

the higher derivatives where the methanol was replaced by *n*-butanol.

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### The Colloid Chemistry of Leaf and Flower Pigments and the Precursors of the Anthocyanins

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In an interesting memoir<sup>1</sup> under the above title, W. D. Bancroft and J. E. Rutzler, Jr., have attributed views to us which we do not hold, the divergence being largely one of emphasis and due to generalization from the particular. These authors contrast the idea of Willstätter that anthocyanins result in nature from the reduction of flavonols with our supposed hypothesis that they normally originate from the oxidation of leuco-anthocyanins.

We are aware that Willstätter (and Everest also) showed that flavonols could be reduced in the laboratory to anthocyanidins, but are not certain that he founded any general theory of biogenesis on these observations. It may be that we have overlooked a statement of this kind. For our own part it has been made abundantly clear that we do not regard the ordinary anthocyanins as derived from leuco-anthocyanins. Here it should be interpolated that by leuco-anthocyanin (idin) we do not mean the colorless pseudo-bases or the readily oxidizable hydrocyanidin of Kuhn, but a special widely distributed class of natural products which are somewhat hard to convert into anthocyanidins, and are recognized by this transformation *in vitro* under certain conditions. Not one of the true leuco-anthocyanins (idins) has been isolated but cyanomaclurin and peltogynol are similar substances. Until they have been purified and characterized by analysis it is idle to speculate on their state of oxidation. A suggestion of a possible structure for a leuco-anthocyanidin having the same state of oxidation as cyanidin has been advanced.<sup>2</sup> Some leuco-anthocyanins undoubtedly are in a lower state of oxidation and it is to these alone that we consider the biogenetic oxidation hypothesis applies.

It is true that we have stated that oxidation *in vitro* is a more likely process than reduction

but this was an argument against the flavonol theory and it was not coupled with adherence to any general oxidation theory, though we can understand how a misconception of our attitude could arise.

As the concentration of leuco-anthocyanins cannot be determined independently, and in view of the possibility of disproportionation, it is hard to devise a sound experimental test for the oxygen requirement in any particular case. In some examples we can see that an oxidizing agent is necessary; the negative results cannot be interpreted unambiguously.

The following citation<sup>3</sup> explains our view of the part played by leuco-anthocyanins:

"Inevitably the transformation of these substances (leuco-anthocyanins) into coloring matters of flavylum type must occur in nature occasionally, but it does not follow that this route represents the standard mechanism. Two dihydroanthocyanidins, namely, cyanomaclurin and peltogynol, have been isolated and characterized; these are stabilized by the possession of a glucose-like oxide ring, and the related anthocyanidins are not found in the form of naturally occurring anthocyanins. The true leuco-anthocyanidins usually afford cyanidin on hydrolysis and autoxidation.

"It is too early to attribute a predominant role to the leuco-anthocyanidins, but it is already safe to assume that their modification represents an auxiliary pigmentation process. It is possibly operative in autumnal reddening and in the coloration following injuries or decortication of leaves."

Analogously we do not wish to exclude even flavonol reduction in *special* cases; we think there is ample evidence that it is not the *usual* mechanism of phyto-synthesis.

We have consistently maintained that the anthoxanthins, and anthocyanins are formed in the plant from a common progenitor by forking of the roads. Furthermore, we discern a C<sub>6</sub> unit and a C<sub>6</sub>-C<sub>3</sub> unit and assuming an origin from carbohydrate have pointed out that cyanidin represents the normal state of oxidation. This hypothesis was first advanced in 1921<sup>4</sup>; it has been developed in various lectures and publications.<sup>5</sup> The

(3) Robinson and Robinson, *Nature*, **137**, 172 (1936).

(4) British Association Report, 89th Meeting, Edinburgh, p. 417.

(5) Ref. 3 and Report of the IXth International Congress of Pure and Applied Chemistry, Madrid, Vol. V, 1934, p. 25. *Proc. Univ. Durham Philosophical Society*, **8**, (i), 17 (1927-1928).

(1) Bancroft and Rutzler, *THIS JOURNAL*, **60**, 2738 (1938).

(2) Robinson and Robinson, *Biochem. J.*, **27**, 207 (1933).

chromogens are doubtless colorless but must not on this account be confused with the leucoanthocyanins.

Finally we remark that attention was pointedly drawn to the importance of colloid association of anthocyanins in the Presidential Address to Section B of the British Association (Leicester, 1933).<sup>6</sup> We showed *inter alia* that cyanin is blue in the acid cornflower cell-sap because it is adsorbed on colloidal particles which stabilize the anions at a *pH* of 4.9.

Co-pigmentation is, however, a different phenomenon and is observable in dilute, strongly acid solutions. In the heterogeneous systems of a flower petal the two factors probably merge and in addition surface phenomena become important in relation to flower color. In this connection it is interesting to note that all the colors of hydrangeas (blue, violet, red) can be exactly reproduced by pouring acid solutions of synthetic delphinidin 3-monoglucoside<sup>7</sup> (now recognized as the hydrangea anthocyanin) on filter paper. The best results are obtained with optimum concentrations of organic acids and in the presence of tannin. Dilute (red) solutions give blue on the paper, more concentrated solutions, red, and the hydrangea violet is intermediate. Blue margins are noted in all cases unless the acid concentration is too high. Hence we reject the view that the blue color is due to ferric salts, physical conditions being obviously paramount. The function of iron salts, etc., added to the soil is probably to initiate physiological disturbances whereby the anthocyanin concentration is diminished. Further relevant facts are reported in the following note.

(6) Cf. Report of the British Association for the Advancement of Science, 1933, and *Nature*, **132**, 625 (1933).

(7) Reynolds and Robinson, *J. Chem. Soc.*, 1039 (1934).

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## Notes on Variable Colors of Flower Petals

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These notes refer to variations not due to change in the basic anthocyanins.

It has already been noted that a factor for flower color is the concentration of the anthocyanin and the ratio of this to the concentration of co-pigments of the tannin and flavonol classes. A good example is the ordinary lilac in which the co-pigment remains substantially constant. The

pale mauve colored flowers contain much less anthocyanin than the red flowers.<sup>1</sup> In the latter case the co-pigment is unable to modify the color of all the anthocyanin present. A second factor is colloid association, probably with polysaccharides (see the preceding note) and in this case too the development of much anthocyanin may produce a new shade. A third factor is virtual change of *pH* due to surface phenomena (diffusion of mobile ions), and fourth and fifth may be dyeing of the fibers and crystallization of the pigment, respectively. Modifications due to traces of elements such as iron are also possible but have not yet been recognized with certainty in any one case. A common phenomenon is that of a red bud which becomes mauve, purple, violet or blue when the flower opens or a little later; changes due to fading and loss of vigor are not under consideration. This sequence has only been observed in flowers characterized by *relatively high pH* (about 6.0). The following are representative observations and some notes on exceptional cases are appended. *pH* was determined after trituration of the material (3 g.) with distilled water (14 cc.).

**Ageratum.**—Young red buds (*pH* 6.0) develop into violet flowers (*pH* 5.8). The anthocyanin is an acylated cyanidin diglucoside. The concentration of anthocyanin is higher in the buds than in the flowers but the ratio of flavone to anthocyanin is higher in the flowers than in the buds.

**Anchusa** (Dropmore var.).—Red buds (*pH* 6.35) become blue flowers (*pH* 6.2). The flavone remains constant but the ratio of anthocyanin in buds to flowers is 34.4:22.5. In this case colloid association in the flowers, as in the cornflower, is indicated.

**Centaurea.**—The concentration of anthocyanin in purple flowers of the annual cornflower is higher than in the blue flowers.

**Cheiranthus mutabilis.**—The color changes are due to the gradual formation of anthocyanin superposed on the yellow ground of the petals; subsequently the anthocyanin is destroyed. This anthocyanin is an acylated glucoside of an acylated cyanidin. The reddish bracts contain unacylated cyanidin diglucoside.

**Delphinium** (vars. Dr. B. Crane, Lorenzo di Medici, Blue Bird).—Blue petals or parts of petals (*pH* about 5.6) contain less anthocyanin and relatively less flavone than violet petals (*pH* about 5.7) of the same plant. Here again colloid association is indicated.

**Hydrangea.**—Red and blue flowers contain the same anthocyanin, namely, delphinidin 3-monoglucoside. There is a little more flavone in the red than in the blue flowers. The ratio of anthocyanin concentrations, red to blue, is usually from 6:1 to 7:1 and using a specially deep-colored blue variety it was still 4:1. The *pH* of hydrangeas is

(1) Robinson and Robinson, *Nature*, **130**, 21 (1932).