

CHEMISTRY OF GOSSYPOL

Part III. Methylation

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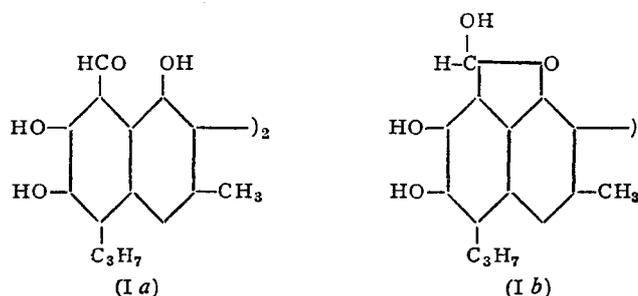
GOSSYPOL is highly reactive and unless the hydroxyl groups are protected, no definite degradation products could be obtained. In the elucidation of the constitution of gossypol, therefore, methylation is an operation of great importance. Adams and co-workers were the first to use it and consequently they were successful in obtaining a large number of degradation products through which much light was thrown on the constitution of the parent compound. However, a study of their work indicates the existence of a number of discrepancies. During the course of their voluminous work several samples of hexamethyl ether of gossypol, differing widely in their appearance and melting point were obtained. In all their experiments, they used dimethyl sulphate and carried out the methylation under various conditions. During the earlier part of the work, by the methylation of gossypol they¹ obtained a red hexamethyl ether melting at 160° and a colourless variety melting at 237°. These were recorded to be interconvertible and their existence was attributed to easy tautomerism. Later, starting from dimethyl gossypol they² obtained a hexamethyl ether which melted at different temperatures depending upon the solvent employed for crystallisation. When methyl alcohol was employed, the melting point was 211° whereas with a mixture of methyl alcohol and acetone it was 223° and with ligroin 173°. At a later stage² gossypol-acetic acid was used as the starting material. When the methylation of this compound was carried out at 10–15° the resulting sample of the hexamethyl ether of gossypol melted at 240° whether crystallised from acetone-methyl alcohol mixture or ligroin; when the temperature employed was 20–25° the melting point of the product was 225°. A third method³ in which the tetramethyl ether was an intermediate stage produced a hexamethyl compound melting at 175°. It is therefore clear that marked differences in the nature of the product were brought about by differences in experimental technique and the solvent employed for crystallisation. However, all samples were found to have the same chemical properties and these authors felt that 'their exact character is not very significant'. Obviously, all these differences could not be explained as due to isomerism. As already

explained in our previous publication⁴ the existence of different forms of gossypol itself does not seem to be so definite. Throughout their work it is noteworthy that Adams *et al.* made no determination of methoxyl value for the reason that they felt that it was unreliable. They found that even gossypol gave considerably high methoxyl value though it contained no methoxyl groups. Consequently they relied entirely on the determination of carbon and hydrogen for controlling the purity of the compounds. This does not seem to be satisfactory since marked difference in the percentages of carbon and hydrogen do not occur with variation in the methoxyl content, as for example the values of carbon and hydrogen for the pentamethyl ether are 71.4 and 6.8% whereas for the hexamethyl ether, the values are 71.8 and 7.0%. Therefore, the possibility of incomplete methylation and other differences in composition as the causes of the above discrepancies are not excluded. Efforts have therefore been made now to prepare the hexamethyl ether in a pure condition with definite and constant properties.

The provisional structure (I a) given by Adams *et al.* for gossypol contains ortho-hydroxy-aldehyde groups. It was first expected that these may offer difficulties in methylation due to chelation. The substance is also unstable in the presence of alkali. For similar cases met with among flavones and flavonols methylation of the acetyl derivatives using dimethyl sulphate in the presence of acetone and potash has been found in these laboratories⁵ to be very successful. The products were completely methylated derivatives. Using gossypol hexaacetate for our present purpose a pure sample of the hexamethyl ether was obtained. It was very pale yellow in colour and melted at 130°. Its methoxyl value was quite normal as also the values for carbon and hydrogen. It has already been mentioned in our earlier publication⁴ that our sample of gossypol gave no methoxyl value.

With a view to find out how far hindrance existed for complete methylation, gentler methods of methylating gossypol were adopted. Methylation using diazomethane and a methyl alcoholic solution of gossypol as well as methylation employing methyl iodide and potassium carbonate in anhydrous acetone medium gave the same hexamethyl ether easily. No partially methylated compounds could be obtained. Thus there seems to be no resistance to methylation. Further it was noted that by employing a mixture of gossypol and dimethyl sulphate in methyl alcoholic solution and adding potassium hydroxide solution a similar result is obtained. Thus complete methylation of gossypol takes place with ease yielding the same hexamethyl ether. The lower melting point of the ether as compared with gossypol is also according to expectations. That this is not due to hydration is clear from the fact that the sample is anhydrous.

The ready methylation of gossypol may be satisfactorily explained by the formula I *b* of Adams *et al.* in which no free aldehyde groups exist. But the methyl ethers of such structures should undergo ready hydrolysis with dilute acids simulating the behaviour of glycosides. No such change was found to take place. The hexamethyl ether was quite stable in boiling dilute sulphuric acid. Consequently the aldehydic form is not excluded and the absence of the effect of chelation may be due to steric factors.



Experimental

Preparation of hexamethyl ether from hexaacetate.—Gossypol hexaacetate (white) melting at 276° was employed. This substance (1 g.) was dissolved in acetone (20 c.c.) and treated with dimethyl sulphate and 20% sodium hydroxide solution (5 c.c. each). After shaking vigorously for some time more of dimethyl sulphate (3 c.c.) and alkali (5 c.c.) were added alternately in small quantities. Vigorous shaking was given after each addition and the solution was kept alkaline. The contents were allowed to stand overnight and then boiled under reflux for one hour. When the solvent was subsequently distilled off, a pale brown solid separated. It was collected and crystallised from dilute acetone.

The methyl ether obtained as given above appeared in bulk as a pale brown crystalline solid; under the microscope, it had the form of light yellow rectangular plates. It melted down to a liquid at 130° with sintering a few degrees earlier. It did not dissolve in aqueous alkali but was readily soluble in alcohol, acetone and other common organic solvents. Alcoholic solution gave no colour with ferric or stannic chlorides. Concentrated sulphuric acid gave light orange colour in contrast to the scarlet red colour obtained with gossypol. (Found: C, 72.0; H, 7.1; OCH_3 , 30.5; $\text{C}_{30}\text{H}_{24}\text{O}_2$ (OCH_3)₆ requires C, 71.8; H, 7.0; OCH_3 , 30.9%. $\text{C}_{30}\text{H}_{26}\text{O}_4$ (OCH_3)₄ requires C, 71.1; H, 6.6; OCH_3 , 21.6%.)

Direct Methylation of Gossypol

(a) *With diazomethane.*—Gossypol (1 g.) was dissolved in absolute methyl alcohol (30 c.c.) and the clear solution was treated with a large

excess of diazomethane (obtained from 10 g. of nitroso-methyl urea) in ether solution. The reagent was added in two lots on two successive days. After adding the first half in small quantities attended with shaking, the mixture was allowed to stand for 24 hours and as much ether as possible distilled off using a hot water-bath. After cooling, the second half of the reagent was slowly added. After standing overnight the solvents, ether and alcohol, were completely distilled off. On adding water to the residue a yellow crystalline solid separated and it was purified by crystallisation from dilute acetone. The crystal structure, melting point and other properties were the same as those possessed by the hexamethyl ether obtained from the acetate. The mixed melting point was unchanged. (Found: C, 71.9; H, 7.0; OCH₃, 30.5; C₃₀H₂₄O₂ (OCH₃)₆ requires C, 71.8; H, 7.0; OCH₃, 30.9%.)

(b) *With methyl iodide.*—A solution of gossypol (2 g.) in anhydrous acetone (50 c.c.) was treated with anhydrous potass. carbonate (10 g.) and methyl iodide (15 c.c.) was added slowly in small quantities at a time. The contents were kept gently boiling under reflux for 48 hours. The solvent was then distilled off and the residue treated with water in order to dissolve the carbonate. The insoluble yellow solid was filtered; washed with water and finally crystallised from acetone-methyl alcohol mixture. This sample of the hexamethyl ether had also the same melting point and other properties as already described. The mixed melting point with previous samples was undepressed. [Found: C, 71.9; H, 7.1; OCH₃, 30.6; C₃₀H₂₄O₂ (OCH₃)₆ requires C, 71.8; H, 7.0; OCH₃, 30.9%.]

(c) *With dimethyl sulphate.*—Gossypol (1 g.) was treated with a mixture of absolute methyl alcohol (5 c.c.) and freshly distilled dimethyl sulphate (5 c.c.) and warmed on a steam-bath. When a clear solution was obtained 10% methyl alcoholic potash (3.5 c.c.) was added in quantities of half c.c. each time and vigorously shaken. The reaction flask was then stoppered and allowed to stand for 24 hours with occasional shaking. The excess of dimethyl sulphate was decomposed by adding more alkali and warming. On adding excess of water a light brown flocculent precipitate was formed. It was filtered and crystallised from dilute acetone. Though a little brown colour persisted even after repeated crystallisations the substance was otherwise identical with samples of hexamethyl ether obtained by the methods given above. (Found: C, 72.0; H, 7.1; OCH₃, 30.4; C₃₀H₂₄O₂ (OCH₃)₆ requires C, 71.8; H, 7.0; OCH₃, 30.9%.)

All the samples of the hexamethyl ether melted without decomposition and gave satisfactory methoxyl values. Further repeated recrystallisations brought about no change in the melting point or analytical values.

The methods which did not employ aqueous alkali (diazomethane or methyl iodide and potassium carbonate) yielded samples which had a pale yellow colour whereas those in which caustic alkali had to be used left a pale brown colour with the samples and it could not be removed completely by repeated crystallisations. Though the hexamethyl ether exhibited sintering below 130° it did not contain any solvent of crystallisation since it lost no weight on drying *in vacuo* and gave satisfactory and consistent analytical values.

Gossypol hexamethyl ether (0.5 g.) was dissolved in dilute alcohol (20 c.c.) and enough of concentrated sulphuric acid (0.8 c.c.) added so as to bring its concentration to about 7%. The mixture was then boiled under reflux for 2 hours. When the alcohol was distilled off a precipitate was formed. When filtered and crystallised from dilute acetone, it was found to be the original hexamethyl ether recovered unchanged.

The experiment was repeated using hydrochloric acid and even in this case no demethylation took place.

However, by boiling with glacial acetic acid containing concentrated sulphuric acid, partial demethylation to gossypol dimethyl ether could be effected (compare Adams, *et al.*²).

Summary

The methods adopted by Adams *et al.* for the methylation of gossypol do not seem to produce a uniform product of the hexamethyl ether having constant properties. The following methods have now been employed: (1) methylation of hexaacetate using dimethyl sulphate and alkali in acetone medium, (2) methylation of gossypol with diazomethane in methyl alcoholic solution, (3) with methyl iodide and potassium carbonate in acetone solution and (4) with dimethyl sulphate and alkali. All the products had the same melting point, 130°, and gave analytical results corresponding to the hexamethyl ether. The methods which do not employ caustic alkali give samples which are less coloured. The methyl ether is unaffected by treatment with hot dilute sulphuric acid and hence does not seem to have the formula corresponding to the structure of glycosides.

REFERENCES

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3. ——— .. *Ibid.*, 1938, **60**, 2967.
4. Murty, Murty and Seshadri .. *Proc. Ind. Acad. Sci.*, A. 1942, **16**, 54.
5. Rao and Seshadri .. *Ibid.*, 1939, **9**, 177 and 365.