

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

**Structure of Gossypol. II. Acylation**BY R. F. MILLER,<sup>1</sup> D. J. BUTTERBAUGH<sup>1</sup> AND ROGER ADAMS

The results of previous investigations on the structure of gossypol, as well as the preliminary experiments in this Laboratory,<sup>2</sup> made it appear doubtful whether satisfactory degradation reactions of gossypol itself might ever be found. The molecule is so sensitive and contains so many reactive groupings that some of them must be protected in order to limit the point of attack of reagents to a single position in the molecule. One of the most obvious derivatives for study is the hexaacetate, previously described by Clark.<sup>3</sup> His directions led to a good yield of crude product but unfortunately only a small amount of pure material can be isolated by repeated crystallizations. By the use of pure gossypol this method is somewhat more satisfactory. The hexaacetate could, by careful purification, be obtained white and non-fluorescent in petroleum ether. As a by-product it is possible to obtain from the filtrate of the white hexaacetate a yellow product, also a hexaacetate, but modified by the loss of water or oxygen.

A more satisfactory synthesis for these products was discovered by the use of acetic anhydride and sodium acetate under mild conditions. Colored by-products are thus eliminated and a quantitative yield of a mixture of the white and yellow products just described is obtained. No modifications of temperature, time and catalyst which were investigated made it possible to obtain merely the white compound. In general, the two were present together in amounts ranging from a slight excess of white to a large proportion of yellow. Fortunately, the two differ markedly in solubility, and consequently are readily separated from each other.

The white hexaacetate, on further treatment with acetic anhydride and sodium acetate or pyridine, is converted into the yellow compound. It thus appears that the yellow is formed from the white as an intermediate in the original acetylation. Failure to find a method for preparing the white compound exclusively may be explained on the basis that even the mildest con-

ditions necessary for conversion of gossypol to gossypol hexaacetate transform the white product to the yellow.

The white hexaacetate is dimorphic, forming rectangular plates from benzene-ligroin solution, and boat-shaped plates from methyl alcohol-ethyl acetate. Both melt at the same point, though the high temperature necessary may cause interconversion before melting. Microphotographs of the two forms are shown in the plates.

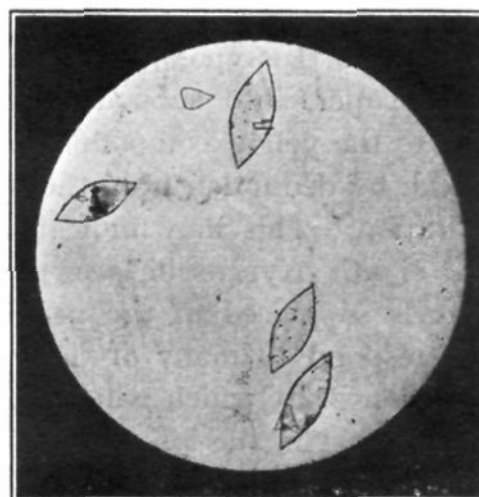


Plate 1.—Gossypol hexaacetate (methyl alc.-ethyl acetate).

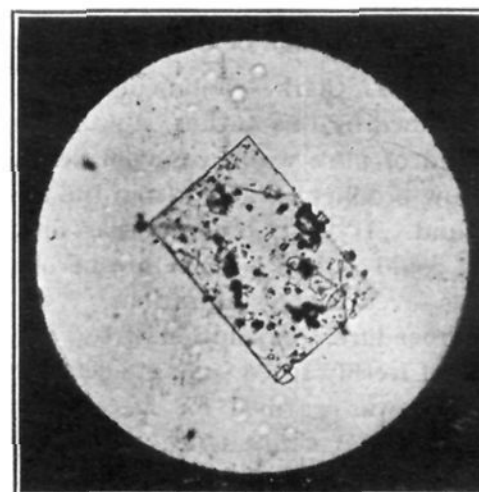


Plate 2.—Gossypol hexaacetate (high boiling petroleum ether).

Both the white and yellow compounds are hydrolyzed to gossypol by means of concentrated sulfuric acid or barium methyrate. The color of each in concentrated sulfuric acid is orange, which changes instantaneously to the scarlet color of gossypol.

(1) A portion of a thesis submitted in partial fulfillment of the requirements for a Ph.D. degree in Chemistry.

(2) Paper I, Campbell, Morris and Adams, *THIS JOURNAL*, **59**, 1723 (1937).

(3) Clark, *J. Biol. Chem.*, **76**, 725 (1927).

Tetraacetyl-gossypolone formed by Clark, by oxidizing crude acetylated gossypol, is obtained in a yield of 57% from the pure white hexaacetate and in a yield of 7% from the yellow.

White gossypol hexaacetate can be ozonized readily and yields an interesting product which is now under investigation.

The yellow product always accompanying the white hexaacetate is a compound with a melting point that is not sharp. It fluoresces in chloroform. The analyses do not indicate with certainty whether it is formed by the loss of an oxygen atom or a molecule of water from the white hexaacetate. It has been observed that gossypol and derivatives frequently give high hydrogen analyses, so that analytical data cannot be relied upon too implicitly. Acetic anhydride converts other derivatives of gossypol, to be described in a subsequent paper, into substances analogous in properties to the yellow compound. The conversion should, by deduction from the procedure, be a dehydration. This may indicate that the seventh and eighth oxygens in gossypol are tertiary hydroxyls so placed in the molecule that they do not respond to many of the commonly expected reactions, but which will under certain conditions take part in the loss of water.

Benzoylation of pure gossypol gave a homogeneous product which by analysis appears to be a hexabenzoyl derivative.

## Experimental

### Acetylation of Gossypol

(a) **Method of Clark.**—Following the procedure exactly as described by this author, it was possible to isolate a 5% yield of pure white gossypol hexaacetate and 52% of a yellow product obtained from the filtrate of the white compound. It was observed that only by using pure gossypol could a pure hexaacetate be obtained, and then only after several recrystallizations.

(b) **New Procedure.**—A solution of 1 g. of pure gossypol and 1.6 g. of freshly fused sodium acetate in 10 cc. of acetic anhydride was warmed for fifteen minutes on a steam-bath, then poured into a beaker containing 100 g. of crushed ice. After standing until all of the excess acetic anhydride was decomposed, the bright yellow product was collected on a filter, washed and dried. The yellow powder was dissolved in 80 cc. of methyl alcohol and after standing for several hours the gossypol hexaacetate crystallized. It was crystallized from benzene-ligroin and formed white rectangular plates, decomp. 276–279° with slight shrinking at about 265°; yield 0.50 g., although in some runs it was as high as 0.75 g. If recrystallized from ethyl acetate–methyl alcohol, it formed boat-shaped plates which gave the same observed decomposition point.

*Anal.* Calcd. for  $C_{30}H_{24}O_2(OCOCH_3)_6$ : C, 65.45; H, 5.46. Found: (from benzene–ligroin) C, 65.28; H, 5.43. Found: (from ethyl acetate–methyl alcohol) C, 65.39; H, 5.40.

The original methyl alcohol solution from which the white hexaacetate crystallized, was evaporated to dryness on the steam-bath and the dry residue extracted with 200 cc. of boiling ligroin (60–110°). The ligroin solution was allowed to cool somewhat; a small amount of product precipitated which removed most of the colored by-product from solution. The solution was then filtered through a hot funnel with suction. The clear yellow filtrate was evaporated to 60 cc. and allowed to stand. A bright yellow microcrystalline mass separated. It began to soften at 138° and melted at about 184–186° with decomposition; yield 0.4 g. This substance could never be obtained in such form that it melted sharply and consequently there was some uncertainty as to its purity. Analysis did not make possible the definite distinction between  $C_{42}H_{40}O_{13}$  and  $C_{42}H_{42}O_{13}$ , but an acetyl number indicated without doubt the presence of six acetyl groups.

*Anal.* Calcd. for  $C_{42}H_{40}O_{13}$ : C, 67.02; H, 5.34; acetyl, 33.4. Calcd. for  $C_{42}H_{42}O_{13}$ : C, 66.84; H, 5.57; acetyl, 34.2. Found: C, 66.67, 66.70; H, 5.91, 6.00; acetyl, 33.3, 32.6.

Both white and yellow products gave an orange color with concentrated sulfuric acid which almost instantaneously changed to the scarlet color typical of gossypol. By dilution of the sulfuric acid, gossypol was recovered.

The preparation of the white hexaacetate and yellow hexaacetyl compound was studied under a variety of conditions. (a) Similar procedure except three hours at room temperature gave the same results. (b) Pyridine and acetic anhydride for one hour at reflux temperature gave variable results with the ratio of white to yellow varying from 1:10 to 1:5. A brown amorphous by-product soluble in methyl alcohol but insoluble in ligroin was obtained in this procedure. (c) Acetic anhydride without a catalyst gave merely gossypol-acetic acid. (d) Acetic anhydride and a few drops of sulfuric acid at 0° gave about the same yields as sodium acetate but the hexaacetate was a little more difficult to purify. (e) Reductive acetylation gave both white and yellow products in about the same ratio as by the sodium acetate method.

**Conversion of White Gossypol Hexaacetate to the Yellow Compound.**—A mixture of 0.1 g. of white gossypol hexaacetate, 3 cc. of acetic anhydride and 0.1 g. of sodium acetate was allowed to react and worked up exactly as described for the acetylation of gossypol; yield, 0.06 g. of white gossypol hexaacetate and 0.04 g. of the yellow hexaacetyl product identical with that previously described.

**Hydrolysis of the Yellow Compound with Barium Methylate.**—Sulfuric acid, as previously mentioned, hydrolyzed both white and yellow products to gossypol. Aqueous sodium hydroxide apparently hydrolyzed both products, but gossypol could not be isolated as it is sensitive to alkali and decomposes.

To a solution of 0.36 g. of the yellow product in a little methyl alcohol was added with stirring 5.95 cc. of cold (ice and salt) 0.469 molar barium methylate in methyl alcohol.<sup>4</sup> A precipitate formed rapidly after addition of

(4) Weltzien and Singer, *Ann.*, **443**, 104 (1925).

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.<sup>5</sup>

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<sup>6</sup> 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

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

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

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.

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## The Structure of Gossypol. III. Gossypol Ethers

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The presence of six hydroxyl groups in gossypol has been demonstrated by the preparation of a white hexaacetate<sup>1</sup> 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.<sup>2</sup>

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 con-

sequently 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

(1) Paper II, Miller, Butterbaugh and Adams, *THIS JOURNAL*, **59**, 1729 (1937); Clark, *J. Biol. Chem.*, **78**, 725 (1927).

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