CONSTITUTION OF GOSSYPIN—PART I

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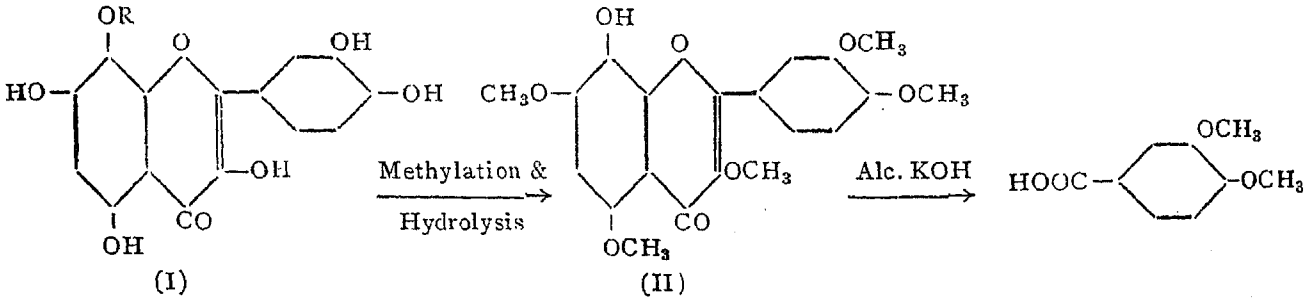
Gossypin, a new glucoside of gossypetin, was originally obtained from *Gossypium indicum.*[1] A detailed study of this substance could not be carried out earlier owing to lack of material. These cotton flowers were variable in composition and did not yield this glucoside in any appreciable amounts in later experiments. A richer and more convenient source has recently been found in the flowers of *Hibiscus vitifolius*[2] and considerable quantities of gossypin have been obtained. A detailed study of it has therefore been possible now.

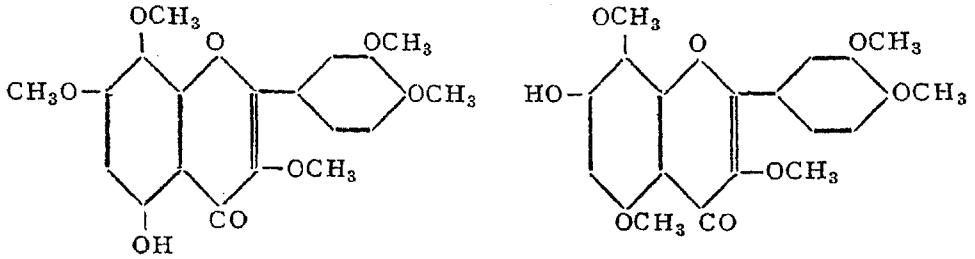
Gossypin is markedly soluble in water and much less soluble in anhydrous organic solvents. Because of this characteristic, it has not been possible to obtain it entirely free of mineral matter. Consequently accurate analytical data could not be obtained; but analysis for carbon and hydrogen and estimation of the products of acid hydrolysis, glucose and gossypetin agreed satisfactorily with a monoglucoside formula for gossypin.

Two significant properties which distinguish gossypin from gossypitrin are as follows: (1) it does not give any prominent colours in alkaline buffer solutions; (2) it does not respond to the gossypetone reaction. These may indicate that in the linking of the sugar group a hydroxyl in the 5- or 8-position is involved, the latter being more probable. In order to locate the position of the glucose group definitely gossypin has been subjected to complete methylation using dimethyl sulphate and potassium carbonate in anhydrous acetone medium. Though the substance is sparingly soluble in this solvent the suspension reacts and complete methylation eventually takes place. This is indicated by the fact that the product does not give any colour with aqueous sodium hydroxide or ferric chloride. Hydrolysis of the methylated glucoside yields a monohydroxy compound (A) which yields veratric acid on fission with alcoholic potash. This reaction shows that the free hydroxyl group is not in the side phenyl nucleus. Four alternative positions still left for its location are 3-, 5-, 7- and 8-. O-Pentamethyl gossypetins with a hydroxyl in the 5- and the 7-positions (formulæ III and IV) are already[3,4] known. The isomeric compound with a free hydroxyl

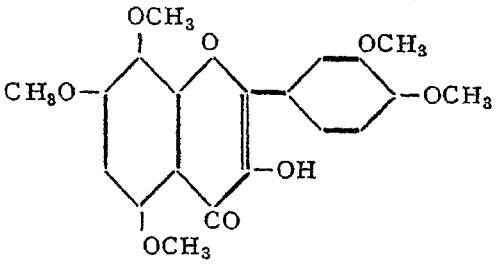
in the 3-position (V) has now been prepared for purposes of comparison. As shown in the table given below these three compounds do not agree with the degradation product of gossypin (A) in properties. Mixed melting points have been taken and have been found to be depressed. Hence it should be concluded that compound (A) bears a hydroxyl in the 8-position (formula II) and that gossypin is a 8-mono-glucoside of gossypetin (I).



(I) (II)



(III) (IV)



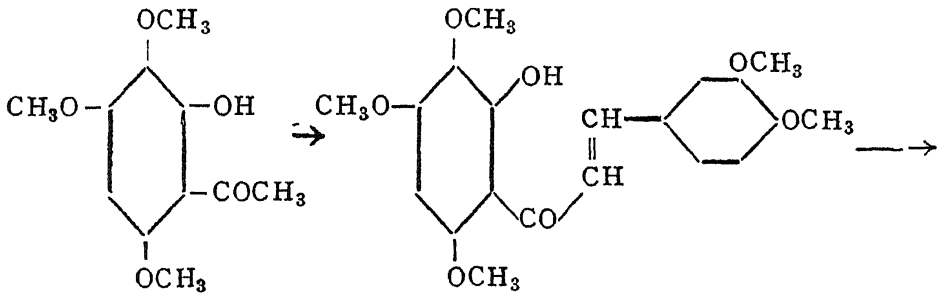
(V)

This constitution satisfactorily explains why gossypin does not respond to the gossypetone reaction with *p*-benzoquinone and why it does not exhibit marked colour changes in alkaline buffer solutions. But how the location of the sugar group in the 8-position gives rise to the markedly high solubility of gossypin in water and its marked insolubility in organic solvents is not yet clear.

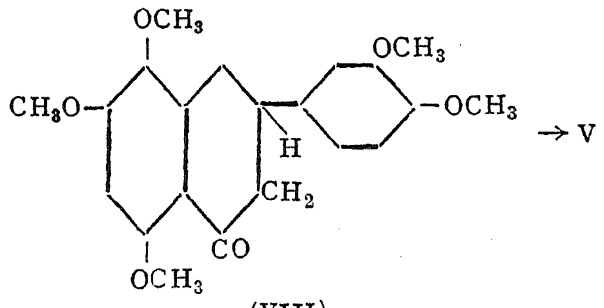
Of the O-pentamethyl gossypetins required for the above comparison the 7-hydroxy compound (IV) has been obtained according to the method of Baker, Nodzu and Robinson,[4] and the 5-hydroxy compound (III) by the partial methylation of gossypetin in anhydrous acetone solution using dimethyl sulphate and anhydrous potassium carbonate. The 3-hydroxy

Name of the substance	Melting point of the substance	Melting point of the acetate	Ferric chloride colour
5-Hydroxy-3:7:8:3′:4′-pentamethoxy flavone (III) ..	166–68°	165–66°	Olive green
7-Hydroxy-3:5:8:3′:4′ pentamethoxy flavone (IV) ..	253–55°	167–69°	Nil
3-Hydroxy-5:7:8:3′:4′ pentamethoxy flavone (V) ..	228–30°	207–8°	Violet brown
Degradation product of gossypin (A)	196–98°	214–16°	Brown

compound (V) was synthesized starting from 2-hydroxy-3 : 4 : 6-trimethoxy acetophenone (VI). This ketone is best obtained according to the procedure of Baker[5] which involves Friedel and Craft's reaction on 1 : 2 : 3 : 5-tetramethoxy benzene. Its constitution is definitely established by its preparation by an alternative method using 2 : 5-dimethoxy resorcinol.[6] It is condensed in alkaline solution with veratric aldehyde and the resulting chalkone (VII) converted subsequently into the flavanone (VIII) and flavonol (V).



(VI) (VII)



(VIII)

Experimental

The sample of gossypin used for the following experiments was purified by repeated crystallisation from hot water. It separated out in the form of bright yellow sheaves of fine needles which melted with vigorous decomposition at 228–30°. The results of analysis for carbon and hydrogen reported in an earlier paper[1] agree closely with the requirements of the formula $C_{21}H_{20}O_{13}$ than the more complex formula originally suggested.[1] (Found in air-dried sample: C, 46·7; H, 4·5; loss on drying *in vacuo* at

110° for 3 hours, 10·1%. $C_{21}H_{20}O_{13}$, $3H_2O$ requires C, 47·2; H, 4·9 and $3H_2O$ loss 10·1%.) Quantitative estimation of glucose and gossypetin was already reported.[2] This also agrees with the monoglucoside formula. (Found: Gossypetin 63·3; glucose 33·4; $C_{21}H_{20}O_{13}$, $3H_2O$ requires $C_{15}H_{10}O_8$, H_2O, 62·9 and $C_6H_{12}O_6$, 33·9%.)

Though the acetyl derivative of gossypin could be obtained only as a colourless powder and could not be crystallised, it gave values for acetyl groups agreeing with the monoglucoside formula. (Found: $COCH_3$, 44·6%; calculated for 9 acetyl groups in $C_{39}H_{38}O_{22}$, 45·1%.) It undergoes hydrolysis on keeping and turns yellow.

Methylation of Gossypin.—A suspension of finely powdered gossypin (1 g.) in anhydrous acetone (200 c.c.) was treated with freshly distilled dimethyl sulphate (8 c.c.) and anhydrous potassium carbonate (20 g.). After refluxing for 30 hours on a water-bath the potassium salts were filtered off and the residue washed with acetone. The filtrate was distilled to recover the solvent. When the reddish brown oily residue was treated with excess of ether a light brown amorphous solid separated out which was easily soluble in water, alcohol and acetone but not in ether or benzene. The alcoholic solution did not give any colour with alkali or with ferric chloride. Attempts to crystallise the methyl ether from various solvents were unsuccessful and it was therefore directly used for hydrolysis.

Hydrolysis of Methylated Gossypin.—The methylated product (1 g.) was refluxed with 7% sulphuric acid (50 c.c.) for 2 hours. The dark red solution was filtered hot through a plug of cotton-wool from a small amount of resinous impurity. On diluting with water (200 c.c.) and cooling in the ice-chest fine silky needles of the hydrolytic product separated out. It was filtered and washed free from acid. It crystallised from alcohol in the form of pale yellow rectangular plates and prisms melting at 196–98°. (Found: C, 58·9; H, 5·3; methoxyl, 38·0; loss on drying *in vacuo* at 110–20° for 3 hours, 4·1; $C_{20}H_{20}O_8$, H_2O requires C, 59·1; H, 5·4; methoxyl, 38·2 for 5 methoxyl groups and loss on drying, 4·4%.) It was easily soluble in alcohol but sparingly in benzene and ethyl acetate. In alcoholic solution it gave a brown colour with a drop of ferric chloride and with a few more drops a reddish brown slimy precipitate separated. With lead acetate it did not give any precipitate. In aqueous sodium hydroxide it dissolved to a reddish brown solution from which it was reprecipitated on saturation with carbon dioxide.

A small quantity of the above hydroxy compound was acetylated by boiling with acetic anhydride and few drops of pyridine for 2 hours. The

acetate crystallised from alcohol in the form of colourless narrow rectangular plates melting at 215–16°. (Found: C, 61·0; H, 5·4; $C_{22}H_{22}O_9$ requires C, 61·4; H, 5·1%.)

On methylation with dimethyl sulphate and potassium carbonate in anhydrous acetone medium the hydroxy compound yielded the methylated product in the form of colourless narrow rectangular plates melting at 170–72°. The mixed melting point with an authentic sample of gossypetin hexamethyl ether was not depressed.

Alkaline hydrolysis of O-pentamethyl gossypetin (II).—The pentamethyl gossypetin (1 g.) was treated with absolute alcoholic potash (30 c.c. of 8% solution) and the dark red solution refluxed for 6 hours in a current of hydrogen. The solvent was removed, the residue dissolved in water (30 c.c.) and the clear solution acidified with concentrated hydrochloric acid. The solution was repeatedly extracted with ether and the ether extract shaken with aqueous sodium bicarbonate. On acidifying the bicarbonate extract a crystalline solid separated out. It was filtered, washed with a little water and crystallised twice from hot water using animal charcoal when it came out in the form of colourless rectangular prisms melting at 180–82°. The mixed melting point with an authentic sample of veratric acid was not depressed.

The residual ether extract on evaporation gave a small quantity of a pale yellow solid which gave a greenish colour with ferric chloride. The quantity was too little for successful purification.

3:7:8:3′:4′-*O*-*Pentamethyl gossypetin (IV).*—The preparation of this has already been described.[3] A more convenient procedure for obtaining it is as follows:

Gossypetin (0·5 g.) was dissolved in a mixture of anhydrous acetone (20 c.c.) and benzene (100 c.c.). The solution was treated with dimethyl sulphate (0·8 c.c.) and potassium carbonate (5 g.) and refluxed for 12 hours. The potassium salts were removed by filtration and the filtrate evaporated. The residual yellow solid was taken up in absolute alcohol, an equal volume of 10% absolute alcoholic potash was added and the mixture cooled in ice. The yellow crystalline solid (potassium salt) was filtered, washed with a little absolute alcohol, dissolved in water and the solution acidified. On extracting it with ether and removing the solvent the pentamethyl ether was obtained as a yellow crystalline solid. It crystallised from alcohol in the form of bright yellow narrow rectangular plates melting at 166–67°. It was sparingly soluble in aqueous alkali and gave a bright olive green colour with a drop of ferric chloride.

3:4:6:3′:4′-*Pentamethoxy-2-hydroxy-chalkone* (*VII*).—A mixture of veratraldehyde (9 g.) and 2-hydroxy-3:4:6-trimethoxy acetophenone (3 g.) was dissolved in alcohol (25 c.c.) and the solution treated with a strong aqueous solution of potassium hydroxide (25 g., in 20 c.c. of water) with cooling. Sufficient alcohol (100 c.c.) was then added to get a clear solution and it was left out of contact with air for 3 days. The dark red reaction mixture was diluted with water (400 c.c.) and extracted with ether twice. The alkaline layer, on being acidified, deposited an orange yellow solid which was filtered and washed with water. Yield 4·3 g. After crystallisation from alcohol it separated in the form of large, orange coloured rectangular plates melting at 143–45°. (Found: C, 64·0; H, 5·8; $C_{20}H_{22}O_7$ requires C, 64·2; H, 5·9%.)

It was readily soluble in alcohol and the alcoholic solution gave a brown colour with ferric chloride. In concentrated hydrochloric acid and sulphuric acid it dissolved to form a blood-red solution.

5:7:8:3′:4′-*Pentamethoxy-flavanone* (*VIII*).—A solution of the above chalkone (1 g.) in aqueous alcohol (25 c.c. alcohol and 25 c.c. water) was treated with concentrated hydrochloric acid (3 c.c.). After refluxing for 24 hours on a water-bath most of the alcohol was removed under reduced pressure and the residue diluted with water (300 c.c.). A pale brown turbid solution with some resinous solid resulted. It was extracted with boiling benzene (300 c.c.) in 3 lots. The combined benzene extract was distilled off to recover the solvent and the residue, on the addition of ether, solidified to a light brown solid. It was macerated with dilute aqueous sodium hydroxide to remove the chalkone, filtered and washed. Yield 0·6 g. On crystallisation from ethyl acetate the flavanone separated out in the form of colourless narrow rectangular plates, melting at 172–74°. (Found: C, 63·8; H, 5·6; $C_{20}H_{22}O_7$ requires C, 64·2; H, 5·9%.) The flavanone was sparingly soluble in alcohol and benzene and more soluble in ethyl acetate. It was insoluble in aqueous alkali and did not give any colour with ferric chloride. In concentrated mineral acid it dissolved to an orange yellow solution. When reduced with magnesium and hydrochloric acid in alcoholic solution a pink colour resulted.

3-*Hydroxy*-5:7:8:3′:4′-*pentamethoxy flavone* (*V*).—A solution of the flavanone (0·5 g.) in alcohol (40 c.c.) was treated at 70–80° with freshly prepared isoamyl nitrite (4 c.c.) in small portions and concentrated hydrochloric acid (4 c.c.) (d. 1·19) was then slowly added little by little maintaining the above temperature. After all the acid was added, the flask was closed and left for 3 hours. It was then diluted with water (200 c.c.) and kept in

the ice-chest overnight when a yellow solid separated out. It was filtered, washed and crystallised from ethyl acetate from which it came out as pale yellow flat needles, melting at 228–30°. (Found: C, 61·8; H, 5·0; $C_{20}H_{20}O_8$ requires C, 61·9; H, 5·2%.) It was sparingly soluble in ethyl acetate, alcohol and benzene and aqueous sodium hydroxide. In alcoholic solution it gave a greenish brown colour with ferric chloride.

On acetylating the compound with acetic anhydride and pyridine the acetate was obtained; it crystallised from alcohol in the form of colourless narrow rectangular plates melting at 207–08°. The mixed melting point of this with the acetate of the pentamethyl gossypetin obtained from gossypin was depressed (180–90°). (Found: C, 61·2; H, 5·0; $C_{22}H_{22}O_9$ requires C, 61·4; H, 5·1%.)

Summary

The analytical data indicate that gossypin is a monoglucoside. As a result of complete methylation and hydrolysis it yields an O-pentamethyl gossypetin. From a study of its decomposition with alcoholic potash whereby veratric acid is obtained and from a comparison of its properties with those of isomeric compounds, it is concluded that it has a free hydroxyl in the 8-position. Consequently gossypin should be 8-monoglucoside of gossypetin. The synthesis of O-pentamethyl gossypetin with a hydroxyl in the 3-position has been described.

References

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3. ———, Reddy and Seshadri .. *Ibid.*, A, 1940, **12**, 496.
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5. ——— .. *Ibid.*, 1941, 666.
6. Sastri and Seshadri .. *Proc. Ind. Acad. Sci.*, A, 1946, **24**, 246.