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COPIGMENTATION OF AURONE AND FLAVONE FROM PETALS OF *ANTIRRHINUM MAJUS*

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Key Word Index—*Antirrhinum majus*; Scrophulariaceae; aureusin; apigenin 7-glucuronide; copigmentation; yellow flower colour.

Abstract—A bathochromic shift of the visible λ_{max} of aureusin was obtained by the copigmentation of this aurone with apigenin 7-glucuronide. The spectra of the copigment complex and that of the petals of Yellow Rocket snapdragons were similar.

INTRODUCTION

MICROSCOPIC examinations of the petals of Yellow Rocket snapdragons showed the yellow color to be in the vacuole of epidermal cells with no evidence of chromoplasts in the cytoplasm, so that carotenoids do not contribute to the yellow color. The principal yellow pigment is the aurone aureusin (aureusidin 6-glucoside). The visible spectrum of aureusin, at a concentration and pH similar to that of epidermal tissue, was different from the spectrum of the intact petal (Fig. 1). The purpose of this investigation was to resolve this difference.

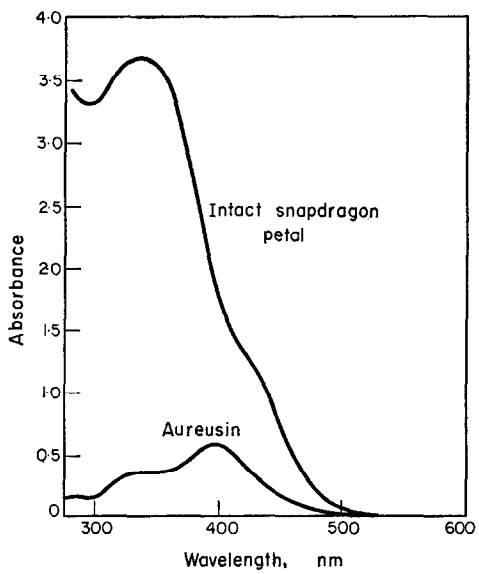

FIG. 1. ABSORPTION SPECTRA OF SNAPDRAGON PETAL AND AUREUSIN 5×10^{-3} M IN CITRATE–PHOSPHATE BUFFER pH 5·35, OPTICAL PATHLENGTH 0·062 mm.

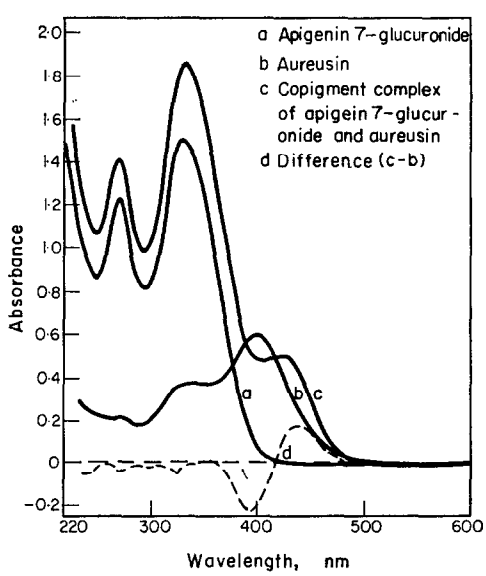

FIG. 2. COPIGMENTATION OF 5×10^{-3} M AUREUSIN AND $1{\cdot}5 \times 10^{-2}$ M APIGENIN 7-GLUCURONIDE AT pH 5·35 AND AN OPTICAL PATHLENGTH OF 0·062 mm.

RESULTS AND DISCUSSION

Apigenin and luteolin 7-glucuronides and aureusin have been previously identified as the major flavones and aurone in yellow flowers of *Antirrhinum majus* L.[1-3] We also found these to be the major flavones and aurone in flowers of Yellow Rocket snapdragons. The pH of the epidermis where the pigments occurred was 5·30.

The long wavelength absorption of aureusin cannot account for the shoulder which is apparent on the absorption curve of the tissue. This shoulder must come from a pigment absorbing at *ca.* 440 nm. The λ_{max} of aureusin varied from 403 to 394 nm as the concentration was increased from 5×10^{-5} to 10^{-2} M. Both the λ_{max} and extinction were independent of pH within the range of 3–6. Increasing pH to 7 shifted the λ_{max} from 416 to 399 nm for the same range of concentrations; however, this pH is considerably higher than that found in the tissue. Changes in the visible λ_{max} due to the increased concentration of aureusin are probably due to self association.

The visible λ_{max} of a 5×10^{-3} M solution (pH 5·35) of aureusin (Fig. 2b) was 395 nm. This visible λ_{max} shifted to 425 nm in a solution (pH 5·35) which contained 5×10^{-3} M aureusin and $1{\cdot}5 \times 10^{-2}$ M apigenin 7-glucuronide (Fig. 2c). This shift in λ_{max} demonstrates copigmentation since the λ_{max} of apigenin 7-glucuronide (Fig. 2a) is 335 nm. Copigmentation resulted in the formation of a new absorber at 440 nm and a decrease in absorption at 395 nm, the visible λ_{max} of aureusin (Fig. 2d). The absorption gained at 440 nm was about equal to the absorption lost at 395 nm.

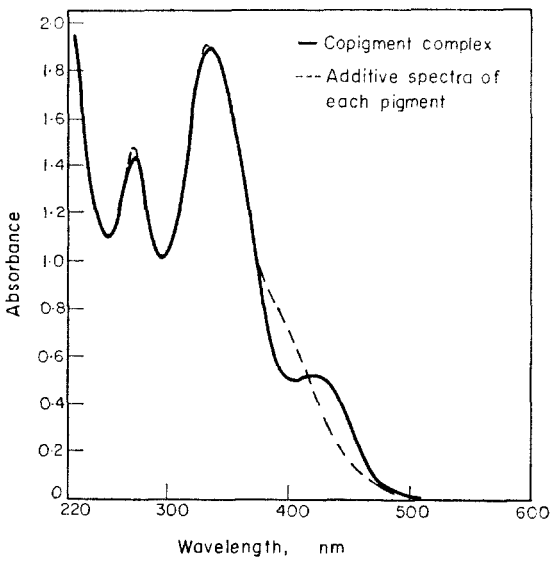

FIG. 3. COMPARISON OF THE SPECTRA OF THE COPIGMENT COMPLEX OF 5×10^{-3} M APIGENIN 7-GLUCURONIDE AND $1{\cdot}5 \times 10^{-2}$ M AUREUSIN AT pH 5·35 AND AN OPTICAL PATHLENGTH OF 0·062 mm WITH THAT OF THE ADDITIVE SPECTRA OF EACH PIGMENT.

A comparison of the mathematical addition of the absorption spectra of 5×10^{-3} M aureusin and $1{\cdot}5 \times 10^{-2}$ M apigenin 7-glucuronide at pH 5·35 with that of the copigment complex is shown in Fig. 3. The visible λ_{max} of the copigment complex cannot be simulated

[1] E. C. JORGENSEN and T. A. GEISSMAN, *Arch. Biochem. Biophys.* **54,** 72 (1955).
[2] T. A. GEISSMAN, E. C. JORGENSEN and B. L. JOHNSON, *Arch. Biochem. Biophys.* **49,** 368 (1954).
[3] J. B. HARBORNE, *Phytochem.* **2,** 327 (1963).

by the mathematical addition of the absorption spectra of any ratio of aureusin and apigenin 7-glucuronide.

The copigment complex provides the absorption needed in the 440 nm region to match that of the tissue, but the tissue absorption is greater in the 360–400 nm region. However, the tissue contains both apigenin and luteolin 7-glucuronides and luteolin 7-glucuronide absorbs in this region. Both apigenin and luteolin 7-glucuronides copigmented with aureusin, but we chose apigenin 7-glucuronide to demonstrate the effect because the visible λ_{max} of this copigment complex was more definitive.

The copigmentation of aureusin with apigenin 7-glucuronide caused a bathochromic shift in the visible λ_{max} of *ca.* 30 nm. This shift in λ_{max} has only a slight effect on the color because the eye is less sensitive to wavelength shifts in this spectral region.[4] This contrasts with the copigmentation of anthocyanins and flavones where an equivalent shift can change the color from red to blue.[5] Many flavonoids copigment with anthocyanins and if the same is true for aurones then it is conceivable that copigments other than apigenin 7-glucuronide might have a greater effect on color. The mechanism by which aurones and flavones copigment and its importance in flower color have yet to be established.

EXPERIMENTAL

Isolation of aureusin and apigenin 7-glucuronide. Fresh Yellow Rocket snapdragon petals were extracted with MeOH and the volume reduced at 40° under reduced pressure. The aqueous residue was taken up in citrate–phosphate buffer pH 3·0, filtered with the aid of celite and then absorbed on a 25 × 400 mm column of purified insoluble polyvinylpyrrolidone (polyclar AT) with the fines removed.[6] The column was washed with 200 ml of H_2O and then the pigments were eluted with MeOH containing 1 ml of 2 N HCl/l. Apigenin 7-glucuronide was eluted within 24 hr, but it was esterified. The methyl ester was hydrolyzed by dissolving the compound in 0·5 N NaOH and then placing it on a steam bath for 30 min. After cooling and acidifying apigenin 7-glucuronide crystallized from the aqueous solution. Aureusin was eluted after 3 days. Final purification was accomplished on a 35 × 450 mm cellulose column with EtOAc–2-PrOH-HCOOH–H_2O (10:5:2:3) as the eluting solvent. Aureusin was crystallized from aqueous alcohol.

Absorption spectra. Stock solutions of apigenin 7-glucuronide (3×10^{-2} M) and aureusin (10^{-2} M) were prepared in citrate–phosphate buffer pH 3·85 and 0·08 N NaOH, respectively. Equal volumes (0·25 ml) of each stock solution were thoroughly mixed and the pH of the solution (5·35) was measured with a combination pH microelectrode. Absorption spectra were determined 15 min after mixing in a spectrophotometer developed in one of our laboratories[7] using a quartz cell with a pathlength of 0·062 mm.

Tissue pH measurement. The pH of epidermal tissue where the pigments are present was measured spectrophotometrically.[8]

[4] G. Wyszecki and W. S. Stiles, in *Color Science*, p. 519, Wiley, New York (1967).
[5] S. Asen, R. N. Stewart and K. H. Norris, *Phytochem.* **11**, 1139 (1972).
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[8] S. Asen, K. H. Norris and R. N. Stewart, *J. Am. Soc. Hort. Sci.* **96**, 770 (1971).