[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

Structure of Gossypol. V.[1] Anilino Derivatives

BY ROGER ADAMS, C. C. PRICE AND W. R. DIAL[2]

Gossypol reacts readily with two molecules of aniline;[3] two molecules of water are eliminated and dianilinogossypol is formed. This product is purified easily, and as a consequence aniline has actually been used for the extraction of gossypol.[3]

The formation of this compound has led to the deduction that two keto groups are present in the gossypol structure. However, in a previous paper from this Laboratory it was pointed out that certain vinyl alcohol groups condense equally well with aniline with the elimination of water. There is reason for believing that gossypol contains groupings of this type.

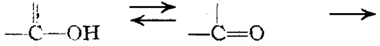
Anhydrogossypol reacts with aniline to give dianilinogossypol quantitatively; moreover, liquid ammonia reacts with gossypol or anhydrogossypol to give diaminogossypol. Both diaminogossypol and dianilinogossypol hydrolyze with unusual ease to give gossypol.

Substituted anilines have now been combined with gossypol. Some of them condense in a manner similar to aniline, β-naphthylamine and *o*-phenylenediamine. The derivative of *o*-phenylenediamine is of particular interest. Karrer and Tobler[4] reported the condensation product of gossypol and *o*-phenylenediamine as consisting of one molecule of gossypol and one of diamine with the loss of two molecules of water. From this they concluded that an *o*-quinone or 1,2-diketone might be present in the parent molecule. All the reliable reactions thus far described and many yet unpublished have conformed to the point of view of a symmetrical molecule for gossypol. An even number of molecules of reagent condense. It is not impossible that two ketone groups are adjacent and are symmetrically located in the molecule but as this seemed unlikely the condensation with *o*-phenylenediamine has been studied again. It has now been established that *o*-phenylenediamine when used in molecular amounts with gossypol gives a non-homogeneous product which agrees in properties with that reported by the above-mentioned authors. On the other hand, if two or more molecules of *o*-phenylenediamine are used, a readily purified, nicely crystalline compound is isolated in quantitative yield which consists of two molecules of *o*-phenylenediamine and one of gossypol with the elimination of two molecules of water. The presence of a 1,2-diketone grouping in gossypol, therefore, appears improbable.

Other substituted anilines condense readily with gossypol. However, in many cases (*o*-, *m*-, and *p*-toluidine, *p*-anisidine, *p*-chloroaniline) the analyses of the products do not conform to a simple derivative similar to the dianilinogossypol. They are more difficult to purify and it appears in some cases that an additional molecule of aniline is present in the product or that an oxygen has entered simultaneously. These compounds, however, all hydrolyze to gossypol. Similarly, the acetates of all but the dianilino compound are difficult to purify and give variable analyses.

Dianilinogossypol acetylates to a hexaacetyl derivative. Pyrolysis of this compound gives acetanilide and as a consequence it seems likely that two acetyls are attached to nitrogen atoms which would be possible only if the dianilinogossypol were so constituted that hydrogens were present on the nitrogen atoms.

Dianilinogossypol also can be methylated. Under certain conditions, a crystalline dimethyldianilinogossypol is sometimes produced. This substance is deep red. It has the methyl groups on the two nitrogens, if the fact that the Zeisel number of the derivatives is almost zero may be used as experimental evidence. Moreover, the compound cannot be hydrolyzed to gossypol under the conditions used for dianilinogossypol. These data and those on the acetylation product make it appear probable that the dianilino compound is tautomeric. The original dianilinogossypol which is yellow possibly may be a Schiff base which upon methylation is converted into a red N-methyl derivative of the hydroxymethylene type. These reactions may be explained by the presence of the following groupings



$$\begin{array}{l} \text{—C—CHO} \\ \ \ \| \\ \text{—C—OH} \end{array} \rightleftarrows \begin{array}{l} \text{—C=CHOH} \\ \ \ | \\ \text{—C=O} \end{array} \longrightarrow$$

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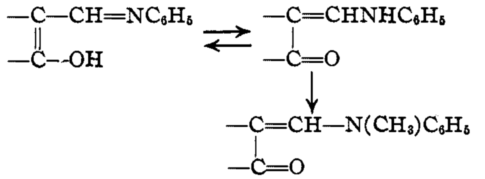
(1) For the previous article in this field see Miller and Adams, THIS JOURNAL, **59**, 1736 (1937).

(2) Dow Chemical Fellow, 1936–38. Portion of a thesis submitted in partial fulfilment for the degree of Doctor of Philosophy.

(3) Clark, *J. Biol. Chem.*, **76**, 229 (1928); Carruth, THIS JOURNAL, **40**, 647 (1918).

(4) Karrer and Tobler, *Helv. Chim. Acta*, **15**, 1204 (1932).



Under slightly modified conditions of methylation, a crystalline dimethyldianilinooxogossypol is readily formed in which it appears that an atom of oxygen has replaced two hydrogens in dimethyldianilinogossypol or possibly merely an atom of oxygen was added. The compound does not hydrolyze to gossypol under the usual conditions. Its structure has not yet been investigated.

Experimental

Dianilinogossypol.—This compound previously described by Carruth[3] and by Clark[5] was prepared by dissolving crude gossypol-acetic acid in ten times its weight of boiling aniline. The orange crystals which separated on cooling were collected and washed thoroughly with ether. It was purified from chloroform–petroleum ether and yielded yellow-orange plates, m. p. 303°, with decomposition.

For hydrolysis, it was dissolved in concentrated sulfuric acid, the solution allowed to heat to 90° on a hot-plate (about two to two and one-half minutes), poured onto ice, filtered, and dried. The product was dissolved in hot glacial acetic acid and water was added dropwise to the boiling solution until crystals started to appear. The solution on cooling deposited gossypol-acetic acid which was usually in practically a pure state as golden platelets.

Dianilinogossypol, when heated on the steam cone for thirty minutes in 40% aqueous alkali, followed by methylation with dimethyl sulfate, gave a 60% yield of apogossypol hexamethyl ether.

By Zeisel methoxyl determination, gossypol is reported to give about 5% methoxyl. A repetition of this determination using a commercial grade of 47% hydriodic acid containing a small amount of preservative (1.5% H_3PO_2) gave less than 0.2% methoxyl. Dianilinogossypol also showed less than 0.2% methoxyl.

Hexaacetyldianilinogossypol.—A solution of 0.5 g. of dianilinogossypol in 10 cc. of pyridine and 5 cc. of acetic anhydride was warmed on a steam-cone for thirty minutes. About 10 cc. of acetic acid was added and the reaction mixture was poured into water. A bright yellow product separated which was purified by crystallization from methanol, m. p. 185°, decomposing vigorously without discoloration at 220°.

Anal. Calcd. for $C_{54}H_{52}N_2O_{12}$: C, 70.40; H, 5.70; N, 3.04; acetyl, 28.0. Found: C, 69.94; H, 5.82; N, 2.89; acetyl, 28.4.

The heat decomposition of this compound led to the isolation of a white crystalline compound, m. p. 112°, which sublimed in the cooler portions of the pyrolysis tube. It proved to be acetanilide. It may thus be deduced that two acetyl groups are probably attached to nitrogens in the starting material.

By hydrolysis with concentrated sulfuric acid, gossypol was recovered. The initial color in the acid is yellow which turns to olive-green in thirty seconds and to scarlet on addition of a drop or two of water.

Di-*o*-phenylenediaminogossypol.—A mixture of 2.5 moles of *o*-phenylenediamine and one of pure gossypol-acetic acid in chloroform solution was refluxed for thirty minutes. On cooling the solution, the product separated as orange crystals. It was recrystallized from chloroform, m. p. 246°.

Anal. Calcd. for $C_{42}H_{42}N_4O_6$: C, 72.13; H, 6.06; N, 8.02. Found: C, 72.01, 71.92; H, 5.84, 6.08; N, 8.18, 7.99.

By the use of one molecule of *o*-phenylenediamine, a noncrystalline substance was obtained which had properties similar to that reported by Karrer and Tobler.[4] An excess of diamine, however, readily converted it to the product reported above.

In the preparation of the di-*o*-phenylenediamine derivative, a product melting at 184° sometimes was obtained. This product, however, on drying and long standing acquires the higher melting point mentioned above.

Di-β-naphthylaminogossypol.—It formed bright orange crystals from chloroform, m. p. 310–313° with decomposition. It hydrolyzed to gossypol with sulfuric acid.

Anal. Calcd. for $C_{50}H_{44}N_2O_6$: C, 78.09; H, 5.97; N, 3.65. Found: C, 77.95; H, 5.78; N, 3.69.

The preparation of similar compounds was attempted from gossypol and *o*-, *m*-, and *p*-toluidine, *p*-chloroaniline and *p*-anisidine. In all these cases, crystalline products were obtained, more difficult to purify than the ones previously described. Analyses did not check the calculated values. In some cases, the analyses indicated that normal condensation took place and an additional molecule of the amine had added. In other cases, addition of one atom of oxygen to the condensation product of two molecules of amine agreed most satisfactorily. All these compounds, however, were hydrolyzed to gossypol. The acetyl derivatives were difficult to purify and did not give the expected values on analysis.

Dimethyldianilinooxogossypol.—To a solution of 1 g. of dianilinogossypol in 100 cc. of chloroform, were added 10 cc. of dimethyl sulfate and 6 cc. of pyridine in portions with shaking. The reaction mixture did not change color but separated into two phases in a few minutes. After standing at room temperature for five days to a week, 15 cc. of pyridine was added to decompose the excess dimethyl sulfate. The chloroform solution was washed twice with water, dried over sodium sulfate, and evaporated. It was purified by recrystallization from benzene as red microcrystals, m. p. 275–280°. It gave a brown color in concentrated sulfuric acid which turned red on standing for five to ten minutes.

Anal. Calcd. for $C_{44}H_{42}N_2O_7$: C, 74.33; H, 5.95; N, 3.94; OCH_3, 8.74. Found: C, 74.22, 74.46; H, 5.96, 5.82; N, 4.00, 3.99; OCH_3, 1.45.

The compound was recovered unchanged after eighteen hours in concentrated sulfuric acid by pouring on ice and recrystallizing from benzene–methanol. The resistance to

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(5) Clark, *J. Biol. Chem.*, **75**, 725 (1927).

hydrolysis, as well as the low Zeisel number, indicates that the methyl groups are probably attached to nitrogens.

Dimethyldianilinogossypol.—By a similar procedure to the preparation of the oxo compound but refluxing overnight rather than allowing the reaction mixture to stand at room temperature resulted in a methylated but unoxidized product. It was purified from benzene and formed red crystals, m. p. 253–258°. It gave a dark red-brown color in sulfuric acid.

Anal. Calcd. for $C_{44}H_{42}N_2O_6$: C, 75.83; H, 6.36; N, 4.02; OCH_3, 8.89. Found: C, 75.70; H, 6.11; N, 4.09; OCH_3, 1.01.

Concentrated sulfuric acid does not hydrolyze this product. This and the low Zeisel number indicate the probability that the methyls are attached to nitrogen.

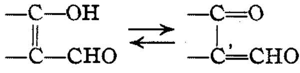
Summary

Gossypol condenses with β-naphthylamine or *o*-phenylenediamine in the same way as with aniline. Two molecules of amine react and two moles of water are eliminated.

Several other aniline derivatives gave products which did not analyze for simple condensation products. All of these hydrolyzed with sulfuric acid to give gossypol.

Dianilinogossypol can be acetylated to a hexaacetate. Two acetyls are attached to nitrogen. Methylation gives sometimes a dimethyldianilinogossypol or more commonly a dimethyldianilinooxogossypol.

It is deduced that there exist in gossypol two of the tautomeric residues



$$\begin{matrix}\text{—C—OH} \\ \| \\ \text{—C—CHO}\end{matrix} \rightleftarrows \begin{matrix}\text{—C=O} \\ | \\ \text{—C=CHOH}\end{matrix}$$

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Structure of Gossypol. VI.[1] Addition Products with Butadienes

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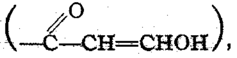
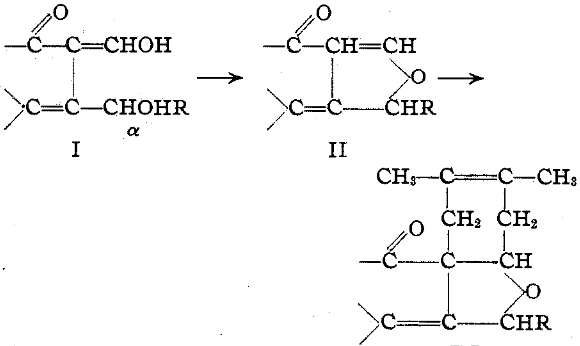
From a study of anhydrogossypol[3] and its reaction products and of the diamino- and dianilinogossypols[1] it was deduced that gossypol contains two similar groupings which are probably vinyl alcohols. Since the most common type of relatively stable vinyl alcohols possesses a carbonyl in place of one of the hydrogens of the methylene group  $\left(\text{—}\overset{\text{O}}{\overset{/\!/}{\text{C}}}\text{—CH=CHOH}\right)$, the possibility of two such residues in gossypol has now been studied. The reaction used for this purpose was the Diels–Alder and the reagent, 2,3-dimethylbutadiene.

Gossypol condenses very readily in alcohol solution with the addition of two molecules of dimethylbutadiene and elimination of two molecules of water to give a beautifully crystalline product. In a similar way, anhydrogossypol or diaminogossypol in benzene condenses with two molecules of dimethylbutadiene to give the same derivative.

In the case of gossypol, the reaction involves the elimination of water; in the case of diaminogossypol the loss of ammonia; in the case of anhydrogossypol, however, the same derivative is formed by simple addition. Since anhydrogossypol adds the diene, it is a safe conclusion that the molecule probably contains α,β-unsaturated carbonyl linkages. The addition product, unlike anhydrogossypol itself, is not hydrolyzed. Consequently, the easily-opened oxygen ring which was postulated previously as being present in anhydrogossypol, must have been stabilized. This is no doubt due to the fact that the carbon–carbon double bond to which the diene adds is part of the oxygen-containing ring of the anhydrogossypol. Suggested nuclei which will account for the changes follow: gossypol (I), anhydrogossypol (II), dimethylbutadiene addition product (III).



I II III

It is probable that gossypol and diaminogossypol

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[1] For the previous article in this field see Adams, Price and Dial, THIS JOURNAL, 60, 2158 (1938).

[2] A portion of a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy by E. C. Kirkpatrick, Solvay Fellow, 1936–1938.

[3] Miller and Adams, THIS JOURNAL, 59, 1736 (1937).