VII. THE FLOWER PIGMENTS OF *ANTIRRHINUM MAJUS*. I. METHOD OF PREPARATION.

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I have previously [Wheldale, 1909, 2, 3; 1910, 2; 1911] made certain suggestions as to the nature of the chemical reactions involved in the formation of anthocyanin. There is little doubt that anthocyanin, as a collective term, in the same sense as protein, sugar, tannin, etc., includes many substances having in general similar properties, but differing among themselves as regards constitution. Evidence, from various sources, has led me to conclude that some anthocyanins may be derived from members of the groups of natural yellow colouring matters, known as the flavones and xanthenes.

The yellow colouring matters are largely present in the plants as glucosides, some, or possibly all the hydroxyl groups, being replaced by sugar. I have suggested that the reactions involved in the formation of anthocyanin may be represented, in very general terms, as follows:—

(Flavone or Xanthone)

The first reaction may be regarded as controlled by one or more glucoside-splitting enzymes and it is conceivable that specific enzymes may act on hydroxyl groups in different positions. When certain hydroxyl groups (position to be determined) are free from sugar, oxidation may take place at these points, or possibly condensation, or both, with the formation of anthocyanin. The residual hydroxyl groups in the anthocyanin molecule would probably be replaced by sugar and hence the anthocyanins would occur as glucosides. There is evidence that the second reaction may be brought about by an oxidase system [Wheldale, 1909, 3; 1910, 2; 1911; Keeble and Armstrong, 1912].

Some of the evidence for the above hypothesis is derived from results obtained in cross-breeding with varieties of *Antirrhinum majus* [Wheldale, 1907; 1909, 1; 1910, 1]. The wild type of this plant has magenta flowers, the colour due to anthocyanin. Among other varieties produced on cultivation are three without anthocyanin, i.e. ivory, yellow and white. Ivory and yellow contain pigments very probably of the flavone class. The white has no such pigment but carries other factors which apparently act on the yellow pigments to form anthocyanin. None of the three varieties alone can produce anthocyanin, but when ivory is crossed with white, a plant having magenta flowers is produced; when yellow with white, a plant having crimson (possibly a mixture of magenta and yellow) flowers.

With a view to testing this hypothesis I commenced a chemical investigation of the pigments of *Antirrhinum* and in the summer of 1911 I succeeded in finding a satisfactory method for obtaining the pigment in quantity in the solid state. Extractions were made from yellow and ivory separately, but all varieties containing magenta anthocyanin (i.e. ivory tinged with magenta, magenta and crimson) were extracted together. In this way all extracts contained mixtures of pigments, as will be seen below on referring to the varieties in detail. When attempting to purify the crude pigment later, I found difficulty in separating some of the pigments. Hence, when I again prepared pigment this year, the precaution was taken, in the case of certain varieties, of extracting portions only of the corolla, containing, to the best of my belief, either one pigment or two, which could be easily separated.

The method of preparation is as follows:—the flowers, picked off the spikes, are boiled with water in saucepans. The pigments are readily soluble in water and the extract is filtered through large funnels into lixiviating jars. The pigments are then precipitated as insoluble lead salts by adding solid crystalline lead acetate until no further precipitate appears. After standing a few hours the greater part of the liquid is decanted from the precipitates, which are then filtered through a large Buchner funnel attached to a filter pump. The lead salts are stirred up with 5–10% sulphuric acid which decomposes the salts with formation of lead sulphate. The lead sulphate is filtered off and the filtrate contains the pigments as glucosides in dilute sulphuric acid solution. These solutions are boiled for several hours in two-litre Jena flasks fitted with simple tube condensers, care being taken to avoid concentration of the solution, since under such circumstances the pigment may become charred. Hydrolysis of the glucosides takes place during boiling and, on cooling and standing about 12 hours, the pigment, which is less soluble than the glucoside, is deposited. The deposit is separated by

filtering through as small a Buchner funnel as possible, a Geryk pump being used. After washing, the pigment is dried in a desiccator over sulphuric acid.

During the current year (1912), the following varieties were extracted. Excellent coloured figures of the varieties are given by Baur [1911]. Figures below refer to Baur's plate.

Ivory. (Fig. 3.) The corolla contains a pale yellow pigment (ivory) and a patch of deeper yellow pigment (yellow) on the lower lip. Extracts from the whole flower or from the lower lip gave an orange-yellow lead precipitate. From the separated upper lip, a canary-yellow lead precipitate. Pigment was prepared separately from both upper and lower lips.

Yellow. (Fig. 2.) The lips of the corolla contain yellow pigment which is chiefly confined to the epidermal cells. The inner tissues of the lips and the entire tube contain ivory pigment. Extracts gave an orange lead precipitate. Pigment was prepared separately from both upper and lower lips.

Ivory tinged with magenta. (Fig. 6.) Flower, ivory, with some development of magenta anthocyanin which is chiefly confined to the epidermis. Extracts gave a yellow-green lead precipitate. Pigment was prepared from entire flowers only and presumably contains ivory, yellow and magenta.

Magenta. (Fig. 7.) Magenta chiefly confined to epidermis; inner tissues contain ivory and there is a yellow patch on the lower lip. Extracts gave a deep green lead precipitate, some pigment apparently remaining in solution in the acetate, forming a deep green solution which is dichroic, red by transmitted and green by reflected light. Pigment was prepared separately from both upper and lower lips. The upper presumably contains magenta and ivory, the lower, yellow in addition.

Crimson. (Fig. 8.) Lips of the corolla are crimson and the tube magenta. It is at present uncertain whether the crimson colour is due to the presence of both yellow and magenta in the cells or to a distinct crimson pigment. Pigment was prepared from entire flowers only.

Rose doré. (Fig. 15.) Corolla contains a "red" anthocyanin [Wheldale 1909, 2]. The inner tissues, ivory, and there is a yellow patch on the lower lip. Extracts gave a reddish-green lead precipitate. Pigment was prepared from entire flowers only.

Bronze. (Fig. 17.) Bears the same relation to rose doré as crimson to magenta and it is again uncertain whether the colour is due to a mixture or to a separate pigment. Extracts gave a deep-red lead precipitate. Pigment was prepared from entire flowers only.

Methods of purification were first carried out on pigment obtained in 1911. As mentioned above, tinged ivory, magenta and crimson flowers had

then been extracted together. The crude pigment which presumably contained ivory, yellow and magenta was finely powdered thoroughly dried, placed in a Soxhlet thimble, and extracted first with warm ether in which the ivory and yellow pigments are soluble, though not readily. The anthocyanins, both magenta and red, are insoluble in ether.

From the ether extract two pigments were obtained by fractional crystallisation from alcohol and ethyl acetate. The less soluble pigment was taken to be ivory, the more soluble to be yellow. It is doubtful whether either pigment was obtained in the pure state.

Ivory pigment. This is readily soluble in alcohol and acetic acid, with more difficulty in ether and ethyl acetate, very slightly soluble in cold, more so in hot water; insoluble in chloroform and benzene. It crystallises from dilute alcohol in plates; M.P. 338°. It underwent combustion with difficulty in oxygen and did not give constant results for carbon. Tested by Zeisel's method for a methoxyl group, some silver iodide was obtained but only in such quantity as to indicate impurity. An acetyl derivative was prepared by boiling with acetic anhydride and pouring the product into sodium acetate solution. The product was purified with difficulty by crystallisation from ethyl acetate. The final product was pure white and crystallised in glistening needles; M.P. 182°. The combustion results were as follows:

C = 63.19 %	H = 4.30 %
63.21	4.22
62.95	4.31

Of the known flavones, the ivory pigment bears most resemblance to apigenin in properties and acetyl derivative. Opinion is reserved as to its identity until further analyses have been made, especially with the ivory pigment (free from yellow) obtained from the upper lip of the ivory variety.

Yellow pigment. Crystallises in plates from dilute alcohol but was not obtained in the pure state; M.P. 290°–300°.

Magenta pigment. After extraction with ether for several weeks, the magenta pigment was obtained free from yellow. It crystallises, but not well, from a mixture of alcohol and ethyl acetate. It decomposes without melting when heated to 340°.

Other results obtained with magenta and yellow are reserved until further work has been done.

The crude pigment was extracted in the laboratory of the John Innes Horticultural Institution, Merton Park, Surrey. Large numbers of plants, the offspring of varieties originally used in breeding experiments, were grown

in the garden of the Institution. The yield of pigment was small and was not ascertained quantitatively because of the labour involved in weighing the flowers.

The work of purification and analysis, as far as it has gone, was carried out in the Biochemical Laboratory of the Institute of Physiology, University College, London. I am much indebted to Dr Aders Plimmer for kind help and suggestions.

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