PIGMENTS OF COTTON FLOWERS.

Part II. Uppam (*Gossypium herbaceum*)

By K. Neelakantam, T. R. Seshadri
and
(in part with) R. H. Ramachandra Rao.
(From the Department of Chemistry, Andhra University, Waltair.)

Received October 22, 1935.

Uppam (*G. herbaceum*) is an important dry-land cotton grown widely in South India since it is well adapted to withstand drought. The flower petal is yellow with deep crimson spot near the base. The petals used in this investigation were collected from the Central Farm, Coimbatore. At the close of the picking season the fresh flowers were collected, the petals removed and dried in the sun to crispness as rapidly as possible and then stored in cloth bags.

A. G. Perkin (1916) made a chemical study of the flower petals of *G. herbaceum* which he called the ordinary Indian yellow cotton flower and found that it contained Gossypitrin as the main component along with some isoquercitrin. It is not possible to find out exactly how his material differed from ours. He seems to have obtained his supply from North India. We are informed by the Cotton Specialist, Coimbatore that the North Indian Herbaceums are of longer duration, bushier in habit, often finer in quality and longer in staple ; the fibres are more difficult to gin than those in Madras. The flower petals, however, seem to be quite similar in appearance.

The results of our investigation are rather different from those of Perkin. We find that the chief components are gossypitrin (9 g. from 2200 g.) and quercetin (10 g.). Besides these we isolated in a fair yield (3 g.) a new glycoside which we find to be a monoglucoside of a new flavonol and small quantities of gossypetin (1 g.). We have not been able to detect the presence of any quercetin glucoside in the flowers. If any should be present, the quantity should be exceedingly small. When an alcoholic extract of the flowers was concentrated and allowed to stand for several days, a good amount of a crystalline solid was deposited. Perkin (1916) obtained a similar solid and found it to be easily soluble in water and inferred that it consisted of alkali metal salts of the pigment. We have observed that though this deposit is easily soluble in hot water, it dissolves rather sparingly in the

cold, that it gives very little residue on igniting in a platinum crucible and that its aqueous solutions are not alkaline to litmus. We find that it contained almost all the gossypitrin of the flowers in a fairly pure state. Our observations with these flowers do not therefore support Perkin's view that the pigments are present in them as alkali metal salts of the glucosides.

A detailed study of the new glycoside will be published later as soon as its nature is known definitely. Our samples of gossypitrin and gossypetin agree closely in properties and reactions with those isolated by Perkin and the aglucone synthesised by Baker, Nodzu and Robinson (1929). We have adopted Robinson's method of examining the colour reactions in buffer solutions of varying p_H and we find that our sample of gossypetin produces the same colour effects as recorded by him. This method gives useful results with gossypitrin also and easily distinguishes between the two. Our samples of gossypitrin and its acetyl derivative melt higher than those of Perkin probably due to their being purer.

Experimental.

Isolation of Gossypitrin.—Dry petals of *G. herbaceum* (2200 g.) were powdered, extracted repeatedly with boiling rectified spirits till the pigments were completely removed and the alcoholic extract concentrated to about 800 c.c. On allowing it to stand for some days a large quantity of deep yellow crystalline solid (needles) was deposited (*cf.* Perkin, 1916). It was not easily soluble in cold water or alcohol but dissolved readily on heating and crystallised out on cooling. The aqueous solution was neutral to litmus (rather faintly acidic). Ignition of the crude substance in a platinum crucible gave a very small quantity of ash which consisted mostly of alkali chlorides. It did not therefore appear to be a metallic salt of a glycoside pigment.

The crystalline sold was filtered, washed with a little alcohol and boiled with a sufficiently large quantity of water (about 800 c.c.). The yellow pigment dissolved and tarry impurities floated on the top. The hot solution was carefully decanted on to a filter and filtered. The resin was washed with a small quantity of hot water and the washings added to the original filtrate. On allowing the solution to cool overnight a large mass of golden yellow crystals was obtained. It was filtered and washed with a small quantity of water. A second crop was obtained by concentrating the mother liquor on a water-bath to 100 c.c. and allowing it to stand for two days. After one recrystallisation from water this sample resembled the previous one closely. The two put together weighed 15 g. It was, however, impure since the melting point was rather indefinite. After two crystallisations from aqueous pyridine it sintered and shrank at about 202° C. and melted

at 237–38° C. (yield 9 g.). It was sparingly soluble in glacial acetic acid and could easily be crystallised from it when mixed with some water. But the best method of further purifying it was to boil it up with a small quantity of dilute acetic acid. This removed some brown impurities and the solid now melted at 250–52° C. (decomp.). One further recrystallisation from boiling dilute acetic acid gave yellow needles melting at 251–52° C. without any previous sintering. (Found in air dried specimen: C, 48·3 ; H, 4·8 ; $C_{21}H_{20}O_{18}$, $2H_2O$ required C, 48·8 ; H, 4·7%). The substance was a hydrate. We could not get any form melting at 203° C. and acetone did not produce any definite change in the pure specimen. We, however, noticed that when the above pure sample was crystallised again from pyridine, the product sintered strongly and showed marked shrinking at about 203° C., though it melted down only at 250° C. The pure substance was sparingly soluble in alcohol, acetone, and glacial acetic acid but dissolved easily in hot dilute acetic acid and in pyridine. The colour reactions of our specimen agree entirely with the observations of Perkin. Aqueous lead acetate gives a deep red precipitate and ferric chloride gives an olive green colour. We find that alkali colour reactions are best observed by using buffer solutions of varying p_H and the reactions of gossypitrin contrast clearly with those of gossypetin (Baker, Nodzu and Robinson, 1929) as shown below :

Colour Reactions.

p_H	Gossypetin	Gossypitrin
6·8	Goes into solution very slowly producing a greenish yellow colour. In about 2 hours a green amorphous solid separates out. After 24 hours a colourless liquid with a dirty green precipitate at the bottom. No further change.	Substance is unaffected.
8·0	Dissolves rapidly to a yellow solution. In 3 minutes becomes pure green, then slowly opaque and dark and in about ½ hour turns brown purple. In 24 hours goes into a pale yellowish green liquid with a green precipitate at the bottom ; After 48 hours colourless liquid with a green precipitate.	Yellow solution rapidly changes (½ minute) to yellowish green and in another ½ minute becomes bright emerald green which slowly fades. After 24 hours it is brown red and after 48 hours very faint brown.

p_H	Gossypetin	Gossypitrin
8·6	Yellow solution rapidly goes green which is fairly stable and slowly turns blue. After 1½ hours signs of purple appear and the solution is rather opaque. After 24 hours pale yellowish green liquid with green precipitate; after 48 hours colourless liquid and a green precipitate.	Yellow solution immediately going into green and then to emerald green which fades rapidly. In 10 minutes all green was lost, it was then brownish yellow fading slowly. After 24 hours pale brown red and no change thereafter.
9·8	Yellow solution. In a few seconds deep green, further 2 minutes pure blue which was stable for about 1½ hours. After 24 hours very faint blue and 48 hours colourless with a small precipitate.	Yellow solution going immediately to green and emerald green and rapidly fading. In 10 minutes loses all green and becomes brownish yellow rapidly fading. After 24 hours pale brown red and no further change.
11·0	Yellow-green-blue-deep blue very rapidly fading to pale blue. In ½ hour pale greenish blue, fading. After 24 hours very pale yellow brown and after 48 hours colourless with no precipitate.	Yellow, immediately goes into yellowish green, after 2 minutes goes yellowish brown and fades rapidly. In 5 minutes pale brownish yellow. In 24 hours faint brown red and no further change.

Gossypitrin is affected only at a higher p_H than the aglucone; its most brilliant colour is emerald green and it does not show any blue or purple whereas the brilliant effect of gossypetin is blue. The glucoside undergoes the changes and loses colour more rapidly than gossypetin. Further the green precipitate that is formed as an end product in the case of gossypetin is not formed with the glucoside.

Our sample of gossypitrin gives with *p*-benzoquinone, a maroon coloured gossypitrone which exhibits the same behaviour as described by Perkin. The acetyl derivative of the glucoside was obtained by boiling with acetic anhydride with or without sodium acetate and the product was purified by crystallisation from acetic anhydride and alcohol mixture. It was obtained as colourless needles, m.p. 240–44° C. (*cf.* Perkin 226–28° C.).

Hydrolysis to Gossypetin.—The glucoside was hydrolysed by boiling with 7% sulphuric acid for 2 hours and a good yield of the crystalline aglucone

was obtained. It was easily recrystallised from rectified spirit and it came out as yellow flat needles which grow dark and decompose at about 310° C. (Found in air dried specimen : C, 48·5 ; H, 4·6 ; $C_{15}H_{10}O_8$, $3H_2O$ required, C, 48·4 ; H, 4·3%). The hexa acetate crystallised from alcohol as colourless rhombohedral plates melting at 228–30° C. agreeing with the melting point recorded by Perkin for the natural specimen and by Baker, Nodzu and Robinson (*loc. cit.*) for the synthetic one. As the other product of hydrolysis of the glycoside, glucose was isolated as the osazone. Our sample of gossypetin gave colour reactions with sulphuric acid, lead acetate, ferric chloride and benzoquinone identical with those recorded by previous workers.

Isolation of a new glycoside.—The alcoholic mother liquor left after the removal of gossypitrin was again distilled on a water-bath in order to remove as much alcohol as possible and then treated with a large volume of water. The whole mixture was poured on to an evaporating basin and most of the remaining alcohol removed by heating on a water-bath. A brown red solution was produced along with a lot of tarry matter. By careful filtration of the hot solution most of the resin was removed and on allowing the filtrate to stand for two days a good amount of a yellow crystalline solid mixed with some resin separated out. It was filtered and twice recrystallised from hot water when it appeared as a greenish yellow solid. After three crystallisations from dilute pyridine, it was quite pure and appeared as yellow needles, m.p. 247–49° C. (yield 3 g.). It was found to be a new glycoside different from gossypitrin and quercimeritrin. A detailed report on this substance will be published later.

The mother liquors obtained in the course of the purification of the new glycoside were concentrated on a water-bath and extracted with ether repeatedly. The extraction was rather slow and the ether layer continued to become yellow even after 5 extractions. On evaporating the ether extract a yellow crystalline solid was obtained which crystallised out of rectified spirits as yellow needles, m.p. 310–12° C. and gave an acetyl derivative melting at 195° C. It was identified as quercetin (0·2 g.). From the aqueous solution left after ether extraction no definite substance could be obtained by concentration. It was therefore boiled with 7% sulphuric acid for 2 hours. The solution was filtered hot in order to separate resin and when it was cooled a yellow crystalline precipitate was formed mixed with some resin. It was, therefore, repeatedly extracted with ether and the ether solution evaporated. A yellow solid was obtained which easily crystallised out of alcohol as needles, m.p. 250–70° C. (yield 0·5 g.). It was obviously a mixture of aglucones. It was therefore acetylated and the product crystallised from alcohol. Of the two fractions which were isolated, the

more soluble fraction consisted of quercetin pentacetate which was rendered quite pure by one further recrystallisation; the less soluble fraction consisted of gossypetin hexa-acetate which was also obtained quite pure by one further crystallisation. Glucose was detected in the solution after removing the aglucones. Whether quercetin obtained here was originally present free or in the form of an easily soluble glucoside is a question which cannot be answered definitely. Since quercetin could be obtained from the solution even before hydrolysis and the quantity of quercetin obtained after hydrolysis in the form of the acetyl derivative was very small (about 0·1 g.), it could be surmised that no appreciable amount of quercetin glucoside could have been present.

Neutral lead acetate fraction: Isolation of Quercetin.—The original aqueous mother liquor left after the separation of the new glycoside was now treated with excess of neutral lead acetate with vigorous shaking. After allowing to stand for a day the orange red precipitate was filtered, washed with water and the pigment liberated by passing hydrogen sulphide into an aqueous suspension of the lead salt. Lead sulphide was filtered off, the precipitate again extracted with hot water and the collected water extracts concentrated on a water-bath. Crystals were collected in four fractions and each fraction separately examined. Each fraction was found to be mixed up with a good amount of resin. Repeated crystallisation from aqueous pyridine produced yellow crystalline substances. The first and second fractions were found to yield the same product—yellow needles, m.p. 310–12° C. which yielded an acetyl derivative, m.p. 197–98° C. Its identity with quercetin was established by the determination of the mixed melting points with quercetin and penta acetyl quercetin obtained from *G. herbaceum* and by a comparison of colour reactions. Yield of quercetin was 10 g. The mother liquor from the crystallisation of these fractions was extracted repeatedly with ether. The ether extract gave a crystalline yellow solid which no crystallisation from alcohol melted indefinitely between 240–70° C. It was obviously an impure aglucone. So it was acetylated and the acetyl derivative crystallised from rectified spirits. Acetyl gossypetin m.p. 228–30° C. (mixed m.p. the same) could be obtained pure (0·2 g.). After ether extraction the aqueous layer was boiled with 7% sulphuric acid for 2 hours and from the highly brown semi-solid that was produced some more gossypetin acetate (0·3 g.) was isolated by acetylation and repeated recrystallisation from alcohol. No other compounds could be separated.

The third and fourth fractions consisting of about 10% of the whole of the neutral lead acetate precipitate were found after two recrystallisations from water and subsequently from dilute pyridine to melt above 290° C.

(yellow needles) but did not give a sharp melting point. They were therefore acetylated, when they yielded readily pure acetyl gossypetin (0·5 g.).

Basic lead acetate fraction.—The filtrate left after isolating the neutral lead acetate precipitate was then treated with a large excess of basic lead acetate. Comparatively small quantity of a lemon yellow precipitate was obtained. When this was decomposed with hydrogen sulphide about 1 g. of a crude pigment was produced. This was identified as quercetin after repeated recrystallisation and comparison with an authentic specimen.

Summary.

The flower petals of the Uppam cotton plant (*G. herbaceum*) contain as their main components gossypitrin and quercetin. An appreciable quantity of a new glycosidic pigment and a small quantity of gossypetin were also isolated. Uppam petals, therefore, differ in their composition from those of *G. herbaceum* examined by Perkin who probably obtained his supply of the petals from the North Indian variety. His results indicated that gossypitrin was the main component and that isoquercitrin was present in small quantities.

REFERENCES.

1 A. G. Perkin, *J. C. S.*, 1909, 2181–93; 1913, 650–662; 1916, 145–154.

2 Baker, Nodzu and Robinson, *J. C. S.*, 1929, 74–84.