
<i>L. purpureum</i> L.	Cy3Glu, Cy3Glu5Glu Cy3Rt, Cy-triGly	petal, leaf, stem	purple
<i>Prunella vulgaris</i> L. subsp. <i>asiatica</i> (Nakai) Hara	CyGly, DpGly	petal, bud	violet
<i>Meehania urticifolia</i> (Miq.) Makino	CyGly, DpGly	petal	violet
<i>Scutellaria laeteviolacea</i> Koidz.	CyGly	petal	pale violet
<i>Glechoma hederacea</i> L. subsp. <i>grandis</i> (A. Gray) Hara	Cy3Glu5Glu, Dp3Glu5Glu	petal	purple
<i>Elsholtzia ciliata</i> (Thunb.) Hylander	CyGly	petal	purple
<i>Salvia japonica</i> Thunb.	CyGly, DpGly, MvGly	petal	purple
<i>Stachys Riederi</i> Chamisso var <i>intermedia</i> (Kudo) Kitam.	Cy3Glu, Cy3Glu5Glu	petal	purple
Scrophulariaceae			
<i>Veronica persica</i> Poir.	Ac-DpGly (Glu, Rham)	petal	blue
<i>V. serpyllifolia</i> L. subsp. <i>humifusa</i> Penn.	CyGly, DpGly	petal	violet
<i>Veronicastrum sibiricum</i> (L.) Pennell var. <i>japonicum</i> (Nakai) Hara	Ac-CyGly	petal	purplish blue
<i>Pedicularis respinata</i> L.	CyGly(Glu, Rham)+CA	petal	reddish purple
Bignoniaceae			
<i>Paulownia tomentosa</i> (Thunb.) Steud.	DpGly, CyGly	petal	pale violet
Caprifoliaceae			
<i>Lonicera Tschonoskii</i> Maxim.	CyGly	petal, bud	violet
<i>L. Morrowii</i> A. Gray	CyGly, PgGly	fruit	red
<i>L. gracilipes</i> Miq. ver. <i>glabra</i> Miq.	Cy3Glu5Glu	petal	pale red
<i>L. sempervirens</i> Ait.	Cy3Glu5Glu	petal	pale red
<i>Weigela hortensis</i> (Sieb. et Zucc.) Koch	CyGly	petal	red
<i>W. floribunda</i> (Sieb. et Zucc.) K. Koch	Cy3Glu	petal	dark red
<i>Linnaea borealis</i> L.*	Cy3Glu5Glu	petal	pink
Campanulaceae			
<i>Campanula punctata</i> subsp. <i>hondoensis</i> Kitam.	CyGlu, CyGly (Glu, Rham) CyGly, Dp3Glu, Dp3Rt	petal	pale pink
<i>Asyneuma japonicum</i> (Miq.) Briq.	CyGlu, CyGly, DpGlu, DpGly, Dp-triGly (Glu, Rham)	petal	violet

Plants	Anthocyanins identified	Plant parts	Coloration
<i>Codonopsis lanceolata</i> (Sieb. et Zucc.) Trautv.	CyGly, DpGly	petal	brown red
Compositae			
<i>Sonchus oleraceus</i> L.	Cy3Glu	leaf	red
<i>Senecio flammeus</i> Turcz. subsp. <i>glabrifolius</i> (Cufod.) Kitam.	CyGly	bud	deep orange
<i>Pertya scandens</i> (Thunb.) Sch. Bip.	CyGly	petal	pale pink
<i>Synurus pungens</i> (Fr. et Sav.) Kitam.	Cy3Glu	petal	dark violet
<i>Hemistepta lyrata</i> Bunge	CyGly	petal	purple
<i>Eupatorium Fortunei</i> Turcz.	CyGly	petal	purple
<i>Ligularia dentata</i> (A. Gray) Hara	Cy3Glu, CyGly	sepal	dull red
Liliaceae			
<i>Heloniopsis orientalis</i> (Thunb.) C. Tanaka*	DpGly (Glu, Rham), PtGlu MvGlu	tepals	pink
<i>Erythronium japonicum</i> Decaisne	CyGlu, DpGlu, Dp3Rt	petal	purple
<i>Hemerocallis fulva</i> L. form. <i>Kwanso</i> (Regel) Kitamura	CyGlu, Cy3Rt	tepals	orange
<i>Veratrum nigrum</i> L. subsp. <i>Maackii</i> (Regel) Kitamura	Cy3Glu, CyGlu, CyGly	flower	black
<i>Trillium Smallii</i> Maxim.*	Cy3Rt	fruit	blue black
<i>Clintonia udensis</i> Trautv. et Mey.	CyGly	fruit	blue
<i>Lilium maculatum</i> Thunb.	CyGly	tepals	red orange
<i>L. Leichtlinii</i> Hooker f. var. <i>Maximowiczii</i> (Regel) Baker	Cy3Rt	tepals	red orange
<i>Liriope platyphylla</i> Wang. et Tang	Cy3Glu, Dp3Glu, Dp3Rt, DpGly, MvGly	fruit	blue black
Araceae			
<i>Symplocarpus foetidus</i> Nutt. var. <i>latissimus</i> (Makino) Hara	Cy3Gly (Glu, Rham), CyGlu, PnGly (Glu, Rham)	spathe	brownish red

Abbreviations :

Pg, pelargonidin; Cy, cyanidin; Dp, delphinidin;

Pn, peonidin; Pt, petunidin; Mv, malvidin; Gly, glycoside;

Glu, glucoside; Rt, rutinoside; So, sophoroside; Ara, arabinoside;

Gal, galactoside; Gly(Glu, Rham, Xy), anthocyanin attached by glucose, rhamnose and xylose; ac-, acylated anthocyanin; +CA, acylated with caffeic acid; +pC, acylated with *p*-coumaric acid; *, alpine and subalpine plant.

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