LETTERS TO THE EDITORS

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Relation of Leuco-anthocyanins to Anthocyanin Synthesis

Among the herbaceous plants which Bate-Smith and Lerner[1] have recently found to contain leuco-anthocyanins were *Impatiens noli-tangere* and *I. parviflora* of the Balsaminaceae. We have been investigating the genetic control of anthocyanin pigmentation in *Impatiens balsamina*, which has been reported previously to produce glycosides of pelargonidin, peonidin and malvidin[2]. In the course of these studies we have obtained evidence that the leuco-anthocyanins may serve as precursors for the anthocyanin pigments. In view of current interest in the leuco-anthocyanins, this aspect of the work is presented here.

In all analyses, fresh or frozen plant parts were first extracted by boiling with 0·1 *N* hydrochloric acid, filtered, then boiled for 3–5 min. after the addition of an equal volume of concentrated hydrochloric acid. This procedure hydrolyses any anthocyanins present and converts leuco-anthocyanins to anthocyanidins. The acid extracts were washed with ethyl ether to remove flavones and certain other substances. After removal of residual ether by boiling, the pigments were transferred by partition to a small volume of amyl alcohol, from which they were spotted or streaked directly on Whatman No. 1 paper for chromatography. Ascending chromatograms were used throughout, and solvents employed were *t*-butanol/acetic acid/water (15 : 2 : 5) and *m*-cresol/acetic acid/water (25 : 1 : 24)[3]. The phenolic phase of the latter mixture served as the mobile solvent, while the aqueous phase was included within the jars in an open dish. Pigments were identified by comparison with extracts of flowers of known pigment content, by Robinson's[4] tests, and by comparison of R_F values with those reported by Bate-Smith. The sequence of spots corresponds to those reported by Bate-Smith, although the actual R_F values differ somewhat as a consequence of differences in technique and solvents employed.

Leuco-anthocyanins occur in balsams which have green stems and pure white flowers. The leuco-pigment of the stems yields cyanidin, whereas the petals and sepals contain in addition a leuco-anthocyanin which yields delphinidin.

Plants having the proper genotype produce violet-purple flowers and contain in both sepals and petals glycosides of malvidin. Buds (sepals and petals together) collected from these plants before any pigment is visible yield cyanidin and delphinidin from leuco-anthocyanins. Both leuco-anthocyanins disappear before the purple flowers mature.

Another combination of genes controls the production of bright red flowers. In this instance, petals contain only pelargonidin glycosides, whereas sepals contain both these pigments and some peonidin glycoside. Young colourless buds of this form contain leuco-anthocyanins which yield not only cyanidin and delphinidin but also pelargonidin. The leuco-pigments which correspond to cyanidin and delphinidin disappear before the flowers mature. While it is probable that the leuco-anthocyanin yielding pelargonidin also disappears during development of the flowers, this leuco-pigment cannot be detected in the presence of pelargonidin by present techniques. The appearance in young, unpigmented buds of a leuco-anthocyanin convertible to pelargonidin is invariably associated with the presence of a specific dominant gene which also governs the production of pelargonidin glycosides in mature flowers.

Pelargonidin, however, is also produced under the influence of still another gene when in the proper genetic background. Since this gene does not govern the production of the corresponding leuco-anthocyanin in early stages of the bud, we conclude that the final stages of pelargonidin synthesis may follow alternative routes.

These results provide two separate indications that leuco-anthocyanins serve here as precursors for the ultimate anthocyanins. (1) Those leuco-anthocyanins which do not correspond directly with the ultimate pigment disappear as anthocyanins develop. Leuco-anthocyanins do not disappear during the maturation of white flowers, hence the disappearance of leuco-anthocyanins and appearance of anthocyanins are controlled by the same genes. Simmonds[5] has reported a somewhat similar situation in developing bracts of the inflorescence of bananas, where non-methylated leuco-anthocyanins disappear as methylated anthocyanins appear. (2) Under the influence of a gene which has the principal effect of producing pelargonidin derivatives in mature flowers, a corresponding leuco-anthocyanin appears in colourless buds.

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Nature of a Hemicellulose extracted from Cellulose with Water

During the course of investigations leading to the development in this laboratory of the phenol–sulphuric acid procedure for the quantitative determination of sugars[1], small amounts of water-soluble carbohydrate impurities were detected in the paper (Whatman No. 1) used for separating mixtures of sugars.

We have now isolated enough of this water-soluble carbohydrate material from Whatman No. 1 filter paper to permit a more detailed examination of it. Smaller amounts of a similar material have been extracted from cotton linters and from purified wood α-cellulose.

The material, which is polysaccharide in nature, may be extracted at room temperature either by chromatographic irrigation or by simple extraction in water. When chromatographic irrigation is used, the water-soluble polysaccharide appears in the very first portion of the eluate. After re-drying in air, the same piece of filter paper will provide more