THE PIGMENTS OF THE COROLLA OF *BRUGMANSIA AUREA* SAFF.*

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(Received for publication on November 9, 1952)

Brugmansia aurea Saff. is a tree datura, native of the warmer parts of America. It bears periodic heavy blossoms of large funnel-shaped flowers with beautiful orange colour. It is grown as an ornamental plant in tropical gardens or inside the green-houses of the temperate regions. With *B. aurea* as the paternal parent and *Datura inoxia* Mill. as the maternal partner, the writer obtained an intergeneric hybrid by culturing the excised embryo *in vitro*. The cross otherwise always proved to be incompatible. The corollas of the flowers of both *D. inoxia* and the hybrid are almost pure white and they stand in sharp contrast to the deep orange colour of *B. aurea*. It was, therefore, thought desirable to investigate what pigments are responsible for the production of the orange colour in *B. aurea* and also to find out if any pigments in small ineffective amounts are present in the white corollas of *D. inoxia* or the hybrid. The present account deals with the investigation of the pigments of the corolla of *B. aurea*.

The young corolla of *B. aurea* while enclosed inside the calyx-tube is of green colour. As it emerges out of the calyx, it turns greenish yellow. Gradually, the green shade disappears and by the time the corolla unfolds, its colour changes to bright yellow. Later on, the limb starts turning orange, from the tip backwards. In the fully mature corolla, the limb is deep orange with prominent green veins and the tube is yellowish green.

INVESTIGATION

The fresh young green corolla and the orange part of the mature corolla were separately crushed and shaken with various solvents. It was found that the colouring matter was insoluble in water or dilute acid (1% HCl). It was more or less soluble in the various organic solvents, like chloroform, acetone, benzene, ethyl alcohol, ethyl ether, etc., known to dissolve plastid pigments. The young green corolla yielded a green solution with red flourescence, while the orange parts gave a deep yellow extract. This indicated the absence of the sap soluble pigments belonging to the groups of anthoxanthins and antho-

* The article is a portion of the thesis written under the direction of Dr. A. F. Blakeslee, Director, Genetics Experiment Station, Smith College, U.S.A., and submitted to the faculty of Smith College in partial fulfilment of the requirements for the Degree of Doctor of Philosophy. The writer is deeply indebted to Dr. Blakeslee and Dr. S. Satina for the guidance and other help rendered during the investigation.

cyanins, and the possible presence of plastid pigments. The green 80% aqueous acetone extract of the young green corolla was subjected to the procedure for the separation of the chlorophyll and carotenoid components, based on their differential solubility in the various organic solvents as described by Haas and Hill (1928). After transferring the colouring matter to ethyl ether, a strong methyl alcoholic solution of caustic potash was added. A transient brown colour was produced at the junction of the two liquids. The colour gradually changed to olive-green and finally back to the original green, giving a positive 'Phase Test' for the presence of chlorophylls. After adding a little water to this liquid and shaking it, two layers separated out, the lower green aqueous containing chlorophylls and the upper yellow ethereal the carotenoid matter. The yellow ethereal layer solution, after being washed properly, was concentrated by evaporation. Later it was diluted with light petroleum and shaken up with 90% methyl alcohol. Both the petrol ether and the methyl alcohol layers showed the presence of the yellow colouring matters. This strongly indicated the presence of both carotin and xanthophyll besides the chlorophylls in the green corolla. A similar treatment of the acetone extract of the orange part of the mature corolla yielded no green layer and gave a negative 'Phase Test' for the presence of chlorophylls while it displayed yellow pigments in both petrol ether and methyl alcohol layers. This showed that in the young green corolla, the chlorophylls and the carotenoids, carotin and xanthophyll, are present. As the corolla matures and turns orange, the chlorophylls disappear leaving behind carotenoids.

For confirmation of the above results, chromatography, an ingenius analytical method discovered by Tswett (1910) and elaborated by several other workers (cited by Zechmeister and Cholnoky, 1944; Strain, 1942) was resorted to. Chromatography enables one to separate out many biological compounds of closely related chemical structure. The separation depends on the property of their being selectively adsorbed to different degrees by the various adsorbents. It is observed that if an extract of the green colouring matter of the higher plants is slowly percolated through an adsorption column made up of the layers of, from above downwards, sugar, calcium carbonate and aluminium oxide, the chlorophylls are held by sugar, xanthophyll by calcium carbonate and carotin by alumina (Harrow, 1944). The writer adopted the following procedure for the chromatographic analysis of the pigments of the corolla of *B. aurea*. In each case 200 gm. of the fresh corolla was crushed and then immersed in a mixture of 45 c.c. petroleum ether (B.P. 70° C.), 5 c.c. benzene and 15 c.c. methyl alcohol for about four hours. The filtrate was transferred to a separatory funnel and the methanol was removed by carefully washing with distilled water. After that the pigment extract was shaken with some anhydrous sodium sulphate to eliminate the traces of water. For the adsorption column a 20 cm. long and 1 cm. wide glass tube, with a constriction at the lower end was employed. A little cotton wool was inserted into the constriction. A uniform, moderately pressed, adsorption column was prepared as follows: lower 4 cm. aluminium oxide (activated by heating at 200° C. for 15 minutes), middle 4 cm. calcium carbonate

(activated by heating at 150° C. for 10 minutes), upper 6 cm. finely powdered dry sucrose covered over with some anhydrous sodium sulphate. The column was made wet with petroleum ether (B.P. 70° C.), run down from a dropping funnel through gentle suction. This was followed by slow percolation of the extract of the corolla through the adsorbent column and afterwards washing it down with a little petroleum ether (B.P. 30–50° C.). Throughout precaution was taken to keep the column at the top constantly covered with a liquid. After the proper development of the chromatogram, the column was sucked dry in a stream of nitrogen. The size and the intensity of the colour of the various pigment bands formed in the different parts of the column were noted. The chromatogram obtained from the extract of the young green corolla showed four distinct bands—two green ones in the sugar layer, a light yellow in the calcium carbonate layer and a reddish one in the aluminium oxide layer. It confirmed the earlier results that in the young stage the corolla contains the usual four pigments of the green parts of the plants—the two chlorophylls, xanthophyll and carotin. After its emergence out of the calyx tube, the bright yellow corolla on chromatography exhibited the presence of the two carotenoids in larger amounts and the complete absence of the chlorophylls. The chromatogram of the extract of the orange part of the fully mature corolla also showed complete absence of the chlorophyll pigments, but it displayed a still greater increase in the red carotenoid content while the xanthophyll amount remained almost the same.

The histological examination of the corolla of *B. aurea* revealed that the pigments are contained inside the epidermal cells only. Extremely small chromatophores could be seen in the epidermal layer of the young green corolla while it was still inside the calyx tube. No plastids, however, could be made out after the corolla emerged out of the calyx and turned yellow. In the epidermal cells of the yellow corolla the carotenoid pigments are seen under the microscope as shiny and oily looking yellow substance, diffused in the cytoplasmic matrix. It leads to the conclusion that the green plastids of the young corolla distintegrate during its later growth setting free the pigments in the cytoplasm. The chlorophylls disappear leaving behind the carotenoids which impart a yellow colour to the organ. The cells of the epidermis of the orange part of the mature corolla, however, showed the presence of numerous fine acicular red crystals besides the diffused yellow substance in the cytoplasm. These crystals are, presumably, of carotin, or some of its isomer, like lycopin or some other allied carotenoid, into which carotin may be easily convertible. The chromatogram of the extract of the corolla at this stage displayed a marked increase in the red carotenoid contents. The appearance of the orange colour of the corolla of *B. aurea*, therefore, results out of the combined effect of the large number of the red crystals and the yellow diffused pigment occurring together in its epidermal cells. The orange colour of the petals of *Trapæolum majus* and several other plants has been reported to be a somewhat similar combined effect of the presence of yellow and red materials in the tissues (Möbius, 1937). The petals of *Trapæolum*

majus, however, contain a red sap and yellow chromatophores in the same cells.

SUMMARY

Brugmansia aurea Saff. is a tree datura, native of the warmer parts of America. The young corolla while enclosed inside the calyx tube is of green colour. Later it gradually turns greenish yellow, yellow, and finally orange. By determining the differential solubility of its pigments in various organic solvents and by the method of chromatographic analysis, it has been found that the young green corolla contains the typical pigments of a green-leaf chlorophylls, xanthophyll and carotin. Later on, the chlorophylls disappear while the carotenoids increase in amount. The mature corolla shows the presence of large amounts of carotenoids inside the epidermal cells in the form of diffused yellow pigment and numerous red needle-like crystals. The combined effect of the yellow and red results in orange colour of the corolla.

LITERATURE CITED

1. HARROW, B. *et al.* 1944. Laboratory Manual of Biochemistry. 2nd Ed. Philadelphia.
2. HAAS, P. AND HILL, T. G. 1928. An Introduction to the Chemistry of Plant Products. 4th Ed. London.
3. MÖBIUS, M. 1937. Pigmentation in Plants, exclusive of Algæ. Bot. Rev. 3: 351–363.
4. STRAIN, H. H. 1942. Chromatographic Adsorption Analysis. New York.
5. TSWETT, M. 1910. Chlorophylls in Plant and Animal World (*Chromofilli restitelnom i schivotnom* Mirje). Warschau: Tipogr. Warshawskago utschebnago Okruga.
6. ZECHMEISTER, L. AND CHOLNOKY, L. 1944. Principles and Practice of Chromatography (Eng. Transl.). New York.