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

Structure of Gossypol. IV.[1,2] Anhydrogossypol and its Derivatives

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A crystalline compound, anhydrogossypol, derived from gossypol by loss of two molecules of water, was described by Carruth and Clark.[3] The dehydration takes place upon heating gossypol to its fusion point. This method, however, is inconvenient if any quantity of the product is desired and a more satisfactory procedure has been sought. It has now been found that heating a toluene solution of gossypol with pyridine hydrochloride results in an excellent yield of anhydrogossypol.

Anhydrogossypol is very rapidly hydrolyzed to gossypol in the presence of acids, even in the cold.

By the action of acetic anhydride it is converted to a mixture of the white gossypol hexaacetate and the yellow hexaacetate derivative which was previously prepared by the acetylation of gossypol. Since the reaction mixture was kept anhydrous at all times, scission of two oxygen bridges in anhydrogossypol must have occurred with the addition of two molecules of acetic anhydride.

Similarly, methylation of anhydrogossypol by the methods employed for gossypol always resulted in the formation of the white tetramethyl ether or the white hexamethyl ether of gossypol, depending upon the experimental conditions. The hexaethyl ether was produced by ethylation with diethyl sulfate. Even when anhydrous conditions were observed carefully, only derivatives of gossypol were obtained.

Methyl alcohol and dry hydrogen chloride converts anhydrogossypol to the tetramethyl ether of gossypol. No tetramethyl ether is obtained from gossypol by similar treatment.

Anhydrogossypol dissolves in liquid ammonia and a yellow product separates. Two molecules of ammonia add, and a diaminogossypol is formed in which two hydroxyls of gossypol have been replaced by amino groups. The diamine is acetylated to an octaacetate in which, presumably, the four hydroxyl hydrogens and the four amino hydrogens have been replaced by acetyl groups. It also forms a hydrochloride. Both the salt and the free base are hydrolyzed rapidly in acid solution and gossypol is recovered. The octaacetate is hydrolyzed to gossypol when treated with sulfuric acid.

Methylamine, dimethylamine and piperidine give products with anhydrogossypol which are too unstable to be purified by the ordinary methods. In the presence of acid hydrolytic agents, they all revert to gossypol. Aniline adds to give the well-known dianilinogossypol.

One of the first derivatives of gossypol discovered was dianilinogossypol, made from aniline and gossypol with loss of two molecules of water. It has now been found that liquid ammonia causes an analogous reaction to take place and gives the same diaminogossypol as that obtained from anhydrogossypol.

The reactivity of the aminogossypols resembles that of vinylamines. This fact and a consideration of the two methods of preparation, from gossypol or from anhydrogossypol, make it seem probable that the amino derivatives belong to the class of vinylamines and correspond to gossypol with two vinyl alcohol groups replaced by amino or substituted amino groups. The vinyl alcohol groups also take part in the formation of anhydrogossypol and are two of the hydroxyl groups in gossypol which are acetylated or alkylated.

No derivative of anhydrogossypol which retains the anhydro structure has as yet been prepared. It may thus be deduced that anhydrogossypol contains two oxygen bridges which are easily cleaved or hydrolyzed. These may be of a lactone type or more probably of a type resembling a lactone in reactivity. It is not impossible that the oxygens might be present as ethylene oxide rings, though the formation of anhydrogossypol is carried out under much milder conditions than can ordinarily be used for preparation of this type of structure.

The two oxygen bridges are formed by elimination of two molecules of water from gossypol. The hydroxyl part of each molecule of water must come from an enolic or vinyl hydroxyl; the hy-

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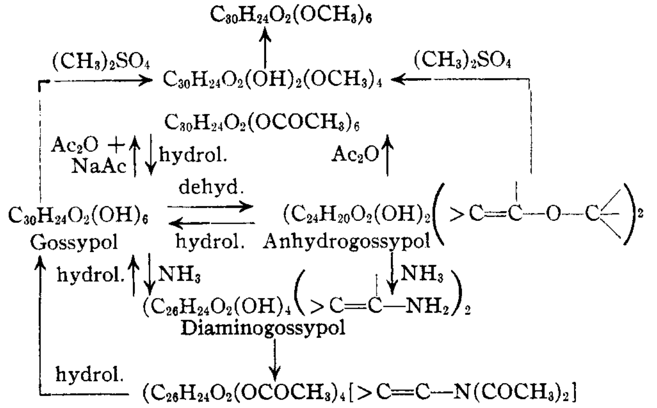
[1] For previous paper (III) in this field, see Morris and Adams, THIS JOURNAL, **59**, 1731 (1937).

[2] A portion of a thesis submitted in partial fulfilment of the requirements of the degree of Doctor of Philosophy in Chemistry.

[3] Carruth, THIS JOURNAL, **40**, 647 (1918); Clark, *J. Biol. Chem.*, **75**, 725 (1927).

drogen part probably comes from another hydroxyl group. The only available experimental evidence for this statement is that anhydrogossypol gives by the Zerewitinoff method two molecules of methane and thus appears to contain only two hydroxyl groups, in contrast to six in gossypol. Although too much reliability cannot be placed on this method when applied to complicated molecules, the conditions used in the experiments were comparable to those which demonstrated six active hydrogens or six hydroxyls in gossypol.[4]

A chart of the various transformations described in this communication is given.



All the experimental evidence thus far accumulated favors a symmetrical formula for gossypol.

Experimental

Anhydrogossypol.—Under anhydrous conditions, dry hydrogen chloride was passed over the surface of a solution of 10 g. of anhydrous pyridine in 50 g. of anhydrous toluene, until all of the pyridine hydrochloride had precipitated. The addition of 2 g. of pure gossypol was now made and nitrogen was passed in while the mixture was heated in an oil-bath at 110° until the toluene had completely distilled. The residue was extracted with two portions of 75 cc. each of boiling toluene and the combined extracts evaporated to 50 cc. Upon cooling, anhydrogossypol crystallized as thick orange rods, m. p. 230°, yield 1.6 g.

Anal. Calcd. for $C_{30}H_{26}O_6$: C, 74.66; H, 5.46. Found: C, 74.83; H, 5.63.

The product was identical with that prepared by the fusion of gossypol.

Anhydrogossypol was allowed to react with excess of Grignard reagent. It gave two moles of methane.

Anal. (Zerewitinoff) 0.0477 g. gave 4.55 cc. methane at 0° and 760 mm. 0.0433 g. gave 3.94 cc. of methane at 0° and 760 mm. Calcd. for two active hydrogens, 4.43 cc. of methane at 0° and 760 mm.; 4.22 cc. of methane at 0° and 760 mm.

Anhydrogossypol upon treatment with concentrated aqueous sodium hydroxide gave apogossypol in excellent yields.

Acetylation of Anhydrogossypol.—A solution of 0.5 g. of anhydrogossypol in 10 g. of freshly distilled acetic anhydride was evaporated to dryness under diminished pressure. The residue was extracted with 20 cc. of anhydrous benzene and the benzene solution treated with 100 cc. of boiling ligroin. A precipitate formed which was filtered through a heated funnel while the solution was still hot. The product was recrystallized from benzene-ligroin and formed colorless plates, m. p. 276–279°, dec.; yield 0.1 g. It was shown by properties and mixed melting point to be gossypol hexaacetate.

Anal. Calcd. for $C_{42}H_{42}O_{14}$: C, 65.45; H, 5.46. Found: C, 65.47; H, 5.47.

The original benzene-ligroin solution was allowed to cool and a yellow precipitate formed. It was collected on a filter and proved to be identical with the yellow gossypol hexaacetate derivative prepared from gossypol.

Methylation of Anhydrogossypol with Dimethyl Sulfate.—To a solution of 0.5 g. of anhydrogossypol in 7.8 g. of dimethyl sulfate and 10 cc. of methyl alcohol was added 8 cc. of 10% methyl alcoholic potassium hydroxide solution in 1-cc. portions. The mixture was allowed to stand at room temperature for twenty-four hours, the excess dimethyl sulfate was decomposed with methyl alcoholic potassium hydroxide, and the excess methyl alcohol was removed under reduced pressure. The residue was extracted with four 50-cc. portions of ether and the ether extracts evaporated to dryness. The pink colored residue was dissolved in 30 cc. of methyl alcohol and allowed to stand. Gossypol hexamethyl ether crystallized in the form of cubes; yield 0.22 g. After one recrystallization from acetone-methyl alcohol it melted at 235–236°. It was identical with gossypol hexamethyl ether prepared from gossypol.

Anal. Calcd. for $C_{36}H_{42}O_8$: C, 71.90; H, 6.98. Found: C, 72.08, 72.00; H, 6.64, 6.87.

The residue from the ether extraction was treated with 50 cc. of *N* hydrochloric acid. The insoluble material was collected on a filter and dried. It was crystallized from methyl alcohol. The product was gossypol tetramethyl ether, m. p. 190°, and was identical with the tetramethyl ether prepared from gossypol. Sometimes it was obtained in the form with m. p. 259°; yield 0.07 g.

By using a 0.5-g. sample of anhydrogossypol, but increasing the amount of methyl alcoholic potassium hydroxide to 15 cc. and refluxing the reaction mixture for one hour on the steam-bath after the preliminary reaction, a yield of 0.41 g. of gossypol hexamethyl ether was obtained. No tetramethyl ether appeared in the reaction product.

Methylation of Anhydrogossypol with Methyl Alcohol and Dry Hydrogen Chloride.—A solution of 0.5 g. of anhydrogossypol in 10 cc. of anhydrous methyl alcohol was saturated with hydrogen chloride and the solution allowed to stand for seven to eight days. At the end of this time a crystalline precipitate was present in the deep blue solution. It was collected on a filter, dried, and recrystallized

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

from methyl alcohol, using norite to decolorize the product. The product was gossypol tetramethyl ether, m. p. 259°, yield 0.05 g.

Anal. Calcd. for $C_{34}H_{38}O_8$: C, 71.08; H, 6.60. Found: C, 70.86; H, 6.39.

Sometimes it was obtained in the form of needles which melted at 190°. It was identical with the tetramethyl ether prepared with dimethyl sulfate.

Ethylation of Anhydrogossypol.—To a solution of 1 g. of anhydrogossypol in 8 g. of pure diethyl sulfate and 20 cc. of absolute alcohol was added 20 cc. of 10% alcoholic potassium hydroxide in 2-cc. portions over a period of thirty minutes. The mixture was allowed to stand for twenty-four hours and then worked up as described for the methylation product of anhydrogossypol. The gossypol hexaethyl ether crystallized as colorless needles from methyl alcohol–acetone solution, m. p. 217°, yield 1.7 g. It was identical with the hexaethyl ether of gossypol made directly from gossypol.

Anal. Calcd. for $C_{42}H_{54}O_8$: C, 73.45; H, 7.85. Found: C, 73.42; H, 7.95.

Diaminogossypol.—To 30 cc. of liquid ammonia in an open Dewar flask was added 1 g. of anhydrogossypol. It quickly dissolved to give an orange solution which immediately turned yellow and a yellow precipitate separated. The ammonia was allowed to evaporate. The light yellow powder was crystallized from benzene and formed orange plates. It turned a deep red at 187–195° with a rapid loss of ammonia, then fused at 228–230° to a black mass.

Anal. Calcd. for $C_{30}H_{32}O_6N_2$: C, 69.76; H, 6.20, N, 5.42. Found: C, 69.56; H, 6.53; N, 5.17.

The diaminogossypol was soluble in ether and methyl alcohol but gradually decomposed in these solvents with liberation of ammonia.

When dry hydrogen chloride was passed over a solution of the diamine in anhydrous ether a precipitate of the hydrochloride formed. It was a yellow powder which decomposed on standing in air.

Diaminogossypol from Gossypol.—A solution of 1 g. of gossypol in 15 cc. of liquid ammonia was allowed to evaporate at room temperature. The brown residue was dried *in vacuo* and recrystallized from benzene. It was diaminogossypol, m. p. 228–230°, dec.

Diaminogossypol and its hydrochloride were dissolved in concentrated sulfuric acid. A yellow solution was produced which upon standing for some time exposed to air or by the addition of a drop of water, became scarlet. Dilution wtih water precipitated gossypol.

The diamino compound was also hydrolyzed merely by warming in dilute acetic acid. Gossypol-acetic acid precipitated.

Acetylation of Diaminogossypol.—A mixture of 0.5 g. of diaminogossypol, 2 g. of fused sodium acetate, and 10 cc. of acetic anhydride was warmed for ten minutes. After cooling for five minutes, it was poured onto crushed ice. When the excess acetic anhydride had decomposed completely, the precipitate was filtered and dried. The yellowish powder was crystallized from 100 cc. of hot benzene. Diaminogossypol octaacetate crystallized in long, white, silky needles, m. p. 282° with decomp.; yield 0.35 g.

Anal. Calcd. for $C_{46}H_{48}O_{14}N_2$: C, 64.90; H, 5.64; N, 3.29; acetyl, 39.4. Found: C, 65.11; H, 5.40; N, 3.61; acetyl, 40.8.

Summary

1. A convenient method for preparing anhydrogossypol is described.

2. It hydrolyzes rapidly. It may be converted under anhydrous conditions to alkylated and acylated gossypol.

3. It reacts with liquid ammonia to give diaminogossypol, which can be acetylated to an octaacetate. Other alkylamines react similarly. All the amines hydrolyze readily in acid solution to gossypol.

4. Gossypol reacts with liquid ammonia to give diaminogossypol.

5. The deduction from the various experiments is that anhydrogossypol contains two oxygen bridges formed by loss of two molecules of water from four hydroxyls in gossypol. Two of the hydroxyls are enolic in character.

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