

CHEMISTRY OF GOSSYPOL

Part II. The Anilino-Derivatives

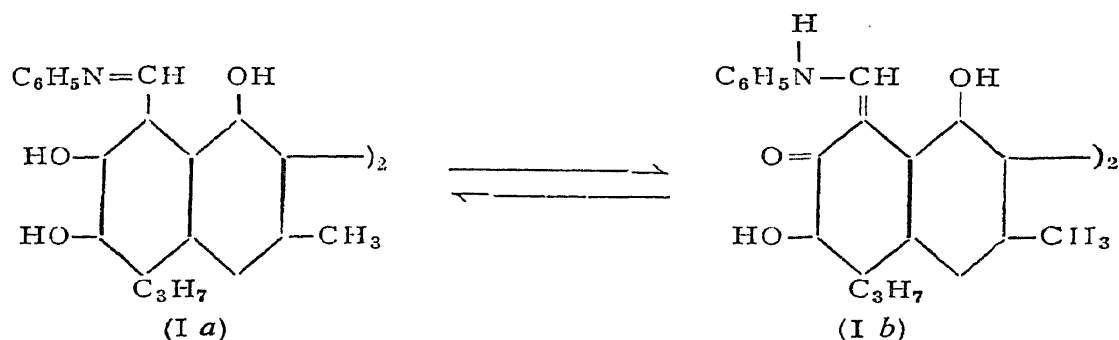
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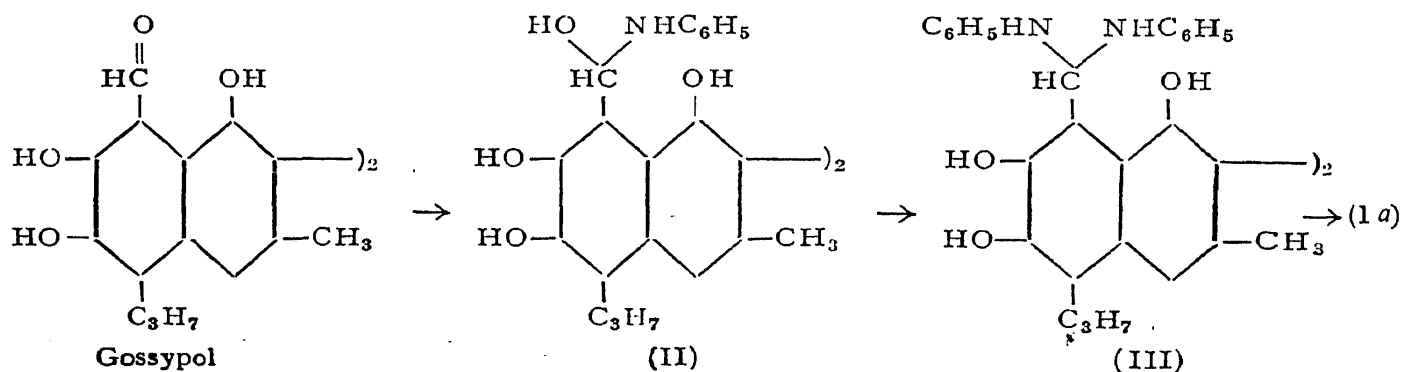
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GOSSYPOL combines with aniline very readily to form a highly characteristic deep yellow anilino-compound. Because of its sparing solubility, it has been used for purposes of estimation of gossypol and also for isolating it from cotton seeds.⁵ Carruth¹ who was the first to prepare it noticed that the nitrogen analyses gave anomalous results. Though the substance which was dried by heating at 100° gave results agreeing with the formula $G, 2 C_6H_5NH_2$ ($G =$ gossypol), the air-dried samples gave uniformly higher values for nitrogen and he represented the air-dried sample by the formula, $2 G, 5 C_6H_5NH_2$. Later workers, Clark² and Adams *et al.*³ did not meet with this difficulty. They considered the compound to be dianilino-gossypol arising as the result of condensation of two aniline molecules with two carbonyl groups present in gossypol with the elimination of 2 mols. of water. As a matter of fact, this was taken as an important confirmation for the existence of two such functional groups. The results of analysis for carbon, hydrogen and nitrogen and the determination of molecular weight were found by them to agree with this view.

In order to get further insight into the constitution of the anilino-compound, Adams *et al.* (*loc. cit.*) subjected it to acetylation and methylation. By the action of acetic anhydride and pyridine they reported the formation of hexaacetyldianilino-gossypol which on pyrolysis yielded acetanilide as one of the products. From this they concluded that in the compound each of the nitrogen atoms was associated with a hydrogen atom which underwent substitution by an acetyl group during the above treatment. This was supported by their experiments on methylation using dimethyl sulphate and pyridine in chloroform solution. A red dimethyl product was isolated which was supposed to have methyl groups attached to the nitrogen atoms since they could not be removed by hydrogen iodide. Further the methylated compound could not be hydrolysed to gossypol under the conditions used for the hydrolysis of dianilino-gossypol (sulphuric acid treatment). They also reported the isolation of another unintelligible compound designated dimethyldianilino-oxygossypol under slightly different conditions of methylation. From these considerations they concluded that the dianilino-compound is not a simple Schiff's base but has constitution represented as below:



In the course of the present investigation it has been observed that an air-dried sample of the anilino-compound, previously purified by crystallization from benzene, melts at 303° with decomposition, but at about 180° evolves considerable quantities of aniline which collects as a pale yellow liquid on the sides of the tube. Though the carbon and hydrogen values are very nearly the same as those required for the dianilino-compound, the nitrogen value is considerably high. This, however, agrees with the one required for a tetraanilino-compound. On heating at 110° for four hours or for much shorter period at 180° , it suffers a loss of weight of about 21% corresponding to the loss of two mols. of aniline. In other respects the oven-dried and air-dried samples behave very similarly. It may be mentioned that combustion values for carbon and hydrogen cannot differentiate between the two substances whereas the values required for nitrogen are capable of indicating the difference. (The dianilino-compound, $\text{C}_{42}\text{H}_{40}\text{O}_6\text{N}_2$ requires: C, 75.4; H, 6.0; N, 4.2 and the tetraanilino-compound, $\text{C}_{54}\text{H}_{54}\text{O}_6\text{N}_4$ requires: C, 75.9; H, 6.3; N, 6.6%.) It is thus obvious that gossypol forms a tetraanilino-compound which on drying undergoes change into the dianilino-compound with the evolution of aniline. In this respect it resembles aromatic aldehydes and the case is parallel to the findings of Hantzsch and Schwab⁴ in regard to benzaldehyde and *p*-nitro-aniline. The simple addition product (formula II) could be obtained by treating gossypol with exactly two molecular proportions of aniline. But it is not quite pure, being accompanied by small quantities of the dianilino-compound (I). The course of the reaction may be represented as below:



On heating dianilinogossypol at its melting point (303°) for sometime further evolution of aniline could be noticed. The residue is a dark uncrystallisable mass and nothing definite could be isolated from it.

In view of the ready decomposition of the anilino compounds with acetic anhydride⁵ the findings of Adams *et al.*³, who claimed to have obtained a diacetyl derivative of the dianil, seemed to be inexplicable. The reaction has, therefore, been repeated using acetic anhydride and pyridine. It is noticed that the main product when carefully purified is the yellow acetyl derivative of gossypol called by them "hexaacetyl-gossypol". Thus the anils have behaved similar to gossypol itself obviously due to ready decomposition in the presence of acetic anhydride. It may be pointed out here that the melting point of the acetylation product mentioned by Adams *et al.* agrees with that of the 'yellow hexaacetyl-gossypol'. It is possible that the presence of nitrogen in their product was due to contamination with acetanilide, a large amount of which is produced particularly from the air-dried tetraanilino-compound. Again, methylation of the anilino compounds by the ordinary method using dimethyl sulphate and alkali yields the hexamethyl ether of gossypol itself, obviously due to the removal of the anilino groups. Consequently the conclusion of Adams and co-workers that the dianilino-compound has constitution I *b* does not seem to be supported.

In this paper the structure assigned by Adams *et al.* for gossypol has been tentatively adopted.

Experimental

Preparation of Tetraanilinogossypol (III).—Gossypol (1 g.) was treated with aniline (10 c.c.) and the mixture was just heated to boiling, whereby a clear solution was obtained. On allowing it to cool an orange-yellow crystalline precipitate was obtained. It was filtered and washed first with alcohol and then with ether. It was finally recrystallised from hot benzene when it was obtained as glistening yellow rectangular plates. After air-drying at the ordinary temperature for over a week, it melted with decomposition at 303° . It was however noticed that a small quantity of a liquid (aniline) condensed on the sides of the melting point tube at about 180° . (Found: C, 76.0; H, 6.3; N, 6.8; $C_{54}H_{54}O_6N_4$ requires: C, 75.9; H., 6.3; N, 6.6%.) The compound could also be prepared by the addition of excess of aniline to a solution of gossypol in ether. It was sparingly soluble in most of the organic solvents and it could be best crystallised from boiling aniline or benzene.

Dianilinogossypol.—The tetraanilinogossypol (1 g.) was heated at 100° to 110° in an air oven for four hours till there was no further loss in weight. This loss amounted to the removal of 2 mols. of aniline. The product was crystallised from boiling benzene and was obtained as bright yellow rectangular plates (Found: C, 75.3; H, 6.1; N, 4.3; $C_{42}H_{40}O_6N_2$ requires: C, 75.4; H, 6.0; N, 4.2%). On heating in a capillary tube it did not evolve any aniline at about 180° but melted with decomposition at 303°. In its solubility and in other reactions it closely resembled the tetranilino-compound. Even the mixed melting point was undepressed. If the decomposition was carried out in a small distillation flask connected to a pump through a condenser and the heating was done at 180° using an oil bath, it was found to be over within an hour and in the distillate aniline could be identified.

Formation of the compound II.—Gossypol (0.5 g.) dissolved in dry ether (10 c.c.) was treated with a solution of aniline (0.2 g.) in the same solvent. On stirring, a crystalline precipitate rapidly formed. After about an hour it was filtered, washed with ether and subsequently with alcohol and allowed to dry at the room temperature. It was a yellow crystalline substance (ill-defined plates) melting with decomposition at 303°. (Found: C, 72.9, H 6.1; $C_{42}H_{44}O_8N_2$ requires: C, 71.6, H, 6.3%). Compound II seems to be therefore mixed with some amount of the di-anilino compound arising as the result of removal of water.

Action of heat on the dianil.—A sample of pure dianilinogossypol was taken into a short test tube provided with a side neck which was connected to a pump through a receiver. It was gradually heated in a metal bath to a temperature of 300° in the course of about an hour. A dark amorphous mass was formed in the test tube and the distillate was identified to be aniline. The residue consisted mostly of dark tarry matter from which a very small quantity of an yellow substance could be extracted with alcohol. The latter gave no test for nitrogen.

Action of acetic anhydride.—The solution of the tetraanilino compound (0.5 g.) in pyridine (10 c.c.) was treated with acetic anhydride (5 c.c.) and heated on a steam-bath for 30 minutes following exactly the method of Adams *et al.* (*loc. cit.*). About 10 c.c. of acetic acid was then added and the mixture poured into water. The solid product was crystallised from methyl alcohol. It was found to be a bright yellow crystalline solid melting at 185° with decomposition and exhibiting most of the properties mentioned by the above authors. It, however, contained no nitrogen and hence could not be a derivative of the anilino-compound. It was also different from

gossypol-acetic acid since it did not give any colour reaction with ferric or stannic chlorides, and was unaffected by cold dilute alkali. It was however, found to be identical with yellow hexaacetyl-gossypol in all respects and mixed melting point with it was undepressed.

The same product was obtained when dianilino-gossypol was employed.

Methylation of the Anilino compound.—The tetraanilino-compound (0.5 g.) was dissolved in 20% alcoholic alkali (5 c.c.) and the solution treated with dimethyl sulphate (3 c.c.) with vigorous shaking and keeping the contents slightly alkaline. After decomposing the excess of dimethyl sulphate by heating the solution for 1 hour, on a water-bath, water was added and the solid precipitate was filtered and purified by crystallisation from acetone-methyl alcohol mixture. It was a pale brown crystalline substance melting at 130° and containing no nitrogen. (Found: OCH_3 , 31.0%; $\text{C}_{30}\text{H}_{24}\text{O}_2$, $(\text{OCH}_3)_6$ requires OCH_3 , 30.9%.) The same result was obtained when dianilinogossypol was used.

Summary

With excess of aniline, gossypol forms tetraanilino-gossypol which decomposes on heating for a long time at 110° or for a short period at 180°, the products being aniline and dianilinogossypol. The two anilino compounds resemble closely except for the fact that the tetraanilino-compound contains more nitrogen and evolves aniline long before its final decomposition point is reached. Acetylation and methylation yield only gossypol derivatives, aniline being removed; acetyl and methyl derivatives of the anilino compounds could not be obtained.

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