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ANTHOCYANIN PATTERN IN THE GENERA *ILEX* AND *EUONYMUS**†

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Abstract-Three kinds of anthocyanin were found in the skins of fruits of seventeen plants belonging to the genera ***Ilex*** and ***Euonymus. They*** were identified as pelargonidin 3-xylosylglucoside, cyanidin 3-xylosylglucoside and cyanidin 3-monoglucoside on the basis of paper chromatographic analysis. Cyanidin 3-xylosylglucoside was commonly found in the genus ***Ilex*** which could be divided into two tribes, onecontaining only cyanidin glycosides, while the other contains glycosides of both pelargonidin and cyanidin. In species of the closely allied genus, ***Euonymus,*** cyanidin 3-monoglucoside was detected as the predominant pigment. In addition a detectable amount of cyanidin 3-xylosylglucoside was also present in the fruits of ***Euonymus japonicus*** and its varieties.

INTRODUCTION

RECENTLY, much attention has been given by phytochemists to the correlation between flavonoid pigmentation and the chemotaxonomy of higher plants. The presence of rare flavonoids and of structural modifications (e.g. hydroxylation, methylation and glycosidation types) in groups of plants are used as chemical markers in classification and phylogeny. Harborne[1] reviewed the relationship between anthocyanidins and their glycosidic patterns and plant systematics, and pointed out that this may well be used, together with other chemical characters, to solve taxonomic problems at the generic level.

Hayashi[2] has previously isolated cyanidin 3-xylosylglucoside (ilicicyanin) from the fruits of *Ilex crenata* Thunb. More recently, the present author has found that the fruits of *Ilex aquifolium* L. contain the 3-xylosylglucosides of both cyanidin and pelargonidin.[3] Therefore, the formation of the 3-xylosylglucoside of anthocyanidins seems to be characteristic of the genus *Ilex*. Therefore the anthocyanin pigmentation of the fruit of plants belonging to other *Ilex* species (Aquifoliaceae) have been examined from the chemotaxonomic point of view in order to try to delineate species relationships in the family. In addition, the anthocyanin pigmentation in several species of *Euonymus* of the Celastraceae, which is closely allied with Aquifoliaceae, has been also examined. But coverage of species has perforce been limited, for only the fruits were used for the examination.

RESULTS

Among the plants examined, *Euonymus tanakae, E. japonicus* and its varieties, and species of Aquifoliaceae except for *Ilex serrata* are evergreen trees. The other species are all

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[1] J. B. HARBORNE, in ***Chemical Plant Taxonomy*** (edited by T. SWAIN), p. 359, Academic Press, New York (1963).
[2] K. HAYASHI, *Acta Phytochim.* (Japan) **13,** 25 (1942).
[3] N. ISHIKURA, *Bor. Mug.* Tokyo **84,** 113 (1971).

deciduous. All fruits of plants examined turn red in the late autumn, except those of *Ilex crenata* and its variety *convexa*, which bear dark purple fruits. These latter fruits have pigmentation in the mesocarp as well as pericarp. The experimental details for characterization of the 3-xylosylglucosides of both cyanidin and pelargonidin present in *I. aquifolium* have been mentioned in the previous report.[3]

Anthocyanins in each plant were extracted from the red or dark-purple fruit skin with cold 1% methanolic hydrochloric acid. Each extract was examined by paper chromatography using four solvents. Three kinds of anthocyanin were detected; cyanidin

TABLE 1. PAPER CHROMATOGRAPHIC AND SPECTRAL PROPERTIES OF THE ANTHOCYANINSINTHEFRUITSOFTHE *Ilex* AND *Euonymus* PLANTS

Anthocyanins	λ_{max} in MeOH–HCl (in nm)	R_f values x 100 in BuH*	AAH*	BAW*	6% HOAc*	Colour of spot
Pelargonidin 3-xylosylglucoside (PXG)	276, 431, 506	66	70	40	46	Orange
Cyanidin 3-xylosylglucoside (CXG)	281, 524	49	65	30	43	Magenta
Cyanidin 3-monoglucoside (CG)	276, 523	38	40	26	22	Magenta

* See text for composition.

TABLE 2. R_f AND R_g VALUESOFAGLYCONESAND SUGARS OBTAINED FROM THE ANTHOCYANINS

Athocyanins	Algycones	R_f values x 100 in BuH*	AAH*	6% HOAc*	Sugars	R_g values in BAW*	EtAPW*	BPW*
PXG†	Pelargonidin	98	30	09	Glucose	100	100	100
					Xylose	160	140	155
CXG†	Cyanidin	87	18	05	Glucose	100	100	100
					Xylose	160	141	155
CG†	Cyanidin	88	18	05	glucose	100	100	100

* See text for composition.
† See Table 1 for constitution.

3-monoglucoside, cyanidin 3-xylosylglucoside and pelargonidin 3-xylosylglucoside. Their chromatographic and spectral data are tabulated in Table 1. And in Table 2 are shown paper chromatographic data of the aglycones and sugars obtained by acid hydrolysis of anthocyanins.

Two of anthocyanins detected were shown to be identical with authentic specimens chrysanthemin (cyanidin 3-monoglucoside) and ilicicyanin (cyanidin 3-xylosylglucoside) respectively. Acid hydrolysis of these glycosides gave cyanidin and glucose or glucose and xylose respectively. The nature of the aglycone was confirmed by degradation with potassium hydroxide to produce phloroglucinol and protocatechuic acid.

The identification of pelargonidin 3-xylosylglucoside was made in the same way, partial hydrolysis giving the aglycone and the 3-monoglucoside. The aglycone was identified by direct comparison with authentic pelargonidin and by potash fusion. The absorption maxima of the pigment are identical with those of the 3-monoglucoside of pelargonidin.

TABLE 3. DISTRIBUTION OF ANTHOCYANINS IN THE GENERA *Ilex* AND *Euonymus**

Families and species	Pelargonidin 3-xylosyl-glucoside	Cyanidin 3-xylosyl-glucoside	Cyanidin 3-mono-glucoside
Aquifoliaceae; *Ilex*			
serrata Thunb.	9	1	—
integra Thunb.	8	2	—
latifolia Thunb.	8	2	—
aquifolium L.	5	5	—
pedunculosa Miq.	—	9	1
rotunda Thunb.	—	8	2
crenata Thunb.	—	7	3
crenata Thunb. var convexa Makino	—	7	3
chinensis Sims.	—	6	4
Celastraceae; *Euonymus*			
japonicus Thunb.	—	Trace	10
japonicus Thunb. var *microphyllus* Sieb.	—	Trace	10
japonicus Thunb. var *aureo-variegatus* Low.	—	Trace	10
japonicus Thunb. var *albo-variegatus* Hort.	—	Trace	10
tanakae Maxim.	—	Trace	10
sieboldianus Blume	—	—	10
alatus (Thunb.) Sieb.	—	—	10
alatus (Thunb.) Sieb. f. *ciliato-dentatus* (Fr. et Sav.) Hiyama	—	—	10

* Figures give approximate ratios (out of 10).

The results of surveying the species and their varieties are presented in Table 3. Species of the genus *Ilex* can be divided into two groups. One contains only cyanidin glycosides, while the other contains the glycosides of both pelargonidin and cyanidin. Cyanidin 3-xylosylglucoside was identified in all *Ilex* species. *Ilex aquifolium* is notable because the glycosides of both cyanidin and pelargonidin are present in the fruits near to the ratio 1: 1. All fruits of the genus *Ilex* contain predominantly the 3-xylosylglucoside. On the other hand, in all plants of the genus *Euonymus* the main pigment was cyanidin 3-monoglucoside. *E. tanakae, E. japonicus* and its varieties form the 3-xylosylglucoside of cyanidin in small amounts.

DISCUSSION

Although *Euonymus sieboldianus, E. alatus* and its form *ciliato-dentatus* have red leaves in autumn and *E. tanakae* has year-round red leaves all the examined species contain marked

amounts of anthocyanin only in the fruits. The comparison of anthocyanin pattern in the fruits thus seems warranted as suggested by Harborne's reviews[1,4,5] on the flavonoid distribution. In the fruit skins of *Ilex crenata*,[2] and of *I. aquifolium*,[3] cyanidin 3-xylosyl-glucoside has been found and the latter has been also shown to contain pelargonidin 3-xylosylglucoside. In view of the rarity of these pigments in nature, it seems of interest to make the comparative study on anthocyanin in the genus *Ilex*.

It is evident that the 3-xylosylglucoside type of anthocyanins might be characteristic of the genus *Ilex*, since all of eight species examined were shown to contain those pigments predominantly and cyanidin 3-xylosylglucoside was found in each. Moreover, species may be divided into two tribes, one containing only cyanidin glycosides and the other containing pelargonidin and cyanidin derivatives. According to the preliminary survey of Hayashi and Abe,[6] pelargonidin 3-hexose-pentoside and chrysanthemin, which are present in about the ratio 9 :1, have been found in the red fruits of *Ilex geniculata.* Although a more detailed survey of this plant is necessary, it is noteworthy that the plant contains a pelargonidin glycoside, because this deciduous species closely resembles I. *serrata* morphologically.

In view of morphological characters, I. *chinensis*, *I. pedunculosa*, *I. rotunda, I. crenata* and its variety *convexa* may be put together in a group. On the other hand, *I. integra, I. latifolia* and *I. aquifolium* resemble each other. The former plants bear a peduncle on the axil formed between stem and annual shoots, while the latter plants have peduncles on the leaf axil of biennial shoots. But I. *serrata* cannot be put in the latter group because it is a deciduous tree and also is somewhat different from the other three morphologically.

Cyanidin 3-monoglucoside was found as the common anthocyanin in the genus *Euonymus.* According to the provisional note of Hayashi and Abe,[6] the fruit of *E. macropterus* Rupr. contains only chrysanthemin. Hayashi[7] also reported that in *E. oxyphllus* Miq., chrysanthemin and a trace of cyanidin glycoside were present whereas in the red leaves of *E. alatus* and *E. sieboldianus* only cyanidin 3-monoglucoside was detected.

It is noteworthy that *E. tanakae, E. japonicus* and its varieties contain a trace amount of cyanidin 3-xylosylglucoside, while some species of *Ilex* contain cyanidin 3-monoglucoside. Wettstein[8] and Hutchinson[9] pointed out the close alliance between Aquifoliaceae and Celastraceae, and they led both families to belong to the order Celastrales. The present information obtained about the anthocyanin constituents in the genera of both *Ilex* and *Euonymus* seems to be compatible with the view of these taxonomists.

The present author has recently found the presence of cyanidin 3-xylosylglucoside in the red fruits of *Euscaphis japonica* (Thunb.) Kanitz belonging to Staphyleaceae.[10] As the close relationship between both families of Celastraceae and Staphyleaceae has been suggested taxonomically,[11] an extensive survey of anthocyanins in the families belonging to Celastrales seems, therefore, to be of much significance to confirm the chemotaxonomical relationships of species in the order.

[4] J. B. HARBORNE, ***Comparative Biochemistry of the Flavonoids,*** Academic Press, New York (1967).
[5] J. B. HARBORNE, in ***Comparative Phytochemistry*** (edited by T. SWAIN) p. 271. Academic Press, New York (1966).
[6] K. HAYASHI and Y. ABE, ***Miscellaneous Reports of the Research Institute for Natural Resources, No. 29, 1 (1955).***
[7] K. HAYASHI, ***Bot. Mag.*** Tokyo 68, 299 (1955).
[8] R. WETTSTEIN, ***Handbuch der systematischen Botanik (4*** Aufl.) p. 838, F. Deutike, Leipzig (1935).
[9] J. HUTCHINSON, ***Evolution and Phylogeny of Flowering Plants,*** p. 348, Academic Press, New York (1969).
[10] N. ISHIKURA, ***Bot. Mag.*** Tokyo **84,** 1 (1971).
[11] H. MELCHIOR (editor), ***Engler's Syllabus der Pflanzenfamilien,*** 12th Edn, Vol. II, p. 292, Borntraeger, Berlin (1964).

EXPERIMENTAL

Plant sources. The present survey of the genera ***Ilex*** and ***Euonymus*** was carried out on ripe fruits of plants growing at the campus of Kumamoto University and at the Kenmotsudai Trees and Shrubs Garden (Kumamoto city), except for ***I. pedunculosa*** which was collected at Mt. Ishizuchi of Shikoku Island in October.

Anthocyanin identifications. The fresh fruit skins were immersed in 1% cold methanolic HCl as soon as the materials were collected, and extracted repeatedly with the same solvent. The ratio of individual pigments in the extracts were estimated by the spot size on the paper chromatograms in four solvents. The solvents used for chromatographic runs were the following: BuOH–HOAc–H_2O (6:1:2) (BAW), BuOH–HCl–H_2O (7:2:5) (BuH), HOAc–HCl–H_2O (3:1:8) (AAH) and 6% acetic acid (6% HOAc). The detailed experiments for identification of 3-xylosylglucosides of both cyanidin and pelargonidin has been mentioned in the previous paper.[3] The results are shown in Tables 1 and 2.

Further confirmation of the structure were made by complete and partial hydrolysis with dilute HCl and by degradation of their aglycones with KOH. Sugars as the hydrolysate were co-chromatographed with authentic specimens on paper in three solvents, which were BAW, EtOAc–pyridine–H_2O (12:5:4) (EtAPW) and BuOH–pyridine–H_2O (6:4:3) (BPW). The detection of sugars was made by using aniline hydrochloride and also alkaline Ag NO_3. Phenolic components obtained by KOH-degradation of aglycone were chromatographed in benzene–HOAc–H_2O (125:72:3) (BzA) and BAW, and detected by spraying with sulphanilic reagent.[12]

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[12] I. SMITH, in ***Chromatographic Techniques*** (edited by I. SMITH), p. ***189,*** Heinemann, London (1958).