CONSTITUTION OF GOSSYPIN—PART II


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Received March 10, 1947

In Part I experiments were described which led to the conclusion that gossypin is the 8-monoglucoside of gossypetin. After complete methylation and subsequent hydrolysis a pentamethyl gossypetin (A) was obtained whose constitution as an 8-hydroxy compound (I) was arrived at by a process of elimination of all other possibilities. Since this type of glucoside is extraordinary and unusual, further study of the degradation product (A) was considered to be necessary to make sure of the position.

![Chemical structure A](image)

(B) II

It has already been mentioned that alkali fission of compound (A) does not proceed well. Though veratric acid could be obtained as a product, the ketonic part could not be isolated. However, the ethyl ether (B) is now found to undergo smooth fission yielding besides veratric acid a ketone whose analysis indicates that it has an ethoxy group in it.

The most interesting results have been obtained by the oxidation of the pentamethyl gossypetin (A) with nitric acid. The reaction takes place readily and a quinone (C) is produced which could be smoothly reduced to a
quinol (D). The same quinone and quinol are formed under the same conditions from pentamethyl gossypetin having a hydroxyl group in the 5-position (V) which could be readily made by the partial methylation of gossypetin. Consequently the quinone (C) and quinol (D) should be represented by formulae (III) and (IV). This definitely supports the 8-hydroxy constitution (I) for pentamethyl gossypetin (A). Confirmation of this has again been obtained by the partial ethylation of the quinol which should be expected to take place in the 8-position (VI). Subsequent methylation yields a pentamethyl-monoethyl ether whose constitution should be represented by formula (II). This substance is found to be identical with the ethyl ether (B) obtained from gossypin.

That no change other than what has been represented above takes place has been proved by the complete methylation of the quinol whereby hexamethyl gossypetin (VII) is obtained. In continuation of the above oxidation experiments it has been found that even this hexamethyl ether could be oxidised to the above mentioned quinone under almost the same conditions. In this respect the ethyl ether (B) also behaves similarly.

Though among the anthoxanthins an 8-glycoside structure has been established for the first time in gossypin, glycosidation of the 8-position need not be considered as unexpected if the related phenomenon of partial methylation is taken into consideration. The possibility was indicated by the existence of wogonin which is a monomethyl ether of 5:7:8-trihydroxyflavone having the methoxyl in the 8-position. A constitution somewhat analogous to gossypin has been suggested for carthamin, the glycosidic pigment of safflower; but there seems to be no definite evidence in support.

![Diagram of Carthamin and Iso-carthamin](image)

**EXPERIMENTAL**

*Ethylation of pentamethyl gossypetin (A) obtained from gossypin; Preparation of the ethyl ether (B):*—

A solution of the substance (A) (1 g.) in anhydrous acetone (50 c.c.) was treated with ethyl iodide (2 c.c.) and potassium carbonate (10 g.). After refluxing for 10 hours the solvent was completely removed and the
residue treated with water. The precipitated ethyl ether was filtered, washed and purified by crystallising twice from alcohol from which it separated in the form of colourless elongated rectangular plates melting at 142-43°. Yield 0·9 g. (Found: C, 61·1; H, 5·9; C₁₂H₂₄O₈, H₂O requires C, 60·8; H, 5·9%). It was not soluble in aqueous alkali and did not give any colour with ferric chloride.

Alkali fission of the ethyl ether (B):—

The ethyl ether (1 g.) was refluxed for 6 hours with absolute alcoholic potash (30 c.c. of 10% solution). The solvent was then completely removed, the residue dissolved in water and the clear brown solution acidified with hydrochloric acid. It was repeatedly extracted with ether and the ether extract shaken with aqueous sodium bicarbonate twice. After washing with water the ether extract was evaporated when the ketonic product was left behind in a crystalline condition. It crystallised from dilute alcohol as almost colourless needles melting at 128-29°. (Found: C, 57·3; H, 6·3; C₁₅H₁₅O₆ requires C, 57·7; H, 6·6%). It was soluble in aqueous sodium hydroxide to give a pale yellow solution. In alcoholic solution it gave a reddish brown colour with ferric chloride.

The bicarbonate extract, on acidification, deposited a crystalline solid which was filtered, washed and crystallised twice from hot water. It melted at 180-82° and the mixed melting point with veratric acid was not depressed.

Gossypetone tetramethyl ether (III, C):—

(a) From gossypetin pentamethyl ether (V).—Gossypetin pentamethyl ether (0·5 g.) was treated with nitric acid (10 c.c., d. 1·25) with stirring while cooling the reaction mixture in ice-water. The solid rapidly dissolved in the acid forming an intense red solution which very quickly deposited a reddish brown solid. After keeping the mixture well stirred at 15-20° for 15 minutes it was filtered and the residue washed free from nitric acid. It was then crystallised from glacial acetic acid from which it came out as brick-red flat needles melting at 245-47°. (Found: C, 58·6; H, 5·0; C₁₉H₁₉O₈, H₂O requires C, 58·5; H, 4·6%). Yield, 0·4 g. It was very sparingly soluble in alcohol and moderately in glacial acetic acid. In aqueous sodium hydroxide it dissolved to a bluish-violet solution.

(b) From the pentamethyl ether (A).—The above reaction was carried out with this pentamethyl ether (0·5 g.). The reaction was very rapid and the quinone separated out in good yield. It was filtered and washed well. It crystallised from glacial acetic acid in the form of brick-red flat needles
melting at 245-47° alone or when admixed with the quinone described above. Its properties were also identical with those of the above.

(c) From gossypetin hexamethyl ether (VII).—Gossypetin hexamethyl ether (0·5 g.) was treated with nitric acid (15 c.c., d. 1·25) with stirring. The solid slowly dissolved producing first a yellow solution which gradually deepened to orange and finally to deep red with the separation of a reddish-brown solid. After allowing the mixture to stand for 30 minutes at a temperature of 15-20° the solid was filtered off; washed first with nitric acid (d. 1·25) and finally with water. It was purified by crystallisation from glacial acetic acid when it separated out in the form of brick-red flat needles melting at 245-47°. It was identical with the quinone prepared by the above two methods.

(d) From pentamethyl-mono-ethyl ether of gossypetin (II, B).—The reaction with this compound proceeded just as in the case of the hexamethyl gossypetin and the quinone was also the same.

3:7:3:4'-O-Tetramethyl gossypetin (IV, D):—

Gossypetone tetramethyl ether (prepared from each one of the above methods) (0·5 g.) was suspended in glacial acetic acid (2-3 c.c.) and treated with a pinch of sodium sulphite. On heating for a few seconds the deep red colour of the solid as well as the solution turned bright yellow. After stirring vigorously for 5 minutes the mixture was diluted with water and the yellow solid filtered and washed with water. It crystallised from glacial acetic acid in the form of golden yellow elongated thick rectangular prisms melting at 251-53°. (Found: C, 66·6; H, 4·6; C_{12}H_{18}O_{6} requires C, 61·0; H, 4·8%). It was sparingly soluble in alcohol and moderately in glacial acetic acid. In alcoholic solution it gave with ferric chloride a transient green colour which quickly changed to brown. When treated with a small quantity of p-benzoquinone in absolute alcoholic solution it was slowly oxidised to gossypetone tetramethyl ether which separated out as a red solid. In aqueous sodium carbonate it was insoluble but in sodium hydroxide it readily dissolved to a deep red solution which changed rapidly on shaking with air to bluish violet.

The dihydroxy compound (0·2 g.) was acetylated by boiling with acetic anhydride (1 c.c.) and a few drops of pyridine for one hour. On adding ether to the cooled reaction mixture the acetate crystallised out. It was filtered and washed with a little more ether. It crystallised from absolute alcohol in the form of colourless long matted needles melting at 227-28°. (Found: C, 60·6; H, 5·1; C_{20}H_{28}O_{10} requires C, 60·3; H, 4·8%). It
was very sparingly soluble in alcohol but was easily soluble in chloroform. It was not soluble in cold aqueous alkali and did not give any colour with ferric chloride.

The dihydroxy compound (0.2 g.) was methylated using dimethyl sulphate (0.5 c.c.) and anhydrous potassium carbonate (5.0 g.) in acetone medium (20 c.c.). After refluxing for 6 hours the solvent was removed, the residue treated with water and the precipitated solid filtered and washed. It was crystallised from alcohol from which it separated out as colourless narrow rectangular plates melting at 170-72° alone or when admixed with the hexamethyl ether of gossypetin.

8-Ethoxy-5-hydroxy-3:7:3':4'-tetramethoxy-flavone (VI):—

A solution of the dihydroxy compound (IV) (0.5 g.) in anhydrous acetone (50 c.c.) was treated with ethyl iodide (0.12 c.c.) and potassium carbonate (5.0 g.) and the mixture refluxed for 6 hours. The solvent was then removed, the residue treated with water and the yellow solid that separated out was filtered and washed with water. After crystallisation from alcohol it was obtained in a crystalline condition but melted indefinitely between 145-55° showing admixture with the fully ethylated compound. It was therefore dissolved in absolute alcohol (10 c.c.) treated with just enough absolute alcoholic potash and the deep orange solution was evaporated quickly on a water-bath to dryness. The orange red crystalline solid thus obtained was washed repeatedly with boiling benzene and filtered. The residue was suspended in water, treated with dilute hydrochloric acid, heated for a few minutes on a water bath and filtered. It crystallised from absolute alcohol in the form of glistening lemon-yellow elongated rectangular prisms melting at 155-56°. (Found: C, 62.9; H, 5.8; C21H22O8 requires C, 62.7; H, 5.5%). It was very sparingly soluble in aqueous sodium hydroxide and in alcoholic solution gave an intense green colouration with ferric chloride. Yield, 0.35 g.

8-Ethoxy-3:5:7:3':4'-Pentamethoxy-flavone (II):—

A solution of the above 5-hydroxy compound (0.2 g.) in dry acetone (20 c.c.) was refluxed with dimethyl sulphate (0.5 c.c.) and potassium carbonate (5.0 g.) for 6 hours. The solvent was distilled off, the residue treated with water and the white solid that separated out was filtered and purified by crystallisation from alcohol whereby it came out as colourless elongated rectangular plates melting at 142-43° alone or in admixture with the ethoxy-pentamethoxy-flavone (B) derived from gossypin.
Constitution of Gossypin—II

SUMMARY

The constitution of gossypin as the 8-monoglucoside of gossypetin is confirmed by a detailed study of the pentamethoxy monohydroxy-flavone obtained by the complete methylation and subsequent hydrolysis of the glucoside. It is different from gossypetin-pentamethyl ether containing a free hydroxyl group in the 5-position but both give on oxidation the same quinone which is transformed into a quinol. Further its ethyl ether is shown to have the ethoxyl in the 8-position by unambiguous synthesis.

REFERENCES

   Hattori and Hayashi .. Ber., 1933, 66, 1279.
   Shah, Mehta and Wheeler .. J. C. S., 1938, 1555.