with alkali, and in general shows many of the reactions of gossypol. It may be remethylated to gossypol hexamethyl ether.

The color reactions and oxidation products of the dimethyl ether are described.

URBANA, ILLINOIS RECEIVED JUNE 27, 1938

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

Structure of Gossypol. VIII.[1] Derivatives of the Ethers of Gossypol

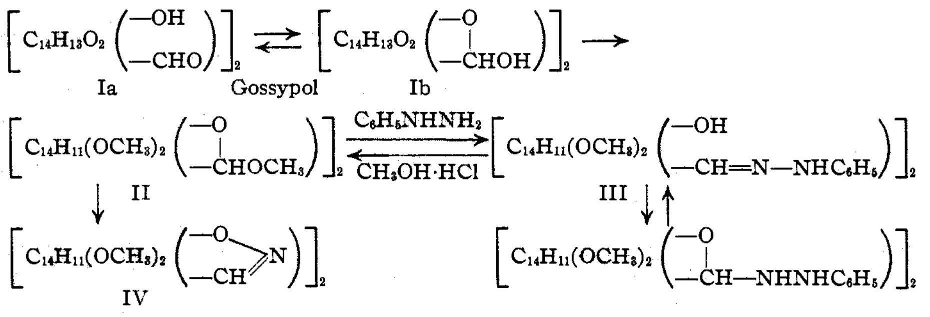
BY ROGER ADAMS AND T. A. GEISSMAN

The non-reactivity of gossypol tetra- and hexamethyl ethers with many carbonyl reagents leaves doubt concerning the presence of aldehyde groups in these molecules in spite of the fact that experiments indicate that six hydroxyls and two aldehyde groups are present in gossypol. This paper presents evidence which points to the belief that in the tetra- and hexaalkyl ethers of gossypol the systems involving the two aldehyde groups and two of the six hydroxyls may be stabilized in the lactol form by alkylation with the formation of two acetal (or lactol ether) linkages (formulas Ia, Ib, II).

Although gossypol (Ia and Ib) and its dimethyl ether form derivatives with aniline and with substituted anilines,[1,2] neither the hexamethyl ethers nor the tetramethyl ether of gossypol have been found previously to form any derivatives which showed unequivocally the presence in the molecule of a carbonyl group. In fact, all attempts to form such a derivative in alkaline or neutral solution have yielded negative results.[3] The white hexamethyl ether (either form) shows a remarkable stability in neutral or alkaline solution, being unattacked by permanganate in acetone or pyridine solution. In the latter case fairly vigorous treatment results in the complete degradation of a small percentage of the starting material with the recovery of the remainder unchanged.

Gossypol hexamethyl ether does not react with malonic acid in alcohol in the presence of piperidine or in pyridine solution.[4] With methylmagnesium iodide in benzene–ether solution some reaction seemed to occur, but a large proportion of starting material was recovered along with a small amount of an uncrystallizable oily product.

In acetic acid solution, however, the hexamethyl ether (II) behaves quite differently. It is attacked instantly by potassium permanganate (no crystalline products isolated), and it forms readily a crystalline condensation product with two molecules of phenylhydrazine (III) or with two molecules of hydroxylamine (IV). These latter condensation products appear to be formed by loss of two methylene groups due to hydrolysis of two methoxyls to hydroxyls followed by the loss of water between the resulting aldehyde groups and the reagents. The dihydroxylamine derivative was found by analysis to have lost an additional two molecules of water, probably through the formation of two orthoxazine rings. The unusual ease with which the methyl groups of two of the methoxyls in gossypol tetramethyl and hexamethyl ethers are eliminated in mildly acid solution leads to the deduction that acetal methoxyls are probably present. The chemical changes just described may be formulated structurally:



The phenylhydrazone can be reconverted to the hexamethyl ether by treatment with methanolic hydrogen chloride, and converted by treatment with ethanolic hydrogen chloride to a diethyl tetramethyl ether which presumably has

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[1] For previous paper see Adams and Geissman, THIS JOURNAL, 60, 2163 (1938).

[2] Adams, Price and Dial, *ibid.*, 60, 2158 (1938).

[3] Morris and Adams, *ibid.*, 59, 1731 (1937).

[4] Kuhn, *Ber.*, 63, 2164 (1930).

two ethoxy groups of the acetal type. Attempts to convert it to a tetramethyl ether by hydrolysis in aqueous or non-alcoholic media were unsuccessful; treatment of the compound with dry hydrogen chloride in acetic acid led to a compound containing chlorine whose constitution has not yet been investigated.

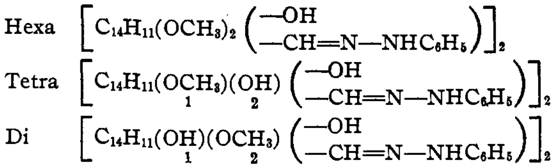
The tetramethyl ether of gossypol reacts with phenylhydrazine under conditions similar to those used for the hexamethyl ether and forms an analogous product, the diphenylhydrazone of a gossypol dimethyl ether. It can be reconverted into the gossypol tetramethyl ether or converted into a gossypol diethyldimethyl ether. The gossypol tetramethyl ether also reacts with two molecules of hydroxylamine. Two methoxyl groups are hydrolyzed and two molecules of water are lost followed by the elimination of two more molecules of water just as occurred in the gossypol hexamethyl ether derivative.

The facts presented in this paper favor the structures written for these compounds; the acetal structure for gossypol hexa- and tetramethyl ethers adequately explains the stability of these compounds toward alkaline reagents, their ready reaction in acid solution *with the hydrolysis of methyl groups from two of the ether linkages*, and the hydrolysis of the hexamethyl ether to the dimethyl ether by sulfuric acid in acetic acid solution.

The dimethyl ether of gossypol also forms a diphenylhydrazone and a dioxime, but as is to be expected no methyl groups are lost in the process. The dioxime, moreover, does not lose water. That acetic acid is not necessary in these condensations is indicated by the ready condensation of the dimethyl ether with aniline in benzene or chloroform solution.

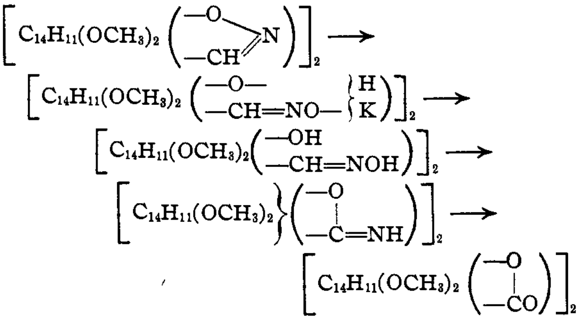
It is significant that the diphenylhydrazone of the dimethyl ether of gossypol is isomeric with the diphenylhydrazone obtained from the tetramethyl ether. As was previously mentioned, two methyl groups are lost in the synthesis of the latter compound. That the two substances are not identical is to be expected from the fact that the two methoxyl groups in gossypol dimethyl ether are the ethers of the two free hydroxyl groups in gossypol tetramethyl ether, as was shown in a previous communication.[1]

The relationship between the phenylhydrazine derivatives of gossypol di-, tetra- and hexamethyl ethers may be formulated as follows



$$\begin{array}{ll}\text{Hexa} & \left[C_{14}H_{11}(OCH_3)_2\begin{pmatrix}\text{—OH}\\ \text{—CH=N—NHC}_6\text{H}_5\end{pmatrix}\right]_2\\ \text{Tetra} & \left[C_{14}H_{11}(\underset{1}{OCH_3})(\underset{2}{OH})\begin{pmatrix}\text{—OH}\\ \text{—CH=N—NHC}_6\text{H}_5\end{pmatrix}\right]_2\\ \text{Di} & \left[C_{14}H_{11}(\underset{1}{OH})(\underset{2}{OCH_3})\begin{pmatrix}\text{—OH}\\ \text{—CH=N—NHC}_6\text{H}_5\end{pmatrix}\right]_2\end{array}$$

The dehydro dioxime or orthoxazine derived from the hexamethyl ether and hydroxylamine forms a crystalline potassium salt when treated with alcoholic potassium hydroxide. This salt on treatment with acetic acid undergoes a change resulting in the formation of a compound which no longer contains nitrogen, formulated as a lactone



The reactions of this compound, which corresponds to the dilactone of a gossypol tetramethyl ether in which the two aldehyde groups are converted into carboxyls and then dehydrated with two hydroxyl groups to lactone residues, will be the subject of a later paper. It is proposed to call the molecule theoretically obtained by oxidizing the two aldehyde groups in gossypol to carboxyls, gossylic acid. This new compound would then be a gossylic acid lactone tetramethyl ether.

The fact that in this molecule there are only eight oxygens, the same number as in gossypol, lends additional weight to the conclusion that the oxygens in gossypol are present in the form of six hydroxyls and two aldehyde groups.

Speculation concerning the structure of the $(C_{14}H_{11})_2$ residue in gossypol may be made. On the basis of the available knowledge, the presence of two naphthyl or analogous residues directly or indirectly linked is indicated. This assumption is founded partly on the work of Schmid[5] and partly on absorption spectra data and on chemical data not yet published which have been accumulated in this Laboratory. Assuming the molecule to be symmetrical and that six hydroxyls and two aldehyde groups may replace eight hy-

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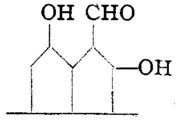
(5) Schmid and Margulies, *Monatsh.*, **65**, 391 (1934).

drogens in the naphthalene nuclei, the residue becomes $(C_4H_9)C_{10}H_2–C_{10}H_2(C_4H_9)$ or an isomer of it, in which each of the C_4H_9 radicals represents more than one group. The two naphthyl groups, however, may be attached through an alkylene residue, thus $C_{10}H_3–(C_8H_{16})–C_{10}H_3$. Still another possible combination is that in which one or more alkyl groups are present in each of the naphthalene nuclei and the two naphthalene residues are connected by one or more methylene groups.

One type of hydroxyaldehyde which may explain many of the reactions of gossypol and its ethers is represented by the partial nucleus.  . A study of a naphthalene derivative with substituents of this type is now being made. An attempt to give a complete formula for gossypol is not yet justified as certain conflicting experimental facts must first be clarified.

Experimental

Condensation of Phenylhydrazine and Gossypol Hexamethyl Ether (a).—To a solution of 0.27 g. of pure gossypol hexamethyl ether (m. p. 239–241°) in 20 cc. of glacial acetic acid was added 1.5 cc. of phenylhydrazine. After heating for several minutes on the steam-bath bright yellow needles began to separate. After thirty minutes the solution was filtered hot and the product washed with acetic acid. It formed bright yellow needles, m. p. 266–267° (corr.) with decomposition, and weighed 0.25 g. No satisfactory method was found for recrystallization of this product.

(b). A solution of 0.25 g. of gossypol hexamethyl ether (m. p. 223–225°) was treated as in (a). The product weighed 0.28 g. In larger runs the yield was nearly quantitative.

Anal. Calcd. for $C_{48}H_{50}O_6N_4$: C, 73.22; H, 6.63; N, 7.43. Found: C, 72.78, 73.09; H, 6.59, 6.58; N, 7.40.

The compound is insoluble in alkali (aqueous or alcoholic) and gives no ferric chloride color. It gives an orange color with concentrated sulfuric acid.

To a suspension of 0.5 g. of the phenylhydrazone in 10 cc. of glacial acetic acid was added 5 drops of concentrated hydrochloric acid. The yellow-brown solution which rapidly formed, began to deposit clusters of yellow needles after standing for an hour at room temperature. The product was collected and washed with acetic acid and water. It could not be recrystallized successfully. It was insoluble in alkali and in ether, acetone and benzene. It was soluble in methanol and slightly so in acetic acid. It contained halogen (Beilstein test) and nitrogen. The compound melted at 257–262° (corr.) with decomposition.

When the solution of this material in methanol was saturated with dry hydrogen chloride a thick mass of short white needles separated. This compound is nicely crystalline, contains halogen and nitrogen, gives a yellow color with concentrated sulfuric acid, dissolves in warm alcoholic alkali to a bluish-red solution, and melts at 274–276° (corr.) with decomposition. It turns brown on standing in air and no method has yet been found for its purification.

Conversion of the Phenylhydrazine Derivatives of Gossypol Hexamethyl Ether to Gossypol Hexaalkyl Ethers

(a) **With CH_3OH–HCl: Formation of Gossypol Hexamethyl Ether.**—A suspension of 0.5 g. of the phenylhydrazone in 20 cc. of absolute methanol was treated with dry hydrogen chloride until the compound had gone into solution. The solution was allowed to stand overnight at room temperature. A crystalline product separated in clusters of stout prisms. It weighed 0.32 g. After recrystallization from ligroin, then from methanol it formed tiny white prisms, m. p. 219–221° (corr.), and mixed with gossypol hexamethyl ether (m. p. 221–223°), it melted at 221–223°.

(b) **C_2H_5OH–HCl: Formation of Gossypol Tetramethyl Diethyl Ether.**—A solution of 0.5 g. of the phenylhydrazone in 20 cc. of absolute ethanolic hydrogen chloride (prepared as in the case of the methanol) was allowed to stand at room temperature. Within two hours white needles began to separate from the solution. The crude product weighed 0.35 g. Recrystallized from ethanol–acetone, it formed white prisms, m. p. 229–230° (corr.).

Anal. Calcd. for $C_{30}H_{24}O_2(OCH_3)_4(OC_2H_5)_2$: C, 72.38; H, 7.30. Found: C, 72.19; H, 7.49.

A mixture of this compound (m. p. 229–230°) with the hexamethyl ether (m. p. 221–223°) melted at 203–208°. The compound gave the usual (for hexaalkylated gossypol) orange color with sulfuric acid.

Condensation of Hydroxylamine with Gossypol Hexamethyl Ether.—To 2.0 g. of gossypol hexamethyl ether dissolved in 30 cc. of hot acetic acid was added a solution of 1.0 g. of hydroxylamine hydrochloride in a very small quantity of water. The solution was heated on the steam-bath (five to ten minutes) until scratching of the walls of the flask caused crystallization to start. After allowing the mixture to stand until cool (overnight is preferable) the product was collected and washed with acetic acid and water. It formed greenish-yellow leaflets weighing 1.5 g.

Recrystallized from dilute acetic acid it forms flat buff needles and from benzene–petroleum ether it forms tiny buff platelets. Both forms when heated rapidly melt with decomposition at 192–204° but when heated slowly merely sinter at this temperature and do not melt below 280°.

Anal. Calcd. for (dioxime) $C_{34}H_{40}O_8N_2$: C, 67.5; H, 6.63; N, 4.64. Calcd. for (dioxime less $2H_2O$) $C_{34}H_{36}O_6N_2$: C, 71.82; H, 6.34; N, 4.92. Found (acetic acid): C, 71.83; H, 6.44. Found (benzene–petroleum ether): C, 72.05; H, 6.51; N, 4.83.

The oxime gives an orange color with concentrated sulfuric acid and no ferric chloride color. It is soluble in hot 10% methanolic potassium hydroxide, and the potassium salt crystallizes from the hot solution in clusters of bright yellow needles.

Degradation of the Hydroxylamine Derivative of Gossypol Hexamethyl Ether to a Lactone.—A suspension of 4.69

g. of the crude hydroxylamine derivative of gossypol hexamethyl ether in 60 cc. of 10% methanolic potassium hydroxide was heated to boiling with stirring. The compound gradually went into solution, then the potassium salt rapidly crystallized as a thick sludge of yellow needles. After being heated for a short time just below the boiling point to complete the reaction the mixture was allowed to cool and the product collected and washed with a small amount of alcoholic potassium hydroxide and with ether. The salt weighed 5.15 g. Purified hydroxylamine derivative gives a better yield but no over-all advantage is gained by such a procedure.

Analysis of the crude product for potassium indicated that it is a dipotassium salt.

Anal. Calcd. for $(C_{17}H_{19}O_4NK)_2$: K, 11.5. Calcd. for $(C_{17}H_{18}O_4NK_2)_2$: K, 20.6. Found: K, 10.8, 10.2.

A solution of 5.15 g. of crude potassium salt in 50 cc. of boiling glacial acetic acid was diluted carefully with water until crystallization started. It was then diluted with several times its volume of water and allowed to cool. The crude, crystalline product was collected and dried. It was found most convenient to recrystallize it from glacial acetic acid in the following way. The crude product was dissolved in 46 cc. (0.5 mole) of boiling acetic anhydride and the acetic anhydride carefully decomposed by the addition of 9 cc. of water dissolved in 10 cc. of acetic acid. As soon as the acetic anhydride had been hydrolyzed the product crystallized in shining leaflets. It weighed 4.05 g. and after recrystallization from acetic acid (in which it is difficultly soluble) or acetic anhydride it melted at 327–328° (corr.).

Anal. Calcd. for $C_{17}H_{17}O_4$: C, 71.58; H, 5.96; 2-OCH_3, 21.75. Found: C, 71.17; H, 6.11; OCH_3, 21.40.

The lactone is difficultly soluble in cold alcoholic alkali but dissolves readily on warming. It gives a yellow color with concentrated sulfuric acid and no ferric chloride color.

Condensation of Phenylhydrazine with Gossypol Tetramethyl Ether.—To a solution of 0.5 g. of gossypol tetramethyl ether in 20 cc. of hot glacial acetic acid was added 1 cc. of phenylhydrazine. On standing overnight the solution deposited 0.61 g. of a bright yellow crystalline product. Recrystallized from acetic acid it forms canary-yellow platelets, from benzene a bright yellow crystalline powder, and from benzene–petroleum ether bright yellow rosettes of scale-like crystals which effloresce on drying at 100° to a yellow powder, m. p. 246–248° (corr.) with decomposition.

Anal. Calcd. for $C_{44}H_{46}O_6N_4$: C, 72.73; H, 6.34. Found (acetic acid): C, 73.07; H, 6.29. Found (benzene): C, 72.76; H, 6.52. Found (benzene–petroleum ether): C, 72.32; H, 6.53.

The compound gives a scarlet color with concentrated sulfuric acid, no ferric chloride color, and is soluble in alcoholic alkali with a reddish-yellow color.

Conversion of the Phenylhydrazine of Gossypol Tetramethyl Ether to Tetraalkyl Ethers

(a) **Gossypol Tetramethyl Ether.**—A solution of 0.5 g. of the phenylhydrazone from gossypol tetramethyl ether in 20 cc. of absolute methanolic hydrogen chloride was allowed to stand at room temperature overnight. A crystalline deposit weighing 0.30 g. was obtained and purified by recrystallization from acetone, m. p. 186–189° (corr.). It gave no depression in melting point when mixed with an authentic sample of gossypol tetramethyl ether.

(b) **Gossypol Dimethyl Diethyl Ether.**—A solution of 0.5 g. of the phenylhydrazone from gossypol tetramethyl ether in 15 cc. of absolute ethanolic hydrogen chloride began to deposit white needles after several hours at room temperature. The product was collected after diluting the solution and weighed 0.32 g. Recrystallized from dilute acetone it formed slender white needles, m. p. 160–162° (corr.).

Anal. Calcd. for $C_{30}H_{26}O_4(OCH_3)_2(OC_2H_5)_2$: C, 71.76; H, 6.98. Found: C, 71.49; H, 6.89.

The compound gives a scarlet color with concentrated sulfuric acid.

Condensation of Hydroxylamine with Gossypol Tetramethyl Ether.—To a solution of 0.5 g. of gossypol tetramethyl ether in 5 cc. of glacial acetic acid was added a solution of 0.25 g. of hydroxylamine hydrochloride in 0.5 cc. of water. After heating on the steam-cone for fifteen minutes, the solution was filtered and cooled. On scratching the walls of the flask the product separated as a yellow powder weighing 0.33 g. It can be recrystallized from benzene or benzene–petroleum ether from which it is obtained as fine canary-yellow platelets or prisms which melted poorly from 140–190° (with decomposition). Analysis indicated that it contained combined solvent. Recrystallized from ligroin (b. p. 90–110°) it formed short yellow needles which melted at 281–283° (corr.) with decomposition.

Anal. Calcd. for (dioxime less $2H_2O$) $C_{32}H_{32}O_6N_2$: C, 71.11; H, 5.92; N, 5.18. Calcd. for $C_{32}H_{32}O_6N_2 \cdot {}^1/_2C_6H_6$: C, 73.78; H, 6.15; N, 4.63. Found (ligroin): C, 70.93; H, 5.97. Found (benzene–petroleum ether): C, 73.56; H, 6.28; N, 4.79.

The compound dissolves in concentrated sulfuric acid to give an orange solution which turns deep wine-red on standing. It gives no color with ferric chloride. It dissolves readily in cold, dilute alcoholic potassium hydroxide to give a yellow solution which quickly turns wine-red. This red color is discharged by sodium hydrosulfite, giving the original yellow color.

Condensation of Phenylhydrazine with Gossypol Dimethyl Ether.—To a solution of 1.9 g. of the dimethyl ether in 20 cc. of glacial acetic acid was added 3 cc. of phenylhydrazine, and the mixture was warmed for fifteen minutes on the steam-bath. The solution was diluted carefully with water, with scratching, until crystallization began. The crude product weighed 2.0 g. Recrystallized from benzene, the compound formed rosettes of yellow needles, m. p. 248–249° (corr.) with decomposition. From dilute acetic acid it forms yellow platelets, m. p. 246–248° (corr.) with decomposition. The melting point varies with the heating rate.

Anal. Calcd. for $C_{44}H_{46}O_6N_4$: C, 72.73; H, 6.34; N, 7.72. Found (benzene): C, 72.89; H, 6.58; N, 7.17.

The compound depressed the melting point of the isomeric phenylhydrazone from gossypol tetramethyl ether (mixed m. p. 232–234° corr.) with decomposition. It gives an orange-yellow color with concentrated sulfuric acid, and with ferric chloride a cherry-red which changes immediately to orange. It is soluble in cold alcoholic alkali.

Attempts to hydrolyze the compound by the procedure described for the phenylhydrazones did not yield a tetramethyl ether.

Condensation of Hydroxylamine with Gossypol Dimethyl Ether.—The product was prepared by treatment of an acetic acid solution of gossypol dimethyl ether with a neutralized solution of hydroxylamine hydrochloride in water, heating for thirty minutes on the steam-bath and diluting and scratching the hot solution. The hydroxylamine derivative was recrystallized from dilute acetic acid, from which it formed buff platelets, m. p. 229–232° (corr.) with decomposition.

Anal. Calcd. for $C_{32}H_{36}O_8N_2$: C, 66.66; H, 6.25; N, 4.85. Found: C, 66.33; H, 6.45; N, 4.38.

In contrast to the hydroxylamine derivative prepared from the hexamethyl ether, this substance has not lost the elements of water to form an orthoxazine.

The compound gives a yellow-orange solution in concentrated sulfuric acid, a green color with ferric chloride, and its yellow solution in aqueous alkali turns red on standing in the air.

Zeisel Determinations

The Zeisel numbers on gossypol and many of its derivatives appear to have very little significance, for the values found are usually too high. They are given in the table.

ZEISEL DETERMINATIONS ON GOSSYPOL ETHER DERIVATIVES

		Found	Calcd.
1	Phenylhydrazone from hexamethyl ether	17.8	16.4 (4-OCH_3)
2	Phenylhydrazone from tetramethyl ether	11.8 12.3	8.5 (2-OCH_3)
3	Phenylhydrazone from dimethyl ether	13.0 11.3	8.5 (2-OCH_3)

Although the found and calculated values are discordant, it is noteworthy that essentially the same values were obtained for the phenylhydrazones of the tetra- and dimethyl ethers and the compounds therefore are isomeric. This coincides with the other analytical data.

Summary

Gossypol hexamethyl ether and tetramethyl ether react with phenylhydrazine in acetic acid solution to give derivatives in which two molecules of water and methyl groups have been eliminated. Treatment of the products with ethanolic or methanolic hydrogen chloride results in the reconversion to hexa- or tetraalkyl ethers of gossypol.

The hexa and tetra ethers also react with hydroxylamine with elimination of four molecules of water and hydrolysis of two methoxyl groups. Apparently the two oxime groups initially formed lose water with formation of orthoxazine rings.

The phenylhydrazine derivatives of gossypol tetramethyl ether and of gossypol dimethyl ether are isomeric, as would be anticipated from the fact that the two methoxyls in gossypol dimethyl ether correspond to the two hydroxyls in gossypol tetramethyl ether.

The hydroxylamine derivative of gossypol hexamethyl ether by treatment with alkali, then with a mixture of acetic acid and acetic anhydride, gives a compound which appears to be a lactone and corresponds to a gossypol derivative in which the aldehyde groups have been converted to carboxylic acid groups.

URBANA, ILLINOIS RECEIVED JUNE 27, 1938

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

Structure of Gossypol. IX.[1] Oxidation and Degradation of Gossypol Hexamethyl Ether; Gossic Acid

BY ROGER ADAMS, R. C. MORRIS AND E. C. KIRKPATRICK[2]

The experimental evidence previously published on the structure of gossypol has given much information concerning the functional groupings present in the molecule and some knowledge concerning the relative arrangement of these groups. On the other hand, it has not been demonstrated that the molecule contains two naphthyl residues or a binaphthyl residue as has been tentatively assumed.

It is only by obtaining degradation products and by identifying them that it may be hoped to clarify the complete structure of gossypol. The search for a convenient degradation reaction has, up to this time, been fruitless. Now, however, a most promising reaction has been discovered by which a smaller molecule can be isolated in a pure state and in reasonable yields.

The white gossypol hexamethyl ether (I) or gos-

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[1] For previous paper in this field see Adams and Geissman, THIS JOURNAL, 60, 2166 (1938).

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