PIGMENTS OF COTTON FLOWERS.

Part III. Karunganni (*Gossypium indicum*).

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**Karunganni (G. indicum)** is a dry-land cotton widely grown in South India. Its lint is better than that of Uppam (*G. herbaceum*) but the flower petals are similar being golden yellow with crimson spots at the base. For purposes of this investigation they were collected from the Cotton Research Station, Coimbatore.

During the cotton season of 1932 one of us (T. R. S.) made a preliminary examination of the petals using about 200 grams of dried material and found that they contained mainly Quercimeritrin and Gossypitrin. After making the usual extraction with alcohol and removing as much of the solvent as possible, the pigment was taken up with water, precipitated with neutral lead acetate and this portion alone was examined since a subsequent precipitation with basic lead acetate yielded very little more. By repeated crystallisation from alcohol Quercimeritrin and Gossypitrin were separated and identified by means of their colour reactions, the preparation of their acetyl derivatives, hydrolysis to the aglucones and the preparation of the acetyl derivatives of the aglucones.

A larger sample was collected in 1933 and examined in detail according to the method already outlined in the previous papers of this series (Neelakantam, Rao and Seshadri, 1935). The pigments were isolated in five different fractions: (1) sparingly soluble in alcohol and coming out on concentrating the alcoholic extract, (2) sparingly soluble in water and getting deposited from the aqueous solution obtained after removing alcohol, (3) obtained by ether-extracting the aqueous solution, (4) the neutral lead acetate fraction precipitated as the lead salt on the addition of neutral lead acetate to the aqueous solution, and (5) the basic lead acetate fraction. From (1) was readily obtained a golden yellow solid which has been found to be a complex glucoside of Gossypetin melting with decomposition at 230° and has been named ‘Gossypin’. It seems to have the formula **C_{68}H_{44}O_{18}**.
Fraction (2) yielded a pale yellow solid, melting at 255-7° with slow decomposition and non-glycosidic in nature. Its formula seems to be C_{16}H_{19}O_{7}. Fraction (3) consisted of Quercetin whereas fraction (4) was found to be composed of Quercetin, Gossypetin, and a new aglucone previously obtained from *Gossypium herbaceum* (Neelakantam, Rao and Seshadri, 1935) and to which we now give the name 'Herbacetin'. The two latter were present mostly as their glucosides Gossypitrin and Herbacintrin which could not be isolated pure but their presence was ascertained from their colour reactions and the detection of glucose as one of their products of hydrolysis. Fraction (5) was very small and was made up of Quercetin only. No Quercimeritrin could be detected in this large sample, though Quercetin was easily isolated; Gossypitrin was present in smaller quantities only, the main bulk of the Gossypetin glucoside being 'Gossypin'.

The details of the constitution of (i) Gossypin, (ii) the new non-glycosidic pigment, and (iii) Herbacintrin are under investigation.

**Experimental.**

**Fraction 1. Isolation of Gossypin.**—Dried and powdered petals of Karunganni (1400 g.) were repeatedly extracted with rectified spirits and the extract concentrated to about 800 c.c. On allowing to stand for over a week a yellow crystalline solid was deposited. It was filtered and washed with a little alcohol (yield 4 g.). It was very sparingly soluble in alcohol, ether or pyridin and dissolved readily in boiling water to form a yellow solution from which sheaves of golden yellow needles separated out on cooling. Though it was easily soluble in hot acetic acid, this solvent could not be used for crystallisation since the substance came out of it as nodules without any definite crystalline shape. It was purified by twice crystallising from water and twice again from dilute alcohol; the pure substance appeared as glistening golden yellow needles, melting at 230° with decomposition. [Found in air dried sample: C, 46.7, H, 4.5 and loss on drying in vacuo at 110°, 10.1%; C_{25}H_{26}O_{18}, 4H_{2}O required C, 46.7, H, 4.4 and H_{2}O (loss), 10.0%. Found in sample dried at 110° in vacuo: C, 51.4, H, 3.7; C_{25}H_{26}O_{18} required C, 51.8, H, 3.7%.]

With dilute sodium hydroxide Gossypin gave a deep yellow solution; the colour faded rapidly on shaking with air and soon assumed a faint yellowish brown tinge. Sodium carbonate produced first yellow which changed into greenish yellow and finally pale yellowish brown. On adding cold concentrated hydrochloric acid to the yellow solid it immediately assumed a deep red colour and on heating went into a clear orange red solution which did not deposit anything on cooling. Strong sulphuric acid produced a similar effect and the solution exhibited no fluorescence. Concentrated nitric
acid dissolved the substance readily to give a pale yellow solution. Neutral lead acetate gave a red precipitate and ferric chloride an olive green colour. The substance did not give prominent colour reactions with buffer solutions of different $P_n$ values. At 6.4 a clear yellow solution was produced which was stable for several days. Between 8 and 9.8 the solutions were first yellow and slowly changed to red in about three days whereas at $P_n$ 11.0 the yellow was lost rapidly (15 minutes) and a very pale brown colour resulted. It did not give the Gossypetone reaction in aqueous alkaline solution on shaking with air or in alcoholic solution on treatment with $p$-benzoquinone.

Acetylation of Gossypin with acetic anhydride alone or with sodium acetate was unsuccessful. The substance dissolved and the yellow solution slowly lost its colour on heating. After boiling for 4 hours the acetyl derivative could not be precipitated by adding alcohol as in the case of Gossypitrin. On pouring the acetic anhydride solution into water and allowing it to stand, a colourless amorphous product was obtained and all attempts to obtain it crystalline resulted in failure. When Gossypin was hydrolysed by boiling with 7% sulphuric acid for 2 hours, Gossypetin and glucose were identified as the products. Gossypetin was identified by a comparison of its melting point, colour reactions and melting point of its acetyl derivative with those of the sample obtained from Uppam ($G. herba-
cenum$). Glucose was identified in the usual way as the osazone.

Fraction 2. Isolation of a New Pigment.—The alcoholic mother-liquor left after the removal of Gossypin was further distilled in order to remove as much alcohol as possible, a large volume of water added (1000 c.c.), and the whole heated in a basin on a water-bath in order to remove the remaining alcohol. A large amount of resin separated out. By careful manipulation the hot aqueous solution was decanted and filtered from the resin and concentrated to about 400 c.c. On cooling, no solid separated out. It was therefore extracted with ether repeatedly (4 times with about 100 c.c. each time) and then allowed to stand for a few days. The ether solution was kept separately for examination. The aqueous solution now deposited a pale yellow crystalline solid which was filtered and washed with small quantities of water. It was sparingly soluble in water but easily soluble in aqueous alcohol or pyridin. After two crystallisations from dilute pyridin and two from dilute alcohol it was quite pure and was obtained as pale yellow needles and rhombic plates, melting at 255–7° with slow decomposition. Yield 1.8 grams. [Found in air dried sample: C, 51.8, H, 5.4, loss on drying in vacuo at 110°, 14·0%; $C_{10}H_{12}O_4$, $3H_2O$ required C, 51.9, H, 4.9 and $H_2O$ (loss), 14·6%. Found in sample dried in vacuo at 110°, C, 60·0, H, 4·4; $C_{18}H_{24}O_7$ required C, 60·7, H, 3·8%.]
The substance was unaffected when boiled for two hours with aqueous or aqueous-alcoholic sulphuric acid and no sugar was produced, and hence it was non-glycosidic. It gave a stable yellow solution with dilute sodium hydroxide or carbonate. Concentrated hydrochloric acid in the cold produced no change whereas on heating a yellow solution was produced. Nitric acid produced a colourless solution. Cold concentrated sulphuric acid gave a yellow solution with a characteristic green fluorescence which became more prominent on diluting with more acid. On pouring this into water, however, a colourless solution resulted. It did not give any noteworthy colour changes with alkaline buffer solutions. Below \( p_n \) 10.0 it dissolved only slowly; at \( p_n \) 11.0 it gave a permanent yellow solution. Neutral lead acetate produced no precipitate whereas basic lead acetate gave a yellow solid. A drop of ferric chloride, added to an aqueous solution of the substance, produced a yellowish green colour. It was readily acetylated with acetic anhydride and sodium acetate. The acetyl derivative was easily soluble in alcohol and was obtained as colourless needles, melting at 186–7°C.

**Fraction 3. Quercetin.**—The ether extract on evaporation gave a brown solid, which after two crystallisations from aqueous pyridin, formed yellow needles (yield 0.5 g.) melting at about 310° with decomposition. Its colour reactions were identical with those of Quercetin and it formed an acetyl derivative melting at 194–5° which was not depressed by admixture with penta-acetyl Quercetin obtained from the Cambodia flowers (G. hirsutum).

**Fraction 4. Neutral Lead Acetate Fraction.**—The original aqueous solution left after the separation of Fractions 2 and 3 was treated with excess of neutral lead acetate. The orange red precipitate produced was filtered, washed with water, suspended in water and decomposed with hydrogen sulphide. By concentrating the aqueous solution thus obtained, the pigment was isolated in three fractions and each examined separately. The first two fractions were more easily purified and, after three crystallisations from dilute pyridin, yielded the same crystalline solid melting with decomposition at about 310° and yielding an acetyl derivative melting at 194–5°. It was identified as Quercetin (yield 1.5 g.).

The third fraction could not be crystallised and obtained pure. The colour reactions were very similar to those of Gossypetin. The sample was hydrolysed with sulphuric acid and the product was crystallised once from dilute acetic acid and a second time from dilute alcohol. Though it now looked quite clean and yellow (yield 1.5 g.) it was still impure, had no definite melting point (260–90°) and could not be purified by further crystallisation. The colour reactions were those of Gossypetin. It was, therefore, acetylated and the acetyl derivative crystallised from a mixture