THE FLORACHROMES: THEIR CHEMICAL NATURE AND SPECTROSCOPIC BEHAVIOUR

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Abstract

This memoir presents detailed studies of the two florachromes discovered by the author and shown by him to be responsible for the colours exhibited by a great many flowers, the two florachromes being present in the petals either separately or together as the case may be. Their characteristic absorption spectra have been photographed and reproduced and also represented as spectrophotometric records of their aqueous solutions and of the acetone extracts of the floral pigments. In appropriate circumstances, the florachromes in solution are found to be quite stable and continue to exhibit their characteristic spectroscopic behaviours permanently. The interesting discovery is recorded that Florachrome B is present as the colouring matter of the fleshy leaves of the shrub *Setcreasia purpurea* and can be readily obtained therefrom. The relation between the structure of the florachromes and of the well-known organic compound flavone which is itself a colourless solid is discussed and an explanation is given why there are two florachromes with distinct spectroscopic behaviours.

1. Introduction

In the literature of organic chemistry and of plant biochemistry, the substances known as the anthocyanins are claimed to be the pigments responsible for the varied colours (other than yellow) exhibited by the petals of flowers in the plant world. The chemical nature of the anthocyanins has been the subject of numerous investigations and it has been shown that they are glycosides of the so-called anthocyanidins, six of which were recognised as being of common occurrence and some ten others have since been added to the list which are less common. The number of anthocyanins derived from the anthocyanidins that can be distinguished from each other by chromatographic or other tests is, of course, much larger.

What concerns us here are the colours exhibited by the flower petals *in vivo*. Since the floral pigments are water-soluble, they can be extracted

from the petals without change and it is found that the aqueous extracts exhibit the same features in their absorption spectra as the petals themselves. But when the aqueous extracts are acidified, drastic changes are observed both in the colour of the extracts and in their absorption spectra, indicating that we are no longer dealing with the same material.

It is well known that the preparation of the anthocyanins involves the preliminary treatment of the floral material with acids. The anthocyanins for this reason are often formulated as oxonium salts or alternatively as carbonium salts. In either case, it is evident that they cannot be identified with the floral pigments present *in vivo* but must be regarded as artifacts resulting from the processes adopted in their preparation. That the colours of flowers cannot be explained in terms of the spectroscopic behaviour of anthocyanins follows as a natural consequence of what has been stated. This is indeed clear from the published data for both the anthocyanins and the anthocyanidins which indicate that their behaviour (as studied in methanol/HCl solution) in the visible region of the spectrum is of the same general nature in all cases, *viz.*, a continuous absorption which extends from the violet end of the spectrum towards greater wavelengths and exhibits a diffuse maximum appearing in the green region, thereafter falling off and becoming insensible in the rest of the spectrum. It is obvious that an absorption of this nature cannot possibly account for the great variety of vivid colours observed in the floral world.

Significantly also, there have been many cases where the same anthocyanin appears in flowers of different colours. A way of escape from these and other difficulties has been sought in the introduction of special assumptions, *viz.*, variations in the pH of the plant sap, metal chelation and so forth. We are justified in rejecting all such *ad hoc* hypotheses, since the real reason for the failures is that stated above, *viz.*, that the floral pigments are not identifiable with the anthocyanins, these latter being only artifacts resulting from the processes adopted in their preparation from the floral material.

2. THE DISCOVERY OF THE FLORACHROMES

That floral pigments possess a distinct chemical individuality of their own is indicated by the highly characteristic absorption spectra which they exhibit *in vivo*. It is appropriate therefore that they are given a specific name which, while reminding us of their floral origin, would also prevent their being confused with the anthocyanins which, as stated above, are chemical artifacts. In his Presidential address to the Indian Academy of Sciences

at the Ahmedabad meeting in December 1968, such a name was proposed by the author. As there were *two* floral pigments revealed by his earlier studies which showed quite different and highly characteristic spectroscopic behaviours, they were referred to in the address as Florachrome A and Florachrome B respectively. It should be remarked that the discovery of of these florachromes was not a chance event but was the outcome of several years of purposeful study of the colours exhibited by the plant world. Reference may be made here to the memoir by the author entitled "Floral Colours and the Physiology of Vision" which was published in Vol. 58 (1963) of the *Proceedings of the Indian Academy of Sciences.*

Following the Ahmedabad address of December 1968 which was duly published in the *Proceedings of the Academy* (Vol. 69, April 1969), the subject has been further pursued and much new information has emerged. It is proposed in the present memoir to give a systematic account of the whole field including the latest findings. It may be useful here at first to sum up briefly the methods adopted and the results which have emerged.

The florachromes present in the petals of flowers can be readily extracted from them, and it thereby becomes possible to exhibit in an objective fashion their distinctive properties as well as the striking differences between Florachromes A and B in their optical and spectroscopic behaviours. The extraction of the florachromes is effected by very simple procedures which leave their basic nature and characteristics unaffected. This may be demonstrated by a comparison of the absorption spectrum of the extract with the spectrum of the light diffused by the petals of the flowers. The close similarity between them makes it evident that the floral pigments remain essentially unchanged by their extraction from the petals. As is to be expected in the circumstances, there is a readily recognisable relationship between the quantity of the pigment which can be taken out from the flower petals and the depth or degree of saturation of the colour exhibited by them *in vivo.*

It is a highly noteworthy fact that while many flowers contain only one or the other of the two florachromes, there are also numerous cases in which both florachromes are present and jointly determine the observed colour of the flower petals. It has been found to be possible in such cases to effect a partial or even nearly complete separation of the two florachromes in the process of their extraction.

One can also obtain extracts of Florachrome A and Florachrome B which continue to exhibit their characteristic optical and spectroscopic behaviour more or less permanently. Their chemical individuality and stability

is thereby demonstrated. By mixing the extracts of the two florachromes, the absorption spectra of the mixtures can be studied. These are found to exhibit the features which may be expected to result from a simple superposition of the absorbing powers of the two florachromes.

It is an interesting discovery that Florachrome B is the colouring matter responsible for the purplish-red hue of the fleshy leaves of the shrub known botanically as *Setcreasea purpurea.* This plant grows luxuriantly and the leaves are vividly coloured on both faces. Flowers having the shape of a trefoil appear at the terminations of the leaf stems. It is not surprising that these terminal flowers also exhibit the absorption spectrum of Florachrome B very conspicuously. *Setcreasea purpurea* is thus a source from which Florachrome B can be obtained in any desired quantity.

2. FLORACHROME A

As illustrative of the spectroscopic behaviour of blue flowers generally and as suitable material for the extraction and preservation of the pigment present in flowers exhibiting that colour, the author has found the plant known botanically as *Clitoria ternata* and more familiarly as the Butterfly-pea to be extremely useful. It is a climber belonging to the botanical order of *Leguminosae.* In one of the two known varieties of the plant, the flowers exhibit a narrow central tract of greenish-yellow hue surrounded by a brilliantly blue margin. This margin is broad enough to permit of its being viewed through a pocket spectroscope, either by transmitted or by reflected light. It is convenient to hold the flower in sunlight and view it by reflected light, a sheet of white paper being placed at some distance below it so that a comparison is possible between the spectra of the light diffused by the latter and by the petals respectively. The most conspicuous feature in the spectrum of the blue flower is a dark band of absorption centred at 630 mμ. A second absorption band which is less intense and rather more diffuse centred at 575 mμ is also fairly conspicuous. A third band which is very weak and diffuse may also be discerned at about 530 mμ. The region of the spectrum between 575 mμ and 630 mμ exhibits a very large reduction in brightness in the spectrum of the blue flower. There is no perceptible dimming of brightness of the short-wave or blue region of the spectrum in the light reflected by the petals. The brightness of the spectrum in the wavelength range between 500 mμ and 560 mμ is diminished but only to a slight extent. The red end of the spectrum beyond 640 mμ continues to be visible with no noticeable decrease of intensity.

By placing the blue areas cut out from the petals of *Clitoria* in a test-tube containing water and squeezing them with a glass rod, we obtain an extract which can be filtered through cotton-wool, resulting in a clear blue solution. This can be placed in a glass cell of suitable dimensions, and its absorption spectrum can be studied. The strength of the extract and the length of the observation column determine the features noticed in the absorption spectrum. They are of the same general nature as those noticed in the spectrum of the flower as observed *in vivo*.

Florachrome A can also be extracted using other solvents, *e.g.*, acetone or alcohol. Placing the blue petals in a beaker containing the solvent followed by a gentle agitation results in an extraction of the pigment. In this manner, we may obtain a solution of Florachrome A from the petals of *Clitoria ternata* exhibiting a deep blue colour. The absorption spectrum exhibits features similar to those obtained with aqueous solutions of the pigment. A photograph of the spectrum is reproduced as Fig. 1 in Plate I.

An aqueous solution of Florachrome A contained in a tube three centimetres long presents a deep blue colour. When viewed end-wise through a pocket spectroscope, it shows a practically complete extinction of the spectral range between 570 mμ and 630 mμ, while the regions of greater and of lesser wavelengths come through more or less freely. But when the absorbing column is of shorter length, say only one centimetre, the discrete absorption bands located at 630 mμ and 575 mμ and 530 mμ are conspicuously visible. A spectrophotometric record of the absorption by a cell of one centimetre depth may thus be expected to display both the bands and the continum very clearly. That this is actually the case will be seen from Text-Fig. 1.

3. Florachrome B

For the extraction and preservation of Florachrome B and for the study of its spectroscopic behaviour, the author has found the flowers of the plant known botanically by the name of *Spathoglottis plicta* to be highly suitable and indeed most convenient. The plant is a terrestrial orchid with elongated leaves, which is quite hardy and can be grown in pots like any other garden flower. *Spathoglottis plicta* is always in bloom, bearing racemes of flowers on long erect scapes which stand out above the leaves of the plant itself. There are two distinct varieties, both of which have useful features. One variety has longer leaves and it flowers quite profusely. The other variety has smaller leaves and flowers less profusely and the plant is therefore much less spectacular. The most noteworthy difference between

them is in the colour of the blooms. We shall refer to the two varieties as Orchid I and Orchid II respectively. The flowers of Orchid I exhibit a purplish-red hue. The flowers of Orchid II are heavily pigmented and have a deep purplish-crimson tint.

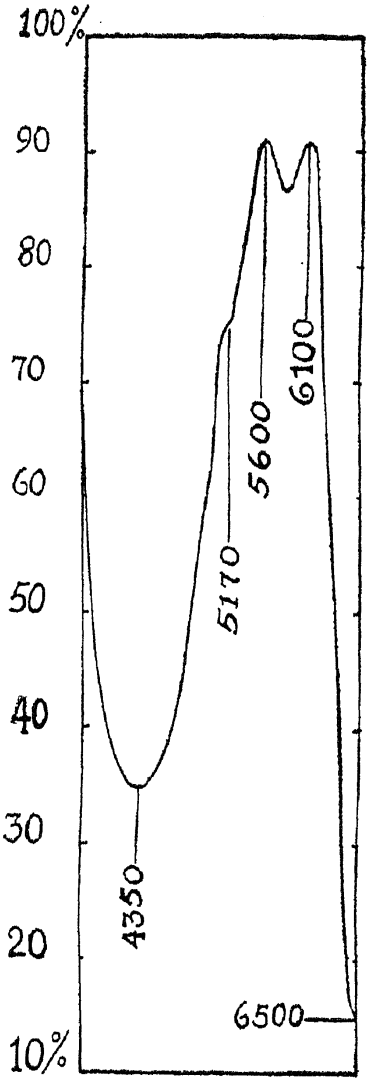


FIG. 1

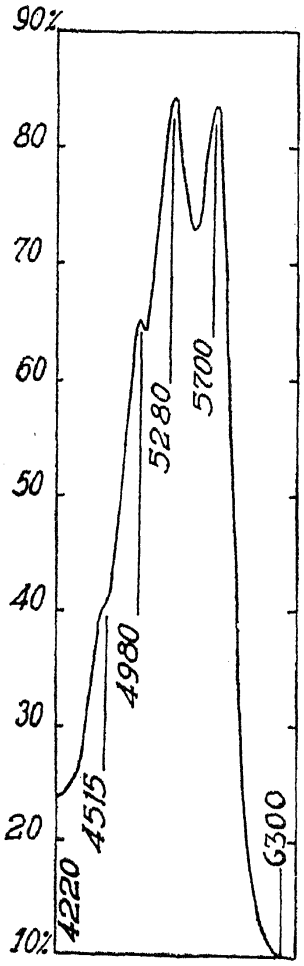


FIG. 2

TEXT-FIG. 1. Spectrophotometric record of aqueous extract from *Clitoria ternata.*

TEXT-FIG. 2. Spectrophotometric record of aqueous extract from *Spathoglottis plicta.*

The spectrum of Florachrome B is strikingly exhibited by the flowers of Orchid I. There are five petals on each flower and when it is held in bright light and any of the petals is viewed through a pocket-spectroscope either by transmission or by reflection, the spectrum is vividly seen. Three absorption bands cut across the spectrum. The first which is the sharpest and darkest is centred at 590 mμ. The second which is less dark and is also somewhat more diffuse appears at about 540 mμ. The third band which is faint and diffuse appears at about 510 mμ. The first band practically

extinguishes the yellow sector of the spectrum. The second band lies in the green sector and effectively reduces its intensity. But there is no absorption or weakening of the red sector of the spectrum which appears in full strength. The blue region of the spectrum is also seen with no noticeable reduction of its intensity.

Orchid II is so heavily pigmented that not much light can penetrate through its petals. But by holding it in the path of a beam of sunlight, the absorption spectrum can be observed. It is found to exhibit essentially the same features as Orchid I, but in a much more pronounced fashion.

Using the petals of *Spathoglottis plicta*, solutions of Florachrome B either in water or in acetone or alcohol can be readily prepared. Surprisingly large quantities of the pigment can be extracted from the petals of Orchid II. The solutions exhibit a rich purplish-red hue. Using absorption cells of appropriate thickness, the spectrum can be readily photographed. Figure 1 in Plate II reproduces the spectrum thus obtained.

When the absorption column is of sufficient length, Florachrome B results in a practically complete extinction of the wavelength range from 500 mμ to 600 mμ in other words of both the green and the yellow sectors of the spectrum. With shorter columns, the three distinct absorption bands can be seen in the positions already stated. Thus, a spectrophotometer record of the absorption by a column of moderate length, as for example one centimetre, can be expected to show the three bands superposed on a continuum covering the green and yellow sectors of the spectrum. That this is actually the case is evident from the record reproduced as Text-Fig. 2.

4. Perception of Floral Colours

No discussion of the subject of floral colours could be meaningful unless it considers both the physico-chemical and the physiological aspects of the problem in relation to each other. The absorption by the floral pigments determines the spectral character of the light emerging from the petals. But the perceived colour is determined by the characteristics of human vision. That these latter play a role of the highest importance will be evident from a few examples.

The large trumpet-shaped flowers of the climbing plant known popularly as the " Morning Glory " and botanically as *Ipomea learii* are borne in profusion and hence are readily accessible for examination. The freshly-opened flowers in the morning exhibit a dark-blue colour. Sunlight

falling on and diffused by the petals when examined through a pocket-spectroscope shows a large diminution of intensity in the region of wavelengths between 575 mμ and 630 mμ which contains the yellow and the orange of the spectrum. The absorption is strongest at 630 mμ and diminishes towards lesser wavelengths. The red of the spectrum in the region of wavelengths greater than 630 mμ appears with full intensity, while the blue violet region shows no diminution of brightness. The green between 500 mμ and 560 mμ shows only a slight dimming. It is highly remarkable in these circumstances that the petals exhibit a dark blue colour which is fully saturated. The observed colour gives no indication that the green and red of the spectrum are present admixed with the blue-violet region.

The blue of *Clitoria ternata* is more brilliant than the blue of the Morning Glory. But it is also a highly saturated colour and gives no indication of the presence in admixture with it of a great deal of green and red light lying outside the regions of special absorption. We are thus led to infer that the blue colour of the flowers containing Florachrome A is a sensation consequent on the elimination of the yellow and orange regions of the spectrum by the two principal absorption bands of the pigment. The green and red rays which are present are masked or prevented from being perceived by the blue of the spectrum which is then the dominant sensation.

The flowers of *Spathoglottis plicta* owe their observed colour to Florachrome B. In the spectrum of the light diffused by the petals, the yellow is totally excluded by the absorption band centred at 590 mμ. But the orange and red sectors with wavelengths greater than 610 mμ is present in full strength. The absorption bands centred at 540 mμ and 510 mμ result in a sensible reduction of intensity of the green sector. But the blue-violet region of shorter wavelengths is present without any noticeable dimming. Red is the dominant colour of the flower. The joint effect of the red and orange parts of the spectrum evidently succeeds in obscuring the sensory effects of green and blue rays which are present as an admixture.

Thus, the shift in the positions of the absorption bands as between Florachrome A and Florachrome B which is not large nevertheless results in transforming the observed colour of the flowers from blue to red.

5. THE COLOURS OF *Cineraria*

We shall now proceed to consider the very interesting effects exhibited by the flowers of the shrubs known as *Cineraria* belonging to the botanical order *Compositae*. *Cineraria* have been described as follows in a book on

ing : " Beautiful pot-plants for the conservatory. Very showy with arge luxuriant leaves which are surmounted by immense panicles of icent flowers of the most brilliant colours. Blooms last for quite time—for nearly a month." The feature of special interest is that lours of the *Cineraria* flowers besides being brilliant are of varied

Spectroscopic examination of the flowers held in sunlight reveals e colours owe their origin to the presence of discrete absorption bands spectrum, their disposition and their relative intensities varying with lour of the flowers. The flowers which are a brilliant blue exhibit ctrum of Florachrome A and those which are a brilliant red, the spec- f Florachrome B. These two spectra of *Cineraria* were observed by thor as far back as the year 1963. They will be found reproduced Plates accompanying Vol. 58 of the *Proceedings of the Academy* for 1963. Excellent spectra of *Cineraria* flowers may be recorded merely ding a petal close to the slit of a spectrograph, the illumination being ed by a tungsten filament lamp. Two such spectra are reproduced gs. 1 and 2 in Plate III.

6. Mixture of the Two Florachromes

ie phenomena exhibited by the flowers of *Cineraria* suggest the ng as an overall picture of the nature and origin of the colours of . Florachrome A present in the petals of flowers gives rise to sensations in which blue is dominant. Likewise, the presence of hrome B gives rise to colour sensations in which red is dominant. In ases, the quantity of the floral pigment present determines the depth gree of saturation of the colour sensation. The simultaneous pre- of both florachromes would result in the production of colours, the sensations in which would be of a composite character, neither blue d but intermediate between them which we may term as purple, the shade depending on the quantities and relative proportions of the rachromes present. The picture here suggested finds strong support facts of observation which emerge from an extensive study of floral s.

e may, first of all, remark on the features of the absorption spectra would result from the presence of a mixture of the two florachromes petals of flowers. The absorption of Florachrome A effectively covers ctral region extending from 575 mμ to 630 mμ. On the other hand, sorption of Florachrome B is most effective in the spectral region n 540 mμ and 600 mμ. The two regions overlap in the spectral

region from 575 mμ to 600 mμ, which is the region in which the yellow in the spectrum visibly manifests itself. We are thus led to associate the purplish colour exhibited by the petals of a great many flowers with the presence of a very strong absorption in the yellow sector of the spectrum. Such an association is indeed a well-attested fact of observation.

The entire sequence of colours resulting from a mixture of the two florachromes in various proportions can be followed by first preparing the aqueous extracts of the two florachromes in the manner already described and then adding A to B (or *vice versa*) in a flat sided glass cell, a little at a time. If A is added to B, the immediate result is to transform the red of B to a purple colour which progressively becomes deeper. If B is added to A, the immediate result is to make the blue a deeper colour, and then progressively to transform it to a purple. The final result is, of course, the same in both cases.

7. SEPARATION OF THE TWO FLORACHROMES

As an illustration of the observable consequences of the simultaneous presence of both florachromes, we may consider the case of the shrub *Meyenia erecta* (also known as *Thunbergia erecta*) which belongs to the botanical group of *Acanthaceae*. This is an erect bushy shrub which bears large funnel-shaped flowers with petals which are a deep purple in colour. The spectrum of the light transmitted through the petals exhibits absorption bands centred at 630 mμ, 570 mμ and 535 mμ. But the first of these bands is much less conspicuous than the one exhibited in the same position by *Clitoria ternata*, and the third band is broader and more conspicuous than is observed with that flower. These and other features indicate that the absorption spectrum of *Meyenia erecta* is a superposition of the absorption spectra of both of the florachromes. The observed colour of the petal as seen by transmitted light is evidently a compromise between the blue and the red sensations produced by the two florachromes.

The foregoing inferences find a striking confirmation in the successful separation of the two florachromes from the petals of *Meyenia erecta*. The petals are first soaked for a few minutes in acetone and then taken out and placed in distilled water. Gentle pressure with a glass rod then results in the extraction of a blue solution which exhibits the characteristic absorption spectrum of Florachrome A. The material left over is then put back into acetone and vigorously stirred up. This results in the production of an extract having a red colour which spectroscopic examination

reveals to be due to the Florachrome B taken out by the second treatment with acetone.

It may be mentioned that similar procedures with the petals of other flowers also results in a more or less complete separation of the two florachromes when both are present. But there are numerous flowers where such attempts do not succeed, thereby indicating that only one or the other florachrome is present and is responsible for the observed colour of the petals.

8. A Survey of the Colours of Asters

Bangalore which has a cool climate is well adapted for the growing of flowers on a commercial scale. There is a large and well-established industry specialising in the production of asters which could serve as table-decorations by reason of their exhibiting brilliant colours. The flowers together with the leafy stalks as the terminals of which they appear are cut and marketed in quantity. It is therefore possible at any time and at a nominal price to obtain a collection of several hundreds of asters exhibiting vivid colours in a fresh condition for scientific study. Each flower consists of a great number of long narrow petals arranged around a common centre. These petals are usually so close together as to overlap, and it is therefore unnecessary to detach the individual petals for observation. The entire flower can be held in bright sunlight and viewed through a pocket-spectroscope held in the hand of the observer, and its spectrum is compared with that of a white card also illuminated by sunlight. A great number of flowers of varied colours can be examined in quick succession. Such a survey is highly instructive, and indeed forms a striking demonstration of the existence of two florachromes and of the roles which they respectively play in the production of floral colours.

Viewing a large collection of the asters offered for sale in the market, it would seem at first sight that they could be grouped in two categories, *viz.*, those of which the colour is predominantly red, and the other of which the colour is predominantly blue. But on closer examination, it is seen that such a description is not complete, since the colour variations between individual flowers are quite pronounced. From a collection of a hundred or more of the flowers, it is easy to pick out a dozen which could be arranged as a sequence exhibiting a regular progression of colour. At one end of the sequence, we have the asters which are a deep red in colour. At the other end, we have those which appear as blue or violet. Intermediately, we have purplish hues of various shades, some which are rather light, but

the majority exhibiting vivid colours tending towards either red or blue. In all cases, there is little or no absorption of the blue-violet region of the spectrum. But the region of wavelengths between 500 mμ and 600 mμ shows a marked diminution in intensity. As we proceed from the red to the blue flowers in the sequence, the region in the spectrum exhibiting the maximum of absorption shifts from the green towards the yellow. A nearly complete absorption of the yellow sector in the spectrum is exhibited by those flowers which are a purplish-blue in colour. They also exhibit a noticeable absorption in the red region of the spectrum, a darker band appearing in the position which is characteristic of Florachrome A.

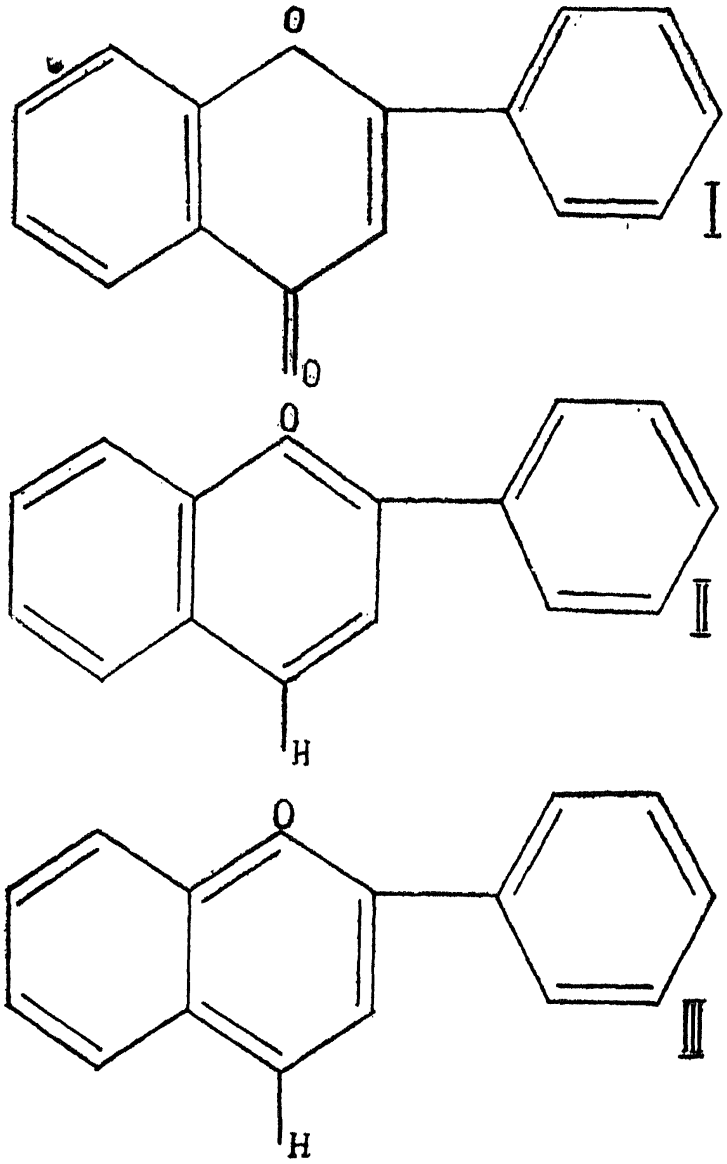
9. CHEMICAL NATURE OF THE FLORACHROMES

If, instead of labelling the floral pigments as oxonium or carbonium salts, we assume them to be neutral molecules having a structure related to that of the well-known organic compound flavone, it is possible to give a satisfactory explanation for the manifestation of two florachromes which differ from each other, as well as for the remarkable spectroscopic properties which they exhibit.

Flavone is itself a colourless crystalline solid melting at 99° C. Replacement of hydrogen atoms by hydroxyl groups results in the substances known as flavonols. The crucial change which transforms flavone and its derivatives into pigments exhibiting vivid colours, in other words, into substances of which the absorption bands appear in the visible region of the spectrum is the replacement of the C=O or carboxyl group which appears in these compounds by a C–H group. The valence bond which is thereby released enters into the closed ring, which in consequence acquires a quasi-aromatic character. In this ring, instead of a carbon atom, we have an oxygen atom which is effectively trivalent. There are two ways in which the rearrangement of the valences in the ring can take place and these are basically different. In one, the quasi-aromatic grouping which resembles naphthalene has a symmetric structure. In the other, it has an asymmetric configuration. These two forms together with the flavone structure from which they are derived are shown in Text-Fig. 3.

The third valence which links the oxygen atom alternately with the two carbon atoms on either side of it would give rise to a spectroscopic behaviour quite different from that of flavone. These oxygen-carbon bonds would be much weaker than the normal carbon-carbon bonds in aromatic compounds, and the characteristic electronic frequencies would therefore move from the ultra-violet into the visible range of the spectrum. In the

symmetric form shown as II in Fig. 3, the ring containing the trivalent oxygen and the ring containing only carbon atoms could be considered as distinct units, whereas in the asymmetric form shown as III in Fig. 3, the two rings would function together as a single entity. Hence, the characteristic frequencies appearing in the visible spectrum should be observably smaller for the form II than for the form III. Hence, we identify II as Florachrome A and III as Florachrome B.



TEXT-FIG. 3. The structure of flavone and of the two florachromes.
I, Flavone Structure ; II, Florachrome A ; III, Florachrome B.

Since the electronic frequencies with which we are concerned are due to the linkages between the oxygen and carbon atoms, we may expect the absorptions associated with the electronic transitions to be accompanied by supplementary bands due to vibrational transitions occurring simultaneously. The vibrational frequencies manifested in such transitions may

be expected to be greater than for the C–O–C group in flavone and less than for the C=O group in the same substance. They may also be expected to be observably different for Florachrome A and for Florachrome B.

As has been remarked earlier, the absorption spectra of the florachromes consist in each case of a succession of three bands in diminishing order of intensity and sharpness. We may therefore identify the first band in each case as due to an electronic transition and the two following ones as due to its combination with vibrational transitions. This interpretation is supported by the measurements made with a wavelength spectrometer of the positions of the absorption bands as observed in aqueous solution. The observed positions of the bands for Florachrome A are 625 mμ, 577 mμ and 531 mμ. The spectral shifts from the first band to the second and from the second band to the second are, expressed in wavenumbers, respectively 1331 cm.$^{-1}$ and 1501 cm.$^{-1}$. The mean of the two, *viz.*, 1415 cm.$^{-1}$ may be taken as the characteristic vibration frequency for Florachrome A. The observed positions of the bands for Florachrome B are 590 mμ, 546 mμ and 511 mμ. The spectral shifts from the first to the second and from the second to the third are respectively 1366 cm.$^{-1}$ and 1254 cm^{-1}. The mean of the two, *viz.*, 1310 cm.$^{-1}$ may be taken as the characteristic vibration frequency for Florachrome B.

The vibration frequency of the C=O or carboxyl group in organic compounds is known from spectroscopic studies and is usually taken as 1720 cm.$^{-1}$, though in some cases it may be as low as 1650 cm.$^{-1}$ or as high as 1800 cm.$^{-1}$, these variations depending on the nature of the substance under study. Thus, the carboxyl frequencies are definitely higher than those exhibited in the absorption spectra of the florachromes. The smaller vibration frequencies deduced from the absorption spectra of the florachromes lend support to the concept of the quasi-aromatic structure of the florachromes indicated in Text-Fig. 3.

10. THE ORIGINS OF FLORAL COLOUR

The presence in the flower-petals of either Florachrome A or Florachrome B or of both florachromes accounts for the colours displayed by them in a great many cases. The justification for this statement is to be found in studies of the spectral character of sunlight diffused by the petals and in observations of the absorption spectra of the floral pigments extracted from them in a state of solution by appropriate methods. We propose here to mention some flowers studied by the author. They fall into three

groups in which we are concerned respectively with Florachrome A alone, with Florachrome B by itself and with both together.

Plumbago capensis.—The garden shrub thus named bears almost throughout the year a profusion of clusters of pale azure-blue flowers. The colour is very weak. But the spectral character of the absorption responsible for its production can be readily recognised by holding a bunch of the flowers together and viewing the light that comes through with a pocket-spectroscope. All the three bands characteristic of Florachrome A can be seen and their wavelengths can be read off on the scale of the instrument, *viz.*, a band at 630 mμ another at 575 mμ, and a faint one at 530 mμ.

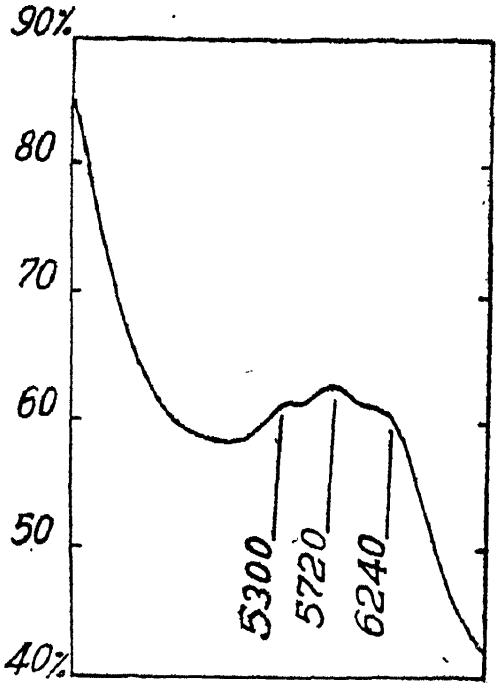
Jacaranda minosiaefolia.—This is a well-known tree which during the flowering season of March to May sheds all its leaves and appears surrounded by a great mass of flowers of a bluish-purple colour which make it a conspicuous object from a distance. The colour of the flowers is not particularly intense. Nevertheless, the absorption bands which give rise to it at 630 mμ and 575 mμ are fairly conspicuous. The third band at 530 mμ is very weak and can easily be overlooked.

Thunbergia grandiflora.—This is a climbing plant which is a native of Bengal and grows well at Bangalore. It is an impressive object with its large pale-blue flowers when allowed to climb and spread freely. The absorption bands characteristic of Florachrome A are readily recognised in the light reflected by the petals. But they are best observed by holding two petals together and viewing the light transmitted through both of them.

Jacquemontia violacea.—This is a small free-blooming creeper which belongs to the botanical order of *Convolvulaceae* and has a habit of growth which makes it suitable for covering trellises or arbours. The flowers are small, bell-shaped and of a light-blue colour, borne plentifully in all seasons. The three absorption bands characteristic of Florachrome A are readily observed with it. By immersing the petals in acetone, the floral pigment is readily extracted. A spectrophotometer record of the absorption by a centimetre column of the extract is reproduced as Text-Fig. 4. The three bands characteristic of Florachrome A are clearly seen in the figure.

Lobelia erinus is a well-known garden shrub which produces blue flowers in profusion. The petals exhibit the characteristic absorption spectrum of Florachrome A by transmitted light and this is readily photo-

graphed. Such a spectrum is reproduced as Fig. 10 B in Plate VIII of the author's book on the *Physiology of Vision.*



TEXT-FIG. 4. Spectrophotometric record of extract of *Jacquemontia violacea.*

Solanum macranthum, also known as the "Potato Tree," is perpetually in bloom. The flowers are arranged in clusters. Their colour which at first is violet progressively fades from day to day and finally becomes white. Seen by reflected or transmitted light, the petals exhibit absorption bands in the red, yellow and green sectors of the spectrum to which and the accompanying general absorption, the observed colour is ascribable.

Larkspur.—This is a well-known favourite in gardens, freely producing spikes of beautiful flowers and of varied colours. The blue larkspurs exhibit the spectrum of Florachrome A very conspicuously. An aqueous extract can be readily prepared from their petals which exhibits the three bands of absorption in the positions characteristic of this florachrome. The pink larkspurs on the other hand show the spectrum of Florachrome B, the bands at 590 mμ and 545 mμ appearing quite clearly.

Delphinium.—This plant, also known as the perennial larkspur, has been much developed in recent years. The varieties available to the author at Bangalore were the blue delphiniums. These exhibit the spectrum of Florachrome A in a conspicuous manner.

Setcreasea purpurea.—As already mentioned earlier, the fleshy leaves of this shrub form a very convenient source of Florachrome B. By scarping off the colour from one face of the leaves, and adding water to the material thus obtained and then filtering the product, it is possible to

obtain a substantial volume of a clear solution of the pigment. Its colour as seen by transmitted light is a rich red. But the spectroscope reveals the three intense bands of absorption centred at 590 mμ, 546 mμ, and 511 mμ respectively which are characteristic of the florachrome. There is also an observable transmission of the blue region of the spectrum appearing beyond these bands. The flowers of *Setcreasea purpurea* also exhibit the absorption spectrum of Florachrome B very conspicuously.

Cineraria.—As has already been mentioned, the flowers bearing this name are exceptionally good material for observing and photographing the absorption spectra of the two florachromes as also the results of their superposition. The brilliancy of the colours which they exhibit and the clarity of the spectra of absorption displayed by the petals stand in the closest relationship to each other.

The Purple Orchids.—Apart from the ground orchids observations with which have been described earlier, other orchids which show a purplish-red colour or isolated spots of that colour has also been studied by the author. They exhibit the characteristic absorption spectrum of Florachrome B very conspicuously.

Roses.—The origin of the red colour of roses is naturally a subject of great interest. That the presence of Florachrome B in the petals is responsible for it is readily established. As is will be seen from the spectrophotometric record reproduced as Text-Fig. 2, the characteristic feature of that florachrome is an intense absorption covering the yellow and green sectors of the spectrum, such absorption falling off steeply to zero as we move into the red beyond 600 mμ, while, on the other hand, there is a readily observable transmission of the blue-violet region of the spectrum. These features are very clearly exhibited in the absorption of light by the petals of roses. The strength of the absorption is determined by the quantity of pigment present, the depth of colour of the petal also increasing *pari passu* with it. By holding together two or three rose petals and examining the spectrum of the light emerging through them, it is readily verified that there is a complete cut-off of wavelengths less than 600 mμ and free transmission of wavelengths greater than 600 mμ, thus reproducing the behaviour of Florachrome B.

Observations of the light transmitted through rose petals do not permit us to recognise the three distinct absorption bands centred at 590 mμ, 540 mμ and 510 mμ which are so conspicuously seen both by reflection and by transmission with the petals of other flowers mentioned above. This suggests

that the spectral behaviour of the florachrome is not altogether independent of the particular circumstances of each case. Fortunately, however, with roses and also with numerous other flowers studied by the author, the discrete structure of the absorption spectrum of Florachrome B is revealed when the pigment is extracted from the petals by the use of acetone as a solvent and light transmitted through the extract is examined spectroscopically. Visual observation of the spectrum reveals the discrete bands very clearly and spectrophotometer records also exhibit the features referred to.

Immersion of rose-petals in acetone enables the floral pigment to be extracted very quickly and efficiently and the nature of the pigment to be determined spectroscopically, irrespective of whether the petals are lightly or deeply coloured. Text-Figure 5 reproduces a spectrophotometer record obtained with an acetone extract of a red rose. It will be seen that while the maximum absorption as spectrophotometrically recorded appears at 530 mμ, distinctive features also appear at 570 mμ and 500 mμ, in the same positions as Text-Fig. 2 reproduced earlier.

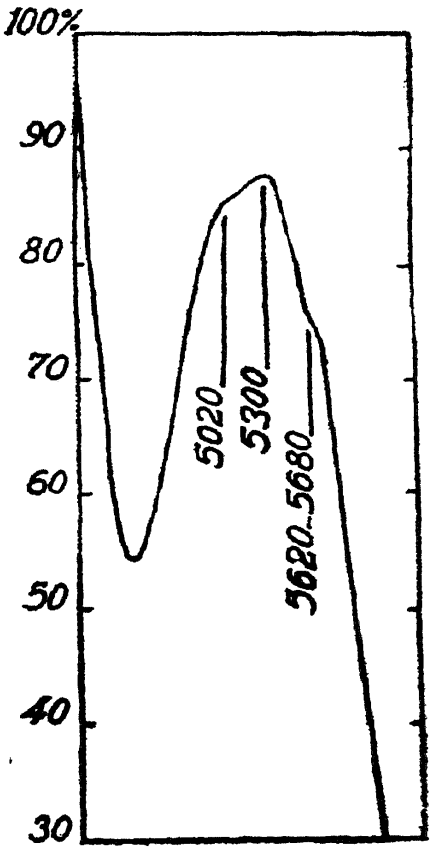


FIG. 5

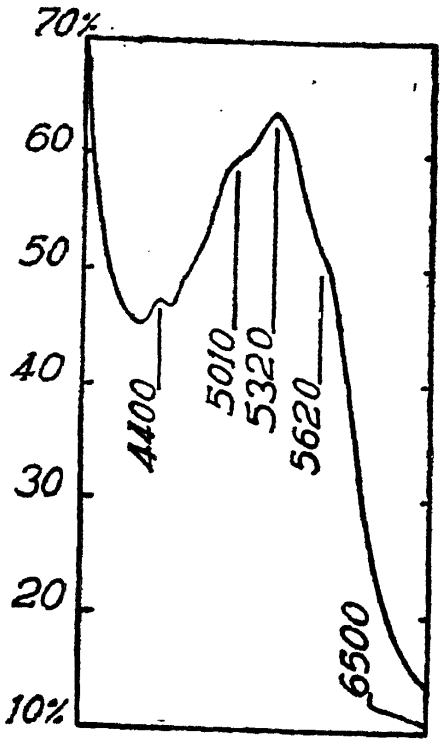


FIG. 6

TEXT-FIG. 5. Spectrophotometer record of acetone extract of a red rose.

TEXT-FIG. 6. Spectrophotometer record of the acetone extract of red oleandre.

The Red Oleanders.—The bushy flowering shrubs known botanically as *Nerium* and commonly as oleanders are a familiar sight in Indian gardens. The red oleanders exhibit a colour resembling that of roses, and spectroscopic examination reveals that the absorption appears in the same region as in the case of roses. The floral pigment is readily extracted by immersing the petals in acetone. A spectrophotometer record of the acetone extract from a red oleander is reproduced as Text-Fig. 6.

Plumeria Rubra.—A spectacular show of floral colour is exhibited by this tree for several months in the year. The flowers appear in large clusters, and a great many such clusters cover the tree against the background of its foliage. The colour of the flowers is a bright crimson surrounding a golden-yellow centre, but only the crimson areas are seen with the partially opened buds. The light transmitted by the crimson-coloured areas, examined through a spectroscope, exhibits an intense absorption of the green and yellow sectors of the spectrum extending upto 600 mμ with free transmission of greater wavelengths. The pigment is readily extracted with acetone. A spectrophotometric record obtained with such an extract is reproduced as Text-Fig. 7.

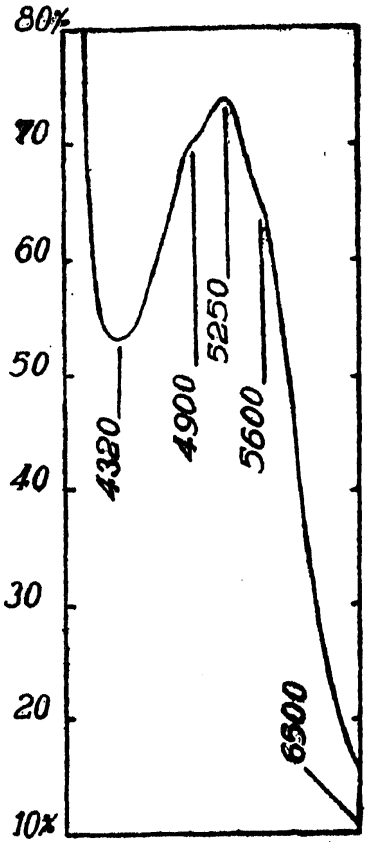


FIG. 7

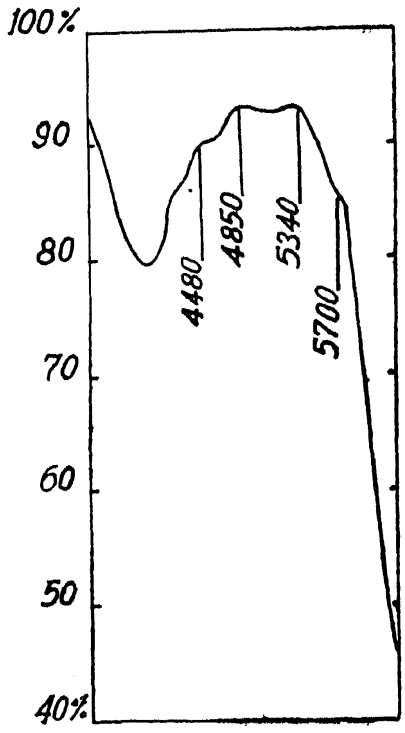


FIG. 8

TEXT-FIG. 7. Spectrophotometer record of acetone extract of *Plumeria rubra.*

TEXT-FIG. 8. Spectrophotometric record of acetone extract of Chinese hibiscus.

Hibiscus rosa sinensis.—There are numerous varieties of the flowering shrub known as the hibiscus, which is much esteemed by reason of its blooms appearing throughout the year, their large size and brilliant colours. Most familiar of them all is the Chinese hibiscus which has large bell-shaped single flowers with a rich red colour, and a pretty column of pistil and stamens projecting from their centres. Spectroscopic examination of its petals reveals a complete extinction of all wavelengths less than 590 mμ. The floral pigment is readily extracted with acetone, and its spectrophotometric record reproduced as Text-Fig. 8 makes it evident that while the absorption of light in the green and yellow regions in the spectrum is due to the presence of Florachrome B in the petals, there is also a powerful absorption of the blue-violet sector due to the presence of a carotenoid pigment which reveals itself by the characteristic absorption maxima in that part of the spectrum.

The efforts of horticulturists have succeeded in the production of hibiscus varieties of the *rosa-sinensis* type but exhibiting other colours and flowering profusely. One of particular interest in the present context is the hybrid exhibiting a rose-pink colour. Unlike the Chinese hibiscus, the petals of this hybrid transmit the blue-violet region of the spectrum freely, and also all wavelengths greater than 600 mμ. The region of absorption between 500 mμ and 600 mμ is thus clearly defined. When the acetone extract of the floral pigment is examined through a pocket-spectroscope, the three absorption bands appearing in this region which are characteristic of Florachrome B stand out very clearly. This is also evident from the spectrophotometric record reproduced as Text-Fig. 9.

Petrea volubilis.—This is the botanical name of a woody climbing plant which with its foliage covers the supports on which it spreads out. Twice a year, the plant envelops itself with wreath-like sprays of purplish-blue stars, making a fine show of colour. The true flowers are rather inconspicuous and drop out, leaving the stars intact. Visual examination through a spectroscope reveals the absorption bands which are responsible for the observed colours. The band at 630 mμ characteristic of Florachrome A can be observed with the true flowers which are a deep-blue in colour.

The floral pigment of the sprays is readily extracted with acetone and the absorption spectrum of the extract can be studied visually. A spectrophotometer record of the same is reproduced as Text-Fig. 10. It shows, besides two bands in the green, also a strong band of absorption covering the yellow region of the spectrum. The record indicates that both Florachrome A and Florachrome B are present in comparable quantities.

The Purple Verbena.—The verbenas are very popular trailing plants of a perennial habit. Books on gardening stress the wide range of colours

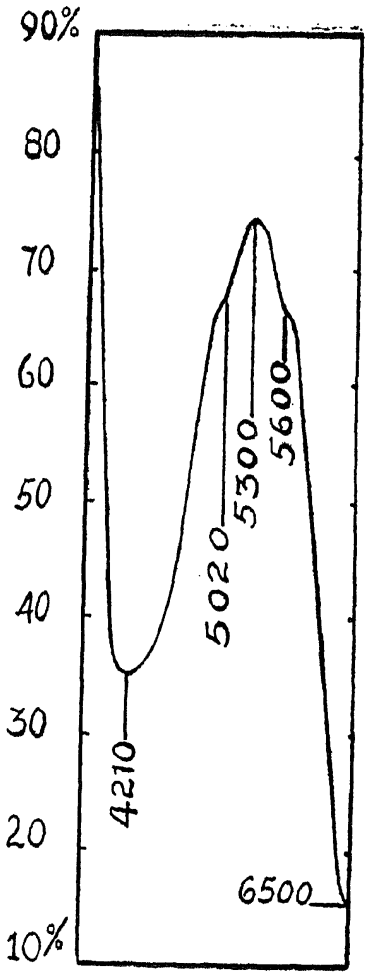


FIG. 9

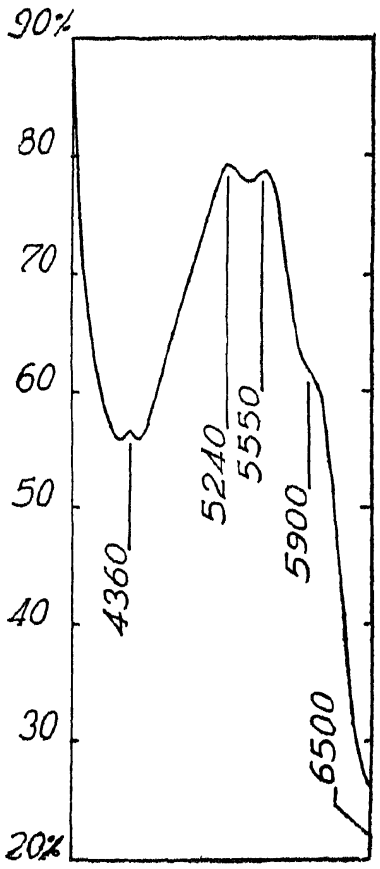


FIG. 10

TEXT-FIG. 9. Spectrophotometer record of acetone extract of rose-pink hibiscus.

TEXT-FIG. 10. Spectrophotometer record of acetone extract of *Petrea volubilis*.

exhibited by them. Intensely blue verbenas and brilliantly scarlet varieties have been described or illustrated. One author goes so far as to say that there are few flowers which can excel the verbenas in the exquisite range of colours displayed.

A purple variety of verbena is frequently noticeable at Bangalore, and when it has been planted over an extensive area, the ground presents the appearance of a carpet of purple colour. Though the individual flowers are small, a bunch of them presents a substantial area, and when this is viewed through a pocket-spectroscope, an absorption band covering the yellow region of the spectrum is a strkiing feature. The green also shows a visible absorption.

The colouring matter of the verbenas comes out easily with acetone, and the absorption spectrum of the extract is readily examined. A spectrophotometric record made with the extract is reproduced as Text-Fig. 11. The positions of the bands and their relative intensities indicate that both Florachromes A and B are present and contribute to the observed colour of the verbenas.

Lagerstroemia indica.—These shrubs (commonly called *Crepe myrtles*) are very pretty in bloom. Their soft-fringed flowers appear arranged in long erect sprays from May to August. There are several varieties, and the one studied by the author had pinkish-purple flowers. Examined through a pocket-spectroscope, the nearly complete extinction of the yellow region in the light diffused by the material is a striking feature. Immersion in acetone readily extracts the floral pigment. Text-Figure 12 reproduces a spectrophotometric record obtained with the extract. This and Text-Figs. 10 and 11 exhibit very similar features.

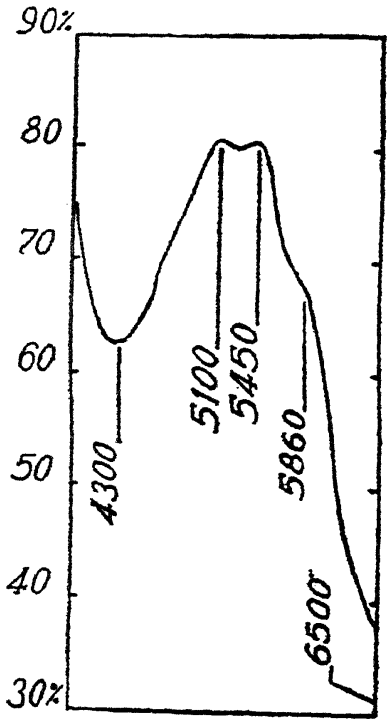


FIG. 11

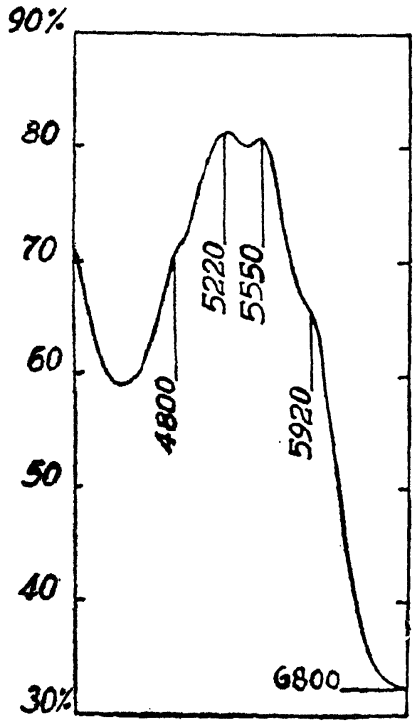


FIG. 12

TEXT-FIG. 11. Spectrophotometric record of acetone extract of the purple verbena.

TEXT-FIG. 12. Spectrophotometric record of acetone extract of *Lagerstroemia indica.*

Hibiscus syriacus.—Sometimes referred to as the blue hibiscus, the specimens of the species found growing at Bangalore exhibit flowers of a pale purple colour. Spectroscopic examination of the light, transmitted through two petals held together, reveals an absorption in the region of the

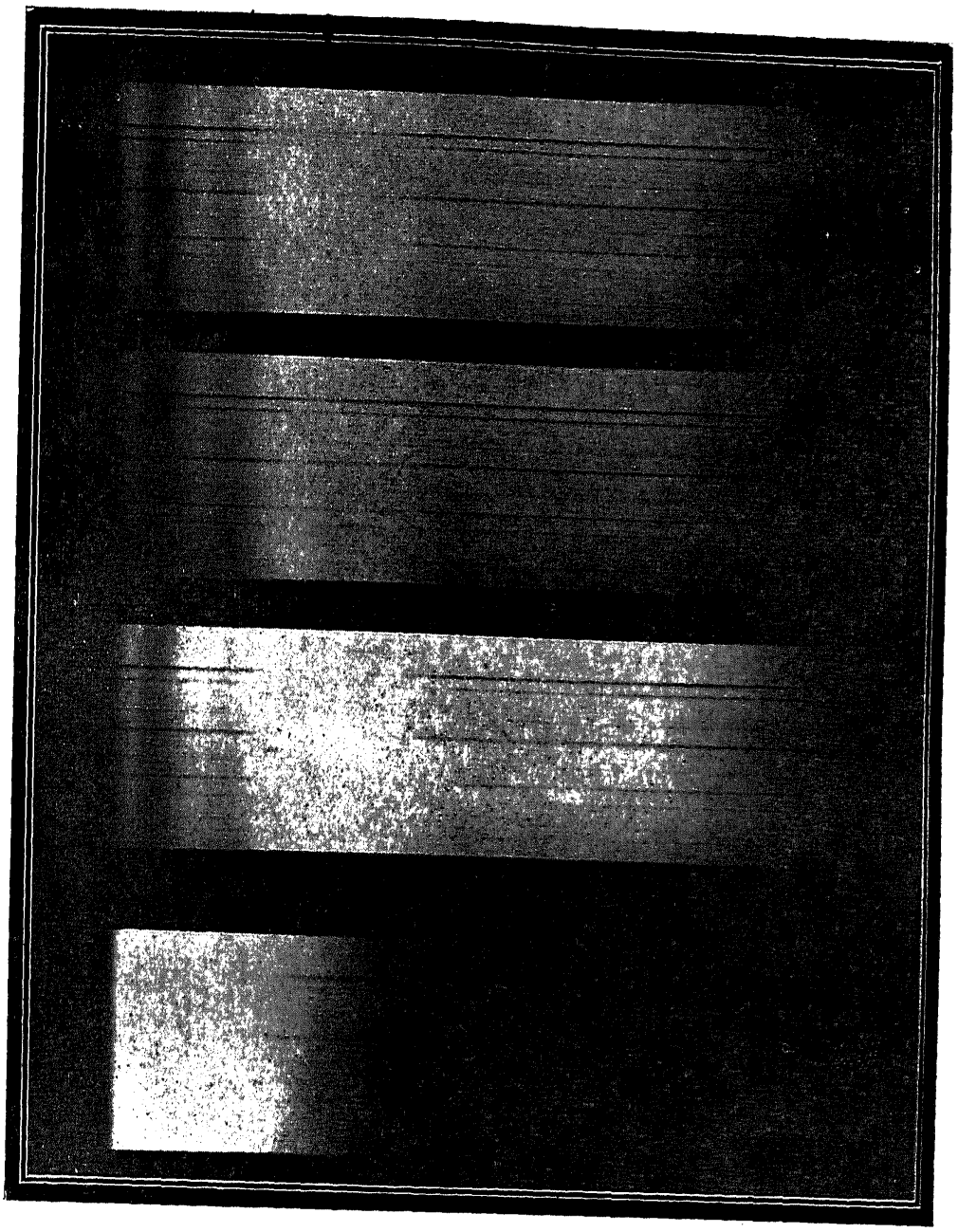


FIG. 1. (*a*), (*b*), (*c*), Absorption Spectra of Florachrome A. (*d*), Comparison Spectrum

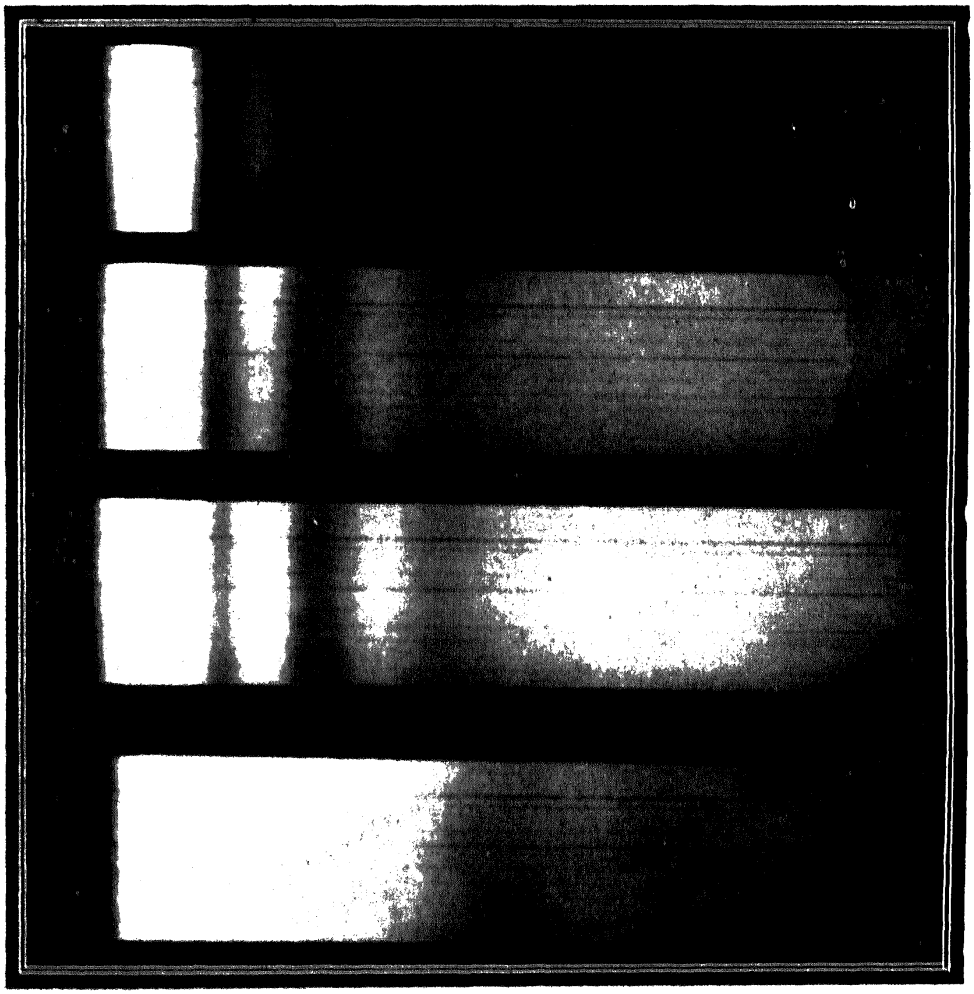


FIG. 1. (*a*), (*b*), (*c*), Absorption Spectra of Florachrome B. (*d*), Comparison Spectrum.

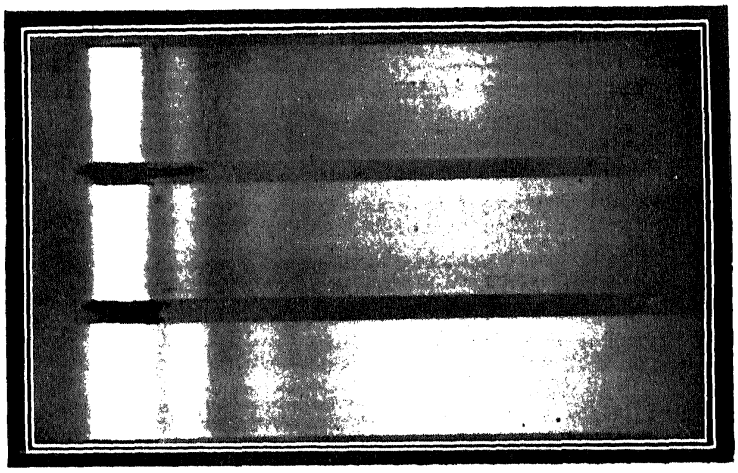


FIG. 1

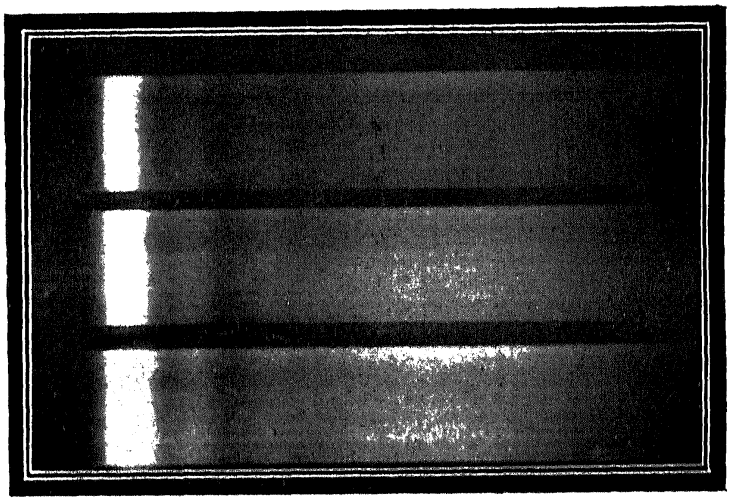


FIG. 2

FIGS. 1–2. Absorption Spectra of petals of the *Cineraria* flowers.

yellow extending from 560 mμ to 590 mμ to be the origin of the observed colour. The acetone extract shows this absorption strongly, and also weaker bands elsewhere in the green and the red.

Iris germonica.—The flowers of the blue iris examined *in vivo* show absorption bands in the green, yellow and red regions in the spectrum. This flower is of particular interest since the existence of both the florachromes in its petals is readily demonstrated by extracting the pigment in two stages, first with acetone and then with water. The acetone extract has a red colour and exhibits the Florachrome B spectrum clearly. The aqueous extract is of a bright blue colour and shows the extinction of the yellow and the orange characteristic of Florachrome A.

ACKNOWLEDGEMENT

The spectrophotometric records reproduced in this memoir were made in the Central Instruments Laboratory of the Indian Institute of Science, to the staff of which the author's cordial thanks are due for their kind assistance.