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SHORT COMMUNICATION

ANTHOCYANINS AND OTHER PHENOLICS IN AUTUMN LEAVES*

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Key Word Index—*Prunus*, *Rhus*, etc.; autumnal colours; anthocyanins; flavanols; gallic and ellagic acids; leaf phenolics.

Abstract—Anthocyanins and other phenolics in young, mature and autumn red leaves of *Prunus*, *Rhus*, *Euonymus*, *Parthenocissus* and *Acer* have been studied. In general, autumnal red leaves contained chrysanthemin, except that *Rhus succedanea* contained, in addition, a small amount of peonidin 3-monoglucoside. In three *Acer* species and one *Prunus* species, red colour of young leaves was due to a mixture of 3-monoglucoside and 3-rutinoside of cyanidin, and some glycosides of peonidin were formed transiently in young leaves of *R. succedanea*. Hydrolysed leaf-extracts contained ellagic and gallic acids, kaempferol and quercetin.

INTRODUCTION

IN THEIR extensive survey of anthocyanins in autumn leaves, Hayashi and Abe[1] have shown that chrysanthemin (cyanidin 3-monoglucoside) is very common. On the other hand, Price and Sturgess[2] noted that a transient red colour appearing in young leaves is due to cyanidin glycosides, in which the sugar moiety is different from that of the autumnal leaves, and that some juvenile leaves contain anthocyanidins other than cyanidin. If this is true, it would seem that the type of pigment changes with the age of a leaf. It was therefore of interest to examine anthocyanins and other phenols at both early and late stages in leaf development in a number of plant species showing autumnal colouration.

RESULTS

Autumnal Leaf Anthrocyanins

The results of the present survey on leaf anthocyanins are summarized in Table 1. Red leaves of all plants examined contained cyanidin 3-monoglucoside, as has been reported previously.[1] Beside this anthocyanin, a small amount of peonidin 3-monoglucoside was also found in the case of *R. succedanea*.

Young Leaf Anthocyanins

Of the plants examined, young leaves of *R. succedanea* and of three *Acer* species (cf., Table 1) produce a transient anthocyanin, which disappears as maturation proceeds. In particular, young leaves of *Acer palmatum* var. *amoenum* become intensely red, and keep their colour until the fall of leaves. As seen from Table 1, young leaves of *R. succedanea*

* Part I of a projected series "The Autumnal Reddening of Leaves".

[1] K. HAYASHI and Y. ABE, *Bot. Mag. Tokyo* **68**, 299 (1955).

[2] J. R. PRICE and V. C. STURGESS, *Biochem. J.* **32**, 1658 (1938).

TABLE 1. SEASONAL CHANGE OF ANTHOCYANINS AND LEUCOANTHOCYANINS OCCURRING IN THE LEAVES OF EIGHT PLANTS

Plant source	Collection date	Leaf colour	Anthocyanin*	Leucoanthocyanidin*
Prunus yedoensis Matsum.	4 June	Green	None	Leucocyanidin (10)
	4 June	Yellow	None	Leucocyanidin (10)
	10 Dec.	Red	Chrysanthemin (10)	
Rhus succedanea L.	12 April	Dull red-brown	Peonidin -glycoside I (4) -glycoside II (6)	
	8 June	Green	None	None
	17 Nov.	Red	Chrysanthemin (9) Peonidin 3-mono-glucoside (1)	
Euonymus alatus (Thunb.) Sieb.	8 June	Green	None	Leucocyanidin (6) Leucodelphinidin (4)
	4 Nov.	Red	Chrysanthemin (10)	
Euonymus alatus (Thunb.) Sieb. f. *ciliato-dentatus* (Fr. et Sav.) Hiyama	8 June	Green	None	Leucocyanidin (6) Leucodelphindin (4)
	4 Nov.	Dull red	Chrysanthemin (10)	
Parthenocissus tricuspidata (Sieb. et Zucc.) Planch.	8 June	Green	None	Leucocyanidin (10)
	17 Nov.	Dull red	Chrysanthemin (10)	
Acer palmatum Thunb. var. *palmatum*	13 April	Red (margin) Green (centre)	Chrysanthemin (8) Keracyanin (2)	
	4 June	Green	None	Leucocyanidin (10)
	4 Nov.	Red	Chrysanthemin (10)	
Acer palmatum Thunb. var. *amoenum* (Carr.) Ohwi	13 April	Red	Chrysanthemin (4) Keracyanin (6)	
	4 Nov.	Red	Chrysanthemin (10)	
Acer buergeranum Miq.	2 April	Yellow-red	Chrysanthemin (6) Keracyanin (4)	
	8 June	Green	None	Leucocyanidin (10)
	4 Nov.	Red	Chrysanthemin (10)	

* Figures in parentheses denote the approximate parts out of 10.

contain two peonidin glycosides in a small amount, the sugars not having been determined. Three species of *Acer* contained the 3-rutinoside of cyanidin (keracyanin) in addition to the 3-monoglucoside. In young leaves of *Prunus sargentii* subsp. *jamasakura*, the 3-monoglucoside and 3-rutinoside are present in the ratio 3:2, whereas in autumn leaves, only the 3-glucoside is present.

TABLE 2. RELATIVE AMOUNTS OF MAJOR PHENOLICS IN LEAF HYDROLYSATES*

Plants	Leuco-antho-cyanins	Quer-cetin	Kaemp-ferol	Caffeic acid	Gallic acid	Ellagic acid
Prunus yedoensis Matsum.	3	2	1	3	0	0
Euonymus alatus (Thumb.) Sieb.	5	4	3	0	0	0
E. alatus (Thunb.) Sieb. f. *ciliato-dentatus* (Fr. et Sav.) Hiyama	5	4	3	0	0	0
Parthenocissus tricuspidata (Sieb. et Zucc.) Planch.	3	2	1	3	0	0
Rhus succedanea L.	0	Trace	2	0	5	5
Acer buergeranum Miq.	1	4	3	0	5	5
A. palmatum Thunb. var. *palmatum*	1	0	0	3	4	4
A. palmatum Thunb. var. *amoenum* (Carr.) Ohwi	—	0	0	5	3	3

* Relative amount was expressed in the range of 5–1, taking the spot area as a measure, while the intensity of anthocyanidin colour was taken for the same purpose in the case of leucoanthocyanin.

Acid Hydrolysates of Mature Leaves

Mature leaves containing no anthocyanin were hydrolysed with 2 N HCl, and major phenolic compounds liberated are shown in Table 2. Leucoanthocyanins detected in the extracts as anthocyanidins are also shown in Table 1.

In all plants, with an exception of *R. succedanea*, leucoanthocyanins were present, with leucocyanidin being predominant. Leucoanthocyanins in the two species of *Euonymus* yielded, upon acid treatment, cyanidin and delphinidin. In all *Acer* plants there is only a minor amount of leucoanthocyanin and *R. succedanea* lacks it; these plants have large amounts of gallic and ellagic acids. Two flavonols, quercetin and kaempferol, were present in all species except *Acer palmatum* var. *palmatum* and var. *amoenum*. In addition, *o*-coumaric acid was detected in the leaves of *Prunus* plants.[3]

DISCUSSION

From these results it is clear that anthocyanins of young leaves may differ from those found in autumn leaves. This is particularly apparent in the case of *Acer* species. Although mature leaves have much leucoanthocyanin, it is unlikely that leucoanthocyanin is converted into anthocyanin in senescent leaves, because there is no correlation in the hydroxylation patterns. Thus in the leaves of two *Euonymus* species, leucodelphinidin and leucocyanidin are present, but no leucoanthocyanin is produced in the case of mature *Rhus* leaves. In contrast, leucocyanidin is formed in yellow leaves of *Prunus*, which fail to form anthocyanin even after degradation of chlorophylls. Also, it is now accepted that leucoanthocyanins are probable precursors of condensed tannins, but not of anthocyanins.[4–6]

From this survey, at least two groups of plants may be distinguished, the one containing both gallic and ellagic acids (*Rhus* and three *Acer*) in large amounts, and the other having none of these acids (*Prunus*, *Parthenocissus* and two *Euonymus*). In the former, the leaves produce a little or no leucoanthocyanin, although they have transient anthocyanin, while in the latter leucoanthocyanin is detected in large amounts. Quercetin and kaempferol have been detected in the leaves of all plants, except *Acer palmatum* var. *palmatum* and var. *amoenum*. According to the previous investigations[7–9] it has been shown that apigenin 7-rutinoside is present in *R. succedanea*, quercetin in *E. alatus*, and vitexin, isovitexin and iso-orientin in *A. palmatum* var. *palmatum*. Therefore, it seems that the anthocyanins of autumnal red leaves are unrelated to the flavonols and leucoanthocyanins (and tannins), which may be present in the same plant.

EXPERIMENTAL

Plant materials. Leaf was collected from trees growing in the campus of Kumamoto University during March–December. In each case, leaf samples at various developmental stages were taken from the sunny side of the same tree.

Identification of anthocyanins. Fresh leaf material was extracted with cold 1% MeOH–HCl. The extract was concentrated *in vacuo*, washed with *n*-heptane, and separated by PC with HOAc–HCl–H_2O (3:1:8) (AAH) and BuOH–HCl–H_2O (7:2:5) (BuH). The glycosides were identified by direct comparison with

[3] K. Takaishi, *Phytochem.* **10,** 719 (1971).
[4] D. G. Roux, *Nature, Lond.* **181,** 1454 (1958).
[5] W. E. Hillis, *Nature, Lond.* **182,** 1371 (1958).
[6] J. R. Troyer, *Phytochem.* **3,** 535 (1964).
[7] S. Hattori and H. Matsuda, *Archs. Biochem. Biophys.* **37,** 85 (1952).
[8] T. Mazaki and M. Aritomi, *J. Pharm. Soc. Japan* **77,** 1353 (1957).
[9] M. Aritomi, *J. Pharm. Soc. Japan* **83,** 737 (1963).

authentic specimens using UV spectrum and co-chromatography in four solvents: AAH, BuH, HOAc–HCl–H_2O (30:3:10) (Forestal), and BuOH–H_2O (6:1:2) (BAW). Further confirmation of the glycosides was made by complete and controlled acid hydrolysis, and also by KOH degradation of their aglycones. The sugars liberated by hydrolysis were identified by comparison with authentic samples by co-chromatography using BuOH–pyridine–H_2O (6:4:3) and EtOAc–pyridine–H_2O (12:5:4). The development of the spots was effected by aniline hydrochloride or alkaline $AgNO_3$.

Acid hydrolysis products of the leaves. Fresh green leaves (1 g) of each plant were placed in a test tube, covered with 2 N HCl (5 ml) and heated in a boiling water bath for 20 min. The hot aqueous solution was decanted and filtered, whereupon cooled filtrate was thoroughly extracted with ether, and aerated to remove remaining ether. The anthocyanidins were transferred to *iso*-amyl alcohol, which was shaken with 5% HCl beforehand, and an aliquot of this extract was examined chromatographically.[10] Anthocyanidins in the alcohol extract were identified by comparison with authentic cyanidin and delphinidin by co-chromatography using three solvents. Absorption max. of the anthocyanidin, purified chromatographically, also agreed with those of the authentic specimens. For the confirmation of ellagic acid and other phenolics, the ether extract obtained above was concentrated, and chromatographed in BAW, Forestal, 6% HOAc and benzene–HOAc–H_2O (125:72:3). The spots were detected in UV light (256 nm) before and after exposure to ammonia vapour, and also by the colour reactions with dizotized sulphanilic acid[11] and/or diazotized *p*-nitroaniline.[12] Additional demonstration of the presence of ellagic acid in the leaves was made by the method of Bate-Smith.[10]

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[10] E. C. BATE-SMITH, *J. Linn. Soc. Bot.* **55**, 699 (1957).
[11] I. SMITH, in *Chromatographic Techniques* (edited by I. SMITH), p. 189, Heinemann, London (1958).
[12] T. SWAIN, *Biochem. J.* **53**, 200 (1953).