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.

sypol tetramethyl diethyl ether[1] can be oxidized with chromic acid, periodic acid or dilute nitric acid. By very short treatment with chromic acid, oxidation occurs to give chiefly a yellow crystalline compound (II) which for convenience may be looked upon as being formed by the hydrolysis of two methoxyl groups followed by replacement of four hydrogen atoms by two oxygens. This product will be called gossypolone tetramethyl ether since it is analogous to the compound described by Clark,[3] designated by him as tetraacetylgossypolone and obtained by the oxidation of gossypol hexaacetate; thus $C_{30}H_{24}O_2(OCOCH_3)_6$ to $C_{30}H_{22}O_6(OCOCH_3)_4$.

Along with the gossypolone tetramethyl ether was invariably found a very small amount of a second oxidation product $C_{30}H_{22}O_4(OCH_3)_4$, III, which proved to be a lactone and identical with the gossylic acid lactone tetramethyl ether described in a previous paper.[1]

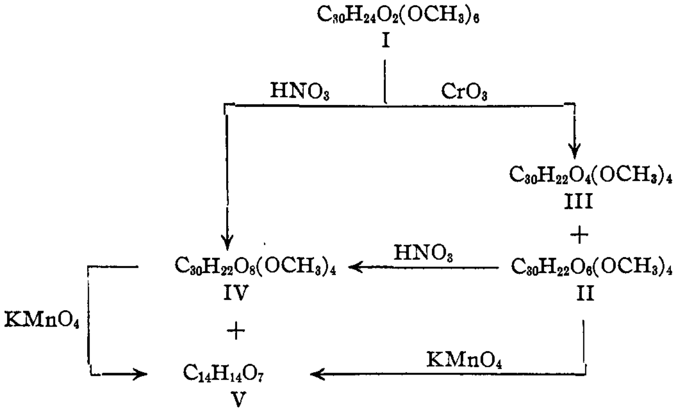
Gossypolone tetramethyl ether probably contains aldehydic carbonyls as shown by the condensation with two molecules of aniline. Preliminary tests indicate that it may also contain quinone groupings. It does not contain carboxyl groups, for the product could not be titrated electrometrically. If gossypol hexamethyl ether is accepted as containing two aldehyde and six methoxyl groups, there may have occurred in this oxidation the hydrolysis of two methoxyls to hydroxyls and the conversion of two phenolic residues to quinones. The product may well be a gossypoloquinone tetramethyl ether.

Gossypolone tetramethyl ether also could be obtained from gossypol hexamethyl ether by the action of periodic acid but this reaction was not nearly as satisfactory as the oxidation with chromic acid.

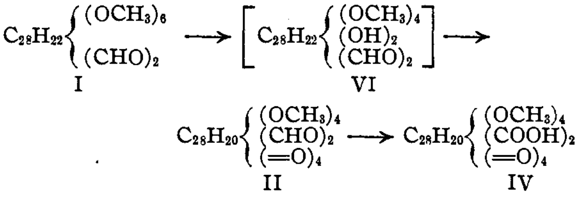
The oxidation of gossypol hexamethyl ether with dilute nitric acid is more deep-seated than with chromic or periodic acid. By this procedure, two products which account for essentially all of the starting material are formed, a yellow crystalline water-insoluble compound (IV), and a white crystalline water-soluble product (V). Approximately equal amounts of each are obtained.

The water-insoluble substance (IV) has a molecular weight comparable with that of the starting material and has two more oxygens than gossypolone tetramethyl ether (II). It is an acid and a tetramethyl ether and some indications are that it is a quinone. It will be called gossypolonic acid tetramethyl ether. Although stable to dilute nitric acid, it is readily oxidized with permanganate to give the same water-soluble compound (V), $C_{14}H_{14}O_7$, which results by the direct dilute nitric acid oxidation of gossypol hexamethyl ether. It is thus obvious that a close relationship in structure between these compounds exists. The oxidation of gossypol hexamethyl ether apparently takes place in two directions, one without degradation and the other with a cleavage to approximately a half molecule.

Moreover, these substances and gossypolone tetramethyl ether (II) are related as shown by the fact that the latter upon treatment with dilute nitric acid readily oxidizes to the water-insoluble product, gossypolonic acid tetramethyl ether (IV), obtained by direct nitric acid oxidation of gossypol hexamethyl ether. By treatment with permanganate gossypolone tetramethyl ether gives the water-soluble product (V) obtained either by the nitric acid oxidation of gossypol hexamethyl ether or by the permanganate oxidation of gossypolonic acid tetramethyl ether. These conversions are shown in the chart



A possible breakdown of these molecules with a postulated intermediate VI may be suggested



$$\underset{\text{I}}{C_{28}H_{22}\begin{cases}(OCH_3)_6\\(CHO)_2\end{cases}} \longrightarrow \underset{\text{VI}}{\left[C_{28}H_{22}\begin{cases}(OCH_3)_4\\(OH)_2\\(CHO)_2\end{cases}\right]} \longrightarrow$$

$$\underset{\text{II}}{C_{28}H_{20}\begin{cases}(OCH_3)_4\\(CHO)_2\\(=O)_4\end{cases}} \longrightarrow \underset{\text{IV}}{C_{28}H_{20}\begin{cases}(OCH_3)_4\\(COOH)_2\\(=O)_4\end{cases}}$$

It is recognized that other tautomeric forms or perhaps closely related isomeric forms of these structures may be the more nearly correct con-

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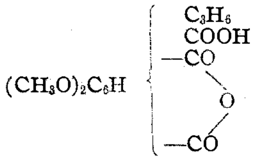
(3) Clark, THIS JOURNAL, 51, 1475 (1929).

figurations but this will have to be determined after further experimentation.

The water-soluble product, because of its ease of preparation and of its molecular size of about 300, is a most promising derivative for immediate study to obtain a further insight into the gossypol structure. It will be called *gossic acid.* This product is a dimethyl ether and is acid in character as shown by its decomposition of aqueous sodium carbonate. It forms a monomethyl ester with diazomethane but titrates in aqueous solution approximately for a tribasic acid. Consequently a dibasic acid anhydride linkage in addition to a carboxyl group may be present. In all respects the compound acts like a substituted benzene. It gives none of the unusual color reactions of the higher molecular weight gossypol derivatives and has entirely normal solubility and crystallizing characteristics. From these meager results a partial breakdown of the gossic acid molecule may be postulated as follows



but the exact constitution must await further investigation.

It is significant that, under similar conditions, gossypol tetramethyl ether is oxidized by dilute nitric acid only to water-soluble acids which have not been obtained in a crystalline state. Neither could crystalline derivatives be obtained by the oxidation with chromic or periodic acid.

Experimental

Chromic Acid Oxidation of Gossypol Hexamethyl Ether or Gossypol Tetramethyl Diethyl Ether: Gossypolone Tetramethyl Ether (II).—To a solution of 2 g. of white gossypol hexamethyl ether in 50 cc. of boiling glacial acetic acid, was added rapidly with vigorous stirring 10 cc. of a 10% aqueous solution of chromic oxide and the mixture was allowed to boil for one minute. It was then poured onto a mixture of 100 g. of ice and 75 cc. of water. The product was filtered with suction and the yellow residue was washed with water until the washings were neutral. The air-dried residue was added to 40 cc. of boiling methanol. The material at first became gummy and then went gradually into solution, but, before solution was complete, a yellow material began to crystallize. By keeping warm on a steam-bath and breaking up the lumps with a stirring rod, all non-crystalline material went into solution. After standing for two hours at room temperature, the yellow crystalline product was filtered; yield 1.5 g. To purify, it was heated with 120 cc. of boiling methanol and filtered hot. The insoluble residue is described below. After cooling and allowing the filtrate to stand for two hours in an ice box, the yellow crystalline plates of gossypolone tetramethyl ether were filtered and washed with 20 cc. of petroleum ether (b. p. 30–60°). After two more crystallizations, the melting point was constant at 156–157°.

Anal. Calcd. for $C_{30}H_{22}O_6(OCH_3)_4$: C, 67.77; H, 5.64; $4CH_3O$, 20.59; mol. wt., 602. Found: C, 67.69, 67.42; H, 5.83, 6.06; CH_3O, 21.0, 20.8; mol. wt. (b. p. acetone), 664, 616.

Exactly the same substance was formed by a similar oxidation of gossypol tetramethyl diethyl ether.

The product gave a dark brown color in concentrated sulfuric acid which changed to a light orange on standing for a half hour. It gave no color with ferric chloride and a deep red with pyroboroacetate.

It was insoluble in 10% aqueous sodium hydroxide but was soluble in a 10% methanolic potassium hydroxide solution with decomposition.

This same product may also be made, though less conveniently, by the oxidation of gossypol hexamethyl ether with periodic acid.

By-Product from Oxidation with Chromic Acid: Gossylic Acid Lactone Tetramethyl Ether (III).—The material insoluble in the 120 cc. of boiling methanol was dissolved in 4 cc. of boiling acetone and the solution filtered. Upon standing, a yellow product separated. By crystallization from 4 cc. of acetone it formed yellow needles. A second crystallization this time from 15 cc. of boiling glacial acetic acid gave light yellow needles, m. p. 315–317°.

Anal. Calcd. for $C_{30}H_{22}O_4(OCH_3)_4$: C, 71.58; H, 5.96; CH_3O, 21.75. Found: C, 70.82; H, 5.84; CH_3O, 21.2.

It proved to be identical by mixed melting point determination with the lactone obtained by treating gossypol hexamethyl ether with hydroxylamine followed by alkali and acetic anhydride.

Oxidation of Gossypol Hexamethyl Ether with Dilute Nitric Acid

Water-Insoluble Oxidation Product: Gossypolonic Acid Tetramethyl Ether (IV).—A suspension of 0.40 g. of white gossypol hexamethyl ether in a mixture of 10 cc. of concentrated nitric acid (sp. gr. 1.42) and 40 cc. of water was refluxed for twelve hours. At the start of the reaction the material was a flocculent suspension, in about two hours it became a reddish-brown gummy mass which floated on the acid. Toward the end of the reaction, the mass had become hard. After cooling, the solid was filtered and the filtrate set aside for isolation of the water-soluble product.

The solid was dissolved in ether and the solution shaken repeatedly with sodium bicarbonate solution until all the alkali-soluble material was extracted. The bicarbonate extract was acidified, the yellow precipitate filtered, washed and dried. On crystallization from benzene, it formed small yellow plates, m. p. 249–251° with decomposition, yield 0.14 g. The product is essentially insoluble in methanol.

Anal. Calcd. for $C_{34}H_{34}O_{12}$: C, 64.4; H, 5.36; $(4OCH_3)$, 21.2; mol. wt., 634. Found: C, 64.33; H, 5.32; OCH_3, 20.9; mol. wt. (acetone), 644.

In concentrated sulfuric acid, the compound gave a deep red color, which rapidly faded to a yellow-brown. An alcohol solution gave no color with aqueous ferric chloride. It was not decolorized with sulfur dioxide or aqueous sodium hydrosulfite.

Although stable when dissolved in aqueous sodium bicarbonate, it was unstable in aqueous sodium hydroxide with the appearance of a green color and precipitation of a gummy mass.

It was unchanged by further refluxing (fifteen hours) with dilute nitric acid and was only very slowly affected by chromic acid in acetic acid solution.

When dissolved in concentrated sulfuric acid and the solution allowed to stand for one-half hour and then diluted with water, there was obtained only a brown amorphous powder which could not be made to crystallize.

Demethylation with constant boiling hydrobromic acid yielded no definite product.

The product can be nitrated with cold 100% nitric acid to give a crystalline nitro derivative which obviously has been oxidized also. Its chemical reactions will be reported later.

Water-Soluble Oxidation Product: Gossic Acid.—The dilute nitric acid filtrate from the oxidation reaction was neutralized with sodium carbonate, then acidified to Congo red with sulfuric acid, saturated with sodium chloride and extracted several times with ether. The crude brown product was crystallized twice from toluene from which it separates slowly in the form of long colorless needles, m. p. 184–186°. It may also be crystallized from petroleum ether (b. p. 60–110°), yield 0.16 g. A preferable method of purification for analysis is one crystallization from toluene, followed by sublimation at 15 mm. and then a second crystallization from toluene or petroleum ether (b. p. 60–110°).

Anal. Calcd. for $C_{14}H_{14}O_7$: C, 57.2; H, 4.76; $2OCH_3$, 21.0; neut. equiv., 98; mol. wt., 294. Found: C, 57.18; H, 4.95; OCH_3, 21.0, 20.6; neut. equiv., 104; mol. wt. (camphor), 316.

When larger amounts of gossypol hexamethyl ether were oxidized it was found an advantage to heat the sodium carbonate solution with potassium permanganate until the purple color had become permanent. The precipitate of manganese dioxide was destroyed with sulfur dioxide, the solution acidified with sulfuric acid and saturated with sodium chloride. Extraction in the way previously described gave a product very easily purified. The yields were equivalent to those obtained without the use of potassium permanganate.

The compound gave no color when dissolved in concentrated sulfuric acid. Such a solution after standing one-half hour gave unchanged product on dilution with water. It gave no color with aqueous ferric chloride solution.

Attempts to nitrate the compound with cold 100% nitric acid were unsuccessful and the product was recovered unchanged. The compound was very stable to chromic acid in acetic acid.

An aqueous solution was not oxidized by periodic acid. Refluxing for eight hours in nitric acid (1:1) did not affect the compound.

Permanganate Oxidation of Gossypolonic Acid Tetramethyl Ether: Gossic Acid.—To a suspension of 0.35 g. of gossypolonic acid tetramethyl ether in 200 cc. of water was added, in portions, 1.5 g. of solid potassium permanganate. The reaction mixture was heated on the steam-bath for five hours, at the end of which time there was still excess permanganate present. The flask was cooled, acidified with dilute sulfuric acid and sulfur dioxide was passed through until the excess permanganate and precipitate of manganese dioxide was destroyed. The solution was filtered, saturated with sodium chloride and extracted with ether. The ether extract was evaporated and the gummy residue crystallized from toluene. It formed long, colorless needles that became opaque on drying, m. p. 184–186°, yield 0.15 g. It proved to be gossic acid.

Oxidation of Gossypol Tetramethyl Ether.—Dilute nitric acid oxidation of gossypol tetramethyl ether, according to the directions used for the hexamethyl ether, gave only a nitric acid soluble product. Upon neutralizing and extracting with ether, an oily product, acidic in nature, was obtained. No crystalline substance was separated.

Methyl Ester of Gossic Acid.—To a solution of 0.10 g. of the acid in 5 cc. of ether was added an ether solution of diazomethane from 0.15 g. of nitrosomethyl urea. The reaction mixture was allowed to stand overnight at room temperature, then evaporated to dryness. The residue was purified by sublimation at 20 mm. and crystallized from petroleum ether (b. p. 30–60°) in long colorless needles, m. p. 106°.

Anal. Calcd. for $C_{15}H_{16}O_7$: C, 58.5; H, 5.19; $3OCH_3$, 30.2; mol. wt., 308. Found: C, 58.86; H, 5.25; OCH_3, 31.2; mol. wt. (Rast), 316.

The ester was saponified back to gossic acid by the following procedure. A suspension of 0.10 g. of the ester was refluxed gently with 5 cc. of 10% aqueous sodium hydroxide. The material was insoluble but gradually went into solution on heating. When solution was complete the reaction mixture was acidified, saturated with sodium chloride and extracted with ether. The ether was evaporated and the residue crystallized from petroleum ether (b. p. 60–110°), m. p. 184–186°.

Nitric Acid Oxidation of Gossypolone Tetramethyl Ether.—A suspension of 0.2 g. of gossypolone tetramethyl ether in 25 cc. of water and 8 cc. of concentrated nitric acid was refluxed for six and one-half hours. The insoluble product was worked up as described in the nitric acid oxidation of gossypol hexamethyl ether; yield 0.15 g. Recrystallized from benzene, it formed yellow needles, m. p. 237–238°. A mixed melting point showed it to be identical with gossypolonic acid tetramethyl ether.

Permanganate Oxidation of Gossypolone Tetramethyl Ether.—To a suspension of 0.1 g. of gossypolone tetramethyl ether in 20 cc. of water was added over a period of thirty minutes, 0.4 g. of potassium permanganate crystals. The mixture was heated under reflux for seven hours. Sulfur dioxide was then bubbled in till the manganese dioxide had dissolved and the solution was colorless. Unchanged gossypolone tetramethyl ether was filtered and 1 cc. of concentrated hydrochloric acid added to the filtrate. The filtrate was then extracted several times with ether, the ether extract dried with sodium sulfate, filtered and evaporated to remove the solvent. The gum obtained was dissolved in 10 cc. of boiling acetone, 10 cc. of petroleum ether (b. p. 60–110°) was added, and the mixture 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).