Anthocyanins and Their Distribution in the Genus *Epimedium*

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Anthocyanins contained in plants belonging to the genus *Epimedium* in Japan are discussed in this study. Two kinds of anthocyanin, delphinidin 3-p-coumaroylsophoroside-5-glucoside (cayratinin) and cyanidin 3-p-coumaroylsophoroside, were identified, and the latter is new to the literature. Only cayratinin was found in the colored petals of the *Epimedium* species, but cayratinin and cyanidin glucoside were contained in the stems, young leaves and autumn leaves of all the species surveyed.

Key words: Anthocyanins — Distribution pattern — *Epimedium*.

The plants belonging to the genus *Epimedium* (Berberidaceae) are perennial and grow in the temperate zone. They are distributed from east Asia through Europe to parts of the Atlas mountains of North Africa. In Japan, the plants are distributed from southern Hokkaido to Kyushu. The plants are well known as medicinal herbs, and are sometimes cultivated as horticultural plants. The plants bear flowers with either purplish-red, white or yellow petals in spring.

Up to the present, there have been many studies describing the compounds contained in the plants (e.g., Akai, 1935; Tomita and Ishii, 1957), and some medicinal compounds have been isolated from the plants. Anthocyanins have been shown to be contained in the petals, young leaves and stems of the plants, but there have been no reports concerning the pigments. Thus, the present study was intended to determine the structure of anthocyanins contained in the plants and to survey the distribution pattern of the pigments in the genus. The paper deals with the paper-chromatographic and spectral identification of the pigments distributed in five species and one variety of the genus *Epimedium*.

**Materials and Methods**

*Extraction of anthocyanins*

Autumn leaves were used as the materials in the present study, since they contain anthocyanin pigments. Autumn leaves of four *Epimedium* species were cut into small pieces, and the pigment was extracted with 0.5% methanolic hydrochloric acid. The extract was concentrated to 1/6 volume, and allowed to stand overnight in the cold. After removal of large amount of precipitate by filtration, the filtrate was shaken with
ethyl acetate. The mother liquor was again concentrated to a small volume. The concentrate was then applied to the column bed packed with insoluble polyvinylpyrrolidone (PVP). The column was washed thoroughly with 0.1% HCl and the pigment was eluted with 50% methanol containing 0.1% HCl. The pigment solution eluted was concentrated \textit{in vacuo} to dryness. The residue was dissolved in a small amount of 0.1% methanolic hydrochloric acid, and five volumes of ether were added to the solution to precipitate the pigment. After decantation of ether from the solution, the pigment precipitate was dehydrated with acetone and collected by filtration.

The pigment powder was dissolved in a small amount of 0.5% methanolic hydrochloric acid and applied to Tōyō No. 50 filter paper. The pigment was developed overnight with the mixture of \textit{n}-butanol/acetic acid/H$_2$O (4 : 1 : 5, designated as BAW) by the descending procedure. Then, two pigment bands separated were cut out and eluted with 80% methanol containing 0.1% HCl. Each eluate was concentrated and dried in a desiccator. The pigment was obtained as homogeneous red powder.

The anthocyanins in the purplish-red petals and the stems were purified by the same method as the case of autumn leaves.

\textbf{Identification of aglycone, sugar and organic acid}

Complete hydrolysis of the pigment was performed by boiling in 6 N HCl for three min. Identification of aglycone, sugar and organic acid of the hydrolyzate were carried out by the same method as described previously (Shibata and Yoshitama, 1969).

\textbf{Partial acid hydrolysis and H$_2$O$_2$-degradation}

Partial acid hydrolysis was performed by the method described by Abe and Hayashi (1956). The hydrolyzate was pipetted out at definite intervals, spotted on Avicel TL-plates, and chromatographed in the solvents BAW and acetic acid/HCl/H$_2$O (15 : 3 : 82, designated as AAH).

H$_2$O$_2$-degradation of the pigments was carried out by the method described by Takeda and Hayashi (1963).

\textbf{Dilute HCl hydrolysis of the anthocyanins}

The pigment was refluxed with 0.2N HCl in a boiling water bath for 1 hr. The hydrolyzate was concentrated \textit{in vacuo} to dryness, and the residue was dissolved in a small amount of methanol. The solution was applied to Tōyō No. 51B filter paper and chromatographed with the solvent BAW. The acyl glucoside on the paper chromatogram was detected by UV-light (365 nm) after treatment with ammonia vapor, and eluted with 80% methanol.

\textbf{Results and Discussion}

Only one anthocyanin was shown to be present in the purplish-red petals of \textit{Epimedium grandiflorum} var. \textit{Thunbergianum}, and the pigment (ED) yielded del-