Phytochemistry, 1969, Vol. 8, pp. 2367 to 2369. Pergamon Press. Printed in England

ANTHOCYANINS IN LEAVES OF *COTINUS COGGYGRIA*

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(*Received* 13 *June* 1969)

Abstract—Leaves of *Cotinus coggygria* are pigmented with delphinidin 3-galactoside, cyanidin 3-galactoside, petunidin 3-glucoside and, tentatively, delphinidin 7-glucoside, and cyanidin 3-glucoside-7-rhamnoside.

ANTHOCYANINS containing sugars in the 7-position are rare in nature, only four having been described previously. Two of these are 7-monoglycosides, viz. delphinidin 7-galactoside in *Bladhia sieboldii*[1] and cyanidin 7-arabinoside in apple skin,[2] while two contain additional 3-substituents, viz. pelargonidin 3-sophoroside-7-glucoside in some *Papaver* species[3] and cyanidin 3,7-diglucoside in *Petunia hybrida*.[4] In a study of the anthocyanins occurring in a purple leaf form of *Cotinus coggygria* Scop. (Anacardiaceae) we have now tentatively identified further anthocyanins of these types.

RESULTS AND DISCUSSION

Preliminary separation of a concentrated leaf extract on Whatman No. 3 paper in BAW (solvent A—Table 1) gave four bands, which were numbered I to IV from the origin. In all the other solvents used, band III split into two bands III_1 and III_2. The amounts of each band decreased in the order $I > III_1 > III_2 > II > IV$. Details of their purification and identification are summarized in Table 1. Band I was a monogalactoside since controlled hydrolysis yielded only the aglycone (delphinidin); its spectral characteristics[3, 5, 6] showed it to be delphinidin 3-galactoside.

From similar considerations Bands II and III_1 were identified as petunidin 3-glucoside and cyanidin 3-galactoside respectively, the identity of the latter being confirmed by comparison of its R_f values with those of an authentic specimen of cyanidin 3-galactoside from copper beech.[7] Band III_2 (a delphinidin glucoside) was rigorously purified since the ratio ($E_{u.v.\ max.}/E_{vis.\ max.}$) of the order of 0·9 had not been described previously and indicated that the anthocyanin was neither a delphinidin 3-glycoside, 5-glycoside nor 3,5-diglycoside but of more unusual structure. Although such a high ratio might be attributed to possible contamination with leucoanthocyanidins, this was considered unlikely since the ratio remained constant despite the variation in the solvents used for purification (see Table 1). The 4′-hydroxyl

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TABLE 1. IDENTIFICATION OF ANTHOCYANINS OF *Cotinus coggygria*

Band	Solvents used†	Absorption spectra* λ_{max} (nm)	$E_{440}/E_{vis.max.}$	$E_{u.v.max.}/E_{vis.max.}$	After total acid hydrolysis: Aglycone	Sugar	R_fs (×100)‡ A	B	C	D	E	F
I	A, D	539, 279	0·18	0·45	Delphinidin	Galactose	20	09	03	16	10	13
	A, E	539, 277	0·19	0·56								
II	A, C	535, 278	0·17	0·60	Petunidin	Glucose	23	06	04	22	15	16
	A, D	536, 278	0·17	0·53								
III_1	A, B	528, 281	0·24	0·60	Cyanidin	Galactose	27	19	08	29	16	33
	A, F, D	528, 281	0·25	0·61								
III_2	A, F, D	540, 277	0·18	0·88	Delphinidin	Glucose	27	31	18	39	24	46
	A, F, D, B	540, 277	0·18	0·92								
	A, F, D, C	540, 277	0·18	0·91								
	A, F, D, E	540, 278	0·18	0·92								
IV	A, D	528, 280	0·22	0·91	Cyanidin	Glucose and rhamnose	33	50	19	41	25	56

* In methanol containing 0·01 per cent conc. HCl.

† A = *n*-BuOH:acetic acid:water (4:1:5, v/v); B = *n*-BuOH:2 N HCl (1:1, v/v); C = water:conc. HCl (97:3, v/v); D = acetic acid:conc. HCl:water (15:3:85, v/v); E = water:acetic acid (98:2 v/v); F = *n*-BuOH:conc. HCl:water (7:2:5, v/v).

‡ Whatman No. 1 paper.

group was also unsubstituted, since addition of aluminium chloride produced a positive wavelength shift, indicative of a free *ortho*-dihydroxyl grouping. While not precluded by this test, substitution in the 3′- or 5′-positions is unlikely since no anthocyanins containing sugars in these positions have yet been described. The only hydroxyl group remaining is in the 7-position. Although glucose was produced on hydrolysis with hydrogen peroxide or acetic acid (10 per cent),[8] suggesting that glucose might be in position 3 and that the anthocyanin might be a 3,7-diglucoside, controlled hydrolysis with hydrochloric acid gave only delphinidin without intermediate formation of its 3-glucoside. Likewise, hydrolysis with 10 per cent acetic acid, which was complete within an hour, gave only delphinidin. Band III_2 was therefore a monoglucoside and probably delphinidin 7-glucoside; the conclusion that it possesses a free 3-hydroxyl group was supported by its rapid decolorization in the presence of ferric chloride[9] compared with Band I. The facile liberation of the sugar from the 7-position by 10% acetic acid (or hydrogen peroxide) contrasts with previous findings.[8]

From its spectral characteristics and positive aluminium wavelength shift, Band IV also appeared to be an anthocyanin of unusual structure, which possessed free 5-, 3′- and 4′-hydroxyl groups. Total acid hydrolysis gave glucose and rhamnose. As anticipated, Band IV was not cyanidin 3-rutinoside which gave lower R_fs in five solvents. Hydrolysis with hydrogen peroxide and acetic acid (10%) indicated that the 3-position contained only glucose. The rhamnose could be accommodated only in position 7; Band IV was accordingly identified as cyanidin 3-glucoside-7-rhamnoside, a conclusion supported by the finding of an intermediate anthocyanin corresponding in R_f to cyanidin 3-glucoside on partial acid hydrolysis. The alternative cyanidin 7-rutinoside was discounted on the grounds that acetic acid hydrolysis would have yielded rhamnose rather than glucose, and that cyanidin 3-glucoside would not have been formed on partial hydrolysis.

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The characteristics of these two new anthocyanins may now be summarized in relation to previous findings.

Anthocyanin 7-monoglycosides. (*a*) Delphinidin 7-glucoside is characterized by a value of ($E_{u.v.\ max.}/E_{vis.\ max.}$) of 0·9 compared with a corresponding value of 1·31 found for cyanidin 7-arabinoside by Sun and Francis.[2] These authors attribute similar findings to Harborne who, however, gives a corresponding value of only 0·51 for pelargonidin 7-glucoside.[3,6] (*b*) The wavelength peaks of delphinidin 7-glucoside are little changed from those of delphinidin 3-glycosides. For other 7-monoglycosides an increase in visible wavelength peak of 2–3 nm has been reported,[2,3,6] with either no change[3,6] or a decrease in u.v. peak of 10 nm,[2] compared with the corresponding 3-glycosides. (*c*) The R_fs of delphinidin 7-glucoside are higher than the corresponding 3-glucoside in butanol-based and in aqueous-acid solvents. Previous authors[2,3,6] show increased R_fs of 7-monoglycosides only in butanol-based solvents.

Anthocyanin 3,7-diglycosides. (*a*) Cyanidin 3-glucoside-7-rhamnoside is also characterized by a ratio of ($E_{u.v.\ max.}/E_{vis.\ max.}$) of 0·9, compared with corresponding values of 0·69 and 0·66 for pelargonidin 3,7-diglucoside and 3-sophoroside-7-glucoside respectively.[3] (*b*) The wavelength peaks of cyanidin 3-glucoside-7-rhamnoside are little changed from those of cyanidin 3-glycosides. In contrast, the 3,7-diglycosides described previously have visible wavelength peaks reduced by 2–8 nm[3,4,6] and u.v. peaks increased by 9–11 nm[3] compared with the corresponding 3-glycosides. (*c*) The 3,7-diglycosides described previously exhibit normal chromatographic behaviour, possessing high R_fs in aqueous-acid solvents and low R_fs in butanol-based solvents.[10] The R_fs of cyanidin 3-glucoside-7-rhamnoside are high in all solvents, but this might be expected of a pigment containing rhamnose.[10]

EXPERIMENTAL

Leaves were extracted with MeOH containing HCl (2–3 per cent) concentrated and chlorophyll was removed by extraction with ether, until the ether layer was colourless. The concentrated extract was streaked on Whatman No. 3 filter papers and chromatographed in successive solvents, the bands being cut out and extracted with MeOH–HoAc–H_2O (94:3:3, v/v). Aglycones and sugars were identified chromatographically by the usual methods after total acid hydrolysis of the purified anthocyanins.[11] The petunidin marker was obtained from the hydrolysate of an extract of *Anchusa* petals.[10] Galactose was distinguished from glucose by co-chromatography in *n*-BuOH–EtOH–H_2O (4:1:2·2, v/v and 10:1:2, v/v) and BAW for 2–3 days.

Acknowledgement—Thanks are due to the British Council for a scholarship for one of us (S. S. Tanchev) and to Dr. A. Pollard for suggesting the topic.

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