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ABSORPTION SPECTRA AND COLOR OF ALUMINIUM-CYANIDIN 3-GLUCOSIDE COMPLEXES AS INFLUENCED BY pH

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Abstract—The effects of pH on the color and the absorption spectra of a solution of cyanidin 3-glucoside ($3{\cdot}5 \times 10^{-5}$ M) complexed with $AlCl_3.6H_2O$ (7×10^{-4} M) were determined. Increasing pH from 2·21 to 6·16 influenced the type of aluminium-anhydro base complex formed and greatly affected the wavelength of maximum absorption of the sample. The color of the solution changed from red to blue–violet as pH increased. The color change was most pronounced in the pH range of 3·00–3·50.

INTRODUCTION

ANTHOCYANINS are responsible for the red and blue color of most flowers. Solutions of free cyanidin 3-glucoside are red at low pH. In the pH range 4–5 the solutions are virtually colorless; at these pH's the anthocyanin is immediately and almost quantitatively converted, via the unstable purple anhydro base, to its colorless carbinol base.[1] Because of the pronounced instability of both colored forms (flavylium salt and anhydro base) at higher pH, the free anthocyanins could not contribute significant color to flowers if the pH of the vacuole (the portion of the cell where the anthocyanin usually occurs) is above 4.

Structural modifications of red anthocyanins to yield relatively stable violet and blue pigments have been reported. Stable violet and blue colors are formed by metal complexes with anthocyanins that contain *ortho*-dihydroxyl systems.[2, 3] We now have measured the effect of very small pH changes on the color and complexing of aluminium with the anhydro base of cyanidin 3-glucoside. Between pH of 3·00–3·50, small pH changes shift the wavelength of maximum absorption (λ_{max}) of the sample from 515 to 545 nm with a dramatic color change from red to blue–violet. This striking color change of the aluminium-anhydro base complexes of cyanidin 3-glucoside due to small pH changes offers a possible explanation for the bluing, with age, of some red pigmented flower petals. A typical example would be flowers of the rose cultivar "Better Times".

RESULTS AND DISCUSSION

Figures 1–5 show the absorption spectra of cyanidin 3-glucoside ($3{\cdot}5 \times 10^{-5}$ M) complexed with $AlCl_3.6H_2O$ (7×10^{-4} M) in the pH range 2·21–6·16. Increasing pH from 2·21 to 2·99 decreased the 510 nm band and increased the absorption in the 560–620 nm. region.

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[1] L. JURD and S. ASEN, *Phytochem.* **4**, 1263 (1966).
[2] E. BAYER, *Chem. Ber.* **92**, 1062 (1959).
[3] E. BAYER, K. NETHER and H. EGGETER, *Chem. Ber.* **93**, 2871 (1960).

This caused the λ_{max} of the sample to shift to a longer wavelength. The λ_{max} at pH 2·77 and 2·99 were 512 and 515 nm, respectively (Fig. 6). The decrease in the 510 nm band is due to a partial conversion of the flavylium salt to the unstable violet anhydro base. Formation of the stable aluminium-anhydro base complex, even at this low pH range, is evident by the appearance of the long-wave band and the presence of an isosbestic point at 550 nm.

Within the range of 2·93–3·25 (Fig. 2), increasing the pH shifted the isosbestic point from 550 to 537 nm. A new long-wave inflection is now evident at approximately 555 nm, due to an aluminium-anhydro base complex different from the one formed at lower pH's. When

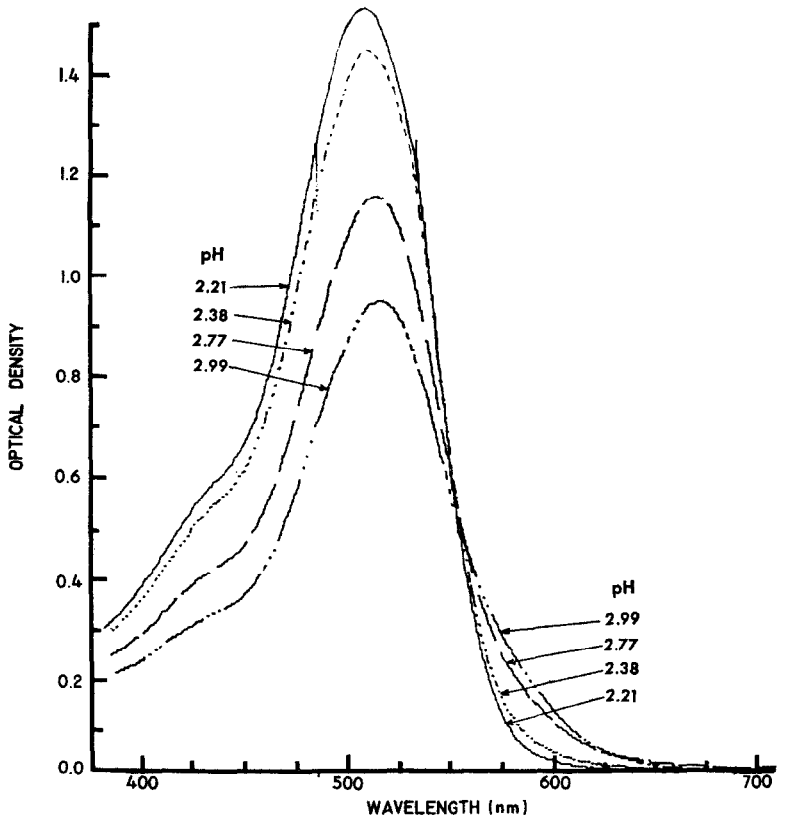

FIG. 1. EFFECT OF pH (2·21–2·99) ON THE ABSORPTION SPECTRA OF CYANIDIN 3-GLUCOSIDE ($3{\cdot}5 \times 10^{-5}$ M) + $AlCl_3 . 6H_2O$ (7×10^{-4} M).

the anhydro base of cyanidin 3-glucoside is complexed with $AlCl_3 . 6H_2O$ in a 1:1 molar ratio at pH 5·50, the solution has a λ_{max} at 570 nm. This complex is insoluble and separates completely as a blue precipitate in a few hours. Increasing the molar ratio to 1:10 resulted in a soluble complex with a λ_{max} at 555 nm. The formation of various types of complexes depending upon the molar ratios of anthocyanin to aluminum previously was shown by Bayer.[4, 5] In our studies, the molar ratio of anthocyanin to aluminium was kept constant so that changes in pH apparently influenced the amount of aluminum available for complexing

[4] E. BAYER, *Chem. Ber.* **91**, 1115 (1958).
[5] E. BAYER, *Angew. Chem. Internat. Edit.* **5**, No. 9 (1966).

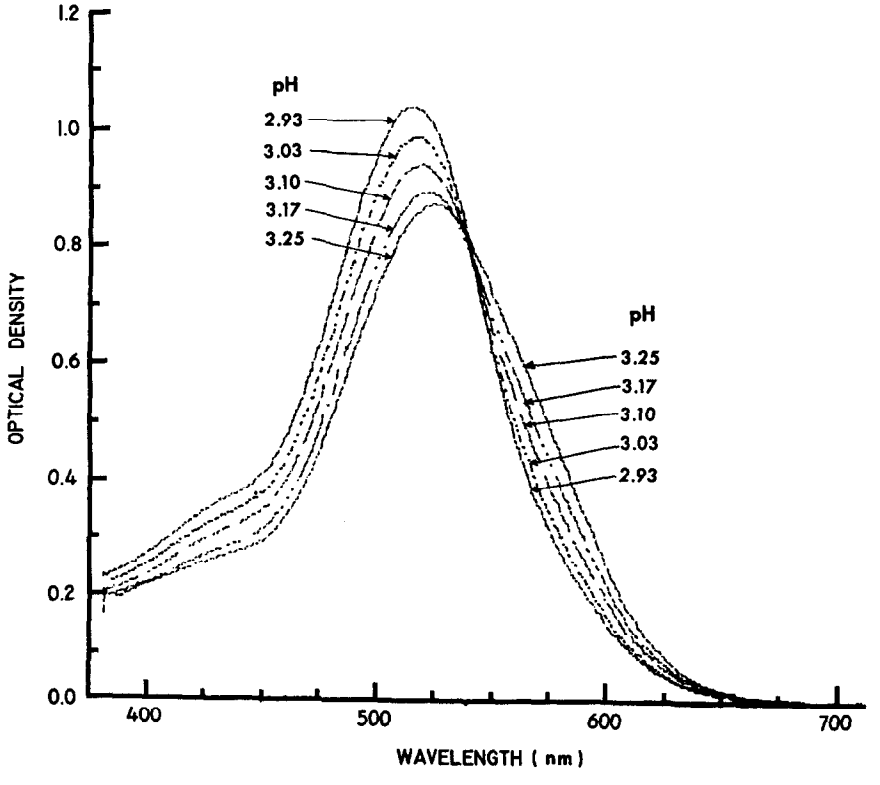

FIG. 2. EFFECT OF pH (2·93–3·25) ON THE ABSORPTION SPECTRA OF CYANIDIN 3-GLUCOSIDE ($3{\cdot}5\times10^{-5}$ M) + $AlCl_3.6H_2O$ (7×10^{-4} M).

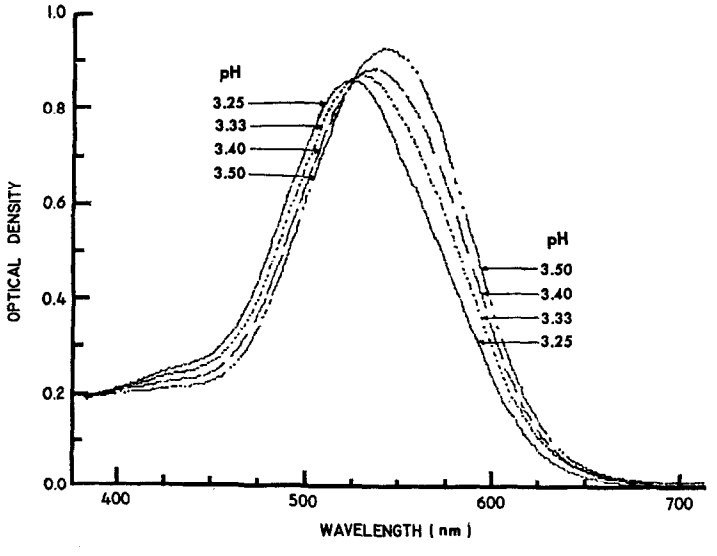

FIG. 3. EFFECT OF pH (3·25–3·50) ON THE ABSORPTION SPECTRA OF CYANIDIN 3-GLUCOSIDE ($3{\cdot}5\times10^{-5}$ M) + $AlCl_3.6H_2O$ (7×10^{-4} M).

as well as the formation of the anhydro base. Therefore, pH also determined the type of aluminum-anhydro base complex formed and drastically affected λ_{max} of the sample (Fig. 6). The result of increasing pH is a color change from red to blue–violet and is most dramatic from pH 3·00 to 3·50.

The absorption spectra in the range of 3·25–3·50 (Fig. 3) show no evidence of additional inflections. The absorption curve at pH 3·25 can be explained as the sum of two bands of equal magnitude with peaks at 510 and 555 nm. Change in absorption as pH increased from 3·25 to 3·50 is the result of a reduction in the 510 nm band and increase in the 555 nm band.

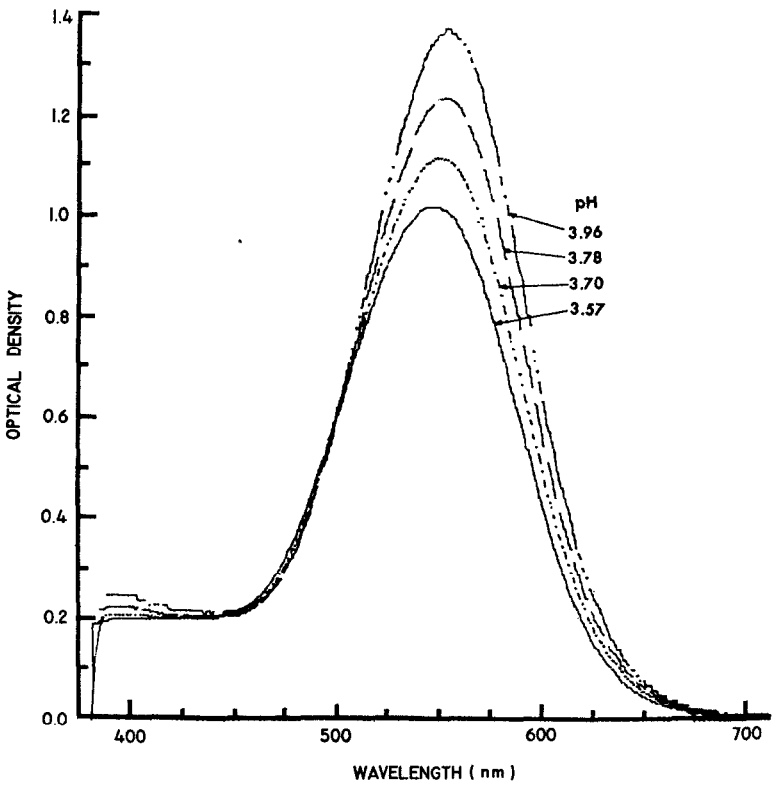

FIG. 4. EFFECT OF pH (3·57–3·96) ON THE ABSORPTION SPECTRA OF CYANIDIN 3-GLUCOSIDE ($3·5 \times 10^{-5}$ M) + $AlCl_3 . 6H_2O$ (7×10^{-4} M).

The shifts in the absorption spectra as shown in Figs. 2 and 3 were closely approximated by theoretical curves constructed from various ratios of two absorption bands, one with λ_{max} at 510 nm and the other with λ_{max} at 555 nm.

In the pH range of 3·57–3·96 (Fig. 4) the further conversion of the 510 nm to the 555 nm form of the pigment caused the λ_{max} of the sample to shift from 546 to 553 nm. The color intensified with an increase in pH and maximum complexing apparently occurred at pH 3·96.

In the pH range of 3·96–6·16 (Fig. 5), there is a decrease in the 555 nm band and an increase in a new band in the region of 630–650 nm. This new long-wave band is probably due to the aluminum-ionized anhydro base complex which has the effect of making the sample bluer. The loss in absorption with the change in pH from 3·96 to 6·16 and the lack of an isosbestic point indicates that all of the pigment is not converted to the new complex.

The dramatic effect of pH on the absorption spectra and the formation of aluminum-anhydro base complexes has been demonstrated. Changes in the λ_{max} in the 510–550 nm region have a greater effect on color than shifts of the same magnitude in other regions of the spectra. One of the common characteristics of senescence in cut roses is the change in petal color from red to violet or purple. Currey[6] reported the cause to be a lack of tannins in cell sap of the petals. Twigg[7] showed that there was little or no change in tannins during aging and he attributed bluing to an increased ratio of anthoxanthin to anthocyanin pigments, to an increased potassium level, and to a higher pH of petal tissue. Weinstein[8] also reported

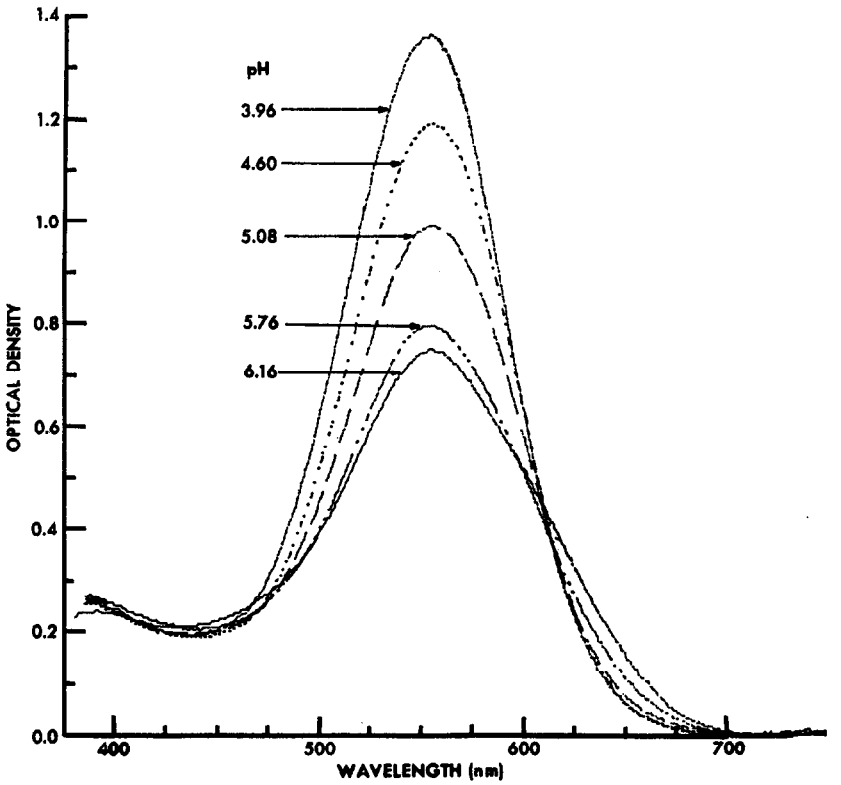

FIG. 5. EFFECT OF pH (3·96–6·16) ON THE ABSORPTION SPECTRA OF CYANIDIN 3-GLUCOSIDE ($3{\cdot}5\times10^{-5}$ M) + $AlCl_3.6H_2O$ (7×10^{-4} M).

that the total quantity of tannins in the petals decreased but slightly during senescence. He found a large increase in free ammonia nitrogen during senescence and suggested that an increase in pH, due to the presence of free ammonia, should be sufficient to induce bluing.

Changes in pH during senescence apparently are important in causing color changes in red roses. Because of the obvious experimental difficulties, pH has not been measured in the vacuoles where the anthocyanin occurs. The pH of the entire rose petal, as measured as a water homogenate, shows a tendency to increase with age. If pH changes are to be considered

[6] G. S. CURREY, *J. Proc. R. Soc. N. S. Wales* **61**, 307 (1954).
[7] M. C. TWIGG, Thesis (Ph.D.), Ohio State Univ. (1952).
[8] L. H. WEINSTEIN, *Contrib. Boyce Thompson Inst.* **19**, 33 (1957).

as the basis for the bluing of red roses, the effect must be on a pigment which is a metal-anhydro base complex. Because of the pronounced instability of the free-anhydro base, it would appear most unlikely that this pigment would be responsible for any bluing effect.

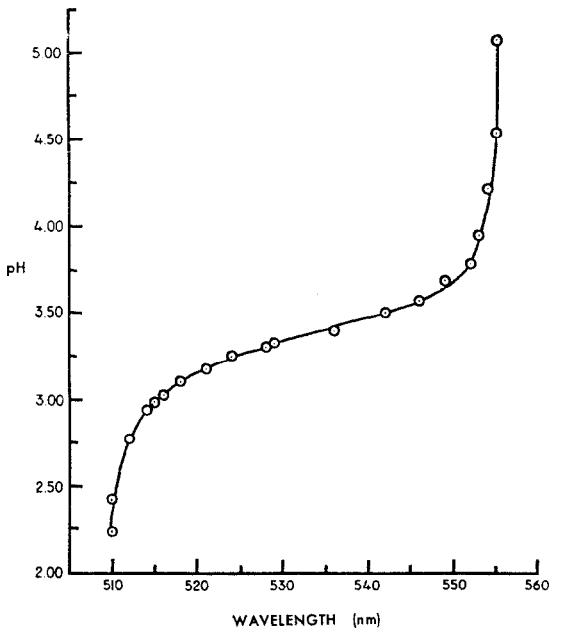

FIG. 6. EFFECT OF pH ON THE WAVELENGTH OF MAXIMUM ABSORPTION OF CYANIDIN 3-GLUCOSIDE ($3{\cdot}5 \times 10^{-5}$ M) + $AlCl_3.6H_2O$ (7×10^{-4} M).

EXPERIMENTAL

Spectral Solutions

The following standard solutions were prepared: Cyanidin 3-glucoside (0·212 g/l. in 0·1% aq. HCl); and aluminium chloride ($AlCl_3.6H_2O$ 0·423 g/l. in water). For spectral measurements 1·0 ml of the standard aluminium chloride solution was added to 1·3 ml of 0·1% aq. HCl followed by 0·2 ml of the standard anthocyanin solution. The pH thereafter was adjusted stepwise with 0·05 or 0·01 M sodium acetate and the absorption spectra at the appropriate pH determined immediately.

Instrumentation[9]

Spectral absorption was measured with a spectrophotometer designed for analysis of dense light-scattering samples.[10] The instrument was operated as a single-beam unit using a digital-storage oscilloscope to provide signal-to-noise enhancement and system correction for a flat baseline. Measurements were made at 1 nm intervals over the range from 380 to 770 nm. The data from the digital-storage oscilloscope were recorded on an X-Y recorder and the figures presented in this paper are photocopies of the original curves. The photometric scale was calibrated with a neutral-density glass previously calibrated on a Cary 14 spectrophotometer. The wavelength scale was checked with a glass of holmium oxide also calibrated with the Cary 14 spectrophotometer. Wavelength error does not exceed ±1 nm and photometric error ±0·005 in absorbance. The baseline is flat from 380 to 770 nm within ±0·005. The spectral bandpass was constant at 3·5 nm for all measurements.

A flat-bottom glass vial 40 mm high with an i.d. of 10 mm was used as the sample cell. This cell was mounted in an upright position directly above the phototube as previously described.[10] The monochromatic beam of

[9] Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may also be suitable.

[10] K. H. NORRIS and W. L. BUTLER, IRE Trans. Bio-Med. Electronics BMS-8, 153 (1961).

collimated light illuminated the central portion of the cell. Beam size was approximately 3 mm in dia. In this type of cell, pathlength is not controlled except by the amount of sample used, and the readings are proportional to the total pigment content rather than to concentration as with fixed-pathlength cells. Therefore, a sample placed in the cell can be diluted with a non-absorbing solvent without changing the readings. Thus, effects of pH on a single pigment sample can be studied by adding non-absorbing buffers and recording the curves after each addition.

The pH was measured directly in the sample cell using a combination electrode (Fisher 13-639-92) coupled with a high-sensitivity electrometer. The readout was made on a digital voltmeter to provide a resolution of 0·001 pH unit. Total drift in reading was less than 0·005 pH unit per hour. The instrument was standardized with calibrated buffers to within $\pm 0{\cdot}01$ pH unit.