COLOURING MATTER OF THE FLOWERS OF *HIBISCUS VITIFOLIUS*

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Hibiscus vitifolius is a tall annual herb common in the hotter parts of India up to a height of 3,000 ft. It bears prominent flowers the petals of which are sulphur yellow in colour with purple eye spots at the base. A preliminary investigation of the flower petals indicated that they are very rich in flavonols possessing high tinctorial properties.

Certain interesting points should be noted regarding the extraction of these flowers. When the dried flower petals were employed, complete extraction could not be achieved by repeated boiling with alcohol. Considerable amount of the colouring matter could be obtained from a subsequent water extract. This indicated that the components present are not so easily soluble in alcohol and are more soluble in water. Consequently for complete extraction it was found necessary to use dilute alcohol. Fresh flowers could be conveniently used for extraction in which case ordinary rectified spirits could be used directly, and the extraction was found to be complete in a much shorter time. A good yield of a crystalline glycosidic substance separated out on concentrating the alcoholic extract and allowing it to stand for a few days. It was very sparingly soluble in alcohol but could be readily crystallised from hot water. It did not give any prominent colours with alkaline buffer solutions, but on hydrolysis it yielded gossypetin and glucose in equimolecular proportions. The acetate of this compound could be obtained as a colourless solid but could not be crystallised. In its composition and in all the properties mentioned above the glucoside agreed closely with gossypin first isolated from the flowers of *Gossypium indicum*.[1] The important point should be noted that whereas the cotton flowers give only a poor yield of gossypin[2] and that too not consistently, the flowers of *Hibiscus vitifolius* form a rich source of this new and interesting glucoside of gossypetin. Further, even for the preparation of gossypetin for various experimental purposes these flowers may be considered to be very handy and very pure specimens of the flavonol could be readily obtained.

The alcoholic mother-liquors left after the separation of gossypin were satisfactorily worked up by precipitation as the neutral lead salt. This

fraction contained besides gossypin a component sparingly soluble in water and capable of being extracted with ether. This was identified as quercetin from its properties and by the preparation of its acetate. It is our experience that gossypetin, the predominantly major flavonol component of these flowers, occurs entirely as the glycoside, gossypin, whereas the minor component quercetin seems to occur almost entirely free. This constitutes a great advantage since the components can be readily separated; gossypin is very sparingly soluble in organic solvents and more readily soluble in water whereas quercetin exhibits just the opposite solubility characters. The association of quercetin with gossypetin in the cotton flowers has been already noted. The persistence of this even in *Hibiscus vitifolius* is significant in connection with the biogenesis of the flavonols as suggested in an earlier publication.

Experimental

Extraction: First Stage (Gossypin).—

The fresh flowers with the calyx removed (2,000) were extracted twice, refluxing each time with alcohol for 3–4 hours. The pigment was completely extracted by this process as was shown by the colourless residue. The dark red alcoholic extract was concentrated to recover most of the solvent whereby a highly viscous dark reddish brown concentrate was left behind. It was kept in the ice-chest for 3–4 days when a large amount of a yellowish brown solid separated out. It was filtered and washed with a little alcohol to remove the darker coloured resinous impurities. It was then dissolved in boiling water (200 c.c.) and the deep red solution filtered through a plug of cotton-wool to remove the waxy matter. The filtrate was cooled to the room temperature, treated with an equal volume of ether and kept in the ice-chest for 24 hours. The pale yellowish brown crystalline solid that separated out was filtered, washed with a little alcohol and ether and dried; yield, 8·0 g. The product was purified by crystallisation from hot water thrice using a little charcoal to remove extraneous colouring matter. From the clear brown filtrate gossypin came out as shining yellow crystals which appeared as narrow rectangular plates under the microscope. On heating in a capillary tube it melted with vigorous decomposition at 228–30°.

When the dry flowers were used (250 g. amounting to roughly 3,000 flowers) they were first moistened with water, left for a few hours and then extracted with alcohol in the above manner. The yield in this case was 10 g.; it is therefore slightly less with dry flowers.

Gossypin was readily soluble in water to a golden yellow solution; it was sparingly soluble in alcohol and pyridine and almost insoluble in ether,

acetone or ethyl acetate. It gave a dark olive green colour with alcoholic ferrric chloride and a brown precipitate separated out soon. In alcoholic solution a bright red precipitate was obtained with lead acetate. It dissolved in aqueous alkali to a stable bright yellow solution. With alkaline buffer solutions, a yellow solution was obtained which changed to a pale pink during the course of 3 days. With *p*-benzoquinone in alcohol it did not produce any reddish brown colour or precipitate even after a long time.

The acetate was prepared by boiling gossypin (0·5 g.) with acetic anhydride (5 c.c.) and a few drops of pyridine for 2 hours. The white solid that separated out on pouring the reaction mixture into water was filtered and washed. It was readily soluble in alcohol or benzene to a brown solution which did not deposit the acetate on cooling. On precipitation from benzene solution by the addition of petroleum ether it could be obtained as a colourless amorphous solid and all attempts at crystallising it were unsuccessful. It melted indefinitely round about 120°.

Hydrolysis of the glucoside.—Gossypin (1 g.) was hydrolysed by boiling with 7% sulphuric acid (30 c.c.) for 2 hours. Bright golden yellow silky needles of the aglucone separated out during the course of the reaction. The mixture was then cooled, the aglucone filtered and washed and the filtrate preserved for the examination of the sugar. The yield of the aglucone was 0·5 g.

It was purified by crystallising from a mixture of ethyl acetate and benzene from which it separated out as bright yellow elongated rectangular prisms decomposing at 300–10°. It was sparingly soluble in water but it readily dissolved in alcohol, ether, acetone or ethyl acetate. In alkaline buffer solution (pH, 9·8) it formed a yellow solution which immediately changed to bright emerald green and then to pure blue; this gradually faded and finally a colourless solution was left. In alcoholic solution it gave a dark red precipitate with benzoquinone, an olive brown colour with ferric chloride and a red precipitate with lead acetate. It was identical with gossypetin in all respects.

A small quantity of the aglucone was acetylated with acetic anhydride and pyridine. The acetate was sparingly soluble in alcohol from which it crystallised in the form of rectangular prisms melting at 226–28°. The mixed melting point with an authentic sample of gossypetin hexa-acetate was not depressed.

By methylating the aglucone with dimethyl sulphate and potassium carbonate in anhydrous acetone medium the hexamethyl ether was obtained.

It was best crystallised from ethyl acetate from which it came out as colourless narrow rectangular plates melting at 170–72°. The mixed melting point with gossypetin hexamethyl ether was not depressed.

The acid filtrate containing the sugar was neutralised with barium carbonate, filtered and the filtrate concentrated to small bulk. The syrup was diluted with a little distilled water and filtered. The filtrate was treated with an excess of a mixture of phenylhydrazine hydrochloride, sodium acetate and acetic acid. On heating for about 30 minutes in a boiling water-bath the osazone separated out as a yellow crystalline solid which had the characteristic crystal appearance of sheaves of needles resembling that of glucosazone. When crystallised from dilute alcohol it melted with decomposition at 205°.

A quantitative estimation of the products of hydrolysis of gossypin from *Hibiscus vitifolius* yielded gossypetin (monohydrate) 63·3% and glucose 33·4%. Similar results were obtained with the gossypin sample from *Gossypium indicum* also.

Extraction: Second Stage (Gossypin).—

The original alcoholic filtrate left after gossypin had been removed, was concentrated further in a large basin and the concentrate treated with excess of water and filtered from waxy and resinous impurities. An equal volume of ether was added to the filtrate and the mixture kept in the ice-chest for a few days. A small quantity of a yellow solid separated out and it was filtered and washed. Yield, 1 g. Its properties indicated that it was gossypin.

The filtrate was diluted with water and extracted with ether repeatedly. On distilling off the ether from the extract a viscous semi-solid residue was obtained. It did not crystallise. Keeping it in the ice-chest for a long time and other attempts at crystallisation using various solvents were not successful. However, it gave an olive green colour with ferric chloride, a reddish brown precipitate with lead acetate and dissolved in aqueous alkali to a stable yellow solution. It seemed to contain a small amount of quercetin in a very impure condition.

Extraction: Third Stage (Gossypin and Quercetin).—

The mother-liquor left after extraction with ether was treated with excess of an aqueous solution of lead acetate when a good yield of a reddish brown precipitate was obtained. It was filtered, washed repeatedly with hot water and alcohol. The lead salt was suspended in hot water and decomposed by passing hydrogen sulphide. The lead sulphide was removed

by filtration and the dark red filtrate was concentrated to small bulk, treated with an equal volume of ether and kept in the ice-chest. A small quantity of a yellow crystalline solid separated out and it was filtered and washed with a little ether. It was found to be identical with gossypin. The filtrate was repeatedly extracted with ether and the combined ether extract was distilled to remove the solvent. The yellow crystalline solid residue was purified by crystallisation from aqueous alcohol twice. It separated out as yellow silky needles decomposing at about 300°. It was sparingly soluble in water but was readily soluble in alcohol, ether or acetone. In alcoholic solution it gave an olive green colour with ferric chloride and a red precipitate with lead acetate. It dissolved in aqueous alkali to a yellow solution and the colour was fairly stable. It did not respond to the gossypetone reaction.

The compound was acetylated using acetic anhydride and pyridine. The acetate was crystallised from a mixture of absolute alcohol and petroleum-ether from which it came out in the form of flat needles melting at 192–93°. The mixed melting point with an authentic sample of quercetin penta-acetate was not depressed.

The filtrate from the neutral lead salt was treated with basic lead acetate. As the precipitate was very small it was not further studied.

Summary

The colouring matter of the flower petals of *Hibiscus vitifolius* consists almost entirely of gossypin along with very small amounts of quercetin. These petals form a very good source of this new and interesting glycoside and eventually of gossypetin also.

Literature References

1. Neelakantam and Seshadri .. *Proc. Ind. Acad. Sci.*, 1936, **4A**, 54.
2. Rao and Seshadri .. *Current Sci.*, 1938, **7**, 227.