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STABLE BLUE COMPLEXES OF ANTHOCYANIN-ALUMINIUM-3-*p*-COUMAROYL- OR 3-CAFFEOYL-QUINIC ACID INVOLVED IN THE BLUEING OF *HYDRANGEA* FLOWER

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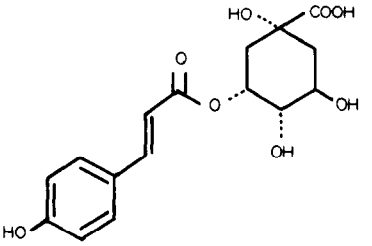
Key Word Index—*Hydrangea macrophylla*; Saxifragaceae; flower; blue complex; anthocyanin; 3-*p*-coumaroylquinic acid; 3-caffeoylquinic acid; aluminium.

Abstract—In a comparison of the behaviour of three esters of *p*-coumaroylquinic acid, only 3-*p*-coumaroylquinic acid formed a stable blue complex with hydrangea anthocyanin (delphinidin-3-glucoside) and aluminium; the 4- and 5-isomers were ineffective. Complex formation was almost identical with that occurring with 3-caffeoylquinic acid. Solutions of both ternary complexes were very stable at pH 3.7 at room temperature. Similar colour augmentation occurred with 3-*p*-coumaroylquinic acid, aluminium and cyanidin 3-glycoside but not with pelargonidin or malvidin glucosides. It is proposed that aluminium conjugates with the *ortho*-dihydroxy group of the anthocyanin B ring and the carboxyl and α-hydroxyl groups of the quinic acid moiety and that the complexes are stabilized in the manner of acylated anthocyanins.

INTRODUCTION

Initial qualitative observations indicated that the blueing of hydrangea sepals can be caused not only by aluminium but also by copigmentation of the anthocyanin present (delphinidin 3-glucoside) by 3-caffeoyl- and 3-*p*-coumaroyl-quinic acids occurring in the blue sepals [1]. 5-Caffeoylquinic acid (chlorogenic acid) found in the blue sepals and the 4-esters of *p*-coumaroyl- and caffeoyl-quinic acids were less effective copigments because they produced purple colour rather than blue [1]. In subsequent quantitative work, blue sepals were shown to contain much more delphinidin 3-glucoside, aluminium and 3-caffeoylquinic acid than red sepals [2]. In contrast, red sepals contained much more 5-caffeoylquinic acid than blue sepals. In *in vitro* experiments using delphinidin 3-glucoside, aluminium and both 3- and 5-caffeoylquinic acids, only 3-caffeoylquinic acid produced a blue complex, the colour with the 5-isomer being red-purple. Neither 3-caffeoylquinic acid nor aluminium independently produced blue colour when mixed with delphinidin 3-glucoside within the range (viz. 1–30 mol ratio) that they were found in blue sepals. It was concluded that the blue ternary complex of delphinidin 3-glucoside-aluminium-3-caffeoylquinic acid is largely responsible for the blue colour in hydrangea sepals and that the function of the aluminium is to stabilize interaction between anthocyanin and quinic ester [2].



In an extension of these studies it was of interest to compare the copigmenting abilities of the three esters of *p*-coumaroylquinic acid, viz. the 3-, 4- and 5-isomers. 3-*p*-Coumaroylquinic acid (**1**) occurs in hydrangea sepals but in much smaller amounts than 3-caffeoylquinic acid and in only slightly greater quantities in blue than red sepals [2].

RESULTS AND DISCUSSIONS

The effects of 3-, 4- *and* 5-p-*coumaroylquinic acids on the absorption spectra of delphinidin* 3-*glucoside without and with aluminium*

In the absence of aluminium chloride, increasing molar equivalents (1–30) of each of the three isomers of *p*-coumaroylquinic acid added to delphinidin 3-glucoside

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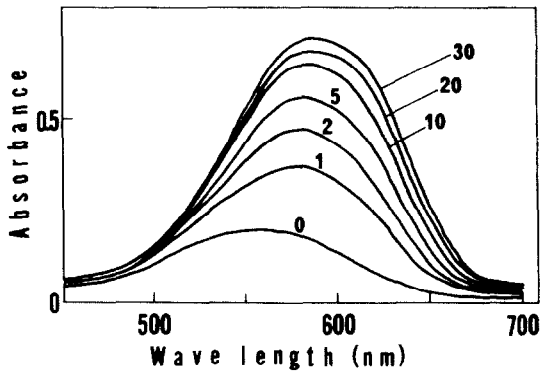

Fig. 1. Effect of 3-*p*-coumaroylquinic acid on absorption spectra of mixtures of delphinidin 3-glucoside (10^{-4} M) and Al (10^{-4} M) in 0.05 M acetate buffer (pH 3.7), light path length 3 mm. The numbers show mol ratio of 3-*p*-coumaroylquinic acid to delphinidin 3-glucoside.

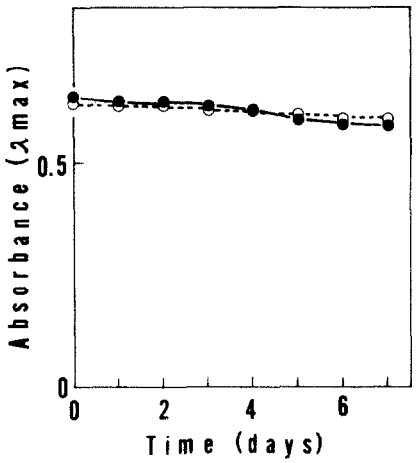

Fig. 2. Stability of the colour of the mixture, delphinidin 3-glucoside (10^{-4} M), Al (10^{-4} M) and 3-*p*-coumaroylquinic acid (10^{-3} M) ●—●, or 3-caffeoylquinic acid (10^{-3} M) ○---○, in 0.05 M acetate buffer (pH 3.7).

(10^{-4} M) in acetate buffer at pH 3.7 produced similar effects. The absorbance increased with increasing concentration of isomer until it was about doubled at 30 molar excess. There were only minor shifts in peak wavelength and the colour remained red.

Under similar conditions but in the presence of aluminium chloride (10^{-4} M), absorbance was little affected by increasing concentrations (1–30 molar equivalents) of the 4- or 5-*p*-coumaroylquinic acid. There were only slight changes in peak wavelength and the colours remained reddish-purple. However, 3-*p*-coumaroylquinic acid gave a most pronounced blueing effect with a 3.5 times absorbance increase (at peak wavelength) and a bathochromic shift of 31 nm, when present in 30 times molar excess (Fig. 1).

Comparison of the effects of 3-p-*coumaroylquinic acid and* 3-*caffeoylquinic acid on delphinidin* 3-*glucoside in presence of aluminium*

Colour augmentation and blueing with increasing molar additions (1–30) of 3-caffeoylquinic acid to delphinidin 3-glucoside (10^{-4} M) and aluminium chloride (10^{-4} M) at pH 3.7 was very similar to that occurring with 3-*p*-coumaroylquinic acid. At 1:1 mole ratio 3-caffeoylquinic acid was a little more effective than 3-*p*-coumaroylquinic acid, but there were no differences between 10 and 20 mole ratios and 3-*p*-coumaroylquinic acid was slightly the more effective at a mole ratio of 30.

Stabilities of the blue complexes

The blue complexes of delphinidin 3-glucoside-aluminium-3-*p*-coumaroyl or 3-caffeoylquinic acid were very stable and equally so at pH 3.7 and room temperature. Colour loss of the complex at 1:1:10 mole ratio was only a few per cent after one week (Fig. 2).

The effect of 3-p-*coumaroylquinic acid on other anthocyanins in presence of aluminium*

Increasing additions of 3-*p*-coumaroylquinic acid, as formerly, to either pelargonidin 3-glucoside or malvidin 3-glucoside and aluminium at pH 3.7 produced little colour change. However, similar additions of 3-*p*-coumaroylquinic acid to cyanidin 3-glucoside (10^{-4} M) and aluminium (10^{-4} M) gave very pronounced colour augmentation with change of hue from rose-red through purple (1–2 mol ratio) to bluish purple (5–30 mole ratio). At a mol ratio of 30, absorbance increase (3.17 times) was slightly less than occurred with delphinidin 3-glucoside (Fig. 1) and the bathochromic shift was slightly greater (33 nm), but colour augmentation was essentially similar with both cyanidin and delphinidin 3-glucosides.

The lack of response with pelargonidin and malvidin glucosides indicates the involvement of the *ortho*-dihydroxy grouping of the anthocyanin B ring in the blueing phenomenon by its well-known conjugation with aluminium. In contrast, the *ortho*-dihydroxy group in the cinnamic part of the quinic acid ester is not essential in the formation of the blue ternary complex since this was produced equally by 3-*p*-coumaroyl- as 3-caffeoylquinic acid. Thus it is highly probable that the aluminium conjugates with the carboxyl and α-hydroxyl groups of the quinic acid part of the ester as happens with iron (Timberlake, unpublished results) and copper [3].

An established mechanism for the blueing and exceptional stability of acylated anthocyanins involves interactions of the acyl groups (caffeic and *p*-coumaric acids) with the positively charged pyrylium nucleus, so preventing further addition of nucleophiles (such as water) to the pyrylium ring [4]. It seems likely that the caffeic and *p*-coumaric moieties of the 3-esters of quinic acid in the ternary complexes discussed here may have a similar intramolecular effect as the anthocyanin acyl groups, but it is only these isomers, and not the 4- and 5-esters, which have the requisite stereochemistry or configuration to bring their aromatic parts into proximity with the pyrylium nucleus.

As far as blueing of hydrangea sepals is concerned the complex anthocyanin-aluminium-3-*p*-coumaroylquinic acid must also contribute to colour but to a lesser extent than the corresponding 3-caffeoylquinic acid complex because of the lower content of 3-*p*-coumaroyl- than 3-caffeoylquinic acid [2].

EXPERIMENTAL

Materials. The anthocyanins used were obtained by standard methods. The quinic acid esters were obtained as described previously [1].

Effects of copigments and aluminium on the absorption spectra of delphinidin 3-glucoside. Experiments were as described previously [2].

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