evaporated to 9 cc. The product was obtained as described previously and purified in a similar way: yield 0.045 g. of white needles; m. p. 183–184.5°. This product was gossic acid.

Anal. Calcd. for $C_{14}H_{14}O_7$: C, 57.1; H, 4.76. Found: C, 56.52; H, 4.85.

Concentrated aqueous ammonia converted gossypolone tetramethyl ether dissolved in a mixture of 5 cc. of acetone and 5 cc. of ethanol to a yellow crystalline material which separated from solution. It could not be recrystallized and was insoluble in all common organic solvents. It melted above 300°. It gave a deep yellow solution in concentrated sulfuric acid.

Reaction of Aniline with Gossypolone Tetramethyl Ether.—A solution of 0.5 g. of gossypolone tetramethyl ether in 100 cc. of methanol and 0.2 cc. of aniline was boiled gently on a steam-bath for about an hour. The solution was concentrated to 50 cc. and allowed to stand. The crude product which separated was crystallized from 20 cc. of acetone: yellow plates; m. p. 213–215°.

Anal. Calcd. for $C_{34}H_{32}O_8(NHC_6H_5)_2$: C, 73.37; H, 5.84; N, 3.72; 4CH_3O, 16.45. Found: C, 73.54; H, 5.86; N, 3.70; CH_3O, 16.8.

The product was stable when dry. A solution, however, when exposed to air discolored.

It gave a light yellow color in concentrated sulfuric acid.

Action of Concentrated Nitric Acid on Gossypol Hexamethyl Ether.—By the action of cold 100% nitric acid, gossypol hexamethyl ether is nitrated and oxidized to a crystalline product, the nature of which will be reported later.

Summary

Gossypol hexamethyl ether has been oxidized by chromic acid, periodic acid and by dilute nitric acid. By the action of chromic acid or periodic acid a yellow crystalline product, gossypolone tetramethyl ether, is obtained which is formed by hydrolysis of two methoxyls followed by replacement of four hydrogens by two oxygens. Dilute nitric acid, however, gives two products, one which is water insoluble of a molecular weight comparable with the starting material, named gossypolonic acid tetramethyl ether and the other water-soluble, called gossic acid, of only about half the size. The former can be oxidized with permanganate to the latter. Gossypolone tetramethyl ether is oxidized with dilute nitric acid to gossypolonic acid tetramethyl ether and with permanganate to gossic acid. A discussion of the relationship of these compounds is given.

Gossic acid, $C_{14}H_{14}O_7$, appears to be a benzene derivative and contains a carboxyl, a dibasic acid anhydride linkage, and two methoxyls.

URBANA, ILLINOIS RECEIVED JUNE 27, 1938

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

Structure of Gossypol. X.[1] Apogossypol and its Degradation Products

BY ROGER ADAMS AND D. J. BUTTERBAUGH[2]

Clark[3] discovered that by the action of hot concentrated alkali, gossypol, $C_{30}H_{30}O_8$, loses two carbon and two oxygen atoms with the formation of two moles of alkali formate and one mole of a white unstable compound, $C_{28}H_{30}O_6$, which he designated as apogossypol. He demonstrated by methylation and acetylation that this substance contained six hydroxyl groups, for the stable hexaacetyl and hexamethyl derivatives were prepared readily.

Clark did not attempt to speculate as to what occurred in the gossypol molecule by the action of alkali but it is quite obvious from the results published in papers V–IX of this series. The evidence is convincing that gossypol is a hexahydroxy dialdehyde and consequently the ready removal of the aldehyde groups by the action of alkali might be anticipated. Examples of the elimination of aldehyde groups from hydroxy aldehydes in this way are well-known. This is especially true also of easily tautomerized hydroxyaldehydes such as the conversion of hydroxymethylene camphor to camphor by alkali.[4]

It has been shown that two of the six hydroxyls in gossypol are more difficult to methylate than the other four. Moreover, two of the acetyls in gossypol hexaacetate are more difficult to saponify than the other four. Attempts to methylate or acetylate apogossypol to a tetramethyl ether or tetraacetate were unsuccessful but it was found by us that benzoylation yielded merely a tetrabenzoate.

Both apogossypol hexamethyl ether and hexa-

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[1] For previous paper see Adams, Morris and Kirkpatrick, THIS JOURNAL, **60**, 2170 (1938).

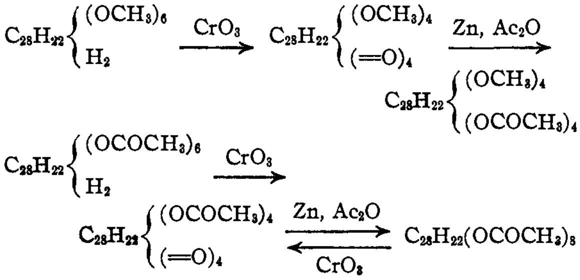
[2] Abstract of a thesis submitted in partial fulfilment for the degree of Doctor of Philosophy in chemistry; Chemical Foundation Fellow, 1936–1938.

[3] Clark, *J. Biol. Chem.*, **78**, 159 (1928).

[4] Bishop, Claisen and Sinclair, *Ann.*, **281**, 314 (1894).

acetate may be oxidized by chromic acid. Clark designated the oxidation product from the hexaacetate as tetraacetylapogossypolone and assigned it the structure $C_{22}H_{16}O_2(OCOCH_3)_4$. To the product from the oxidation of the hexamethyl ether he gave the formula $C_{28}H_{22}O_4(OCH_3)_4$ and named it tetramethoxypseudogossypolone. Our results have demonstrated that these two compounds are analogous and the proper formula for Clark's tetraacetylapogossypolone is actually $C_{28}H_{22}O_4(OCOCH_3)_4$. It is proposed to avoid further confusion by naming both these compounds as apogossypolone derivatives, apogossypolone tetramethyl ether and apogossypolone tetraacetate. The analytical data on the oxidation product of the hexaacetate are equally satisfactory for Clark's assumed compound and the structure assigned it in this Laboratory.

These oxidation products were considered by Clark to be quinones but he offered no experimental evidence for this assumption. It has now been found that they are quinones, since both apogossypolone tetramethyl ether and tetraacetate may be reductively acetylated and the reduction product of the tetraacetate may be reoxidized to the quinone. The formulas, therefore, may be written as follows



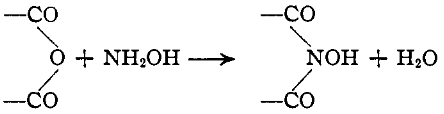
$$C_{28}H_{22}\begin{cases}(OCH_3)_6\\ H_2\end{cases} \xrightarrow{CrO_3} C_{28}H_{22}\begin{cases}(OCH_3)_4\\ (=O)_4\end{cases} \xrightarrow{Zn,\ Ac_2O} C_{28}H_{22}\begin{cases}(OCH_3)_4\\ (OCOCH_3)_4\end{cases}$$

$$C_{28}H_{22}\begin{cases}(OCOCH_3)_6\\ H_2\end{cases} \xrightarrow{CrO_3} C_{28}H_{22}\begin{cases}(OCOCH_3)_4\\ (=O)_4\end{cases} \underset{CrO_3}{\overset{Zn,\ Ac_2O}{\rightleftarrows}} C_{28}H_{22}(OCOCH_3)_8$$

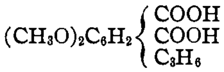
It is obvious that two methoxyl or two acetoxy groups are replaced by oxygens and two additional hydrogens are replaced by oxygens; or, interpreted in another way, two methoxyphenyl or acetoxyphenyl residues in the molecules are converted to quinones. Moreover, it is significant that these molecules differ from the corresponding gossypolone tetramethyl ether or tetraacetate by two carbon and two oxygen atoms. Since the apogossypolone derivatives are unquestionably quinones, this is indirect evidence for the assumed quinone structures of gossypolone derivatives. The analysis of the reductively acetylated apogossypolone tetraacetate does not agree with a formula derived from Clark's postulated structure of apogossypolone tetraacetate.

The apogossypolone tetraacetate is much more sensitive to reagents than the tetramethyl ether and consequently only the latter has been given an exhaustive investigation. Clark[5] oxidized apogossypolone tetramethyl ether with cold permanganate to a white acidic substance which he called apogossypolic acid. He believed this to have the formula $C_{20}H_{24}O_9$ and to be the trimethyl ether of a tricarboxylic acid, which on heating above its melting point lost one molecule of water to produce a trimethyl ether of a ketonic dibasic acid. The latter compound formed a hydrazone and a semicarbazone and hence the presence of a ketonic group was deduced.

A careful study of apogossypolic acid has led us to different conclusions concerning its structure. It proved by titration in aqueous solution to be a dibasic acid. Upon sublimation it lost water to give an anhydride and upon treatment with diazomethane it gave a dimethyl ester. Moreover, the molecular weight of the anhydride gave values which indicated the molecule to be most probably a simple benzene derivative. The molecular formula may be written as $C_{13}H_{16}O_6$ or $C_9H_8(OCH_3)_2(COOH)_2$. The formation of a semicarbazone of the anhydro product with the loss of a molecule of water does not necessarily imply the presence of a ketone group. It readily can be explained by analogy to other dibasic acid anhydrides which react with ketone reagents. Phthalic anhydride, for example, with hydroxylamine gives hydroxyphthalimide[6]



Apogossypolic acid therefore may be given the structural formula



$$(CH_3O)_2C_6H_2\begin{cases}COOH\\ COOH\\ C_3H_6\end{cases}$$

The problem is to determine the disposition of the C_3H_6 residue. It is obvious that in order to explain the facile formation of an anhydride, the two carboxyls must either both be in the *ortho* positions to each other in the ring or one may be in the ring *ortho* to a side-chain holding the carboxyl on the first carbon atom. This limits the structure to (1) a *n*-propyl or isopropyl group in the ring, (2) an ethyl and a methyl group in the ring, (3) a side-chain of the type $—CH(C_2H_5)$-

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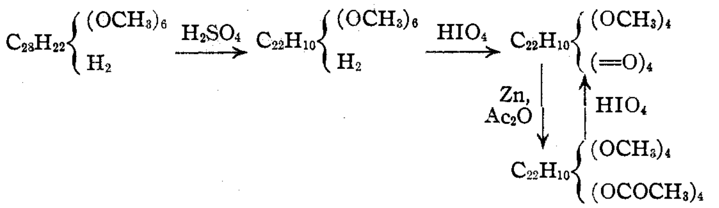
(5) Clark, *Oil and Fat Industries*, **6**, July (1929).

(6) Orndorff and Pratt, *Am. Chem. J.*, **47**, 88 (1912).

COOH or —C(CH_3)$_2$COOH, (4) a side-chain —CH(CH_3)COOH with a methyl group in the ring, (5) a side-chain —CH_2COOH with one ethyl or two methyl groups in the ring. The determination of its exact structure will be the subject of a subsequent communication.

Apogossypol hexamethyl ether undergoes a very unusual reaction under the influence of concentrated sulfuric acid at room temperature. A white stable crystalline substance is formed containing no sulfur and still retaining the six methoxyl groups. This substance will be designated as desapogossypol hexamethyl ether. The molecular weight and analyses agree unusually well for the loss of the residue C_6H_{12} from the starting material and this assumption was confirmed by a study of its oxidation products which apparently are analogous to those of apogossypol hexamethyl ether. Desapogossypolone tetramethyl ether is obtained by a periodic acid oxidation and this product may be reductively acetylated. This latter substance may be reoxidized to the quinone. Both the quinone and its reduction product have C_6H_{12} less than the corresponding apogossypol hexamethyl ether derivatives, and the reaction thus may be formulated as follows



$$\begin{tikzcd}
C_{23}H_{22}\begin{cases}(OCH_3)_6\\ H_2\end{cases} \arrow[r, "H_2SO_4"] & C_{22}H_{10}\begin{cases}(OCH_3)_6\\ H_2\end{cases} \arrow[r, "HIO_4"] & C_{22}H_{10}\begin{cases}(OCH_3)_4\\ (=O)_4\end{cases} \arrow[d, shift right, "{Zn, Ac_2O}"'] \\
 & & C_{22}H_{10}\begin{cases}(OCH_3)_4\\ (OCOCH_3)_4\end{cases} \arrow[u, shift right, "HIO_4"']
\end{tikzcd}$$

Assuming that apogossypol is a symmetrical molecule just like gossypol, the elimination of C_3H_6 from each half of the molecule has taken place. Since the reagent for this degradation is cold sulfuric acid, it appeared likely that propyl or isopropyl acid sulfate might be the by-product. The diluted sulfuric acid reaction mixture therefore was distilled and the distillate treated with potassium dichromate and mercuric sulfate. After boiling for one to two minutes, an orange precipitate separated similar to that obtained by the use of acetone. Moreover, the iodoform test of the original distillate is positive. The quantities of material available were so small as to lack the desired convincing proof, but tentatively it has been postulated that two isopropyl groups have been removed from the original molecule. No exact parallel for such a reaction has been found but it is interesting to note that isopropylnaphthalene can be dealkylated by means of hydriodic acid.[7]

The orange quinone, desapogossypolone tetramethyl ether, undergoes oxidation by means of cold permanganate to give a water-soluble dibasic acid which was identified as *m*-hemipinic acid, 1,2-(CH_3O)$_2$$C_6H_2$(COOH)$_2$-4,5, by comparison of it, its anhydride and its N-ethylimide with synthetic samples.

The isolation of *m*-hemipinic acid proves definitely that apogossypol hexamethyl ether and therefore gossypol hexamethyl ether contain two pairs of *ortho* methoxyl groups.

Since both *m*-hemipinic acid and apogossypolic acids are formed by permanganate oxidation of quinones, it may be inferred that the *o*-methoxyl groups are not in the quinone rings of the apogossypolone or desapogossypolone tetramethyl ethers since the quinone portions of the molecules probably account for two carboxyl groups found in the oxidation products.

The analogy between the degradation reactions of apogossypol and desapogossypol and the possible identification of acetone in the formation of desapogossypol hexamethyl ether make the assumption inviting that two isopropyl groups have been removed and, hence, that apogossypolic acid and *m*-hemipinic acid differ only by an isopropyl residue.

Apogossypol hexaethyl ether can be prepared readily, but it did not undergo the transformation to the desapogossypol hexaethyl derivative under the conditions used. The discussion of the reagents and conditions employed for preparing these different compounds is given in the experimental part. Suffice it to say here that the yields in the degradation reactions are usually not good and only if the conditions are carefully controlled can crystalline, easily purified compounds be obtained.

Experimental

Apogossypol.—The method employed for the preparation of apogossypol was that described by Clark[3] except for minor modifications. In place of pure gossypol as a raw material, it was found possible to use crude gossypol-acetic acid. To the alkaline solution sodium hydrosulfite, to the extent of about one-fifth the weight of the gossypol-acetic acid, was added to inhibit air oxidation. The apogossypol is unstable and turns dark on standing even in a nitrogen atmosphere in a desiccator. It must be converted immediately after preparation to a hexamethyl ether or a hexaacetate.

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(7) Meyer and Bernhauer, *Monatsh.*, **54**, 721 (1929).

Freshly prepared apogossypol gives a yellow color in concentrated sulfuric acid, a dark green with ferric chloride and an orange-red with pyroboroacetate. The nickel acetate, diethyl malonate, thiophene-sulfuric acid and stannic chloride tests were all negative. Attempts to prepare an anhydro compound by the method employed for preparing anhydrogossypol were unsuccessful.

Apogossypol Hexamethyl Ether.—The apogossypol was converted to its hexamethyl ether by the method of Clark.[8] A 40% yield of crystalline apogossypol hexamethyl ether or a 60% yield of methanol-insoluble material suitable for oxidation purposes could be obtained from crude gossypol-acetic acid. Pure apogossypol hexamethyl ether was purified by dissolving in the smallest possible volume of benzene, concentrating to one-half the volume and adding three volumes of methanol, m. p. 259°. Clark also reports 259°.

Apogossypolone Tetramethyl Ether.—This product was prepared from crude gossypol hexamethyl ether by oxidation with Kiliani's reagent according to the directions of Clark[3] (called by him tetramethoxy pseudogossypolone).

It could also be obtained by the oxidation with periodic acid. To a solution of 2.0 g. of (methanol-insoluble) apogossypol hexamethyl ether in 90 cc. of dioxane was added 30 g. of periodic acid in 20 cc. of water. The mixture was refluxed for four hours. During this period a 5-g. portion of periodic acid was added at the end of two hours and another at the end of three hours. The bright red solution changed to light yellow. After cooling, the solution was poured into 300 to 400 cc. of water and part of the dioxane removed by a current of air. The orange solid separating during this concentration was collected on a filter, dried and treated with just enough methanol to cover the solid. A bright orange solution resulted from which apogossypolone tetramethyl ether precipitated as a bright yellow solid. For further purification the material was recrystallized from a small volume of methanol in preference to an ether-acetic acid mixture recommended by Clark: m. p. 212–214°; yield 0.37 g.

Anal. Calcd. for $C_{28}H_{22}O_4(OCH_3)_4$: C, 70.3; H, 6.23; mol. wt., 546. Found: C, 70.30, 70.20; H, 6.27, 6.27; mol. wt., 538.

No crystalline products could be obtained by the action of *o*-phenylenediamine or by demethylation with hydrobromic acid in acetic acid.

It gave in concentrated sulfuric acid a dark orange color changing rapidly to bright purple. With the diethyl malonate–ammonia reagent it gave a blue-green.

Reductive Acetylation of Apogossypolone Tetramethyl Ether; Hydroapogossypolone Tetramethyl Ether Tetraacetate.—A mixture of 0.02 g. of pure apogossypolone tetramethyl ether, 0.02 g. of anhydrous sodium acetate, 1.0 g. of zinc dust and 2.0 cc. of acetic anhydride was refluxed for twenty-five minutes. Small additions of zinc dust were made intermittently during this time. The mixture was filtered hot, the residue washed twice with small portions of acetone and the filtrate poured into water to decompose the acetic anhydride. A few drops of glacial acetic acid aided materially in this decomposition. The white hydroapogossypolone tetramethyl ether tetraacetate was purified by crystallization from anhydrous ethanol, m. p. 229–230.5°, yield 0.015 g.

Anal. Calcd. for $C_{28}H_{22}(OCOCH_3)_4(OCH_3)_4$: C, 66.85; H, 6.41. Found: C, 66.93; H, 6.44.

Apogossypolic Acid.—This product was prepared according to unpublished directions of Clark.[9] A solution of 1.0 g. of crude apogossypolone tetramethyl ether (methanol-insoluble material) in 100 cc. of acetone distilled from potassium permanganate was cooled to 0° in an ice-bath. To this was added with stirring in three portions one hour apart 1.7 g. of potassium permanganate. The mixture was stirred, kept cold for twenty-four to thirty hours, after which time the manganese dioxide was collected on a gravity filter and washed free of any excess permanganate with acetone. After *thorough* air drying of the manganese dioxide precipitate, it was covered with water to which two or three drops of ethanol were added to decompose any permanganate that might not have been removed. It was then heated on a steam-bath for fifteen minutes. The manganese dioxide was removed by filtration and the alkaline solution acidified with dilute sulfuric acid. The yellow semi-solid material which separated was extracted with ether. The ether solution was dried and then concentrated to a few cubic centimeters. After addition of 15 cc. of dry benzene and concentration until the product started to separate (about 5–8 cc.), the solution was allowed to stand a day for crystallization. The apogossypolic acid obtained is essentially pure but may be purified further by dissolving in dry ether, pouring into benzene and concentrating till crystallization begins: white crystals, m. p. 162–165°, but the value obtained is very dependent on the rate of heating.

Anal. Calcd. for $C_{13}H_{16}O_6$: C, 58.2; H, 5.97; neut. equiv., 2COOH, 134. Found: C, 58.10, 58.00; H, 5.89, 5.90; neut. equiv., 134.

Dimethyl Ester of Apogossypolic Acid.—To an ether solution of 0.07 g. of apogossypolic acid was added an ether solution of diazomethane prepared from nitrosomethyl urea. At the end of twenty-four hours at room temperature, the solution was filtered and concentrated by evaporation. The oil obtained required about twenty days in an ice box before it became crystalline. For purification, it was twice sublimed at 25 mm.: white crystals; m. p. 45–46.5°.

Anal. Calcd. for $C_{15}H_{20}O_6$: C, 60.8; H, 6.76. Found: C, 60.49; H, 6.77.

Apogossypolic Acid Anhydride.—Sublimation of apogossypolic acid at 25 mm. with a bath temperature of 170–180° gave the anhydride in quantitative yields. After a second sublimation at 90–100°, a white product, m. p. 93–94° (Clark, 95°), resulted.

Anal. Calcd. for $C_{13}H_{14}O_5$: C, 62.40; H, 5.60; neut. equiv., 2COOH, 125; $2OCH_3$, 24.8. Found: C, 62.10; H, 5.39; neut. equiv., in H_2O, 123; OCH_3, 23.6.

The Action of Semicarbazide Hydrochloride on Apogossypolic Acid Anhydride.—A mixture of 0.075 g. of apogossypolic acid anhydride, 0.075 g. of semicarbazide hydrochloride, 0.112 g. of sodium acetate and 1.2 cc. of 95% ethanol was immersed in a bath of hot water and left while

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(8) Clark, This Journal, 51, 1475 (1929).

(9) Private communication from E. P. Clark.

the bath cooled. After standing for five hours, a few drops of water was added and the sides of the container scratched. The product crystallized and was purified by dissolving in a little methanol and adding an equal volume of water. Fine white crystals separated in the course of a few hours. m. p. 221–223°, yield 0.055 g. Clark reported 233°.

Anal. Calcd. for $C_{14}H_{17}O_5N_3$: C, 54.8; H, 5.54; N, 13.68. Found: C, 54.91; H, 5.60; N, 13.58.

Action of Concentrated Sulfuric Acid on Apogossypol Hexamethyl Ether; Desapogossypol Hexamethyl Ether.—To 80 cc. of concentrated sulfuric acid was added with vigorous stirring to aid solution, 4.0 g. of apogossypol hexamethyl ether. The mixture was allowed to stand for thirty minutes at room temperature and then poured onto ice. A fine purple-tinted amorphous material precipitated which was extremely difficult to filter. The dilute sulfuric acid suspension was therefore shaken with 25 to 50 cc. of ether, the two layers separated and the ether suspension poured into water. As the ether was evaporated on the steam-bath, a gray-white granular precipitate was obtained; yield 3.3 g. The product was purified by dissolving it in the smallest possible volume of boiling benzene, concentrating to about 40% of its original volume and adding two volumes of methanol. Crystallization was complete in about three hours; yield 1.6 g. An additional treatment in benzene with Norite followed by concentration and addition of methanol as previously described gave a white crystalline product, m. p. 295–296°.

Anal. Calcd. for $C_{28}H_{30}O_6$: C, 72.70; H, 6.50; mol. wt., 462; OCH_3, 40.2. Found: C, 72.62, 72.73, 72.68; H, 6.79, 6.56, 6.56; mol. wt., 425; OCH_3, 36.1, 35.8, 36.3, 37.8, 37.7, 35.8, 36.7.

In preparing the above compound in larger quantities, crude (methanol-insoluble) apogossypol hexamethyl ether proved satisfactory. Once recrystallized sulfuric acid compound was satisfactory for degradation purposes.

The product gave an orange-yellow color in concentrated sulfuric acid.

Periodic Acid Oxidation of Desapogossypol Hexamethyl Ether; Desapogossypolone Tetramethyl Ether.—A solution of 2.0 g. of desapogossypol hexamethyl ether in 140 cc. of dioxane was prepared by heating to boiling. After cooling to 40–50°, a saturated aqueous solution of 30 g. of periodic acid was added. The mixture was heated gently for a half hour during which time the solution refluxed vigorously and turned a dark red-brown color. The solution was then heated till it refluxed and after another thirty minutes a second 30-g. portion of periodic acid was added. After two more hours of refluxing, during which period the solution generally changed to orange in color, the solution was cooled to 60–70° and poured into 400 cc. of water. An orange flocculent precipitate formed. The major portion of the dioxane was removed by a current of air and the orange powder filtered; yield 1.5 g. This crude product was digested with 10 cc. of warm methanol for thirty minutes during which time all the lumps were crushed thoroughly with a glass rod. After standing for one hour, an orange powder weighing 0.53 g. was obtained. This was further purified by dissolving in a small volume of acetone, adding two volumes of methanol and concentrating till crystals appeared. The product formed orange spikes, softening at 199° and melting at 236–238° with decomposition. This product retained a small amount of solvent which was very difficult to remove; consequently, it was dissolved in benzene and concentrated to one-half volume, diluted with benzene and again concentrated. Petroleum ether (b. p. 60–110°) was then added, the mixture concentrated to one-half and this procedure repeated till practically all the benzene was removed. On cooling, an orange microcrystalline product separated, m. p. 245–248° with decomposition. This was used for analysis.

Chromic acid and Kiliani's reagent could not be substituted for periodic acid. With these reagents an orange product resulted but could not be obtained crystalline.

Anal. Calcd. for $C_{26}H_{22}O_8$: C, 67.5; H, 4.76; $4OCH_3$, 26.8. Found: C, 67.20; H, 4.98; OCH_3, 23.5, 23.3.

It gave a permanent dirty brown color in concentrated sulfuric acid. The *o*-quinone test with thiophene-sulfuric acid was negative.

Reductive Acetylation of Desapogossypolone Tetramethyl Ether; Hydrodesapogossypolone Tetramethyl Ether Tetraacetate.—A mixture of 0.4 g. of *pure* desapogossypolone tetramethyl ether, 5 cc. of acetic anhydride, 0.4 g. of fused sodium acetate and 2 g. of zinc dust was refluxed gently for twelve minutes. Small additions of zinc dust were made from time to time during this period. The solution was then filtered, the zinc washed with acetone and the filtrates poured into water. The fine white crystalline powder was purified by crystallization from methanol, m. p. 264–266°, yield 0.36 g.

Anal. Calcd. for $C_{34}H_{34}O_{12}$: C, 64.40; H, 5.37; $4CH_3$-CO, 27.1. Found: C, 64.03, 64.28; H, 5.50, 5.18; CH_3-CO, 25.8, 25.8.

Periodic Acid Oxidation of Hydrodesapogossypolone Tetramethyl Ether Tetraacetate.—Excess (2 to 3 g. in 2 cc. of water) periodic acid was added to a dioxane solution of 0.030 g. of hydrodesapogossypolone tetramethyl ether tetraacetate and the solution warmed gently on a steam-bath for fifteen to twenty minutes. During this time a colorless to yellow to orange-red change was observed. The orange product, precipitated by the addition of water to the dioxane solution, was collected on a filter, dried, digested with a small volume of methanol and crystallized from a hot mixture of benzene and methanol. The orange crystals so obtained proved to be identical with desapogossypolone tetramethyl ether obtained by the periodic acid oxidation of desapogossypol hexamethyl ether.

Permanganate Oxidation of Desapogossypolone Tetramethyl Ether; *m*-Hemipinic Acid.—To a cold (0°) solution of 0.5 g. of desapogossypolone tetramethyl ether in 130 cc. of acetone (distilled from permanganate) was added portionwise over a period of five hours, 1 g. of potassium permanganate. The solution was stirred and maintained at 0° for forty-eight hours. The manganese dioxide was filtered and washed to remove excess permanganate. The manganese dioxide precipitate was air-dried, then covered with water to which two to three drops of ethanol and 0.5–1 cc. of 10% aqueous sodium bicarbonate had been added. After heating on a steam-bath for fifteen minutes, the manganese dioxide was filtered and a dark orange solution remained. Dilute sulfuric acid precipitated a tan flocculent material which was extracted with ether. After drying the ether solution with sodium sulfate, a little benzene was added and the mixture concentrated. After the ether was removed, a light colored crystalline product began to separate. After standing for several hours, it was filtered and purified by a Norite treatment in acetone solution, followed by concentration after the addition of benzene, m. p. 191°, yield 0.13 g.

To complete the purification of this slightly colored material, it was sublimed at 60 mm. in a bath at 195°. A perfectly white crystalline product resulted, m. p. 179–181°, which apparently was the anhydride of the unsublimed material. This substance proved to be *m*-hemipinic acid anhydride. Carr and Pyman[10] report m. p. 176° for this substance.

Anal. Calcd. for $C_{10}H_8O_5$: C, 57.70; H, 3.85; mol. wt., 208. Found: C, 57.82; H, 4.14; mol. wt. (Rast), 215.

This anhydride was dissolved in 5% aqueous sodium hydroxide and the solution acidified with acid, keeping the mixture below 20°. The white insoluble *m*-hemipinic acid was extracted with ether, the ether solution dried and mixed with an equal volume of petroleum ether (b. p. 30–60°). Upon evaporation at room temperature long white needles of *m*-hemipinic acid separated, m. p. 191°. Carr and Pyman report a melting point of 192°.

Anal. Calcd. for $C_{10}H_{10}O_6$: C, 53.20; H, 4.43; neut. equiv., 113; mol. wt., 226. Found: C, 53.29; H, 4.65; neut. equiv., 115; mol. wt. (Rast), 224.

N-Ethylimide.—The acid just described was dissolved in an excess of ethanolic ethylamine containing 30–40% water. The product obtained by evaporating the solution to dryness on a steam-bath was sublimed at 60 mm. and a bath temperature of 235–240°. The fine white needles were then recrystallized from methanol, m. p. 229–231°. Carr and Pyman report a variety of melting points of the N-ethylimide of *m*-hemipinic acid ranging from 228–234°.

Anal. Calcd. for $C_{12}H_{13}O_4N$: C, 61.35; H, 5.54; N, 5.96. Found: C, 61.49; H, 5.68; N, 5.92.

m-Hemipinic acid, its anhydride and N-ethylimide were synthesized from veratric aldehyde[11] by Perkin's[12] method. The melting points are recorded in Table I.

TABLE I

COMPARISON OF *m*-HEMIPINIC ACID FROM APOGOSSYPOL AND BY SYNTHESIS

	Acid	Anhydride	N-Ethylimide
Synthetic	190–191	176–177	228–231
Mixture	190–191	176–178	229–231
From apogossypol	190–191	179–181	229–231

Apogossypol Dimethyl Ether.—To a small pressure bottle filled with nitrogen was added 12 cc. of 30% methanolic potassium hydroxide and 0.5 g. of gossypol dimethyl ether.[13] The flask was closed and heated at 100° for four hours after which period it was cooled and the contents poured into 8 cc. of water and acidified immediately with concentrated sulfuric acid. A light cream colored precipitate formed which could not be separated by filtration. The suspension was extracted with ether, dried and evaporated to yield a light brown amorphous solid. No method of purification was discovered. Methylation of this product in 20% aqueous alkali gave apogossypol hexamethyl ether, m. p. 259°.

Apogossypol Hexaethyl Ether.—To a solution of 2.0 g. of freshly prepared dry apogossypol in 10 cc. of methanol was added 12 cc. of pure diethyl sulfate and then dropwise with stirring a saturated methanolic solution of potassium hydroxide until the mixture was alkaline to moist litmus. After standing for several hours, the mixture was poured into water and the brown powder filtered. It was purified by dissolving in the smallest possible amount of benzene followed by addition of four volumes of methanol. This was repeated several times until the melting point was constant, 176–180°; yield 0.8 g.

Anal. Calcd. for $C_{28}H_{24}(OC_2H_5)_6$: C, 76.15; H, 8.57. Found: C, 75.82; H, 8.36.

Apogossypol Hexaacetate.—This product was prepared by the method of Clark[8] in yields of 62% of crystalline material, using crude gossypol-acetic acid for the conversion to apogossypol.

Apogossypolone Tetraacetate.—Clark's[8] procedure was used in this preparation. It was found that crude apogossypol hexaacetate gave satisfactory results. The product was purified from methanol, m. p. 230–232°. Clark reports 230°.

It gave in concentrated sulfuric acid a dark orange color changing rapidly to bright purple; with diethyl malonate–ammonia reagent, it gave a reddish-purple.

Reductive Acetylation of Apogossypolone Tetraacetate; Hydroapogossypolone Octaacetate.—A mixture of 0.62 g. of apogossypolone tetraacetate, 10 cc. of acetic anhydride, 0.3 g. of fused sodium acetate and 2 g. of zinc dust was refluxed gently for twelve minutes. The product was isolated as described for hydroapogossypolone tetramethyl ether tetraacetate; yield quantitative. After crystallization from ethanol it formed white crystals, m. p. 225–229°.

Anal. Calcd. for $C_{44}H_{46}O_{16}$: C, 63.8; H, 5.32. Found: C, 63.74; H, 5.33.

Oxidation of hydroapogossypolone octaacetate gave apogossypolone tetraacetate. To a hot solution of 0.2 g. of octaacetyl product in 10 cc. of glacial acetic acid was added 1 cc. of 10% aqueous chromic acid. The mixture was heated for one minute and poured onto ice. The bright orange apogossypolone tetraacetate melted at 230–232°.

Apogossypol Tetrabenzoate.—To 45 cc. of a cooled 20% aqueous sodium hydroxide solution of 2.5 g. of apogossypol was added in portions with stirring and cooling 18 cc. of benzoyl chloride. A light yellow product separated and was purified by dissolving in a small volume of hot benzene, concentrating one-half, adding an equal volume of methanol and allowing to stand. The benzoate crystallizes as white crystals with a faint pinkish tint: m. p. 314–316°; yield 1.9 g.

Anal. Calcd. for $C_{28}H_{24}(OCOC_6H_5)_4$: C, 76.55; H, 5.24. Found: C, 76.42; H, 4.89.

Gossypol Tetrabenzoate.—In a previous article[14] gossypol hexabenzoate was described. The analysis of this sub-

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[10] Carr and Pyman, *J. Chem. Soc.*, **105**, 1630 (1914).

[11] The preparation of veratric aldehyde was best accomplished by adding simultaneously concentrated aqueous sodium hydroxide and dimethyl sulfate to fused vanillin, *Org. Syn.*, **16**, 91 (1936).

[12] Perkin and Robinson, *J. Chem. Soc.*, **91**, 1073 (1907).

[13] Adams and Geissman, THIS JOURNAL, **60**, 2163 (1938).

[14] Miller, Butterbaugh and Adams, *ibid.*, **59**, 1729 (1937).

stance was poor for the hexa but it conforms satisfactorily to a tetrabenzoate which we now believe it to be. This opportunity is therefore taken to correct the error appearing in the earlier paper.

Summary

Apogossypol formed by the action of concentrated aqueous sodium hydroxide on gossypol yields a stable hexaacetate and a stable hexamethyl ether.

Both these products are oxidized to quinones, apogossypolone tetraacetate and apogossypolone tetramethyl ether. During this reaction, two acetoxy groups in the acetate and two methoxyl groups in the ether have been eliminated.

The presence of two quinone nuclei in each was proven by reductive acetylation. Hydroapogossypolone octaacetate and hydroapogossypolone tetramethyl ether tetraacetate are thus produced and the former could be reoxidized readily to apogossypolone tetraacetate.

The apogossypolone tetramethyl ether is oxidized with permanganate to a water-soluble acid, called apogossypolic acid, $C_{13}H_{13}O_6$. It contains two methoxyls and two carboxyl groups.

Apogossypol hexamethyl ether by the action of cold concentrated sulfuric acid loses the residue C_6H_{12} with the formation of a product called desapogossypol hexamethyl ether. This product is oxidized with periodic acid to desapogossypolone tetramethyl ether which corresponds to apogossypolone tetramethyl ether in being a quinone but with C_6H_{12} lost. Reductive acetylation gives hydrodesapogossypolone tetramethyl ether tetraacetate which can be reoxidized to desapogossypolone tetramethyl ether. This reductively acetylated product also analyzes for C_6H_{12} less than hydroapogossypolone tetramethyl ether.

When apogossypolone tetramethyl ether is oxidized with permanganate, *m*-hemipinic acid is formed as proved by a comparison with an authentic sample. It is thus demonstrated that two methoxyls are adjacent and since gossypol or apogossypol are symmetrical molecules, two pairs of *o*-methoxyls must be present.

Since apogossypolic acid and *m*-hemipinic acid differ by a C_3H_6 or half the C_6H_{12} lost in conversion of apogossypol tetramethyl ether to desapogossypol tetramethyl ether, it is a reasonable assumption that the difference is merely an isopropyl or propyl residue. The difference between the apo and desapo series would then be two isopropyl or propyl residues. Evidence is presented to indicate these groups to be isopropyl.

URBANA, ILLINOIS RECEIVED JUNE 27, 1938

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

Structure of Gossypol. XI.[1] Absorption Spectra of Gossypol, its Derivatives and of Certain Dinaphthalene Compounds

BY ROGER ADAMS AND E. C. KIRKPATRICK[2]

Previous work on the structure of gossypol has involved the preparation of derivatives and degradation products. While much information has thus been obtained concerning the functional groups present, little evidence is available as yet to indicate the nature of the basic nucleus of gossypol. Ultraviolet absorption offers a method of procuring such knowledge.

An examination of the spectra of gossypol[3] and many of its derivatives shows them all to be characterized by an absorption maximum at approximately 2500 Å. with the logarithm of the molal extinction coefficient close to 5 and a maximum or point of inflection near 3000 Å. with a log *e* of 4. Thus, gossypol is a very intense absorbent which can be explained only by assuming the presence of aromatic rings.

A comparison of the absorption spectrum of gossypol with those of substituted naphthalenes indicates that it is of the same general shape but with subdued detail.[4] The peaks of the gossypol curve are regularly more intense. If this intensity were due merely to auxochromes or other

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[1] For previous paper, see Adams and Butterbaugh, THIS JOURNAL, **60**, 2174 (1938).

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

[3] Grünbaumowna and Marchlewski, *Biochem. Z.*, **286**, 295 (1936); see also Marchlewski, *J. prakt. Chem.*, **60**, 84 (1899); Tobler, Dissertation "Arbeiten über Gossypol," Zürich, 1932; Podolskaja, *Biochem. Z.*, **284**, 401 (1936); Zamyshlyaeva and Krivich, *J. Gen. Chem.* (U. S. S. R.), **7**, 1936 (1937).

[4] De Laszlo, *Z. physik. Chem.*, **118**, 369 (1925); *Proc. Roy. Soc.* (London), **111**, 355 (1926); Henri and de Laszlo, *ibid.*, **A105**, 355 (1936); Ley, *Handbuch der Physik*, **21**, 176 (1929).