

# The florachromes: their constitution and optical behaviour

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## 1. Introduction

The nature of the pigments or colouring matters which are present in the petals of flowers and which are responsible for the hues which they exhibit is a subject of great interest. An important first step towards the determination of their nature makes use of a very simple procedure, which is to view the flowers held in bright sunlight through a pocket spectroscope. The spectrum of the light emerging through the petals or diffusely reflected by them when thus examined exhibits characteristic features. Certain regions in the spectrum are observed to be much weakened or even totally extinguished, while other regions do not exhibit any noticeable diminution in their intensity. The spectral regions which exhibit the absorption are indicative of the nature of the floral pigments, while those regions of the spectrum which escape such absorption and are present in the emergent diffused light determine the observed colour of the petals.

Another procedure for the study of the floral pigments which suggests itself is their extraction from the petals with the aid of suitable solvents and the spectroscopic examination of the solutions thus obtained. In following this procedure, it is obviously of the highest importance that the solvent employed does not produce any noticeable change in the constitution or optical behaviour of the pigment. Whether such a change is actually produced becomes evident on a comparison of the colour of the petals and their spectroscopic behaviour *in vivo* with the colour and spectroscopic behaviour of the solution of the extracted material. In most cases, the pigments are so firmly embedded in the structure of the petals that an attempt to extract them by placing them in a vessel containing water or methyl alcohol or ethyl alcohol, followed by vigorous shaking produces no observable result. Much more successful is the use of acetone as a solvent. In numerous cases, immersion of the petals in acetone and vigorous shaking results in a nearly complete extraction of the colouring matter. Other cases which resist such simple treatment may be successfully dealt by first grinding the petals in an agate mortar and adding a little acetone to the pulp thus obtained. Filtering the acetone extract yields a clear solution which can then be spectroscopically examined.

There are several advantages in thus studying the floral extracts instead of the flowers themselves *in vivo*. By regulating the quantity of the petals used for the

extraction and the volume of the solvent employed, the concentration of the solution can be adjusted to that best adapted for spectroscopic examination. The length of the absorbing column and the intensity of the light-beam can also be chosen suitably. Further, it is only by using the extracted material in solution that it is possible to obtain spectrophotometric records of the transmitted light with the instruments generally available. Still another advantage is that the extraction can be carried out in successive steps, thereby enabling it to reveal whether we are concerned only with one pigment or with more pigments than one differing in their solubility in various liquids.

## 2. The florachromes

Studies by the author adopting the techniques described above resulted in the discovery that there are two distinct categories or species of floral pigment characterised by quite different spectroscopic behaviours. In the publication announcing this discovery, the author ventured to name these categories as florachrome A and florachrome B respectively. The presence of one or of the other pigment, in greater or less quantity, is found to be responsible for the observed floral colours in numerous cases. The simultaneous presence of the two categories of pigment in diverse proportions is also found to be the explanation of many other floral colours met with in practice.

The case with which the spectral behaviour of individual flowers *in vivo* can be recognised would naturally depend on the particular circumstances of the case and especially on the quantity of the pigment present in the petals. It is evident that either too little or too much of the pigment would be unfavourable for a direct observation of the absorption spectrum in the light emerging from or diffused by the petals. It is therefore useful to mention some cases in which the characteristics of the florachromes are exhibited with the maximum clarity by the flowers themselves.

The example chosen for special mention here is *Clitoria ternata*, which bears flowers of some size. In one variety, these exhibit wide margins of a bright blue colour. The plant belongs to the botanical class *Leguminosae* and is a hardy perennial by nature, though it may be raised and grown as an annual from seeds. Commonly known as the Butterfly-Pea, it is to be found in many Indian gardens. Viewed through a spectroscope in bright light, the blue areas exhibit a characteristic three-banded spectrum, the most striking feature of which is an intense absorption at about  $630\text{ m}\mu$ , followed by another distinctly less intense absorption at about  $575\text{ m}\mu$ , and a very weak and diffuse band at about  $530\text{ m}\mu$ . As the result of the first two absorptions, the entire spectrum between  $630$  and  $575\text{ m}\mu$  is seen with much reduced intensity. It is thus evident that the observed bright blue colour of the flower is a consequence of the reduced intensity of the orange and the yellow regions in the spectrum of the light diffused by its petals.

The pigment responsible for the observed blue colour of *Clitoria ternata* is readily extracted by simple immersion of its petals in acetone. A tube 5 cm long containing the blue extract shows the same features in its spectrum as the petals themselves, but more conspicuously.

A particularly fine example of the absorption spectrum of florachrome B exhibited with the maximum of clarity is furnished by the terrestrial or ground orchid known botanically as *Spathoglottis plicata*. This is a very hardy plant which may be grown in pots like any other garden plant. It flowers profusely at all seasons of the year, bearing racemes of flowers on erect spikes of great length. The material is therefore available in quantity for examination at all times. The petals exhibit a purplish-red colour, and the spectrum exhibits a three-banded structure but this differs from that of florachrome A. The most intense of the three absorption bands is located around 590 m $\mu$  and completely obscures the yellow region in the spectrum. A second band of distinctly lesser intensity is located in the green around 540 m $\mu$ . A third band which is rather faint appears in the blue-green region of the spectrum around 510 m $\mu$ . The region of still smaller wavelengths exhibits no noticeable absorption. It is evident that the extinction of the yellow and the weakening of the green regions in the spectrum are responsible for the observed colour of the flower.

The floral pigment is readily extracted from the petals of the orchid *Spathoglottis plicata* by immersing them in a beaker containing acetone. The extract exhibits a purplish-red colour and the spectrum of the light transmitted by it exhibits features similar to those of the flowers *in vivo*. But there is an observable change in the relative intensities of the bands. The band in the green appears somewhat more intense than the band in the yellow as seen in the spectrum of the acetone extract.

Spectrophotometric records of the absorption spectra of the acetone extracts from *Clitoria ternata* and from *Spathoglottis plicata* are reproduced respectively as figures 1 and 2. The wavelengths shown were roughly estimated from their positions on the recorded chart, and lay no claim to precision. But the reproduced curves should be sufficient to convince any one that two florachromes actually exist exhibiting different spectrophotometric behaviours.

As an example of a flower of which the observed colour is due to the co-existence of both the florachromes, we may cite the case of the blue iris (*Iris germanica*). The curiously shaped flowers of this plant have petals of a purplish-blue colour. But this is not a deep or saturated colour, and it is therefore not surprising that spectroscopic examination of the light diffused by the petals shows only weak and ill-defined bands. Examination of the light transmitted through two petals held together however exhibits the bands more distinctly. In particular, the absorption band in the yellow region can be seen, and the presence of an absorption band in the red region is also evident. Immersion of the petals of the blue iris in acetone results in the immediate extraction of a solution which exhibits a purplish-red colour, which the spectroscope reveals as due for the most

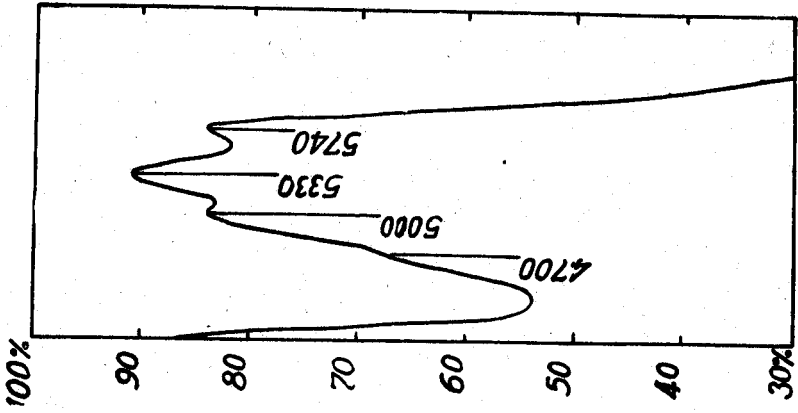


Figure 2. Absorption spectrum of *Spathoglottis plicata*.

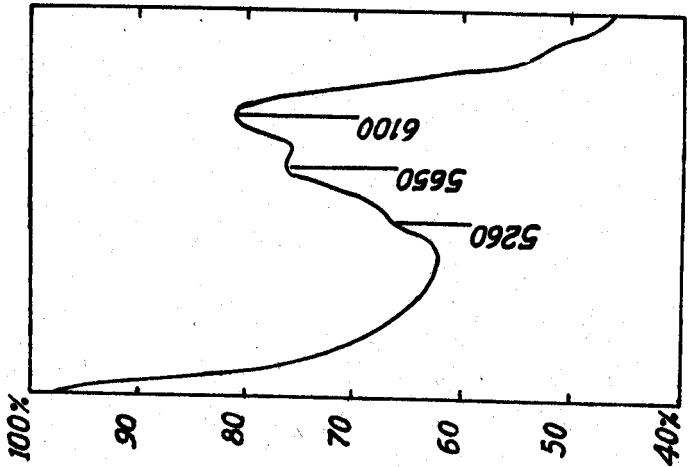


Figure 1. Absorption spectrum of *Clitoria ternata*.

part to the presence of florachrome B. The residue left after the acetone extraction when stirred in water yields a blue solution which the spectroscope reveals as due to the presence of florachrome A. Similar results have been obtained with many other flowers. In these cases, the acetone extract from the petals either in the natural state or after being ground to a pulp in an agate mortar is a purplish-red solution exhibiting the absorption spectrum of florachrome B, while the residue when stirred up with water gives a blue solution showing the absorption spectrum of florachrome A.

We now proceed to consider the explanation of the spectra of the two florachromes in terms of their chemical constitution. Though the spectra are quite different, they exhibit a measure of similarity which indicates that they are substances of the same general nature which differ only in the specific configuration of the centres responsible for the absorption of the light by the molecules of the pigment. The triplet band observed in both the cases is readily interpreted as due to the electronic absorption by a CO group in the structure giving the most intense line of the triplet, while its two other weaker and more diffuse companions represent its combinations with vibrational transitions. The three possible structures of a molecule of the pigment are represented as (a), (b) and (c) respectively in figure 3. If (a) and (b) are superposed on each other, we have an absorbing centre where the CO group remains in the same position throughout. On the other hand, if (b) and (c) are superposed, the CO occupies two positions alternately. The electronic energy of the CO group would obviously be

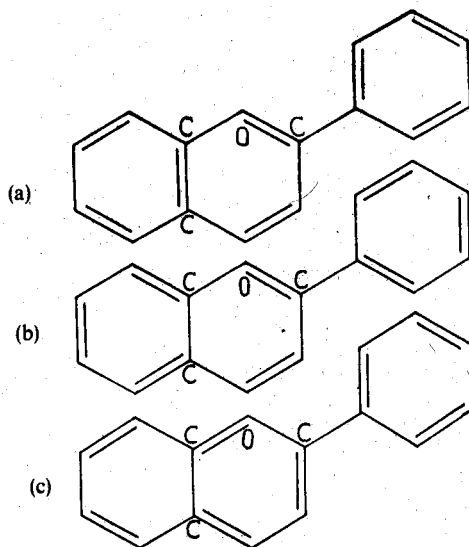


Figure 3. Chemical constitution of the florachromes.

different in the two cases. We may identify the superposition of (a) and (b) as giving us florachrome A, and the superposition of (b) and (c) as giving florachrome B, which has the higher electronic energy.

The spectrophotometric records reproduced as figures 1 and 2 were made in the Instruments Section of the Indian Institute of Science to whose authorities the thanks of the author are due.