FLORAL COLOURS AND THEIR ORIGINS

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ABSTRACT

A new orientation is given to the subject of floral colours by the author's discovery that these colours may be placed into two distinct spectral categories, which have been designated by him respectively as the spectrum of florochrome A and of florochrome B. Typical of these two categories are the colours of Delphinium ajacis (larkspur) in the blue and pink varieties respectively, the former showing the spectrum of florochrome A and the latter that of florochrome B. As a general rule, all blue flowers exhibit the spectrum of florochrome A which consists of three distinct and clearly separated bands of absorption appearing respectively in the red at 630 mμ, in the yellow at 580 mμ and in the green at 540 mμ. The spectrum of florochrome B also consists of three distinct bands of absorption, but these now appear in the orange-yellow at 590 mμ, in the green at 545 mμ and in the blue-green at 505 mμ. Spectra exhibiting these features are reproduced with the paper. Their explanation is discussed and it is shown that they owe their origin to an electronic absorption frequency located at the first of the three bands combining with vibrational transitions, the oscillator being the CO group present in the structure of the florochrome.

1. INTRODUCTION

The colours which flowers exhibit when held in bright sunlight represent the physiological perception of the radiation which emerges from their petals after suffering diffusion and absorption within their substance. The spectral characters of the light emerging from the petals and the physiological characteristics of human vision which determine the sensation produced by the composite radiation have alike to be taken into consideration. Any discussion of floral colours which ignores either of those determining factors, would not only be futile but may also lead one to erroneous conclusions.

The processes of absorption and diffusion suffered by light in its passage through the petals and its emergence therefrom are determined by the nature

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and condition of the materials present within the living substance of the flower. It follows that no inference regarding these materials can be valid or sustainable unless it is based on their optical and spectroscopic behaviours observed in vivo. If one attempts to extract from the flowers the materials responsible for the observed colours, it is necessary to use processes which do not fundamentally alter their nature. In particular, if a solvent is used for the extraction, it should be such that it does not produce an observable change in the optical properties or spectroscopic behaviour of the pigment.

When one examines the voluminous literature in which the subject of floral colours and their origin has been dealt with, one fails to find any recognition of the fundamental considerations set forth in the two preceding paragraphs. The identification of the materials responsible for the colours of flowers as “anthocyanins” and the explanations put forward for the great differences in colour exhibited by various flowers are thereby rendered highly dubious. In treatises on plant biochemistry, the anthocyanins are placed in the general category of “flavonoids”. The parent substance which gives the name to this group of organic compounds is flavone and it is a significant fact that this substance is itself a colourless solid which melts at 97°C. How such a substance can be transformed into the brilliantly coloured floral pigments merely by hydroxylation and combination with glucose or other sugar residues is a mystery which one seeks in vain for an elucidation in the chemical literature.

From what has been stated above, it is evident that the origin of the vivid colours exhibited by many flowers in vivo has so far remained an unsolved problem. The present investigation addresses itself to finding an answer to the highly interesting questions arising in this field.

2. FLORACHROME A

A new orientation is given to the subject of floral colours by the author’s discovery that the spectral character of the light emerging from the petals of flowers is related to the observable hue of the flowers in a highly characteristic fashion. We may illustrate this finding by a reference to some cases studied by him in earlier years.

The well-known avenue tree known botanically as Jacaranda mimosifolia bears numerous clusters of bluish-purple bell-shaped flowers. The climbing plant Thunbergia grandiflora bears large and widely expanded flowers of a pale blue colour. The well-known shrub Plumbago capensis commonly used as a hedge plant bears clusters of small flowers which are azure-blue
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in colour. Examination through a pocket spectrooscope reveals that the spectra of the light diffused by or transmitted through the petals in all these three cases are very similar, viz., three distinct absorption bands well-separated from each other, one in the red region of the spectrum at about 630 m\(\mu\), another in the yellow region at about 580 m\(\mu\) and a third in the green at about 540 m\(\mu\). Holding two petals of the Thunbergia flowers together and viewing the light transmitted through them with the spectroscope, all the three bands are very clearly seen, the bands in the red and in the yellow being very conspicuous and the band in the green much less so. The absorption by the flowers of Plumbago capensis is very weak, but the bands are well seen when a bunch of the flowers held together is viewed through the spectroscope. It should be mentioned that in none of the three cases is there any sensible weakening of the blue region of the spectrum.

We next consider three other cases in which the observed colours are more vivid. The first is of the tree Solanum grandiflorum which bears large flowers of a deep bluish-mauve colour. The next is the climbing plant Clitoria ternata (also known as the Butterfly Pea), which bears curiously shaped flowers which in one variety are deep blue in colour. The third case is that of the plant Meynia erecta, which bears funnel-shaped open-mouthed flowers. One variety of this shrub bears flowers which are a deep purplish-violet in hue. In all three cases, the absorption spectrum as viewed either by the transmitted or by diffused light exhibits three bands which appear respectively in the red, yellow and green regions of the spectrum. As is to be expected, the bands as seen with these flowers are more conspicuous than with the less vividly coloured flowers mentioned earlier.

It is unnecessary here to list more flowers displaying absorption spectra of the same kind, as they will be referred to later on. The examples cited are sufficient to justify the statement that the origin of the colours displayed is the presence in the flowers of a material with the stated spectral behaviour, viz., three distinct absorption bands appearing respectively in the red, yellow and green regions of the spectrum. The three bands in its absorption spectrum are very clearly seen in Fig. 1 in Plate V. Figure 1 (a) is the comparison spectrum of white light, while Fig. 1 (b) and Fig. 1 (c) recorded with different exposures are absorption spectra of an aqueous extract from the blue larkspur obtained in the manner later to be explained.

3. Florachrome B

The author's study of floral colours in other cases led to the discovery of another material which will be here designated as florachrome B which
also exhibits three absorption bands, but these now appear respectively in the orange-yellow, green and blue-green regions of the spectrum. A particularly fine example of a flower exhibiting the three absorption bands in the stated region is the terrestrial orchid known botanically as *Spathoglottis plicata*. This orchid is very hardy and may be grown in pots like any other plant. It has elongated leaves and bears a great many racemes of flowers on erect spikes of considerable length. Being always in bloom, it is available at all times for observation of the very striking nature of the absorption spectra of the flowers. Each flower has five petals which are well-separated from each other, so that any one of them can be viewed without being detached from the rest. The colour of the petals is a vivid purplish-red and the absorption spectrum which gives rise to this colour is most conveniently studied by holding the flower in a strong light and viewing the selected petal through a pocket spectroscope held not too far away from it. The spectrum of the light diffused by the petal as thus observed exhibits a nearly complete extinction of the orange-yellow region of the spectrum from 580 mp to 600 mp. Another dark band is conspicuous between 540 mp and 550 mp, but it is not one of complete absorption. A third and comparatively faint absorption is visible between 500 mp and 510 mp. The red region of the spectrum is seen with full strength. There is also no observable weakening of the blue region of the spectrum. Figure 2 in Plate V reproduces the absorption spectra of a petal of *Spathoglottis plicata* recorded by placing the petal before the slit of a wavelength spectrometer. Figure 2 (a) and Fig. 2 (b) reproduce the spectra thus obtained with different exposures, while Fig. 2 (c) is the comparison spectrum of white light.

4. SOME COLOURFUL GARDEN PLANTS

The role played by the two florachromes in the production of the observed colours of flowers is very well illustrated by the case of *larkspur*. This is a favourite garden plant belonging to the botanical category *Ranunculaceae*. Gardening books describe the larkspur as “a very showy annual, freely producing spikes of beautiful flowers, available in blue, lilac, purple, white and pink shades”. The plant grows well at Bangalore and the flowers which are available fall into two clearly defined groups. One of the groups may be described as exhibiting blues and bluish-purples of various shades. The other group exhibits a pink colour ranging from a bright to a very light shade of that hue. Spectroscopic examination of the light diffused by the petals of the first group reveals that they owe their colour to florachrome A. Likewise, it is found that the flowers of the other group owe their colour to florachrome B. In either case, the deeper the colour, the more pronounced
are the absorption bands, thereby indicating that the intensity of the perceived colour is determined by the quantity of florachrome present in the petals.

The spectra of florachrome A and florachrome B are vividly exhibited by the flowers of *Cineraria* which is classed botanically as belonging to the *Compositae*. Gardening treatises describe the *Cineraria* as "beautiful pot plants which are showy with their large luxuriant leaves surmounted by immense panicles of magnificent flowers of most brilliant colours; the blooms last for quite a long time, nearly a month." The colours are observed to fall into two groups, one group ranging between a light blue and a dark violet, and another from a light to a deep purplish-red. Spectroscopic examination reveals that the flowers of the first group exhibit the spectrum of florachrome A and those of the second exhibit the spectrum of florachrome B. As is to be expected in view of the brilliance of the colours, the absorption bands are highly pronounced and are indeed very striking.

*Delphinium* is another garden plant which is of great beauty. Its flowers appear arranged along spikes, the colours ranging from a delicate blue to a dark purple or violet. In every case, the petals exhibit the spectrum of florachrome A, the strength of the absorption bands increasing with the depth of the colour exhibited by the flower.

*Iris Germanica*, also known as Flag Iris, has sword-like leaves and bears flowers on erect stalks. It is a hardy and vigorous plant and the flowers which are curiously constructed have gorgeous colours, one of the most attractive varieties being that in which flowers are purplish-blue. The petals of the flower are very thin, and hence it is desirable to hold two of them together and examine the light transmitted by them to observe the absorption bands. Here again, we notice three well-separated bands and recognise the spectrum as that of florachrome A.

*Petrea volubilis* is classed botanically amongst *Verbenaceae*. It is a climbing plant which requires a support on which it can spread out. It bears purple star-like flowers in large elegant wreath-like sprays. Racemes of flowers crowd the plant covering it with a mass of purple-blue colour. Holding a bunch of the flowers in sunlight and comparing the spectrum of the light diffused by their petals and by a sheet of white card held below, it becomes evident that there is practically complete extinction of the yellow part of the spectrum, besides a noticeable weakening of the red region. The
blue sector, on the other hand, shows up quite strongly. It is evident that
the bluish-purple colour exhibited by the sprays of Petrea volubilis is due
to florachrome A present in the petals.

Asters belong to the botanical group known as the Compositae. They
bear very showy flowers, exhibiting numerous petals arranged around a
common centre. The varieties which are grown extensively at Bangalore
as a commercial proposition for export to other parts of India exhibit brilliant
colours. These flowers are readily available for study. They show a great
range of hues. A considerable proportion exhibit colours ranging from a
light pink to a deep red or crimson. There is another group of flowers whose
colours range from a light bluish-purple to darker shades of purple and to a
deep colour which may be termed as violet. Holding the flowers in sunlight
and viewing them through a pocket spectroscope, their optical behaviour
may be compared with that of a white card below, and several remarkable
facts come to notice. Particularly interesting is that there is no noticeable
difference between the two groups of flowers in respect of the blue sector of
the spectrum which is conspicuous in the spectrum of the diffused light in
both cases. But the two groups differ markedly in other respects. With the
blue flowers, the red sector of the spectrum is much weakened and it also
shows an indication of being divided into two by an absorption band running
through it. A dark absorption band also covers the yellow sector while
the green remains fairly bright. On the other hand, the flowers which range
in colour from pink to crimson show the red sector of the spectrum in full
strength. But the yellow and green sectors are both much weakened. These
features are readily understood when it is recognised that the colours of the
blue asters are ascribable to florachrome A and those of the red asters to
florachrome B.

5. The Isolation of the Floral Pigments

Treatment by immersion either in dilute acids or in dilute alkalies has
a destructive effect on the florachromes. This becomes evident when, for
eexample, the blue petal of Delphinium is immersed in dilute hydrochloric
acid. The petal changes to a bright red colour, and examination through a
pocket spectroscope reveals that the discrete absorption bands due to
florachrome A have disappeared. The discoloured petal shows a spectrum
in which the green sector is nearly extinguished and the yellow is weakened,
while the red sector appears in full strength. Immersion of a petal of Del-
phinimum in dilute ammonia results in its changing colour from blue to a
greenish-blue. The discrete bands disappear and the spectral regions of
red and yellow appear with greatly reduced intensities, while the green continues to be in full strength. The purplish-red petals of *Spathoglottis plicata* which exhibit the absorption spectrum of florochrome B change colour to an orange-red on immersion in dilute HCl. The discrete absorption bands disappear and the green sector of the spectrum is nearly extinguished, while the yellow and red sectors appear with normal strength. The petal of the same orchid when treated with dilute ammonia changes colour from a purplish-red to a greenish-blue. The discrete absorption bands disappear and while the green of the spectrum retains its full strength in the transmitted light, the red and yellow sectors become extremely weak. Examples of this kind may be indefinitely multiplied to show that the effect of acids and alkalis on flower petals is fundamentally to alter their spectroscopic behaviour.

In view of the facts stated above, it is scarcely possible to accept the view that the anthocyanins prepared by the methods usually adopted by organic chemists are the pigments responsible for the colours exhibited by flowers *in vivo*. It is, of course, of great interest to isolate these pigments from the flowers so that their behaviour can be studied *in vitro*. But for these purposes, as has already been stated in the introduction, it is necessary to adopt methods which do not fundamentally alter the optical properties and spectroscopic behaviour of the pigments. Such methods are indeed available.

In many cases, it is possible to obtain an extract of the floral pigment merely by grinding a few moist petals to a fine paste in an agate mortar and then adding a little water and filtering the product through a loose plug of cotton-wool into the observation cell. The aqueous solution of the floral pigment thus obtained is often of considerable strength and may have to be diluted to obtain the desired degree of transparency. Using a longer or shorter cell to hold the extract also enables the strength of the transmitted light to be controlled.

A second and generally useful method is to place a sufficient quantity of the flower petals in a flask and to pour in some acetone to cover the petals. Vigorous shaking results in the pigment being extracted by the acetone and a solution of adequate strength being obtained which can be transferred to an observation cell. In those cases where the extraction by the acetone proceeds too slowly, it can be speeded up by warming the flask, or if necessary by heating it till the acetone begins to boil.

The extraction of the floral pigment by the method first described is evidently to be preferred as it is a purely physical process which would not
result in any alteration of the structure and properties of the florachrome. Indeed, the aqueous solutions obtained by that process show a spectroscopic behaviour similar to that of the petals themselves. It should be mentioned however that the extract may include some colloidal material which has passed through the filtering plug of cotton-wool and diminishes its transparency to light.

The extraction of the pigment with the aid of acetone is generally both quick and convenient, and if the petals used are quite clean, the solution obtained is free from colloidal matter and exhibits the maximum transparency. The method has the advantage that by adjusting the quantity of material put in and the volume of acetone made use of, it is possible to obtain extracts which show the absorption bands in satisfactory strength, viz., more petals and less acetone for weakly pigmented flowers, and fewer petals and more acetone for strongly pigmented ones. The spectroscopic behaviour of the pigment can then be observed in more satisfactory conditions than with the petals themselves. It should be mentioned, however, that the acetone extracts do not always reproduce exactly the spectral behaviour of the petals. It is noticed that the relative intensities of the absorption bands are altered, and their positions may also exhibit observable shifts.

6. VISUAL PERCEPTION OF COLOUR

We proceed to consider how the observed colours of the flowers are related to the spectral characteristics of the florachromes. A few remarks are necessary here regarding the composition of white light and the visual effects produced by the different parts of its spectrum. The relative luminosities of the various regions of the spectrum depend to a great extent on the absolute level of brightness at which the observations are made. At the fairly high levels with which we are here concerned, the most luminous part of the spectrum is the yellow sector, the limits of which may be indicated as from 560 mμ to 600 mμ. Its great influence on our visual perceptions is readily demonstrated by viewing a brilliantly illuminated area of white light through a filter of glass doped with neodymium oxide which cuts out the spectral region between 570 mμ and 600 mμ completely, but has very little effect on the rest of the spectrum. An enormous reduction of visual brightness is found to result from holding such a filter before the eye. It is a consequence of this characteristic of our visual sensations is that the exclusion of the yellow sector in the spectrum by absorption is necessary for the other parts
of the spectrum to manifest themselves strongly in the perception of colour. Indeed, extensive studies demonstrate that no object can appear brilliantly green or blue or red unless the yellow of the spectrum has been much weakened or totally excluded from appearing by absorption.

In view of what has been stated, the role of the florachromes in the production of visible colour is readily understood. Both florachrome A and florachrome B exercise a strong absorption on the yellow sector of the spectrum. Further, florachrome A also exercises a noteworthy absorption in the red sector of the spectrum, while florachrome B does not exhibit such absorption, but on the other hand has an absorption of significant strength in the green sector of the spectrum. A large reduction in the strength of the yellow sector and the enfeebling of the red and green sectors would enable the blue sector to manifest itself and by masking the rest of the spectrum to become the dominant sensation. *Per contra*, a large reduction in the strength of the yellow and green sectors would enable the red sector to mask the rest of the spectrum and become the dominant colour. For such effects to be manifested to the maximum extent respectively by florachrome A and by florachrome B, it is necessary that they should be present in the petals in adequate quantities.

That the absorption of light appearing in the yellow sector of the spectrum plays a highly important role in the perception of floral colours becomes evident when a flower is viewed through a neodymium glass filter. In all cases where the absorption of the yellow by the flower itself is incomplete, the introduction of the filter before the observer’s eye completes the process and the result is an enrichment of the observed colour. For example, a pink larkspur viewed through the filter appears red. Such enrichment of the observed colour becomes a spectacular phenomenon when a tree enveloped by a mass of its flowers is viewed through the neodymium filter. Two examples of such trees may be mentioned here. One of them is *Milletia ovalifolia*, which in the flowering season bears great masses of tiny flowers of a lilac colour. They make the tree a conspicuous object even from a distance. The interposition of the filter results in a very striking intensification of the massed colour of the flower-laden branches when then appear of a reddish-purple colour. Another case is that of *Tabebuia guayacan* which is one of the loveliest of ornamental trees, bearing lilac-coloured flowers in the form of bunches or bouquets. Seen through the filter, these flowers turn to a rich red hue. Similar effects are exhibited by all flowers in which florachrome B functions but is not present in sufficient quantity to produce its maximum effect. Likewise, in all cases where florachrome A functions
but the resulting colour is not very intense, the introduction of the filter produces a readily observable effect.

7. **THE SPECTRA OF THE FLORACHROMES**

It may be stated as a general rule that flowers which exhibit a blue colour contain florachrome A which is responsible for that colour being perceived. The more intense the colour, the greater is the quantity of the florachrome present, as may be demonstrated by the methods of extraction described above. In such cases, also, the three bands of absorption in the spectrum which are characteristic of florachrome A are conspicuously visible in the light diffused by the petals. Their positions can be approximately determined by observation through a direct vision spectroscope provided with a wavelength scale. A few such measurements are listed below:

- **Blue larkspur**  
  630 m\(\mu\), 580 m\(\mu\), 540 m\(\mu\)

- **Delphinium**  
  630 m\(\mu\), 580 m\(\mu\), 540 m\(\mu\)

- **Solanum grandiflorum**  
  630 m\(\mu\), 580 m\(\mu\), 540 m\(\mu\)

- **Meyneia erecta**  
  630 m\(\mu\), 580 m\(\mu\), 540 m\(\mu\)

It will be noticed that the positions of the bands in the four cases listed are not observably different, and this is clear evidence that we are concerned with a material with specific properties appearing in flowers of totally different origin. In all four cases, the first band appearing in the red is the most conspicuous, while *per contra* the third band appearing in the green is weak and diffuse. These observations suggest that the band of the greatest wavelength represents the principal absorption of the material and that the other two bands arise from combinations of the principal electronic frequency with vibrational transitions. The differences in the wave-number of the successive bands are about the same and may be put as 1325. This may be regarded as the vibration frequency of the florachrome A.

As has already been stated, the complete spectrum of florachrome B is shown with admirable strength and sharpness by the terrestrial orchid *Spathoglottis plicata*. It is also shown vividly by several orchids of a purple colour and sundry other plants which have come under observation by the author. The bands of florachrome B are shown with special intensity by the flowers of *Cineraria* which display a purplish-red colour. The first two of the three bands can also be recognised in the spectrum of the pink larkspurs. They are as noted below:
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Spathoglottis plicata . . . 590 mµ, 545 mµ, 505 mµ
Cineraria (purplish-red) . . . 600 mµ, 550 mµ, 510 mµ
Larkspur (pink) — 590 mµ, 545 mµ.

As in the case of florachrome A, the first band in florachrome B which here appears in the orange-yellow region of the spectrum is the most conspicuous, while, per contra, the third band appearing in the green-blue region is weak and diffuse. The same suggestions as those made above for florachrome A can also be put forward regarding the relationships between the three bands exhibited by florachrome B. The differences in the wavenumber of the successive bands are about the same and may be put as 1425, which is the vibration frequency of florachrome B.

Since the absorption bands of florachrome B appear in the yellow and green sectors of the spectrum, these sectors appear with much reduced intensity, and as already mentioned, the result is that the red sector of the spectrum becomes dominant and determines the observed colour of the flower. The quantity of the florachrome B present determines the strength, in other words the degree of saturation of that colour. This is well-illustrated by the case of Spathoglottis plicata, the commonest variety of which bears flowers which are of a vivid purplish-red colour. There is another variety of the same plant, the flowers of which are of a deep purplish-crimson colour. Spectroscopic examination of these flowers shows the same absorption bands but intensified in strength, so much so that the green and yellow are almost completely extinguished. It should be noted that neither variety of Spathoglottis plicata shows any observable weakening of the blue sector of the spectrum. It is therefore not surprising that the colour of these flowers is not a pure red or crimson but exhibits a purplish hue.

Many flowers which appear pink or red or crimson show a strong absorption of light in the yellow and green parts of the spectrum. That such absorption determines the observed colour is evident from the fact that it is deeper in the varieties which exhibit the absorption most strongly. The three bands which characterise florachrome B are not usually seen as distinct and separate regions in the absorption spectra of the petals of such flowers. We are nevertheless justified in assuming that the florachrome is present and is responsible for the observed colour. Red or crimson roses may be mentioned as an example of such a situation. Definite proof that we are concerned in such cases with florachrome B is forthcoming when the spectrum of white light transmitted through a column of the acetone extract of the
floral pigment is examined. The presence of distinct bands of absorption in the yellow, green and green-blue regions of the spectrum then becomes evident to observation.

8. THE STRUCTURE OF THE FLORACHROMES

The configuration of the anthocyanin molecules envisaged by the organic chemists is based on a grouping of atoms similar to the aromatic hydrocarbon naphthalene joined by a single bond to another grouping similar to benzene. There is however a noteworthy difference, viz., that instead of ten carbon atoms arranged round the periphery as in naphthalene, we have only nine carbon atoms and one of oxygen. Since oxygen is divalent, it is not possible to have a regular succession of alternating single and double bonds around the periphery as in naphthalene. This disturbance in the order characteristic of an aromatic molecule results in a profound modification of the structure and optical behaviour of the grouping. One of the four valences of carbon atom adjacent to an oxygen atom is engaged in the formation of what may be described as a pseudo-double bond between this carbon atom and the adjacent oxygen. This pseudo-bond is necessarily much weaker than the double bond appearing in a carboxyl group, and as a consequence, its characteristic absorption is thereby shifted and falls within the range of the visible spectrum. The observable consequence is the production of visible colour. The vibration
Fig. 1. Absorption Spectra of Florachrome A.

Fig. 2. Absorption Spectra of Florachrome B.
frequency of the pseudo-double bond will also be less than for a carboxyl group which is 1800 in wave-numbers. Such lowering of the vibration frequency will manifest itself in a closer approach of the vibration bands to the characteristic electronic frequency. As the oxygen atom is located between two carbon atoms occupying different positions in the group, there are two distinct situations of the kind described above which are possible. These are shown separately in the diagram in the text. One of the two situations corresponds to florachrome A and the other to florachrome B. In the latter case, the pseudo-double bond lies closer to the centre of the group and therefore presumably results in a somewhat stronger binding.