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PARTICIPATION OF *p*-COUMAROYL GLUCOSE RESIDUE OF AWOBANIN IN SYNTHESIS OF THE COMPLEX MOLECULE OF COMMELININ*

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Key Word Index—*Commelina communis*; Commelinaceae; commelinin; synthesis; metallo-anthocyanin.

Abstract—*In vitro* synthesis of commelinin-like metallo-anthocyanins was attempted using flavocommelin, metals and some anthocyanins having structures similar to awobanin, i.e. delphin, violanin, shisonin and cyanin. Only shisonin formed stable metallo-anthocyanins (with Mg, Zn, Cd, Mn, Ni and Co); the blue pigments were homologues of those prepared from awobanin. The ineffectiveness of delphin (deacylated awobanin) and cyanin (deacylated shisonin) suggests that the *p*-coumaroyl glucose residues of awobanin and of shisonin play an important role in the formation of rhamnosylglucoside) indicates that the length and nature of the sugar chain are critical factors for the formation of The ineffectiveness of violanin, similar to awobanin except for a different *p*-coumaroyl sugar chain (*p*-coumaroyl rhamnosylglucoside) indicates that the length and nature of the sugar chain are critical factors for the formation of commelinin and similar complex pigments.

INTRODUCTION

In the preceding papers [1,2] it was shown that commelinin [3–5], a blue metallo-anthocyanin from the flowers of *Commelina communis*, was synthesized *in vitro* from delphinidin 3-(6-*O*-*p*-coumaroylglucoside)-5-glucoside (awobanin) [6–8], swertisin 4′-*O*-glucoside (flavocommelin) [9,10] and Mg, and that similar blue metallo-anthocyanins were formed using bivalent metals such as Mn, Co, Ni, Zn, Cd in place of Mg. In the present work attempts were made to synthesize commelinin-like complex compounds from flavocommelin, metals and some anthocyanins other than awobanin. The anthocyanin components having structures similar to awobanin were: delphinidin 3,5-diglucoside (delphin) corresponding to deacylated awobanin; delphinidin 3-[4-*O*-(*p*-coumaroyl)rhamnosylglucoside]-5-glucoside (violanin or delphanin) [11–13]; cyanidin 3,5-diglucoside (cyanin); and cyanidin 3-(6-*O*-*p*-coumaroylglucoside)-5-glucoside (shisonin) [7, 13, 14].

RESULTS AND DISCUSSION

Synthesis of some blue complexes having delphinidin derivatives as their anthocyanin moiety

As shown in Table 1, quinoidal bases of both delphinidin derivatives, delphin and violanin, exhibited a deep blue colour upon the addition of flavocommelin and each of the metals. When these reaction mixtures were

Table 1. Complex pigments formed from mixtures of delphinidin derivatives, flavocommelin and metals

	Anthocyanin					
	Delphin		Violanin		Awobanin	
Metal component	Colour of reaction mixtures	Complex pigment*	Colour of reaction mixtures	Complex pigment*	Colour of reaction mixtures	Complex pigment†
Mg	blue	—	blue		blue	+
Zn	blue	—	blue		blue	+
Cd	blue	—	blue	-	blue	+
Mn	blue	--	blue	--	blue	+
Ni	blue	—	blue	--	blue	+
Co	blue		blue	--	blue	+

† Stable blue complex pigment separable on Sephadex column.

*Part III in the series of "Metallo-anthocyanins". For Part II, see ref. [2].

passed through a Sephadex LH 20 column, the blue colour changed into purplish red and gradually faded as the pigment moved down the column. That is, no stable blue pigment such as commelinin was obtained. The blue colours of these reaction mixtures can be attributed to the co-pigment effect of flavocommelin on the anthocyanin, and also by the pH change due to the metal acetate added to the solution. On the other hand, awobanin used as control formed stable blue complexes with flavocommelin and metals, as previously reported [1, 2].

Delphin differs from awobanin only in the absence of *p*-courmaric acid linked with a glucose residue at the 3-position of delphinidin. Violanin has a *p*-coumaroyl-rhamnosyl-glucose in the 3-position, so that its side chain is longer than that of awobanin by the additional rhamnose moiety. Accordingly, it seems that the *p*-coumaric acid residue in awobanin may take part in the formation of the commelinin molecule, and that the chain length of the *p*-coumaroyl-sugar residue at the 3-position is critical for the formation of a stable blue complex.

Synthesis of blue complexes of some cyanidin derivatives

As shown in Table 2, the complexes formed by the quinoidal base of cyanin upon admixture with flavocommelin and each of the metals used were coloured but unstable. In contrast, the quinoidal base of shisonin gave rise to stable blue complexes with certain metals. Stable pigments as such were not obtained in the absence of either metal or flavocommelin. It is significant that six bivalent metals, Mg^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+} and Co^{2+}, were effective for the formation of stable complexes with flavocommelin, while the metals, Ca^{2+}, Ba^{2+}, Sr^{2+} and K^{+}, were not, quite similar to that observed with awobanin [2].

Properties of blue complexes of shisonin

The blue pigments obtained above were purified repeatedly by Sephadex LH 20 column chromatography using water as eluant. On paper chromatography developed with a neutral solvent, each pigment gave a single blue spot, and no liberation of shisonin and flavocommelin was observed. When chromatographed with acidic solvents, however, spots of shisonin (red) and flavocommelin appeared. The existence of the metal in each blue pigment was confirmed by means of atomic absorption spectrophotometry. On paper electrophoresis, the metallo-anthocyanins such as commelinin [1–4] and protocyanin [11] have been characterized previously by rapid migration toward the anode. Likewise, the synthesized blue pigments now moved toward the anode (+11.6 mm) as a single spot without perceptible colour change and without any traces of shisonin and flavocommelin liberated during this procedure. Absorption spectra of both commelinin and the blue synthetic products derived from awobanin, flavocommelin and metals exhibit two characteristic peaks at 591–603 and 647–661 nm [2], whereas the analogous pigments synthesized from shisonin exhibit a single peak at 566–580 nm and an inflection at 613–636 nm (Table 3). Spectral differences as such may be due to the difference of hydroxylation pattern between shisonin and awobanin. It is noteworthy that the positions of the absorption maxima are slightly different from each other according to the metal present. The maxima shift to longer wavelengths in the order of Mg, Zn, Cd, Mn, Ni and Co, behaviour quite similar to that of the blue pigments synthesized from awobanin, flavocommelin and metals [2].

These findings strongly suggest that the blue pigments are homologues of commelinin. Since shisonin consists of cyanin acylated with *p*-coumaric acid, it is conceivable that the *p*-coumaric acid residue attached to 3-standing glucose in shisonin should participate in the production of complex pigments. Crystallization and further analysis of this shisonin complex are in progress.

The results obtained here show that *p*-coumaroyl glucose residue in awobanin provides a key role for our correct understanding of the stable blue complex molecule of commelinin.

Table 2. Complex pigments formed from mixtures of cyanidin derivatives, flavocommelin and metals

Metal component used	Anthocyanin			
	Cyanin		Shisonin	
	Colour of reaction mixtures	Complex pigment*	Colour of reaction mixtures	Complex pigment*
K	purple	–	purple	–
Ca	purple	–	purple	–
Sr	purple	–	purple	–
Ba	purple	–	purple	–
Mg	purple	–	blue	+
Zn	dark blue†	–	blue	+
Cd	dark blue†	–	blue	+
Mn	purple	–	blue	+
Ni	dark blue†	–	blue	+
Co	dark blue†	–	blue	+

*Stable complex pigment separable on Sephadex column.
†Sparingly soluble in water.

Table 3. Absorption maxima of complex pigments synthesized from shisonin, flavocommelin and metals

Metal component used	λ_{max} (nm)*
Mg	273, 316, 566, 613 sh
Zn	273, 316, 569, 616 sh
Cd	273, 316, 570, 616 sh
Mn	273, 316, 573, 617 sh
Ni	273, 316, 575, 627 sh
Co	273, 316, 580, 636 sh

*In 0.05 M acetate buffer, pH 4.8.

EXPERIMENTAL

Anthocyanin. Awobanin chloride was prepared from a crystalline specimen of commelinin, as reported previously [1]. Delphin was obtained by deacylation of awobanin chloride (120 mg) by treatment with 8% NaOH (in 60% MeOH) for 45 min in N_2 and acidified with 5% MeOH–HCl. The pigment was then pptd with Et_2O. The red ppt. was dissolved in hot H_2O (4 ml), filtered and 20% HCl (0.2 ml) added, whereupon the pigment separated as lens-shaped leaflets. Recrystallization was repeated twice in a similar manner (50 mg). On partial hydrolysis, the pigment gave delphinidin 3- and 5-monoglucosides as intermediates. The pigment was chromatographically identical with authentic delphin. Crystalline violanin chloride which was isolated previously [12] was used as starting material. Cyanin chloride was obtained from the deep red petals of the rose cultivars, Tassin and Josephine Bruce, and crystallized in the form of rhombic platelets according to the usual procedure [13]. Shisonin chloride was isolated from red leaves of *Perilla ocimoides* (dried, 200 g) by extraction with 1% MeOH–HCl, precipitation as lead salt, conversion into chloride, precipitation with Et_2O, and purification by Sephadex LH 20 column (2.7 × 35 cm) using 20% EtOH as eluant. The column chromatography was repeated using 50% EtOH as eluant. For final purification, the pigment was dissolved in 0.1% EtOH–HCl and kept for several days under an atmosphere of *iso*-PrOH vapour, whereupon shisonin chloride separated as deep red granules. This process was repeated twice, yield 350 mg. Saponification gave rise to *p*-coumaric acid and cyanin, and oxidative degradation to 6-*O*-*p*-coumaroyl glucose. Shisonin obtained here showed a single spot on paper chromatograms. R_fs of shisonin and cyanin in three solvent systems were: 0.60, 0.56 (AAH); 0.65, 0.17 (BuH); 0.40, 0.21 (BAW), respectively.

Flavocommelin. Isolation and purification of this glycoside were made according to the method reported previously [9].

Synthesis of complex pigments from anthocyanins, flavocommelin and some metals. The quinoidal base of each anthocyanin (5–10 mg), which was prepared from its chloride as before [14], was mixed with an aq. soln of flavocommelin and each of the metals (as their acetates) in molar ratios of 1:2:1, respectively. The mixture was evapd to dryness *in vacuo*, and the residue was re-dissolved in a small vol. of H_2O. Evapn of the soln and re-soln. of the residue in H_2O were repeated several times; finally, the aq. soln was passed through a Sephadex LH 20 column (0.8 × 15 cm) using H_2O as eluant. A blue fraction of complex pigment most rapidly moving and easily separable from others was collected and evapd *in vacuo*. Further purification was made by chromatography on a Sephadex LH 20 column (0.8 × 35 cm) using H_2O as eluant.

PC. Tôyô No. 51 filter paper and the following solvents were used: 60% EtOH, AAH (HOAc–HCl–H_2O, 3:1:8), BuH (*n*-BuOH–HCl–H_2O, 7:2:5), BAW (*n*-BuOH–HOAc–H_2O, 4:1:5).

Electrophoresis. Paper electrophoresis was run with 0.1 M acetate buffer (pH 4.8) on Tôyô No. 50 filter paper at 250 V, 0.59 mA/cm at room temp.

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