PIGMENTS OF COTTON FLOWERS.

Part IV. Constitution of Herbacitrin and Herbacetin—New Glucoside and Aglucone (Flavonol).

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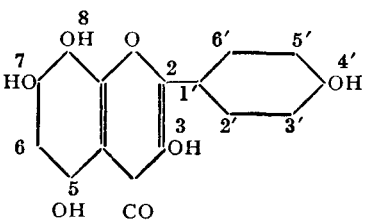
The isolation of a new flavonol glucoside from *Gossypium herbaceum* (Uppam) was recorded in a previous paper.[1] Though its constitution was known at that time the publication of our results was postponed till they could be confirmed by synthesis. It has been named Herbacitrin since it was first obtained from *Gossypium herbaceum* and since there already exists another similar substance with the name Gossypitrin. The corresponding aglucone which is a flavonol has received, therefore, the name Herbacetin. These have been found to occur in *Gossypium indicum* (Karunganni) also.[2]

Herbacitrin is a monoglucoside having the formula $C_{21}H_{20}O_{12}$ and it gives on hydrolysis with acids glucose and an aglucone (Herbacetin) of the composition $C_{15}H_{10}O_7$. Since they undergo oxidation in alkaline solution exposed to air, they belong to the flavonol series. They are isomeric with Quercimeritrin and Quercetin respectively to which they bear some resemblance particularly in their melting points and those of their derivatives, and in their colour reactions with ferric chloride and lead acetate. On closer examination, however, the differences become evident. The following table sums up these characteristics:

	Herbacitrin	Quercimeritrin
1. Melting point	247–49°	246–48°
2. Melting point of acetyl derivative ..	214–16°	216–17°
3. $FeCl_3$ colour reaction	Olive green	Olive green
4. Lead acetate colour reaction ..	Red precipitate	Red precipitate
5. Formula of hydrate	$C_{21}H_{20}O_{12}$, $2H_2O$	$C_{21}H_{20}O_{12}$, $3H_2O$

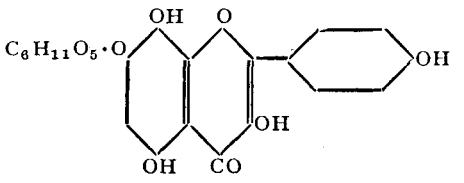
	Herbacetin	Quercetin
1. Melting point	280–83°	310–12°
2. Melting point of acetyl derivative ..	192–93°	197–98°
3. $FeCl_3$ colour reaction	Olive green	Olive green
4. Lead acetate colour reaction ..	Red precipitate	Red precipitate
5. Formula of hydrate	$C_{15}H_{10}O_7$, H_2O	$C_{15}H_{10}O_7$, $2H_2O$

The most remarkable difference between the isomeric pairs exists in their reactions towards alkaline buffer solutions of different pH values. Quercimeritrin and Quercetin give only yellow colours which fade slowly whereas Herbacitrin produces first yellow rapidly changing to emerald green just like Gossypitrin, and Herbacetin gives a bright blue after the initial yellow just like Gossypetin. In these colour reactions the resemblance between Gossypitrin and Herbacitrin, and between Gossypetin and Herbacetin is very close. They resemble also in their colour reactions with ferric chloride and lead acetate. Further a very useful observation in connection with the constitution of the new anthoxanthins is that they give the gossypetone reaction quite similar to Gossypitrin and Gossypetin. From the above reactions and the fact that Herbacitrin occurs in the cotton flowers side by side with Gossypitrin, it was deduced that the new glucoside and aglucone should have constitutions similar to Gossypitrin and Gossypetin as far as the benzopyrone portion of the molecules were concerned, that is, three of the five hydroxyl groups in Herbacetin should be in positions 5, 7 and 8. Since Herbacetin is a flavonol and the 4′-position in the hydroxy-phenyl nucleus is the most favoured among anthoxanthins and anthocyanins, the following constitution was assigned to Herbacetin.



This was confirmed by the oxidation of the glucoside by air in aqueous alkaline solution whereby *p*-hydroxy-benzoic acid was produced and isolated after methylation as anisic acid and identified.

The position of the glucose residue in Herbacitrin is tentatively fixed as 7 by analogy with Gossypitrin and Quercimeritrin (see Attree and Perkin[3]) which it resembles. Position 4′ was not considered probable in view of the fact that no instances are known among anthoxanthins in which the hydroxyphenyl nucleus carries the sugar residue, and Herbacitrin yields *p*-hydroxybenzoic acid on oxidation in alkaline solution. From the colour reaction with lead acetate and the difficulty with which it undergoes hydrolysis it could be concluded that Herbacitrin is not a 3-glucoside. Its ready oxidation to give the gossypetone reaction and its reformation with sulphurous acid show that positions 5 and 8 are free to form a *para*-quinonoid structure. The *ortho*-quinonoid structure does not seem to be so easily formed since Quercetagetin which has hydroxyl groups in the 5, 6 and 7 positions does not give the gossypetone reaction. Position 7 therefore is left as the most probable one for the glucose residue.



The yield of Herbacitrin is at best very small and it varies from sample to sample of the flower petals. Frequently its separation from the accompanying Gossypitrin and Quercetin and further purification become laborius thus reducing the yield considerably. This has to a great extent restricted the scope of our investigations on its constitution, and the best method under the circumstances of placing it on a secure basis was its synthesis. The compound 3 : 5 : 7 : 8 : 4′-pentahydroxy flavone has now been synthesised in the Dyson Perrins Laboratories in Oxford (private communication from Professor R. Robinson, F.R.S.). He has very kindly compared the synthetic flavonol and its acetyl derivative with the specimens obtained from the natural source and found them to be identical in all respects including the colours given in alkaline buffer solutions. Our thanks are therefore due to him for this kindness.

Experimental.

The first isolation of Herbacitrin has already been described (see Neelakantam, Seshadri and Rao[1]). In order to get more of it about 2 kg. of the dry Uppam petals kindly supplied to us by the Cotton Specialist, Coimbatore, last season (1936) were extracted with methylated spirits as usual and the extract concentrated to small bulk (800 c.c.). On allowing it to stand for several days, only a small amount of Gossypitrin (2 g.) separated out, contrary

to what was observed previously. However, on distilling off the alcohol, adding water, removing the remaining alcohol from a water-bath and separating the resin, there slowly crystallised out a considerable amount of a yellow solid (15 g.) which was found to be a mixture of Gossypitrin, Herbacitrin and the aglucones including Quercetin. For separating this mixture an elaborate process of fractional crystallisation had to be adopted using at first boiling water and subsequently dilute alcohol. Gossypitrin was found in the more sparingly soluble fractions and the aglucones Gossypetin, Herbacetin and Quercetin in the more easily soluble fractions whereas Herbacitrin came in between. Consequently the yield of Herbacitrin was considerably reduced (0·7 g.). From the lead acetate and basic lead acetate fractions only the aglucones were obtained.

Crude Herbacitrin was purified by crystallising twice from boiling water and subsequently three or four times from dilute pyridine, when it came out as yellow needles, melting at 247–49°. Further purification did not raise the melting point. This is almost the same as the melting point of Quercimeritrin, but on mixing with Quercimeritrin the melting point was indefinite and lower (232–38°). [Found in air dried specimen : C, 50·5 ; H, 4·9 ; $C_{21}H_{20}O_{12}$, $2H_2O$ required C, 50·4 ; H, 4·8 per cent. Found in specimen dried at 105° *in vacuo* : C, 53·9 ; H, 4·5 ; $C_{21}H_{20}O_{12}$ required C, 54·3 ; H, 4·3 per cent.] The pure substance was insoluble in water and fairly soluble in ethyl alcohol. But the best solvent was pyridine. When aqueous lead acetate was added to an alcoholic solution a red precipitate was obtained which was unchanged on standing or boiling. Ferric chloride gave an olive green colour when added to an alcoholic solution of the pigment and this turned into yellowish green on adding water. When an alkaline solution of the pigment in 50 per cent. potassium hydroxide was left exposed to air for 24 hours, it gave no precipitate on acidification thus showing that it is a flavonol derivative. It gave the Gossypetone reaction with *p*-benzoquinone. When its alcoholic solution was mixed with a similar solution of the quinone, a maroon coloured solid was obtained. This was sparingly soluble in hot water ; the solution was at first blue and subsequently turned brown and on the addition of alkali gave an orange colour rapidly fading to yellow. Lead acetate gave a yellowish brown precipitate and sulphur dioxide regenerated the original pigment.

The colour reactions of *Herbacitrin* in alkaline buffer solutions were as below :

pH	Colour changes
6·8	The solid did not dissolve and remained unaffected for 24 hours.
8·0	Slowly dissolved to a light yellow solution which slowly turned cloudy and green. This was stable for 1 hour. Thereafter it slowly lost colour and after 24 hours was clear and pale brown.
8·6	Solution was complete in five minutes. The yellow changed into a deep emerald green which was rapidly fading. In 10 minutes there was perceptible loss. In ½ hour it turned pale brown and there was no further change for 24 hours.
9·8	Immediately dissolved to a deep yellow solution; in ½ minute became pure emerald green; the colour faded fast and in 10 minutes was pale greenish yellow. After ½ hour it was yellowish brown and after 24 hours very pale brown.
11·0	The changes were much quicker thana bove. The deep green was not obtained. The initial yellow very rapidly (1 minute) went into yellowish green which faded and the solution became almost colourless in ten minutes. No further change was noticed.

The above reactions are fundamentally different from those of Quercimeritrin which are recorded below. However, the resemblance to Gossypitrin (see Neelakantam, Seshadri and Rao[1]) is striking. Probably the loss in colour is a bit faster in the case of Herbacitrin and the absence of deep colour with pH 11·0 is noteworthy.

Quercimeritrin.

pH	Colour changes
6·8	Substance did not dissolve and remained unaffected.
8·0	Dissolved very slowly. After about 24 hours the solution became golden yellow.
8·6	Slowly dissolved (24 hours) to give a golden yellow solution. In 48 hours faded to a very pale yellow.
9·8	Rapidly dissolved. The golden yellow colour was stable for 24 hours. In 48 hours faded to a very pale brown.
11·0	Dissolved immediately to a deep yellow solution. Lost colour in 24 hours.

The above reactions are similar to those given by Quercetin (see below) but the final brown is much weaker.

Herbacitrin octa-acetate was prepared by boiling the pigment with acetic anhydride and sodium acetate. It crystallised from rectified spirits as colourless flat needles and rectangular plates, melting at 214-16°. Mixed melting point with the acetyl derivative of Quercimeritrin was 200-205°. [Found : C, 54·8 ; H, 4·7 ; $C_{37}H_{36}O_{20}$ required C, 55·4 ; H, 4·5 per cent.]

Hydrolysis of Herbacitrin. Preparation of Herbacetin.—Unlike Gossypitrin and Quercimeritrin, Herbacitrin does not give a clear solution on boiling with 7 per cent. aqueous sulphuric acid. So 50 per cent. ethyl alcoholic solution was employed. 0·5 g. of the glucoside was dissolved in 80 c.c. of 50 per cent. ethyl alcohol and enough concentrated sulphuric acid added so as to render the solution 7 per cent. acid and boiled for 3 hours. The turbid solution soon became clear. Alcohol was distilled off and the crystalline yellow precipitate filtered and recrystallised from alcohol. It was thereby obtained as bright yellow flat needles melting at 280-83° darkening a few degrees earlier ; mixed melting point with Quercetin was 250-75°. [Found in air dried specimen : C, 55·6 ; H, 3·9 and loss on drying 5·3, $C_{15}H_{10}O_7$, H_2O requires C, 56·2 ; H, 3·8 ; H_2O, 5·6 per cent. Found in specimen dried at 105° *in vacuo* : C, 58·9 ; H, 3·4 ; $C_{15}H_{10}O_7$ required C, 59·6 ; H, 3·3 per cent.] It gives a deep red precipitate with neutral lead acetate and a dull green colour with ferric chloride. It forms a yellow solution in sulphuric acid devoid of fluorescence and it gives the Gossypetone reaction with *p*-benzoquinone exactly as Gossypetin. The alkali colour reactions were as below:

pH	Colour changes
6·8	The solid was unaffected. After 24 hours there was a tinge of brown in the solution though the solid was apparently undissolved.
8·0	Dissolved very slowly to a light yellow coloured solution. In a few minutes the whole went into a dirty green and then slowly into blue. After ½ hour solution was complete and it was violet blue. This was rather stable and was slowly lost in 24 hours to a pale blue.
8·6	Rapid solution to give a light yellow colour. In a minute it was olive green, in another minute deep violet blue and was fairly stable ; even after 1 hour showed no change. After 24 hours it was colourless.

pH	Colour changes
9·8	Immediate solution to a deep yellow. Faster change (½ minute) to deep blue which was fairly stable ; no change for 1 hour. It lost all colour in 24 hours.
11·0	Much faster changes. Immediate solution to deep yellow, went in a moment to green and then in a second on shaking with air, to blue. No perceptible change for ½ hour. In an hour started fading and in 24 hours complete loss of colour.

There was, therefore, a close resemblance between Herbacetin and Gossypetin (see Neelakantam, Seshadri and Rao[1]). The loss of colour was probably a bit faster with Herbacetin. These reactions are fundamentally different from those given by Quercetin as shown below :

pH	Colour changes
6·8	Unaffected even after 48 hours.
8·0	Slowly dissolved to a yellow solution. Remained cloudy even after 48 hours. After 24 hours still yellow ; after 48 hours yellowish brown.
8·6	Dissolved somewhat faster to a yellow solution, still cloudy. Yellow even after 24 hours. After 48 hours cloudy and faint brown.
9·8	Rapidly dissolved to a clear deep yellow solution. After 24 hours unchanged and after 48 hours yellowish brown.
11·0	Rapidly dissolved to a yellow solution. After 2 hours was deep brown-red and thereafter stable. The colour changes were not prominent. No blue or green colour could be noticed. It closely resembled Quercimeritrin.

The acetyl derivative of Herbacetin (*Herbacetin penta-acetate*) was obtained by boiling the aglucone with acetic anhydride and sodium acetate. It crystallised easily from alcohol as colourless needles and long rectangular plates, melting at 192–3°. [Found : C, 58·0 ; H, 3·9 ; $C_{25}H_{20}O_{12}$ required C, 58·6 ; H, 3·9 per cent.] Mixed melting point with penta-acetyl quercetin was 165-70°.

Oxidation of Herbacitrin. Isolation of Anisic acid.—Herbacitrin (0·5 g.) was treated with 50 per cent. caustic potash (8 c.c.). It dissolved immediately

to a blood red solution which was frequently shaken and kept exposed to air for 48 hours. It became finally opaque and brown. It was acidified and extracted with ether. The ether extract gave a very small quantity of a solid which gave a red colour with ferric chloride. The aqueous solution still contained most of the product and it was therefore again rendered alkaline and shaken with excess of dimethyl sulphate. After shaking for 1 hour it was heated to 100° for a few minutes, acidified and ether extracted, and the ether solution evaporated. A crystalline solid was thereby obtained. It was purified by dissolving in aqueous sodium carbonate and precipitating with hydrochloric acid. When crystallised from aqueous alcohol it was obtained as colourless needles melting at 182–83°. On comparison, it was found to be identical with anisic acid and a mixture of the two melted at the same temperature (182-83°). The mixed melting point with veratric acid was indefinite and lower (145–55°).

Summary.

Herbacitrin, the new flavonol glucoside obtained from *Gossypium herbaceum* (Uppam) has the formula $C_{21}H_{20}O_{12}$. Though isomeric with Quercimeritrin it differs from it in many properties. On hydrolysis it gives glucose and Herbacetin having the formula $C_{15}H_{10}O_7$ which differs from Quercetin in its reactions. In their colour reactions with alkaline buffer solutions and with *p*-benzoquinone Herbacitrin exhibits a very close resemblance to Gossypitrin and Herbacetin to Gossypetin. From these and other considerations Herbacetin has been given the constitution 3 : 5 : 7 : 8 : 4′--pentahydroxy-flavone and Herbacitrin is represented as its 7-glucoside. This has been confirmed by the oxidation of Herbacitrin to *p*-hydroxy-benzoic acid. The flavonol has now been synthesised in the Dyson Perrins Laboratories in Oxford and found to be identical with the natural specimen.

REFERENCES.

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2. Neelakantam and Seshadri, *Proc. Ind. Acad. Sci.*, A, 1936, **4**, 54.
3. Attree and Perkin, *J.C.S.*, 1927, 234.