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A FURTHER SURVEY OF ANTHOCYANINS AND OTHER PHENOLICS IN *ILEX* AND *EUONYMUS**

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Key Word Index—*Ilex*; Aquifoliaceae; *Euonymus*; Celastraceae; anthocyanins; flavonols; chlorogenic and isochlorogenic acids; chemotaxonomy.

Abstract—A survey of 27 plants of *Ilex* and *Euonymus* revealed that the distribution of anthocyanins and cinnamic acid esters in their fruits is correlated with accepted taxonomic classification. In the skin of the fruit, the 3-xylosylglucoside of cyanidin and pelargonidin and the 3-monoglucoside of cyanidin were identified, and the hydrolysed fruit-extracts were found to contain quercetin, kaempferol and caffeic acid. The genus *Ilex* has been shown to be distinguishable from the genus *Euonymus* by their anthocyanins; *I. micrococca* was exceptional in having only chrysanthemin. Additionally, chlorogenic and *iso*chlorogenic acids and caffeylglucose occur in *Ilex* but not in *Euonymus*. The microspectrophotometric examination of the pigment cells of the black- and red-*Ilex* fruits revealed that the position of absorption maxima in the visible region is mainly related to the relative amounts of anthocyanin and flavonol present.

INTRODUCTION

The Aquifoliaceae includes four genera *Ilex*, *Nemopanthus*, *Byronia* and *Phelline* [1], with the majority of species being in *Ilex*. There are nineteen native species of *Ilex* and one cultivated plant, *I. aquifolium*, in Japan, but no other genera [2]. A previous survey of anthocyanins [3] has now been extended to 17 *Ilex* plants including three varieties. Eleven of the 15 *Euonymus* species growing in Japan were also examined. In the previous work, cyanidin 3-xylosylglucoside (ilicicyanin) was found as common anthocyanin in *Ilex*, whereas chrysanthemin was the common pigment in *Euonymus*. Moreover, the presence of chlorogenic acid was found to be confined to *Ilex*. Thus, the present paper describes the distribution of anthocyanins and some other phenolics including flavonols present in the fruit skins of the genera *Ilex* and *Euonymus*. And in addition, the absorption spectra *in vivo* of the red- and black-*Ilex* fruits were compared.

RESULTS

The results of surveying *Ilex* and *Euonymus* plants for anthocyanins are presented in Table 1. Cyanidin 3-xylosylglucoside (ilicicyanin) was identified as the common anthocyanin in all *Ilex* except *I. micrococca*, which contained only cyanidin 3-monoglucoside (chrysanthemin). Pelargonidin 3-xylosylglucoside was found in those *Ilex* plants with peduncles on the leaf axil of biennial shoots. On the other hand, species bearing a peduncle on the axil formed between stem and annual shoots have only the cyanidin glycosides, chrysanthemin and ilicicyanin. Exceptionally, two deciduous plants, *I. macropoda* and *I. micrococca*, in the first group lacked pelargonidin glycoside. Cyanidin 3-monoglucoside was predominantly present in all *Euonymus*. When the fruits of *Euonymus* plants were hydrolysed with 2 N HCl, a large amount of quercetin and kaempferol was detected. Some *Ilex* also yielded both flavonols but

* Part II in the series 'The Distribution of Anthocyanins in the Aquifoliaceae and Celastraceae'. For Part I see (1971) *Phytochemistry* **10**, 2513

Table 1. Additional data of the distribution of anthocyanins in the genera *Ilex* and *Euonymus**

Families and species	Pelargonidin 3-xylosylglucoside	Cyanidin 3-xylosylglucoside	Cyanidin 3-monoglucoside
Aquifoliaceae			
Ilex macropoda Miq.	—	10	—
I. geniculata Maxim.	9	1	—
I. geniculata Maxim. var. *glabra* Okuyama	7	3	—
I. micrococca Maxim.	—	—	10
I. nipponica Makino	9	1	—
I. buergeri Miq.	7	3	—
I. kiusiana Hatusima	8	2	—
I. sugeroki Maxim. var. *brevipedunculata* (Maxim.) S. Y. Hu	—	9	1
Celastraceae			
Euonymus fortunei (Turcz.) Hand.-Mazz. var. *radicans* (Sieb. et Miq.) Rehd.	—	—	10
E. sieboldianus Blume var *sanguineus* Nakai	—	—	10
E. oxyphyllus Miq.	—	—	10

* Figures give approx ratio (out of 10).

Table 2. Absorption spectra (in visible region) of the pigments in the black- and red-fruits of the *Ilex* plants

Plants	Fruit colour	λ_{max} of intact cell spectra		λ_{max} in 0·01% MeOH–HCl		$\Delta\lambda$†
Ilex crenata	black	550	(670 sh)*	534		16
I. rotunda	red	549	(649)	533		16
I. chinensis	red	537	(446)	533		4
I. integra	red	532	(429 685)	517	(422)	15

* sh = inflection.
† $\Delta\lambda = \lambda_{max}$ (intact cell spectrum) − λ_{max} (0·01% MeOH–HCl).

the quantities were in general very low; *I. chinensis*, *I. macropoda* and *I. geniculata* completely lacked flavonols. All the fruit-hydrolysates of *Ilex* plants contained caffeic acid, whereas this acid was completely absent from *Euonymus*. Chlorogenic and isochlorogenic acids and caffeylglucose were found in the direct extracts of *Ilex* fruits.

Of the *Ilex* plants examined, *I. crenata* and its variety *convexa* have black fruits, whereas all of the others have red fruits. In order to examine such a colour variation, intact cell spectra were measured with a microspectrophotometer. As shown in Table 2, there were no significant differences in the visible maxima between the red-fruited *I. rotunda* and the black-fruited *I. crenata*, both of which contain the same anthocyanins, flavonols and cinnamic acid esters. However, the absorption maximum *in vivo* of *I. crenata* showed a difference of 13 nm in visible region as compared with that of the red-fruited *I. chinensis* which lacked flavonols. When the maxima of intact cells were compared with those of 0·01% MeOH–HCl extracts, *I. chinensis* showed only a small difference of 4 nm, while the other three species having flavonols revealed a difference of 15–16 nm (*cf* Table 2). Anatomical observations indicate that the red-fruit skin of *I. rotunda* is composed of a single layer of the light red cells (epicarp), whereas the black-fruit skin of *I. crenata* is composed of several layers of deep red cells.

EXPERIMENTAL

Plant sources. The survey of the genera *Ilex* and *Euonymus* was carried out on ripe fruits of plants. The plants other than species reported previously were collected at different places, e.g. Kumamoto, Fukuoka, Nagasaki, Hiroshima, Ishikawa, Toyama, Aichi, Yamagata, Miyagi and Akita prefectures in Japan.

Anthosyanins and other phenolics identification. Anthocyanin identification was carried out as described earlier [3]. Fla-

vonols and caffeic acid in the 2 N HCl hydrolysate of the fruit-skin were identified using well-known procedures [4]. Chlorogenic and *iso*chlorogenic acids and caffeylglucose in the methanolic extract of the *Ilex* fruit skins were separated by PC in 6% HOAc and then BuOH–HOAc–H_2O (6:1:2). Each compound was identified by spectral and chromatographic comparison with authentic samples. Caffeylglucose was hydrolysed by 2 N aqueous NaOH [5].

Intact cell spectral method. Fresh epicarp containing anthocyanin was carefully peeled off from the fruits of *Ilex* plants, stuck on a quartz slide (0·8 mm), and mounted with distilled water. Light absorption was measured with the recording microspectrophotometer of Olympus DMSP-II. The observation diameter of photometric spot on the surface of fruit cell was 5·6μ (pinhole dia., 0·4 mm; object lens, MO X30).

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