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

Structure of Gossypol. VII. Gossypol Dimethyl Ether

BY ROGER ADAMS AND T. A. GEISSMAN

Evidence has been presented in previous papers[1] that gossypol is a symmetrical molecule containing six hydroxyl groups and two tautomeric hydroxyaldehydic residues. Of the six hydroxyls, two are much less acidic than the other four. The stability of two of the ether linkages in gossypol hexamethyl ether should be different from that of the other four and this now has been tested experimentally.

The white and red hexamethyl ethers of gossypol are demethylated upon treatment with sulfuric acid in acetic acid with the formation of a dimethyl ether. This new product possesses many of the properties of gossypol. It dissolves in alkali to give solutions which oxidize in the air. It gives a green color with ferric chloride; it forms a compound with one molecule of acetic acid; it gives an orange-red color with pyroboroacetate and an orange-red color with stannic chloride. With concentrated sulfuric acid it gives the orange color characteristic of the hexamethyl ethers of gossypol in contrast to the tetramethyl ether which gives a scarlet color similar to that given by gossypol. This is assumed to mean that the two unmethylated hydroxyl groups in gossypol tetramethyl ether are those which are methylated in the dimethyl ether. This assumption has been supported further by a study of the demethylation of gossypol tetramethyl ether under conditions similar to those used in the demethylation of the hexamethyl ethers. Gossypol is the resulting product.

The hexamethyl and tetramethyl ethers of gossypol do not react with aniline but the new dimethyl ether does react to give a condensation product. Although this derivative appears from analysis to be one of complicated type similar to those obtained from gossypol and certain substituted anilines, it can be hydrolyzed by acids to the original dimethyl ether of gossypol.

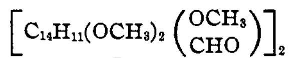
The dimethyl ether does not give an anhydro derivative under the conditions used for preparing anhydrogossypol from gossypol, and could not be converted to the red hexamethyl ether of gossypol either under the conditions used for preparing this compound from gossypol or under conditions which were modifications of these.

Gossypol dimethyl ether undergoes the apo reaction in a manner similar to gossypol, which again is in contrast to the tetra- and hexamethyl ethers which are stable under similar conditions.[2]

Remethylation of the dimethyl ether leads to the lower-melting form of the white hexamethyl ether.

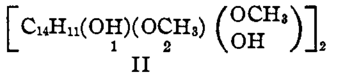
The white hexaethyl ether of gossypol can be deethylated to a gossypol diethyl ether analogous in structure and similar in properties to the dimethyl ether.

If gossypol hexamethyl ether in condensed form is accepted as having the structure indicated in I, and the tetramethyl ether II, then it may be concluded that III would be the structure for the dimethyl ether.



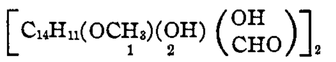
$$\left[C_{14}H_{11}(OCH_3)_2\begin{pmatrix}OCH_3\\CHO\end{pmatrix}\right]_2$$

I



$$\left[C_{14}H_{11}\underset{1}{(OH)}\underset{2}{(OCH_3)}\begin{pmatrix}OCH_3\\OH\end{pmatrix}\right]_2$$

II



$$\left[C_{14}H_{11}\underset{1}{(OCH_3)}\underset{2}{(OH)}\begin{pmatrix}OH\\CHO\end{pmatrix}\right]_2$$

III

The methylation of gossypol was reported to give a tetramethyl ether, two white hexamethyl ethers which were dimorphic and a red hexamethyl ether. It has now been found that the methylation conditions must be controlled very carefully in order to obtain the tetramethyl ether in a pure state. Moreover, it is possible to methylate gossypol-acetic acid to yield either the tetramethyl ether or the two white hexamethyl ethers.

Three different white gossypol hexamethyl ethers have now been isolated, m. p. 188, 225, 238–240°. The first two are apparently dimorphs but the highest melting appears to be diastereoisomeric or isomeric with the other two, if the depressed mixed melting point of the 225 and 240° forms is considered a proper criter-

---

(1) (a) Campbell, Morris and Adams, THIS JOURNAL, **59**, 1723 (1937); (b) Miller and Adams, *ibid.*, **59**, 1729 (1937); (c) Morris and Adams, *ibid.*, **59**, 1731 (1937); (d) Miller and Adams, *ibid.*, **59**, 1736 (1937); (e) Adams, Price and Dial, *ibid.*, **60**, 2158 (1938); (f) Adams, Friedman, Price, Morris and Kirkpatrick, *ibid.*, **60**, 2160 (1938).

(2) The apogossypol dimethyl ether could not be isolated in a pure state but upon methylation yielded apogossypol hexamethyl ether. This work will be reported in a subsequent paper.

ion. Fortunately, the chemical reactions of all these forms lead to the same products, so that their exact character is less significant.

The gossypol dimethyl ether can be oxidized in alkaline or acid medium. A solution in aqueous alkali when treated with air gives a deeply colored crystalline compound. This substance forms a violet salt the solution of which is decolorized by sodium hydrosulfite. The properties of this product indicate it to be a quinone. The dimethyl ether is also oxidized by ferric chloride in acetic acid or by dilute nitric acid to a deep orange crystalline compound. The mechanism by which these substances are produced has not yet been determined.

Experimental

Gossypol Dimethyl Ether.—To 1 g. of gossypol hexamethyl ether (m. p. 238–240°) dissolved in 40 cc. of hot acetic acid was added a solution of 10 drops of concentrated sulfuric acid in 10 cc. of acetic acid. The solution turned a deep red. After heating for thirty minutes on the steam-bath, 23 cc. of water was added carefully, with stirring and scratching of the walls of the flask. The initially flocculent precipitate soon became crystalline. It was filtered, washed with acetic acid and water and dried. The product was a yellow-green crystalline material and weighed 0.82 g. This crude product melted at about 155–165° with decomposition and obviously contained acetic acid. It was difficult to purify directly by further crystallization from acetic acid. By the action of aqueous alkali, a product was obtained which crystallizes from benzene–petroleum ether (b. p. 30–60°) as soft yellow needles, m. p. 191–193° (corr.), with decomposition. This is acetic acid free. The acetic acid also may be removed from the crude product merely by crystallization from benzene–petroleum ether without previous treatment with alkali. Recrystallization of this yellow crystalline form, m. p. 191–193° (corr.), from dilute acetic acid, results in the formation of a pure acetic acid derivative of gossypol dimethyl ether, m. p. 230–232° (corr.), with decomposition. The melting point varies considerably with the rate of heating.

Anal. Calcd. for $C_{30}H_{28}O_6(OCH_3)_2$: C, 70.33; H, 6.23. Found: (benzene–petroleum ether) C, 69.96; H, 6.01. (Removal of acetic acid by alkali) C, 70.48; H, 6.22. Calcd. for $C_{30}H_{28}O_6(OCH_3)_2 \cdot CH_3COOH$: C, 67.33; H, 6.26; OCH_3, 10.20. Found: C, 67.56; H, 5.99, 6.30; OCH_3, 14.2, 12.4, 13.5, 14.4.[3]

Aniline Derivative of Gossypol Dimethyl Ether.—A solution of 0.1 g. of the dimethyl ether of gossypol and 0.25 cc. of aniline in 4 cc. of benzene, was refluxed for thirty minutes. On dilution with petroleum ether (b. p. 30–60°), the aniline compound separated as orange prisms weighing 95 mg. Recrystallized from chloroform–petroleum ether (b. p. 30–60°), it formed bright orange prisms which melted at 268–270° (corr.) with decomposition.

Anal. Calcd. for $C_{44}H_{44}N_2O_8$: C, 75.86; H, 6.3; N, 4.0. Found: C, 74.92, 74.78; H, 6.30, 6.22; N, 5.20.

Deanilination of the Gossypol Dimethyl Ether–Aniline Compound.—To about 50 mg. of the aniline compound dissolved in 2 cc. of hot acetic acid was added one drop of concentrated hydrochloric acid. The clear orange solution was diluted drop by drop with water until crystallization started. The product was recrystallized from benzene–petroleum ether (b. p. 30–60°) (soft yellow needles, m. p. 187–189° (corr.) with decomposition), and then from dilute acetic acid (shining yellow leaflets, m. p. 229–230° (corr.) with decomposition). The latter sample showed no depression in melting point when mixed with a sample of dimethyl ether–acetic acid compound, m. p. 230–232° (corr.) with decomposition.

Gossypol Diethyl Ether.—To 0.1 g. of hexaethyl ether of gossypol in 4 cc. of hot acetic acid was added 1 cc. of acetic acid containing one drop of concentrated sulfuric acid. After heating on the steam-bath for fifteen minutes the solution was diluted with 2 cc. of water and cooled. The crude product was a yellow crystalline powder which weighed 95 mg. After one recrystallization from dilute acetic acid and a second from benzene–petroleum ether (b. p. 30–60°), it formed bright yellow needles, m. p. 193–195° (corr.) with decomposition.

Anal. Calcd. for $C_{34}H_{38}O_8$: C, 71.08; H, 6.62. Found: C, 71.17; H, 6.36.

The compound dissolves in alkali to give a yellow solution which slowly turns violet on standing in air. It gives a bright orange color with concentrated sulfuric acid and a brownish-green color with ferric chloride.

The fact that the material after a crystallization from dilute acetic acid melted at the same point as that from benzene–petroleum ether, indicates that an acetic acid compound analogous to the gossypol dimethyl ether derivative may not form.

Methylation of Gossypol Dimethyl Ether.—To a solution of 1.49 g. of the dimethyl ether in a mixture of 20 cc. of methanol and 20 cc. of dimethyl sulfate, 30 cc. of 10% methanolic potassium hydroxide was added slowly with cooling and the mixture was allowed to stand for forty-eight hours. The excess dimethyl sulfate was decomposed with 10% aqueous sodium hydroxide and the product collected. It formed white needles, weighing 1.5 g. Recrystallization from methanol gave white prisms, m. p. 209–211° (corr.).

Recrystallization from acetone–methanol gave small prisms which could be obtained melting as high as 221–223° (corr.), although it usually was possible to raise the melting point over 219° only by numerous recrystallizations. Recrystallization from ligroin (b. p. 90–110°) or from acetone–water gave long, crisp white needles which melted at 173° when heated quickly to this temperature, or at 223° when heated slowly (with shrinking at about 173°).

Anal. Calcd. for $C_{30}H_{26}O_2(OCH_3)_6$: C, 71.76; H, 6.97; OCH_3, 30.9; mol. wt., 602. Found: (prism form) C, 71.85; H, 6.93; OCH_3, 31.9, 33.3; (needle form) C, 71.78; H, 6.71; mol. wt. (Rast), 620.

This compound was proved by mixed melting point to be identical with the hexamethyl ether, m. p. 225°, obtained by methylating gossypol–acetic acid under suitable condi-

---

[3] Gossypol and most of its derivatives invariably give results from one to five per cent. high in the Zeisel determination. Compare Clark, THIS JOURNAL, **51**, 1479 (1929).

tions, and different from the hexamethyl ether, m. p. 240°, obtained by the methylation of the tetramethyl ether of gossypol (mixed m. p. 210–218° (corr.)).

Methylation of Gossypol–acetic Acid. (a) **Gossypol Hexamethyl Ether** (240° **form**).[4]—A solution of 10 g. of pure gossypol–acetic acid in a mixture of 50 cc. of dimethyl sulfate and 50 cc. of methanol was treated with 75 cc. of 10% methanolic potassium hydroxide with efficient cooling in an ice-bath. The solution was allowed to stand at 10–15° for about thirty-six hours and the excess dimethyl sulfate decomposed by the addition of 10% aqueous sodium hydroxide with ice cooling. The precipitate was washed well with water and with methanol, and consisted of buff needles weighing 5.95 g. and melting at 162–164°. It gave a scarlet color with concentrated sulfuric acid, indicating it to be crude tetramethyl ether.

This was remethylated to the hexamethyl ether according to the previously described procedure.[1c] It was recrystallized from acetone–methanol, when it formed colorless prisms, m. p. 238–240° (corr.). A sample recrystallized from ligroin (b. p. 90–110°) also formed prisms, m. p. 239–241° (corr.).

(b) **Gossypol Hexamethyl Ether** (225° **form**).—The proportions were the same as those just described for the first methylation, but the reaction mixture was cooled under the tap (*ca.* 20–25°) during the first addition of alkali and the final decomposition with 10% aqueous sodium hydroxide, and was allowed to stand for thirty-six hours at room temperature. The first product, methanol washed, weighed about 7.5 g., gave a deep orange color with concentrated sulfuric acid and melted at about 213°. Remethylated in the usual way,[1] it gave a product which melted (crude) at 211–213°, after one recrystallization from acetone–methanol at 216–218°, and after a second crystallization at 224–225° (corr.). A mixture of this with the product melting at 238–240° (corr.) melted at 212–217°.

This compound crystallized from ligroin (b. p. 90–110°) in clusters of white needles which sintered at about 179° and melted to a clear liquid at 221–224°. Plunged into a preheated bath at various temperatures it sintered at 185° and melted at 188°. At 198° it melted instantly, then resolidified, and remelted at 223–225° (corr.). The melted and resolidified samples of the *needle* form were found to have been converted to the *prism* form.

Both forms (m. p. 240° and 225°) were identical in their reactions.

Oxidation Products of Gossypol Dimethyl Ether. (a) **Air Oxidation in Alkaline Solution.**—Air was bubbled through a warm solution of 0.1 g. of the dimethyl ether in 25 cc. of 4% aqueous sodium hydroxide. After about five minutes the color became red and sodium hydrosulfite caused the reappearance of the initial yellow color. Continued treatment with air caused the solution finally to turn a deep violet and a purple sodium salt separated. The solution was acidified with sulfuric acid, extracted with ether, the ether solution dried and concentrated. The addition of petroleum ether caused the separation of 60 mg. of a compound crystallizing in tiny rosets. Recrystallized from ether–petroleum ether (b. p. 30–60°), the compound formed rosets of maroon prisms, m. p. 246° (corr.) with decomposition.

Anal. Calcd. for $C_{30}H_{30}O_8$: C, 69.50; H, 5.79. Found: C, 69.46, 69.51; H, 6.02, 6.10.

The compound gives a deep purple solution in sulfuric acid which changes through red to orange to yellow on dilution. The purple-red solution of the compound in dilute aqueous alcoholic alkali is changed to a yellow color by sodium hydrosulfite. Air reoxidizes the solution rapidly.

(b) **Oxidation with Ferric Chloride.**—To 0.8 g. of the dimethyl ether of gossypol dissolved in 80 cc. of hot glacial acetic acid was added 60 cc. of a 10% aqueous solution of ferric chloride. The solution turned deep greenish-brown and a small amount of tarry substance separated and was removed. The solution was heated on the steam-bath for a few moments and scratching of the walls of the flask caused orange crystals to form. These were collected and washed with acetic acid. They weighed 0.52 g. Recrystallized from acetic acid or dilute acetone the compound formed shining orange leaflets, m. p. 215–216° (corr.).

Anal. Calcd. for $C_{32}H_{30}O_{10}$: C, 66.90; H, 5.23. Found (acetone): C, 66.56; H, 5.19; (acetic acid): C, 67.02; H, 5.12.

The compound gives a red-brown color with ferric chloride and a red-brown color with concentrated sulfuric acid. It is soluble in dilute alkali, giving a red-violet solution which is unaffected by sodium hydrosulfite. It leaves no residue on ignition (not a Fe^{+++} salt).

(c) **Oxidation with Nitric Acid.**—Two attempts to isolate crystalline products after prolonged heating of the dimethyl ether with dilute nitric acid (1:4) were unsuccessful.

In another experiment 0.5 g. of the compound was dissolved in 10 cc. of benzene and 50 cc. of nitric acid (1:4) was added. The mixture was heated until the benzene had distilled off, the solid remaining suspended in the aqueous layer as a reddish, spongy mass. The heating was continued (gentle boiling) until this solid had turned a brick-red color. It was collected and washed free of nitric acid and crystallized three times from slightly diluted acetic acid; orange leaflets, m. p. 213–214° (corr.). Mixed with a sample of the ferric chloride oxidation product (m. p. 212–213°) it melted at 212–214°. Its color reactions with ferric chloride, concentrated sulfuric acid, and alkali were also identical.

Anal. Calcd. for $C_{32}H_{30}O_{10}$: C, 66.90; H, 5.23. Found: C, 66.70; H, 5.22.

Summary

Gossypol hexamethyl ethers are demethylated to gossypol dimethyl ether by the action of a drop of sulfuric acid in glacial acetic acid. Gossypol tetramethyl ether under similar conditions gives gossypol, which leads to the deduction that the two methoxyls in the dimethyl ether correspond to the two hydroxyls in the tetramethyl ether.

This dimethyl ether, in contrast to the tetra- and hexamethyl ethers, reacts with aniline or

---

[4] This product was reported in a previous paper to melt at 235°.

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

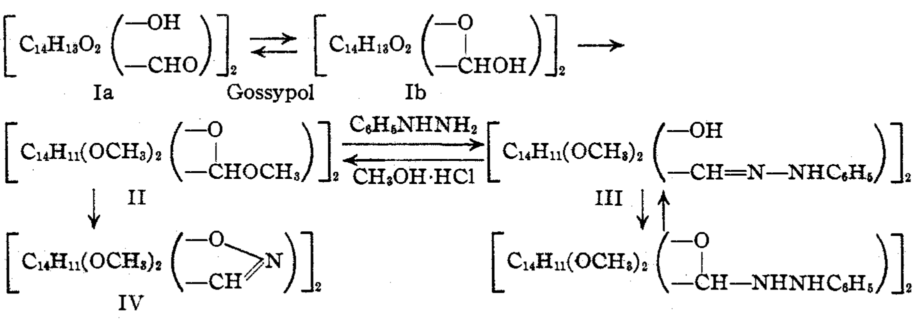
BY ROGER ADAMS AND T. A. GEISSMAN

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

---

(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).