CONSTITUTION OF GOSSYPITRIN. AN ATTEMPT TO DEFINE THE POSITION OF THE GLUCOSE RESIDUE.

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Received May 25, 1937.

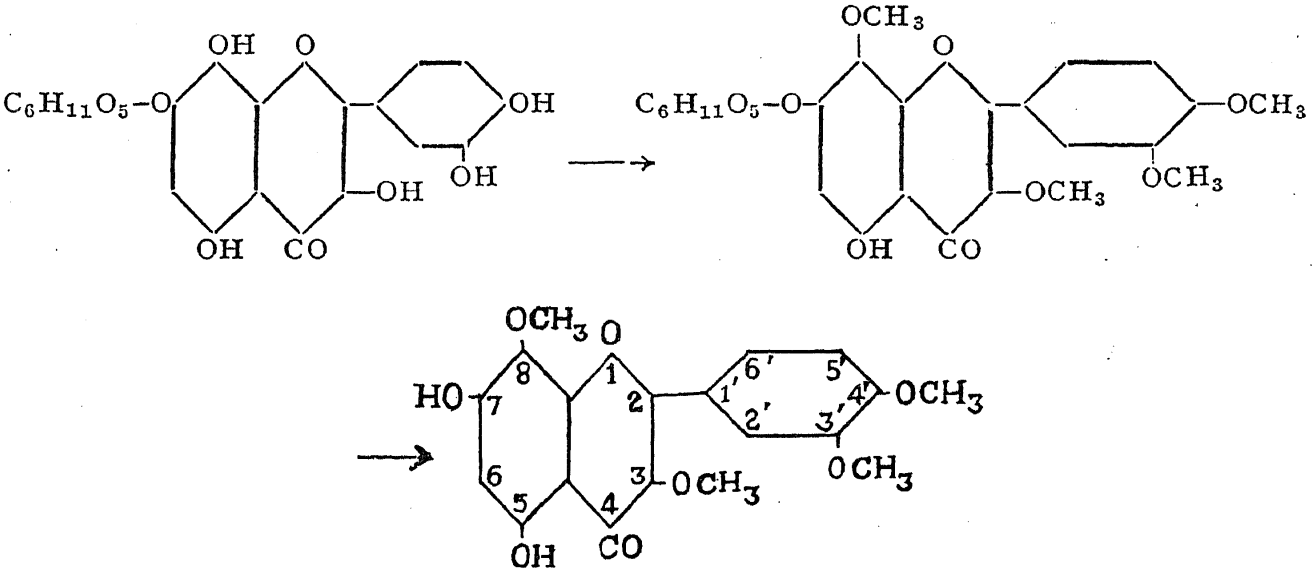
Gossypitrin, a glucoside of the anthoxanthin group of pigments, has been isolated from the Indian (*G. herbaceum* and *neglectum*) and the Egyptian *G. barbadense*) cotton flowers.[1,2] It is a monoglucoside of the flavonol gossypetin whose constitution was arrived at from its decomposition products by Perkin[3] and was established by synthesis by Baker, Nodzu and Robinson.[4] No attempt has till now been made to locate the position of the sugar group, but there exists sufficient information relating to the reactions of gossypitrin that enables a surmise to be made as to the probable position.

All known glycosides of the anthoxanthins fall into two definite groups : (1) the 7-glycosides and (2) the 3-glycosides. As a rule the sugar nucleus has not been found to be present in the hydroxy-phenyl portion of the molecule. Position 5 which seems to be considerably favoured in the anthocyanin group has not been found to be occupied by the sugar residue in the anthoxanthins. In his work on the constitution of the quercetin glycosides Perkin has shown that there exist definite differences between the 3- and 7-glycosides in regard to the following properties :—(1) reaction with lead acetate, (2) rate of hydrolysis with acids and (3) shades obtained with different mordants. Gossypitrin gives a deep red precipitate with lead acetate, is hydrolysed with difficulty by acids and gives deep shades with mordants thus showing that it is a 7-glucoside. Further it gives the gossypetone reaction, thereby indicating that the *p*-hydroxyl groups in 5 and 8 positions are free. The possibility of 7 and 8 positions being free thereby giving an orthoquinone does not seem to exist since quercetagetin which has hydroxyl groups in ortho-positions does not give this reaction.

An attempt has now been made to define the position of the glucose residue by methylating gossypitrin with diazomethane and after hydrolysing the methylated glucoside with acid obtaining a pentamethyl-gossypetin which could be identified. This method has been adopted very successfully for other cases by Herzig and Schönbach,[5] and Attree and Perkin.[6] At the beginning the question seemed to be simple since 7-hydroxy-3 : 5 : 8 : 3′ : 4′-pentamethoxy-flavone which should be expected as the product, if gossypitrin were a 7-glucoside, had already been made by Baker *et al.*[4] But

gossypitrin is now found to undergo only partial methylation with diazomethane though under the same conditions quercimeritrin undergoes complete methylation. One of the hydroxyl groups obviously remains unattacked since on hydrolysis a tetramethyl-gossypetin is obtained. The nature of this compound has been determined by the analysis of the compound itself by the preparation and analysis of the diacetyl derivative, and by the preparation of hexamethyl-gossypetin by further methylation.

Our present attempt has, therefore, not been successful. It is known that during methylation with methyl iodide or diazomethane of flavonols the hydroxyl in the 5 position is the most difficult to methylate.[7,8] It may, therefore, be reasonably assumed that the unreactive hydroxyl group in gossypitrin is the one situated in that position and it is possible that the final tetramethyl-gossypetin obtained by us has hydroxyl groups in the 5 and 7 positions as shown below :



Experimental.

Methylation of gossypitrin.—Gossypitrin (0·5 g.) was finely powdered, dissolved in excess of hot methyl alcohol (250 c.c.), cooled to room temperature and treated with an ethereal solution of diazomethane (2·0 g.) in small quantities at a time, more methyl alcohol being added simultaneously.[6] The solution became cherry-red, there was a slight evolution of nitrogen and the colour gradually disappeared. After leaving overnight, the ether was removed by distillation on a water-bath, more methyl alcohol added, cooled and again treated with an ethereal solution of diazomethane (2·0 g.) in the above manner. After leaving overnight, the ether and alcohol were removed completely on a water-bath. On adding a small amount of water an amorphous pale yellow solid separated out. Attempts to crystallise this solid from water or from dilute alcohol were unsuccessful.

Gossypitrin is sparingly soluble in alcohol. (300 c.c. of methyl alcohol are insufficient to keep 1·0 g. of it in solution in the cold.) With a view to test if the high dilution of the solution was responsible for the incompleteness of the methylation it was considered desirable to repeat the experiment with gossypitrin made into a thin paste with methyl alcohol. In this case, however, there was a little more rapid evolution of nitrogen than in the previous case. Partial methylation which was effected thus increased the solubility of the product in methyl alcohol. Methylation was then completed as above. The product, however, was identical with the one obtained above and gave rise to the same substance on hydrolysis.

The diazomethane required for these experiments was prepared from nitrosomethylurea and potash.[9]

Hydrolysis of the methylated glucoside. Isolation of a tetramethyl ether of gossypetin.—The uncrystallised product was refluxed with 2 per cent. sulphuric acid for four hours. The solid went into solution and deposited on cooling a yellow crystalline precipitate which was filtered and purified by dissolution in very dilute potash (deep yellow solution) and reprecipitation by passing in carbon dioxide. The product was left overnight, filtered and recrystallised from dilute acetic acid when it was obtained as yellow needles. It was recrystallised twice again from alcohol when it was obtained as needles which sintered at 225° and melted at 227–30°. (Found in air dried sample : C, 60·8, H, 4·9 and methoxyl, 32·8 ; $C_{19}H_{18}O_8$ requires C, 61·0, H, 4·8 and methoxyl, 33·2 per cent.)

Acetylation of the product of hydrolysis. Preparation of a diacetyl derivative.—A portion of the tetramethyl-gossypetin was dried and acetylated by boiling with acetic anhydride and sodium acetate and the product obtained recrystallised twice from dilute alcohol. It was obtained as fine colourless needles which sintered at 135° and melted at 142–43°. (Found in air dried sample : C, 60·5, H, 5·1 ; $C_{23}H_{22}O_{10}$ requires C, 60·3, H, 4·8 per cent.)

Methylation of the products of hydrolysis. Preparation of the hexamethyl ether of gossypetin.—Another portion of the hydrolytic product was dissolved in excess of potash and methylated with excess of dimethyl sulphate in the usual manner. In order to destroy the excess of dimethyl sulphate it was finally heated on a water-bath for half an hour. The product was then cooled and repeatedly extracted by shaking with cold ether. On removing the ether, a yellow solid was obtained which on crystallising twice from aqueous alcohol gave almost colourless fine needles, m.p. 170° (Perkin : Hexamethyl-gossypetin, m.p. 170–72°).

Summary.

An attempt has been made to fix the position of the glucose residue in gossypitrin by methylation with diazomethane and hydrolysis of the product. It has been found that methylation is incomplete, one hydroxyl group remaining unaffected, and thereby giving after hydrolysis a tetra-methyl-gossypetin. This compound probably has the two hydroxyl groups in the 5 and 7 positions.

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