PIGMENTS OF COTTON FLOWERS

Part VII. Position of the Glucose Residue in Gossypitrin

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Gossypitrin which is the most important component of the Indian cotton flowers is a monoglucoside of the flavonol, Gossypetin. It was shown in a previous publication[1] from these laboratories that though the position in which the sugar group is situated could be surmised from various considerations, it has not yet been definitely established. An attempt described in the above paper to settle this question by methylating Gossypitrin with diazomethane was not successful since one of the nuclear hydroxyls (probably the one in position 5) was resistant to methylation by this reagent. The partially methylated Gossypitrin on hydrolysis produced a new compound which was tentatively given the constitution of 3′ : 4′ : 3 : 8-tetramethyl gossypetin.

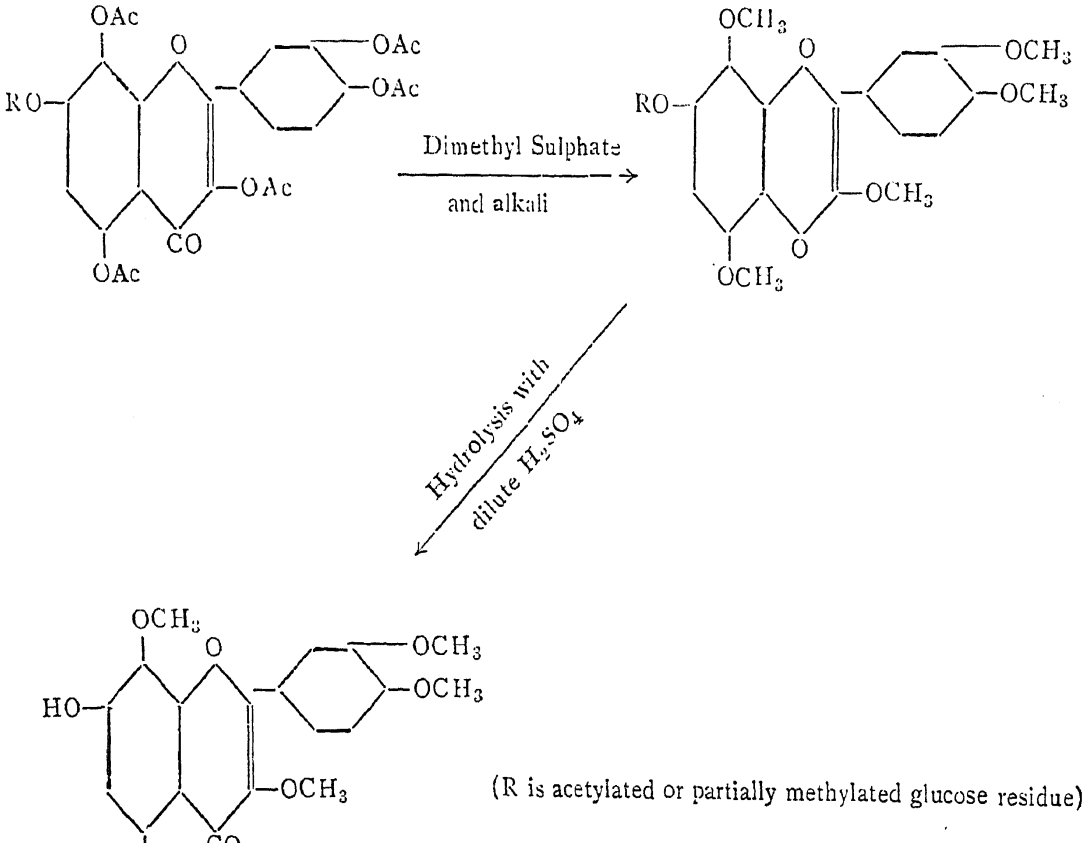
Methylation of the glucoside using alkali and dimethyl sulphate or methyl iodide was not feasible since the pigment is highly sensitive in the presence of alkali and readily undergoes oxidation. Amongst the other possible methods, utilisation of the acetyl derivative as the starting material seemed to be worth trial. This method was employed by Freudenberg and Cohn[2] for preparing the methyl ether of quercetin and subsequently improved by Anderson[3] to obtain better yields. They used a methyl alcoholic solution of the acetyl derivative and methylated it with dimethyl sulphate and alkali. The completely methylated compound was not the exclusive product; the partially methylated tetramethyl ether was also obtained in considerable amounts. We have now found that the substitution of acetone for methyl alcohol as the solvent effects considerable improvement in yielding the completely methylated ether exclusively. The superiority of acetone over methyl alcohol is due to its specific influence in the methylation of the hydroxyl group in position 5 as was observed by Baker and Robinson.[4] We have applied this method for the methylation of Gossypitrin through its acetyl derivative. Though the acetate is rather sparingly soluble in alcohol it dissolves more easily in acetone. The product undergoes deacetylation and methylation smoothly. All the phenolic hydroxyl groups and one hydroxyl in the sugar residue are methylated by this process. On hydrolysis with dilute sulphuric acid, this methylated glucoside gives rise to a pentamethyl

gossypetin which agrees in all its properties with 3 : 5 : 8 : 3′ : 4′-pentamethyl gossypetin obtained by Baker, Nodzu and Robinson[5] during the course of the synthesis of gossypetin.

	3 : 5 : 8 : 3′ : 4′-pentamethyl gossypetin	The pentamethyl gossypetin obtained by the hydrolysis of the methylated glucoside
Melting point	253–54°	252–54°
Colour with ferric chloride	No characteristic colour	No characteristic colour
Solubility in sodium hydroxide	Easily soluble to give yellow solution	Easily soluble to give yellow solution
Solubility in concentrated HCl	Soluble to give yellow solution	Soluble to give yellow solution
M.P. of the acetyl derivative	....	167-69°

On boiling with acetic anhydride and anhydrous sodium acetate the pentamethyl gossypetin gives rise to the monoacetate of the pentamethyl ether. On further methylation with dimethyl sulphate and alkali, it yields the hexamethyl gossypetin.

It is therefore concluded that the hydroxyl group in position 7 of the aglucone which has been left free at the end of the methylation and subsequent hydrolysis of the glucoside is involved in the glucoside formation. Hence Gossypitrin is a 7-glucoside of Gossypetin.



(R is acetylated or partially methylated glucose residue)

Experimental

Gossypitrin acetate (3 g.) was dissolved in acetone (120 c.c.) and dimethyl sulphate (30 c.c.) and 20% aqueous sodium hydroxide (30 c.c.) were added to the solution. On shaking, the contents assumed a lemon yellow colour which faded away gradually. Thereupon more quantities of methyl sulphate (15 c.c.) and 20% sodium hydroxide (15 c.c.) were added alternately in small amounts using 3 c.c. each time and shaking vigorously after each addition. Finally the medium was kept definitely alkaline by the gradual addition of 30 c.c. more of the above alkali. There was gradual development of heat throughout the process and during the later stages the mixture became so hot that there was actual boiling. After leaving overnight it was gently boiled under reflux on a water-bath for an hour. Then almost all the solvent was driven off. At this stage the methylated glucoside separated out as a crystalline solid on cooling. But sometimes the contents deposited a small amount of a brown coloured pasty mass along with the beautifully crystalline solid. In such cases the supernatant liquid was decanted off and the residue treated with a small amount of ether when it changed into a colourless crystalline solid. The decanted liquid deposited some more of the same substance. The methylated glucoside was then crystallised twice from acetic acid and was obtained as tiny needles which were almost colourless. It melted at 290° with decomposition, becoming dark a few degrees earlier. [Found : OCH_3, 31·8 ; $C_{21}H_{14}O_7$ $(OCH_3)_6$, H_2O requires OCH_3, 32·0%.]

The above methylated glucoside (1 g.) was boiled under reflux with 7% sulphuric acid (100 c.c.) when it went into solution. After an hour a faintly coloured solid separated out giving rise to slight bumping. Boiling was continued for one hour more to ensure complete hydrolysis of the glucoside. The pale yellow solid which separated out on cooling the contents was filtered and then crystallised from alcohol using a little animal charcoal. The product came out in the form of fine golden yellow needles melting at 252–54°. It dissolved in hydrochloric acid and sodium hydroxide to form yellow solutions. It imparted no characteristic colour to ferric chloride in alcoholic solution. [Found : C, 61·6 ; H, 5·1 ; OCH_3 39·7 ; $C_{15}H_5O_3$ $(OCH_3)_5$ requires C, 61·9, H, 5·2, OCH_3, 40·0%.]

7-Acetyl-3 : 5 : 8 : 3′ : 4′-pentamethyl gossypetin.—The above pentamethyl ether (100 mg.) was boiled under reflux with anhydrous sodium acetate (1 g.) and acetic anhydride (20 c.c.) for 4 hours. On diluting the contents with 200 c.c. of cold water and leaving overnight, a colourless fibrous solid separated out. It was obtained as a hydrate in the form of fine needles,

when crystallised from alcohol or acetic acid. It sintered from 100 to 105° (dehydration) and melted completely at 167–69°. It lost all the water of crystallisation when kept at 125° for 2 hours. [Found : OCH_3, 32·8 ; loss of H_2O on drying, 10·8 ; $C_{15}H_4O_2$ (CH_3 COO) $(OCH_3)_5$, $3H_2O$ requires OCH_3 32·0 ; loss on drying, 11·2%.]

3 : 5 : 7 : 8 : 3′ : 4′-*Hexamethyl gossypetin*.—The above pentamethyl gossypetin when methylated with dimethyl sulphate and alkali yielded the hexamethyl ether. Addition of some methyl alcohol was helpful in getting a clear solution and in obtaining a good yield. The hexamethyl gossypetin crystallised from alcohol as colourless fibrous needles melting at 170–72°.

Summary

The constitution of Gossypitrin has been established as the 7-glucoside of the flavonol Gossypetin by complete methylation of the glucoside through the acetyl derivative and the isolation of 7-hydroxy-3 : 5 : 8 : 3′ : 4′-pentamethoxyflavone by the hydrolysis of the above methylated glucoside. The acetyl derivative of the pentamethyl gossypetin has also been prepared.

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REFERENCES

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