the yellow hexaacetate. The mixture was kept cold for four hours and then the calculated amount of approximately 0.7 *N* sulfuric acid was added to precipitate the barium. This was followed by dilution with water and extraction with ether. The ether solution was concentrated, dried and the gossypol-acetic acid precipitated by addition of glacial acetic acid until the odor of the latter persisted.

Oxidation of White and Yellow Products.—White gossypol hexaacetate was oxidized to tetraacetylgossypolone by 10% aqueous chromic acid in glacial acetic acid.[5]

The yellow product under similar oxidation gave a yield of only 7.5%.

Gossypol Hexabenzoate.—The Schotten–Baumann reaction was used. The detailed procedure employed was similar to that described by Carruth[6] except that 0.5 g. of sodium hydrosulfite was added to the sodium hydroxide solution and pure gossypol-acetic acid was used. From 5.0 g. of pure gossypol-acetic acid, 4 g. of product, insoluble in methyl alcoholic alkali, was obtained. It was purified from hot ethyl alcohol and formed a yellow powder, m. p. 202–204°, dec.

Anal. Calcd. for $C_{30}H_{24}O_2(OCOC_6H_5)_6$: C, 75.5; H, 4.73. Found: C, 74.6; H, 4.70.

Ozonization of White Gossypol Hexaacetate.—A mixture of ozone and oxygen (about 4%) was allowed to pass through an ice-cold solution of 0.5 g. of pure gossypol hexaacetate in 70 cc. of dry chloroform for forty-five minutes. The ozonide was decomposed by evaporating the chloroform on the steam-bath in the presence of water, acetic acid and zinc. The oily residue was dissolved in 50 cc. of ether, the solution dried with anhydrous sodium sulfate, and petroleum ether (b. p. 30–60°) added until precipitation started. A yellow product separated in the course of an hour as a microcrystalline powder, m. p. 140°.

Anal. Calcd. for $C_{19}H_{20}O_8$: C, 60.20; H, 5.33. Found: C, 60.35, 60.02; H, 5.33, 5.44; acetyl, 25.0, 26.2.

The product was insoluble in water and 10% aqueous sodium hydroxide but soluble in all organic solvents except petroleum ether.

Summary

1. A pure white hexaacetate of gossypol and a yellow hexaacetyl derivative have been prepared by Clark's method. A more satisfactory synthesis of these two products is described.

2. The white product may be converted to the yellow. Both on oxidation give tetraacetylgossypolone, the former in much better yields.

3. Both derivatives are readily hydrolyzed to gossypol.

URBANA, ILLINOIS

RECEIVED JUNE 26, 1937

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(5) Clark, THIS JOURNAL, **51**, 1475 (1929).

(6) Carruth, *ibid.*, **40**, 647 (1918).

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

The Structure of Gossypol. III. Gossypol Ethers

BY R. C. MORRIS AND ROGER ADAMS

The presence of six hydroxyl groups in gossypol has been demonstrated by the preparation of a white hexaacetate[1] and a yellow hexaacetyl derivative. The former compound especially has served to a limited extent as a base material for degradation reactions. However, the susceptibility to hydrolysis of the acyl derivatives might be avoided if the corresponding ethers were prepared. Previous investigators of gossypol have been unsuccessful in their attempts to obtain crystalline ethers.[2]

Owing to the sensitivity of gossypol to alkali, the more common procedure for methylation fails. However, by dissolving the gossypol in dimethyl sulfate with or without the addition of methanol or sulfuric acid, and then treating with alkali, the reaction mixture is at all times during the alkylation kept essentially neutral and consequently proceeds smoothly. Crystalline ethers are thus obtained readily.

A solution of gossypol in a mixture of dimethyl sulfate and methanol gave on treatment with alkali a white tetramethyl ether. On the other hand, if dimethyl sulfate containing 25% of sulfuric acid (98%) was used to dissolve the gossypol and alcoholic alkali was then added, essentially a quantitative yield of a red hexamethyl ether was obtained. Gossypol in dimethyl sulfate upon treatment with alkali yielded a mixture of the two ethers just described.

The tetramethyl ether was not sensitive to alkali or to many of the reagents which decomposed gossypol. It was neutral in character. It could be acetylated to a diacetyl derivative or methylated further with dimethyl sulfate and alkali to a white hexamethyl ether. This latter product was unaffected by concentrated sulfuric acid as it readily dissolved in the acid and could

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(1) Paper II, Miller, Butterbaugh and Adams, THIS JOURNAL, **59**, 1729 (1937); Clark, *J. Biol. Chem.*, **75**, 725 (1927).

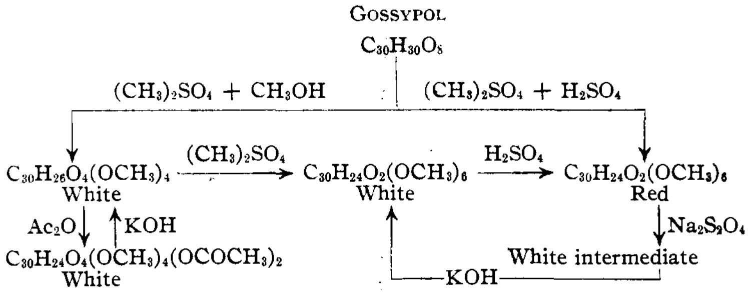
(2) Ewald Tobler, Dissertation, Zurich, 1932.

be reprecipitated unchanged by addition of water. If, however, an excess of aqueous alkali was used for the precipitation from the concentrated sulfuric acid solution, the previously described red hexamethyl ether was isolated.

The red hexamethyl ether could in turn be converted to the white hexamethyl ether. The red form is stable to alcoholic alkali. By treating it in a methyl alcoholic solution with a little aqueous sodium hydrosulfite until the solution had become light yellow, addition of water gave a cream-colored precipitate. This quickly reverted to the red hexamethyl ether if warmed in a solvent such as methyl alcohol. If, however, a cold methyl alcoholic solution of the precipitate was treated in an atmosphere of nitrogen with alcoholic alkali and allowed to stand, the white hexamethyl ether was obtained by addition of water.

The red hexamethyl ether was also decolorized by hydrogen in the presence of a catalyst but the solution rapidly turned red as soon as the hydrogen was removed. Treatment of the colorless solution with alcoholic alkali did not give the white hexamethyl ether.

These derivatives are shown schematically below.



It appears from these results that the two hexamethyl ethers are interchanged easily and confirms previous indications that gossypol contains groups which readily tautomerize. The interchange of the ethers apparently takes place without involving the six methoxyl groups. Both ethers give an orange color in sulfuric acid in contrast to the scarlet given by gossypol and both are stable to aqueous or alcoholic alkali. They differ markedly from each other in solubility and in many other properties. The red, for example, does not melt sharply and is changed by standing in cold concentrated sulfuric acid solution, whereas the white melts sharply and is stable in cold sulfuric acid. The white tetramethyl ether gives the scarlet gossypol color in sulfuric acid. The corresponding diacetyl tetramethyl ether gives an orange color which quickly changes to scarlet due probably to the hydrolysis of the two acetyl groups.

By the use of diethyl sulfate and following the procedures previously mentioned with dimethyl sulfate, red and white hexaethyl ethers were formed. No white tetraethyl ether analogous to the white tetramethyl ether was isolated.

Throughout the many experiments performed during this investigation no pure substances were found which gave a direct clue as to the functional groups holding the seventh and eighth oxygen atoms in gossypol. It was observed, however, that the white tetramethyl ether or its diacetate upon extended acetylation gave a product very soluble in organic solvents and difficult to purify; similarly, the white hexamethyl ether on treatment with acetic anhydride gave a product analogous in properties. Variable analyses resulted for both these substances and it is, therefore, impossible at the present time to be certain of their empirical formulas. The method of preparation and the preliminary analyses indicate that these compounds may be anhydro products. Treatment with alkali converted the former to gossypol tetramethyl ether and the latter to gossypol hexamethyl ether. They are of a type similar to the yellow hexaacetyl derivative reported in the last paper.

When the white hexamethyl ether was treated with methylmagnesium iodide, methane was evolved but owing to the insolubility of the initial reaction product, a quantitative determination was impossible. This result, together with those just previously described, indicates that the two remaining oxygens may possibly be hydroxyls, tertiary in character and so situated in the molecule that the usual procedures for identification cannot be used. It must be mentioned here that Schmid[3] obtained evidence by the Zerewitinoff method that six active hydrogens and hence six hydroxyls were present in gossypol. If two additional hydroxyls are in the molecule it is not surprising that they cannot be detected by this method.

The white tetra- and hexamethyl ethers and the white hexaethyl ether are all dimorphic

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[3] Schmid and Margulies, *Monatsh.*, **65**, 391 (1934–1935).

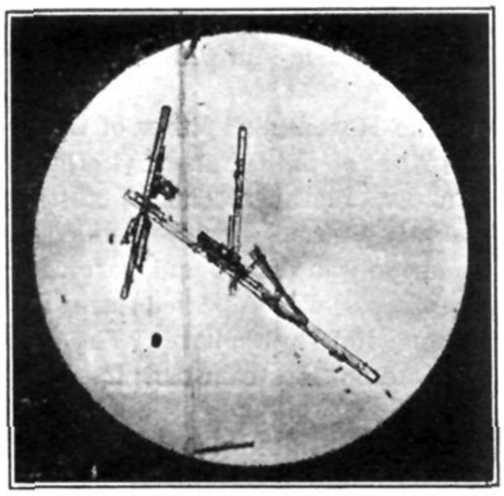

Plate 1.—Gossypol tetramethyl ether, m. p. 190°.

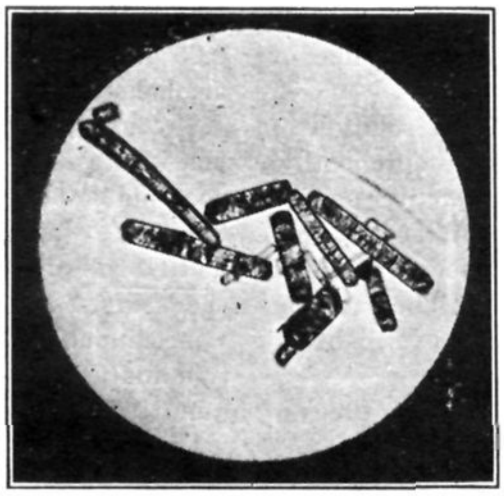

Plate 2.—Gossypol tetramethyl ether, m. p. 259°.

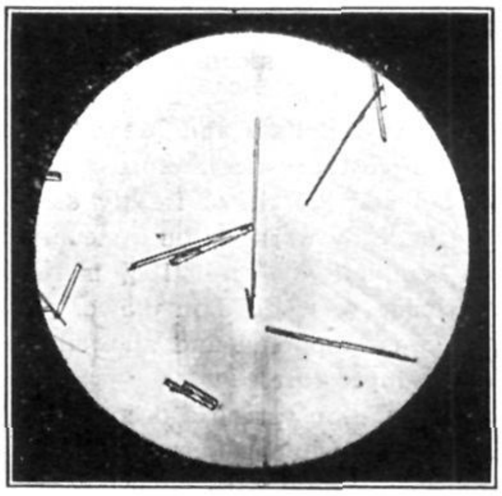

Plate 3.—White gossypol hexamethyl ether, m. p. 221°.

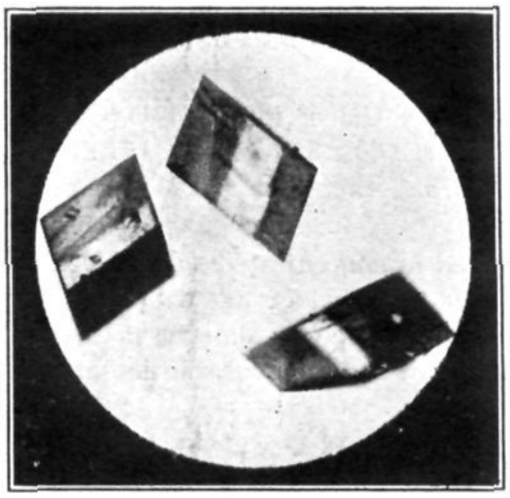

Plate 4.—White gossypol hexamethyl ether, m. p. 235–237°.

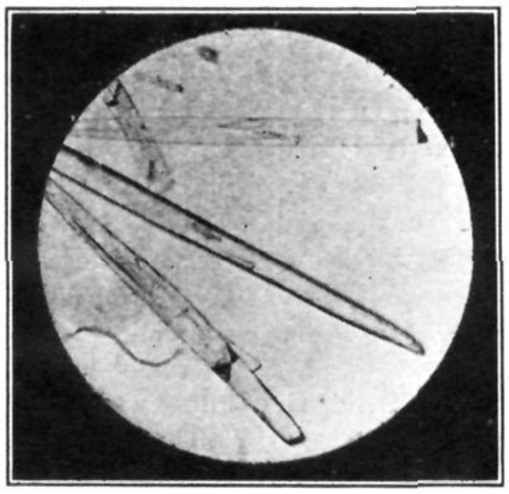

Plate 5.—White gossypol hexaethyl ether, m. p. 217°.

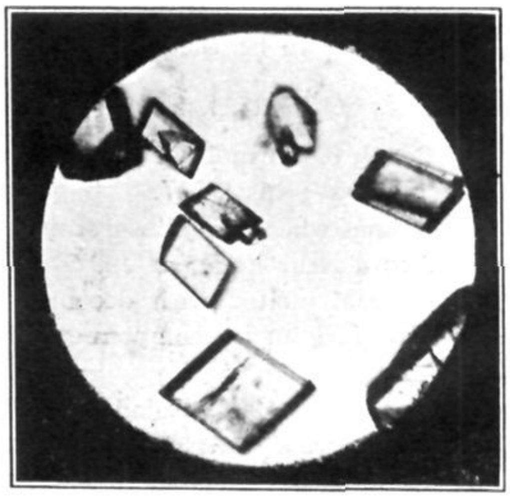

Plate 6.—White gossypol hexaethyl ether, m. p. 231°.

and in this respect resemble gossypol which is polymorphic. Microphotographs of the forms are given herewith.

At present the various chemical reactions of the ethers are being studied, particularly those reactions which may lead to degradation products capable of identification.

Experimental

Methylation of Gossypol

Dimethyl Sulfate and Sulfuric Acid.—A solution of 0.5 g. of gossypol in a mixture of 1.2 cc. of sulfuric acid (98%) and 3.8 cc. of freshly distilled dimethyl sulfate was made by warming on a steam-bath. When solution was complete, the flask was cooled and 25% aqueous sodium hydroxide was added in portions, with shaking, until the dimethyl sulfate had decomposed completely and the reaction mixture was alkaline. The flocculent red precipitate was filtered, washed and dried; yield 0.5 g. This was purified from methyl alcohol in which it was quite soluble. It proved to be a red hexamethyl ether of gossypol. The melting point of the pure material was not sharp. It started to fuse at 140° and was completely melted at 158–160°.

Anal. Calcd. for $C_{30}H_{24}O_2(OCH_3)_6$: C, 71.9; H, 6.97. Found: C, 71.93; H, 6.83.

This compound dissolved in concentrated sulfuric acid to give a brilliant orange color distinctly different from the characteristic scarlet gossypol color.

A solution in concentrated sulfuric acid after standing overnight gave a green amorphous powder upon dilution with water. This product gave an orange color with concentrated sulfuric acid. It was very soluble in organic solvents and no satisfactory method has yet been found for its purification.

A methyl alcoholic solution when diluted with 10% hydrochloric acid yielded a grayish product which analyzed the same as the original. It immediately reverted to red hexamethyl gossypol by dissolving in methyl alcohol or other solvents.

On catalytic reduction with platinum black in an alcohol solution, the material took up one mole of hydrogen in a few seconds and the solution became colorless. If stopped at this point the solution quickly turned red on filtering the catalyst and the original red hexamethyl ether of gossypol was recovered.

A methyl alcohol solution was decolorized immediately by the addition of a few drops of a saturated aqueous solution of sodium hydrosulfite.

On treatment with acetic anhydride, a brown amorphous powder was obtained which could not be crystallized.

The red hexamethyl ether of gossypol was not affected by the following reagents: (1) 40% aqueous potassium hydroxide for thirty minutes on the steam-bath, (2) 30% methyl alcoholic potassium hydroxide for thirty minutes on the steam-bath, (3) *o*-phenylenediamine or phenylhydrazine.

Dimethyl Sulfate and Methyl Alcohol.—A solution of 0.5 g. of gossypol in a mixture of 2.5 cc. of absolute methyl alcohol and 2.5 cc. of freshly distilled dimethyl sulfate was made by warming on a steam-bath. When solution was complete, 3.5 cc. of 10% methyl alcoholic potassium hydroxide was added in 0.5-cc. portions. The reaction flask was stoppered and allowed to stand overnight at room temperature.

The solution turned a deep brown color and a precipitate of potassium sulfate formed. The excess dimethyl sulfate was decomposed with 10% aqueous sodium hydroxide solution. The light brown flocculent precipitate that formed was filtered, washed and dried. It was insoluble in cold methyl alcohol. On crystallization from acetone, it yielded colorless crystals, m. p. 259–260°, which proved to be the tetramethyl ether of gossypol; yield 0.4 g.

Anal. (m. p. 259–260°) Calcd. for $C_{30}H_{26}O_4(OCH_3)_4$: C, 71.2; H, 6.63; mol. wt., 574. Found: C, 70.80; H, 6.90; mol. wt. (Rast), 557.

Sometimes when crystallized from acetone, the material melted to a clear liquid at 190°. On heating further it solidified and melted with decomposition at 259°. No method for forming the compound of m. p. 190° at will was found.

Anal. (m. p. 190°) Calcd. for $C_{30}H_{26}O_4(OCH_3)_4$: C, 71.2; H, 6.63. Found: C, 71.15; H, 6.70.

The tetramethyl ether, m. p. 259–260°, gave the characteristic gossypol color in concentrated sulfuric acid. When a solution of tetramethyl ether of gossypol in concentrated sulfuric acid was allowed to stand overnight at room temperature and was then diluted with water, the product was recovered unchanged.

The tetramethyl ether of gossypol was not affected by the following reagents: (1) diazomethane in ether; (2) phenylhydrazine; (3) a 30% methyl alcoholic solution of potassium hydroxide on the steam-bath for one and one-half hours; (4) a 50% aqueous sodium hydroxide solution on the steam-bath for one-half hour; (5) no color with alcoholic ferric chloride.

The tetramethyl ether of gossypol was oxidized readily with various reagents such as alkaline or acid permanganate, potassium permanganate in acetone, hydrogen peroxide in acetic acid, chromic acid in acetic or sulfuric acid. Thus far, products of acid character have always resulted, none of which has yet been obtained in a crystalline state.

Dimethyl Sulfate.—To a solution of 0.5 g. of gossypol in 5 cc. of freshly distilled dimethyl sulfate was added 3.5 cc. of 10% methyl alcoholic potassium hydroxide in 0.5-cc. portions. When addition was complete the flask was stoppered and allowed to stand overnight at room temperature.

The excess dimethyl sulfate was destroyed with 10% aqueous sodium hydroxide solution. The flocculent precipitate which formed was filtered, washed and dried. This material was extracted with methyl alcohol and two fractions, a soluble and insoluble, were obtained. The soluble proved to be the red hexamethyl ether of gossypol and the insoluble was the white tetramethyl ether; yield, 0.25 g. of methyl alcohol insoluble and 0.2 g. of methyl alcohol soluble.

Preparation of the White Hexamethyl Ether of Gossypol.—To a solution of 0.20 g. of the tetramethyl ether of gossypol in 3 cc. of warm 30% methyl alcoholic potassium hydroxide was added slowly 2 cc. of freshly distilled dimethyl sulfate. The solution was shaken and warmed on the steam-bath. This process was repeated using the same amount of alkali and dimethyl sulfate. The reaction mixture was then heated on the steam-bath for fifteen minutes.

On pouring into a large volume of water a flocculent precipitate formed. This was filtered, washed and dried. The product formed colorless prisms from a mixture of acetone and methyl alcohol, m. p. 235–237°. If crystallized from petroleum ether (b. p. 60–110°) it formed fine white needles, m. p. 221°. These forms were interchangeable by crystallization from the proper solvents; yield 0.18 g.

Anal. (m. p. 235–237°) Calcd. for $C_{30}H_{24}O_2(OCH_3)_6$: C, 71.9; H, 6.97; mol. wt., 602. Found: C, 71.67; H, 6.94; mol. wt. (Rast), 573. *Anal.* (m. p. 221°) Found: C, 71.42; H, 7.05.

The material dissolved in concentrated sulfuric acid to give the brilliant orange color, which did not change on standing. It was not decomposed by allowing it to stand overnight in a concentrated sulfuric acid solution and diluting with water.

The white hexamethyl ether of gossypol remained unchanged when treated as follows: (1) further methylation with diazomethane or dimethyl sulfate; (2) phenylhydrazine; (3) a solution of 30% methyl alcoholic potassium hydroxide for one-half hour on a steam-bath; (4) a solution of 40% aqueous sodium hydroxide for one-half hour on a steam-bath; (5) no color with alcoholic ferric chloride.

It was much more stable to oxidative reagents than the tetramethyl ether.

The hexamethyl ether on gentle refluxing with acetic anhydride and fused sodium acetate for half an hour was recovered unchanged as shown by melting point and mixed melting point. On boiling, however, for one and one-half hours or longer, a product was obtained by decomposing with ice water, which after filtering and drying was easily soluble in cold methyl alcohol and other organic solvents. It began to soften at 160° and melted completely at 179–181°. It was exceedingly difficult to purify and the analyses were variable. There appeared to be a dehydration taking place.

Preparation of the Diacetyl Derivative of the Tetramethyl Ether of Gossypol.—A solution of 0.20 g. of the tetramethyl ether of gossypol and 0.10 g. of freshly fused sodium acetate in 5 cc. of acetic anhydride was refluxed very gently for thirty minutes.

The solution was poured into ice water and after the excess acetic anhydride had decomposed, the flocculent precipitate was filtered, washed and dried. It crystallized from acetone in colorless needles, m. p. 264–265°; yield 0.153 g.

Anal. Calcd. for $C_{30}H_{24}O_2(OCH_3)_4(OCOCH_3)_2$: C, 69.30; H, 6.38. Found: C, 69.43; H, 6.90.

The product dissolved in concentrated sulfuric acid to give an orange color which quickly changed to the bright red color characteristic of gossypol.

If the tetramethyl ether or the diacetyl tetramethyl ether of gossypol was boiled for one hour or longer with acetic anhydride and fused sodium acetate, decomposition with water gave a white flocculent precipitate. After filtering and drying, the product proved to be very soluble in cold methyl alcohol. The substance started to shrink at about 140° and was completely melted at 188°. Purification to constant melting point has not yet been successful and the analyses are variable. It appears to be a dehydration product of the diacetyl tetramethyl ether. It gave a color similar to the diacetyl tetramethyl ether in concentrated sulfuric acid.

Saponification of the Diacetyl Tetramethyl Ether of Gossypol.—To a solution of 0.030 g. of the diacetyl tetramethyl ether of gossypol in 5 cc. of alcohol was added 3 cc. of 10% methyl alcoholic potassium hydroxide. The solution was refluxed gently for one hour and then evaporated to approximately one-half its volume. It was diluted with water, acidified with hydrochloric acid and the suspension extracted with ether. The ether extract was evaporated and the residue crystallized a number of times from acetone–methyl alcohol, m. p. 259–260°. The mixed melting point with the tetramethyl ether of gossypol showed no depression; yield 0.020 g.

In a similar manner, the compound of indefinite melting point produced by more extensive acetylation of the tetramethyl ether, was hydrolyzed to the tetramethyl ether of gossypol.

Conversion of the Red Hexamethyl Ether of Gossypol to the White Hexamethyl Ether.—To a solution of 1 g. of the red hexamethyl ether in 25 cc. of methyl alcohol was added *dropwise* a saturated aqueous solution of sodium hydrosulfite until the solution had become light yellow in color. Excess water was added and the light brown flocculent precipitate was filtered, washed and air dried; yield 1 g. The product could not be purified in the usual way, as heating in a solvent such as methyl alcohol exposed to air caused the compound to turn red; isolation of solid material proved it to be red hexamethyl ether. The substance could be best obtained from cold methyl alcohol by precipitation with water. The product was a cream-colored powder. It did not have a sharp melting point; it started fusing at 110° and was completely melted to a red liquid at 126°. The analysis indicated the possible addition of two hydrogens but of this there is some doubt.

Anal. Calcd. for $C_{30}H_{26}O_2(OCH_3)_6$: C, 71.5; H, 7.3. Found: C, 71.45; H, 7.17.

In an atmosphere of nitrogen, 0.70 g. of the above compound was dissolved in 25 cc. of methyl alcohol. To this solution was added 25 cc. of 10% methyl alcoholic potassium hydroxide. The flask was stoppered and allowed to stand twenty-four hours at room temperature. The solution was flooded with water and the light red flocculent precipitate filtered, washed and dried. The product was digested with methyl alcohol and the methyl alcohol insoluble fraction purified by crystallization from acetone–methyl alcohol; yield 0.40 g. The melting point of the product was 235–237° and proved by properties and mixed melting point to be identical with the white hexamethyl gossypol previously described.

Anal. Calcd. for $C_{30}H_{24}O_2(OCH_3)_6$: C, 71.9; H, 6.97. Found: C, 71.55; H, 6.92.

Conversion of the White Hexamethyl Ether of Gossypol into Red Hexamethyl Ether.—To a solution of 0.050 g. of white hexamethyl ether of gossypol in 5 cc. of concentrated sulfuric acid was carefully added an excess of 25% aqueous sodium hydroxide. The flocculent red precipitate was filtered, washed and dried. On purification from methyl alcohol it gave a product which proved to be identical with the red hexamethyl ether of gossypol by melting point, other physical properties and analyses.

Anal. Calcd. for $C_{30}H_{24}O_2(OCH_3)_6$: C, 71.9; H, 6.97. Found: C, 71.55; H, 6.92.

Ethylation of Gossypol

Diethyl Sulfate and Sulfuric Acid.—To a solution of 0.5 g. of gossypol in a mixture of 1.2 cc. of concentrated sulfuric acid and 3.8 cc. of freshly distilled diethyl sulfate was added an excess of 25% aqueous sodium hydroxide. The reaction flask was cooled and shaken during the reaction. The flocculent precipitate that formed was filtered, washed and dried. It was purified from methyl alcohol, yield 0.4 g. The material fused at 118° and was completely melted at 128–130°. It proved to be a hexaethyl ether of gossypol.

Anal. Calcd. for $C_{30}H_{24}O_2(OC_2H_5)_6$: C, 73.4; H, 7.86. Found: C, 72.80; H, 7.56.

The product dissolved in concentrated sulfuric acid to give an orange color.

Diethyl Sulfate and Ethyl Alcohol.—The procedure employed in the preparation of the tetramethyl ether of gossypol was used. A sample of 0.5 g. of gossypol dissolved in a mixture of 2.5 cc. of absolute ethyl alcohol and 2.5 cc. of freshly distilled diethyl sulfate was treated with 3.5 cc. of a 10% alcoholic potassium hydroxide solution; yield 0.3 g. When crystallized from acetone–methyl alcohol, the material formed colorless cubes which melted without decomposition at 231–232°; from petroleum ether (b. p. 60–110°), long needles, m. p. 211–212°. The product proved to be, not a tetraethyl ether but a hexaethyl ether of gossypol.

Anal. (m. p. 231–232°) Calcd. for $C_{30}H_{26}O_2(OC_2H_5)_6$: C, 73.4; H, 7.86. Found: C, 73.35; H, 7.65. *Anal.* (m. p. 211–212°) Found: C, 73.8; H, 7.90.

The compound dissolved in concentrated sulfuric acid to give the orange color characteristic of the derivatives in which the six hydroxyls had reacted. It gave no color with alcoholic ferric chloride.

Summary

1. Gossypol has been converted to a white tetramethyl ether, a white hexamethyl ether, a red hexamethyl ether, a white and a red hexaethyl ether.

2. The white hexamethyl ether can be converted to the red hexamethyl ether and *vice versa*.

URBANA, ILLINOIS RECEIVED JUNE 26, 1937