Experimental

The absorption spectra were taken on a Bausch and Lomb ultraviolet spectroscope using a hydrogen discharge tube operating under 15,000 volts as a light source. The calibration of wave length was made from the lines Hα, Hβ and Hγ from this source. A quartz cell exactly 1 cm. thick was used throughout with a slit width of 60 μ and appropriate exposure. Stock solutions 5×10^{-4} molar were diluted to yield a series of seven concentrations between this and 5×10^{-6} molar. These concentrations covered the critical concentrations in all cases.

Gossypol and all its derivatives except anhydrogossypol were dissolved in alcohol for these determinations. Anhydrogossypol was dissolved in absolute alcohol and the absorption spectrum taken immediately. Of the α,α-binaphthyl, β,β-binaphthyl, *sym*-di-α-naphthylethane and *sym*-di-β-naphthylethane only the first two were sufficiently soluble in alcohol; as a consequence purified dioxane[7] was used as a solvent for all four. That dioxane gives absorption spectra comparable to those taken in alcohol was shown by the fact that α,α-binaphthyl gave identical curves in both solvents.

Summary

On the basis of the comparison of the ultraviolet absorption spectra of gossypol and its derivatives with known spectra of aromatic ring systems and with four dinaphthalene compounds, α,α-binaphthyl, β,β-binaphthyl, *sym*-di-α-naphthylethane, *sym*-di-β-naphthylethane, it was concluded that gossypol probably contains a binaphthyl nucleus.

The following deductions were drawn from a study of the absorption spectra of gossypol derivatives. (a) Gossypol and gossypol dimethyl ether have a third absorption maximum not found in gossypol tetramethyl ether, hexamethyl ether or hexaacetate. (b) The difference between the absorption spectra of the oxidation products of gossypol hexamethyl ether or apogossypol hexamethyl ether resembles the difference between the naphthalene and 1,4-naphthoquinone. Quinone structures for the oxidation products were thus deduced.

URBANA, ILLINOIS RECEIVED JUNE 27, 1938

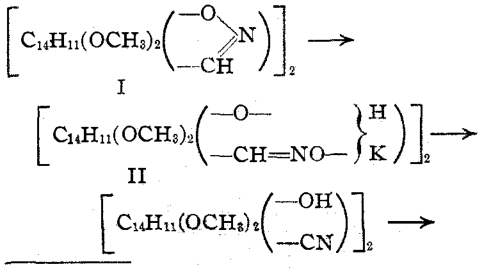
(7) Oxford, *Biochem. J.*, **28**, 1325 (1934).

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

The Structure of Gossypol. XII.[1] Gossylic Acid Lactone Tetramethyl Ether

BY ROGER ADAMS AND T. A. GEISSMAN

When the compound (I) obtained by treatment of gossypol hexamethyl ether with hydroxylamine hydrochloride in acetic acid is dissolved in methanolic potassium hydroxide, a crystalline potassium salt (II) is obtained. This salt when heated in acetic acid solution undergoes a change which is assumed to follow the course formulated below, resulting in the formation of a lactone (III)[2]



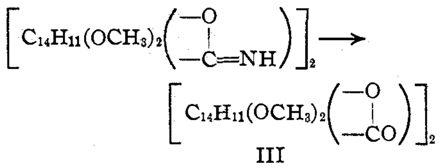
$$\left[C_{14}H_{11}(OCH_3)_2\left(\begin{matrix}-O\diagdown\\ \quad\;\; \gg N\\ -CH\diagup\end{matrix}\right)\right]_2 \longrightarrow$$

I

$$\left[C_{14}H_{11}(OCH_3)_2\left(\begin{matrix}-O- & \\ -CH{=}NO- & \end{matrix}\begin{matrix}\}H\\ \}K\end{matrix}\right)\right]_2 \longrightarrow$$

II

$$\left[C_{14}H_{11}(OCH_3)_2\left(\begin{matrix}-OH\\ -CN\end{matrix}\right)\right]_2 \longrightarrow$$



$$\left[C_{14}H_{11}(OCH_3)_2\left(\begin{matrix}-O\\ \mid\\ -C{=}NH\end{matrix}\right)\right]_2 \longrightarrow \left[C_{14}H_{11}(OCH_3)_2\left(\begin{matrix}-O\\ \mid\\ -CO\end{matrix}\right)\right]_2$$

III

The same compound is obtained in small yields as a by-product in the oxidation of gossypol hexamethyl ether with chromic acid in acetic acid.[3]

In this communication evidence is presented that this substance not only is a lactone but that it is formed from a carboxyl and a ***phenolic*** hydroxyl group. This information along with the results reported in previous papers and additional evidence since accumulated in this Laboratory leads to the conclusion that a lactone structure between the ***peri*** positions of a naphthalene nucleus (IV) is actually in hand.

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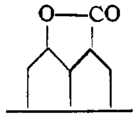
(1) For previous paper see Adams and Kirkpatrick, THIS JOURNAL, **60**, 2180 (1938).

(2) Adams and Geissman, *ibid.*, **60**, 2166 (1938).

(3) Adams, Morris and Kirkpatrick, *ibid.*, **60**, 2170 (1938).

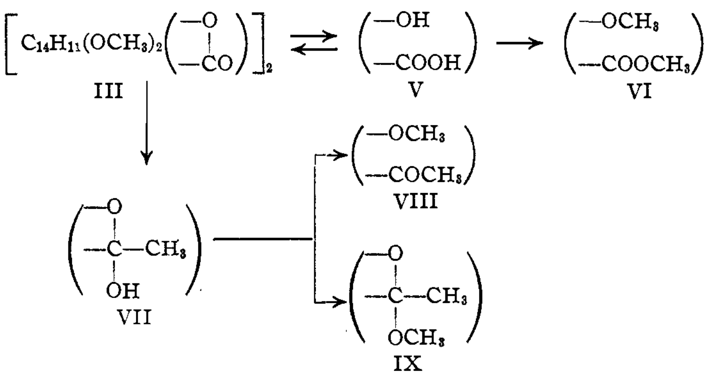


IV

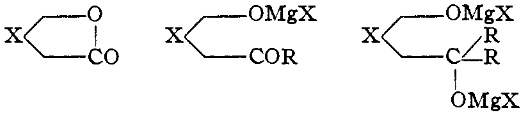
The lactone ring can be opened by warming the compound in 10% methanolic potassium hydroxide solution; acidification of the alkaline solution gives the hydroxy acid (V). It can be converted into the lactone merely by warming in acetic anhydride.

By treatment of the hydroxy acid in methanolic alkali with dimethyl sulfate, the hydroxyl and carboxyl groups are methylated leading to the dimethyl ester of gossylic acid hexamethyl ether (VI). Moreover, the hydroxy acid may be methylated in ether solution with diazomethane to give the same compound (VI). This latter reaction is significant in that alcohols do not ordinarily methylate with this reagent and, hence, it may be inferred that the hydroxyls are phenolic groups. Attempts to hydrolyze the ester (VI) to the corresponding acid were unsuccessful, lending support to the conclusion that ortho substituents to the carbomethoxy groups are present, which hinder saponification. Added confirmation of this is found in the fact that attempts to esterify the hydroxy acid (V) in methanol–sulfuric acid or methanol–hydrochloric acid were equally unsuccessful. Attempted esterification by the methanol–mineral acid procedure resulted in the formation of the lactone.

Methylmagnesium iodide reacts with the lactone to give a compound in which each lactone residue has added one mole of reagent (VII); this substance will be called homogossypol tetramethyl ether since it is homologous with a tautomeric form of one of the possible gossypol tetramethyl ethers. The product is soluble in methanolic alkali and can be methylated in two ways with the formation of isomeric methyl ethers. Upon treatment of an alkaline solution of homogossypol tetramethyl ether (VII) with dimethyl sulfate, the ether produced is formulated as a methoxy methyl ketone (VIII), while treatment with methanol containing a few drops of sulfuric acid gives an isomeric ether probably of the acetal type (IX).



Considerable significance can be attached to the fact that a large excess of Grignard reagent did not bring about the addition of more than one mole to each of the two lactone linkages. The usual course of the reaction between a lactone and a Grignard reagent is as follows

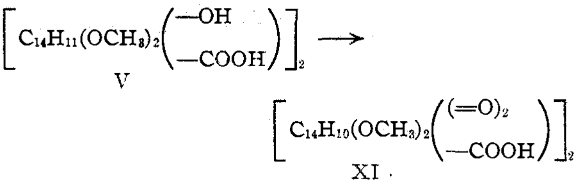


Xa Xb

Since in the present case the keto group represented by step Xa does not undergo further addition (Xb), a reasonable assumption is that it is present in the molecule with ortho substituents on each side as in a 2-substituted 1-naphthyl methyl ketone. The same explanation accounts for the resistance of the ester (VI) to saponification.

The lactone (III) was surprisingly stable to oxidizing agents; it was unattacked by potassium permanganate under most conditions, severe treatment resulting in complete destruction of the molecule. Chromic acid did not oxidize the molecule, which indicates that in the chromic acid oxidation of gossypol hexamethyl ether,[3] the lactone is formed as a by-product and not as an intermediate. The lactone was not affected by dilute nitric acid of a strength which readily oxidized gossypol hexamethyl ether. It was found impossible also to reduce the lactone although a variety of reagents was employed.

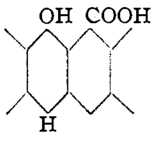
The hydroxy acid (V) differs from the lactone in that it can be oxidized by dilute nitric acid ($1HNO_3{:}4H_2O$) and the product (XI) is gossypolonic acid tetramethyl ether, the water-insoluble compound obtained by a similar oxidation of gossypol hexamethyl ether. It is probable that the ring containing the *peri* hydroxyl is converted to a quinone and since only two hydrogens for each quinone ring formed are lost in the process, a hydrogen in the



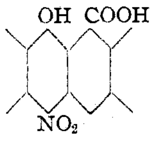
$$\left[C_{14}H_{11}(OCH_3)_2\left(\begin{matrix}-OH\\-COOH\end{matrix}\right)\right]_2 \longrightarrow \left[C_{14}H_{10}(OCH_3)_2\left(\begin{matrix}(=O)_2\\-COOH\end{matrix}\right)\right]_2$$

V XI

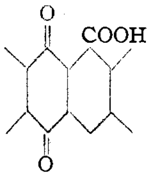
ortho position or more probably in the para position to the hydroxyl in the original ring must be unsubstituted (XII). The para hydrogen seems the more likely since the series of quinones in the gossypol and apogossypol series are all yellow in color while the vast majority of *o*-quinones are red or at least not yellow. The quinone produced by this oxidation may be assumed to have the structure shown in XIII.



XII



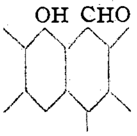
XIV



XIII

The lactone, however, when treated with the proper concentration of nitric acid was readily nitrated and two nitro groups were introduced (XIV). The corresponding diamine was formed by reduction and this was characterized through the acetyl derivative. The nitro group generally enters a 1-naphthol in the 4-position, if unoccupied, and this is in agreement with the results of the oxidation of the hydroxy acid.

Since the carboxyl group in the lactone which has been studied unquestionably has been formed from the corresponding aldehyde, three positions in each of the naphthalene rings in gossypol can be considered as definitely established (XV): 1-aldehydo, 5-hydrogen, 8-hydroxyl.



XV

Experimental

Gossylic Acid Lactone Tetramethyl Ether.—This was prepared by the method previously described.[3]

Gossylic Acid Tetramethyl Ether (V).—To a solution of 1.10 g. of gossylic acid lactone tetramethyl ether in 30 cc. of 10% methanolic potassium hydroxide was added about a gram of zinc dust and 10 cc. of water. The solution (deep red at first) was boiled until the color had faded to yellow and filtered into dilute sulfuric acid. The hydroxy acid was extracted with ether, the ether solution dried over anhydrous sodium sulfate and concentrated. Dilution with petroleum ether (b. p. 30–60°) caused the compound to crystallize in soft white needles weighing 0.85 g. Recrystallized from ether–petroleum ether, the compound formed soft white needles which, when heated slowly, melted at the melting point of the lactone, but when plunged into a bath preheated to 290° melted with vigorous decomposition, resolidified and then remelted at the melting point of the lactone.

Anal. Calcd. for $C_{28}H_{22}(OCH_3)_4(OH)_2(COOH)_2$: C, 67.33; H, 6.27. Found: C, 67.34; H, 6.20.

Dimethyl Gossylate Tetramethyl Ether (VI)

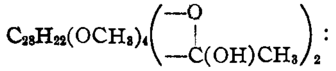
(1) **With Dimethyl Sulfate.**—To a hot solution of 0.5 g. of gossylic acid lactone tetramethyl ether in 10 cc. of 10% methanolic potassium hydroxide was added slowly 1.65 cc. of redistilled dimethyl sulfate. Then 15 cc. of potassium hydroxide and 1.7 cc. of dimethyl sulfate were added in that order and the mixture heated on the steam-bath for five minutes longer. After dilution with water, the mixture was allowed to stand for an hour and filtered. The air-dried solid obtained was recrystallized twice from dilute acetic acid, yielding 0.46 g. of white plates, m. p. 215–216.5°, to a yellow liquid which did not decompose when heated to 300°.

Anal. Calcd. for $C_{28}H_{22}(OCH_3)_6(COOCH_3)_2$: C, 68.89; H, 6.94; OCH_3, 37.5. Found: C, 69.18; H, 6.80; OCH_3, 35.6, 35.4.

The compound gives a pale yellow color in concentrated sulfuric acid. It is insoluble in alkali.

(2) **With Diazomethane.**—A solution of 0.18 g. of gossylic acid lactone tetramethyl ether in 10 cc. of 10% methanolic potassium hydroxide was diluted with 80 cc. of water and acidified with hydrochloric acid. The precipitated hydroxy acid was taken up in ether, extracted from the ether solution with sodium carbonate solution, and after acidification of the sodium carbonate solution extracted with 10 cc. of ether. The ether solution was added to 10 cc. of a solution of diazomethane in ether prepared from 0.2 g. of nitrosomethyl urea. Nitrogen was evolved for about ten minutes. The solution was allowed to stand overnight and then evaporated on the steam-bath. The yellow gum remaining was crystallized from dilute acetic acid by careful dilution of an acetic acid solution held at the boiling point. Recrystallized from 62% acetic acid, the compound formed colorless crystals which gave no depression in melting point when mixed with the compound obtained by methylating the lactone with dimethyl sulfate.

Homogossypol Tetramethyl Ether (VII).—To a solution of methylmagnesium iodide prepared from 10 g. of methyl iodide and 1.7 g. of magnesium in 50 cc. of dry ether was added a suspension of 3.5 g. of gossylic acid lactone tetramethyl ether in 40 cc. of dry ether. Reaction took place immediately with moderate refluxing of the solution, which acquired an orange color. Refluxing was continued for thirty minutes, at the end of which time the lactone had all dissolved. The solution was poured into an iced solution of ammonium chloride, and the ether layer was separated and concentrated. The product separated as a white crystalline powder as the ether evaporated. After washing with fresh ether the product weighed 3.07 g. Recrystallized from chloroform–petroleum ether (b. p. 90–110°) it formed a white crystalline powder, and from dilute pyridine it formed rosets of pinkish-white prisms, m. p. 308–309° (corr.), with decomposition.

Anal. Calcd. for  $C_{28}H_{22}(OCH_3)_4\left(\begin{array}{l}—O\\ \ |\\ —C(OH)CH_3\end{array}\right)_2$: C, 71.76; H, 6.98. Found: C, 71.97; H, 7.10.

It gives a deep yellow color with concentrated sulfuric acid and no ferric chloride color. It is readily soluble in alcoholic alkali to a rose-pink solution which is decolorized by sodium hydrosulfite. The compound is insoluble in sodium carbonate solution.

Phenylmagnesium bromide and gossylic acid lactone tetramethyl ether gave only oily products.

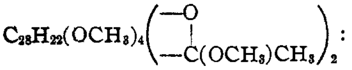
Methylation of Homogossypol Tetramethyl Ether

(1) **Dimethyl Sulfate and Alkali (VIII).**—To a solution of 0.5 g. of homogossypol tetramethyl ether in 5 cc. of 30% ethanolic potassium hydroxide, dimethyl sulfate was added until the solution was no longer alkaline. Alcoholic alkali and dimethyl sulfate were each added in slight excess several times; the solution was finally made alkaline, diluted and filtered. The crude product was white and crystalline and weighed 0.5 g. After two recrystallizations from methanol and two from dilute dioxane the compound was obtained as tiny clusters of white needles, m. p. 242–243° (corr.), to a colorless liquid which was stable up to 270°.

Anal. Calcd. for $C_{28}H_{22}(OCH_3)_6(COCH_3)_2$: C, 72.38; H, 7.30. Found: C, 72.61; H, 7.58.

The compound is insoluble in alcoholic alkali and gives a yellow color with concentrated sulfuric acid.

(2) **Methanol–Sulfuric Acid (IX).**—To a suspension of 0.2 g. of homogossypol tetramethyl ether in 10 cc. of methanol was added 8 drops of concentrated sulfuric acid. On warming, the compound dissolved with the formation of an emerald green solution and after a few moments' refluxing, colorless prisms began to separate from the solution. After cooling, the product was collected. It was obtained as colorless flat prisms and weighed 0.2 g. After several recrystallizations from dilute acetone it was obtained as flat white prisms, m. p. 255–256° (corr.), to a pink liquid. The same product was obtained by methylation in methanol with dimethyl sulfate and alkali but with an excess of dimethyl sulfate. In this case to a solution of 0.5 g. of homogossypol tetramethyl ether in 4 cc. of methanol and 4 cc. of dimethyl sulfate was added 6 cc. of 10% methanolic potassium hydroxide and the mixture was allowed to stand overnight. The solid (product and methyl potassium sulfate) was collected on a filter, the potassium salt washed out with water, and the water-insoluble portion recrystallized from dilute acetone. It formed flat white prisms, m. p. 255-256°, and gave no depression in melting point when mixed with the product obtained by the use of methanol–sulfuric acid.

Anal. Calcd. for  $C_{28}H_{22}(OCH_3)_4\left(\begin{array}{l}—O\\ \ |\\ —C(OCH_3)CH_3\end{array}\right)_2$: C, 72.38; H, 7.30. Found: C, 72.52; H, 7.23.

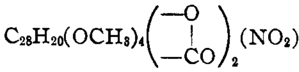
A mixture of IX (m. p. 255–256°) and VIII (m. p. 242–243°) melted at 221–236°.

The compound is insoluble in alcoholic alkali and gives a deep yellow color with concentrated sulfuric acid.

Oxidation of Gossylic Acid Tetramethyl Ether.—The directions followed for this oxidation were those previously described for the oxidation of gossypol hexamethyl ether. One gram of the lactone was converted to the hydroxy acid as described above, and the latter compound was refluxed for four hours with a mixture of 10 cc. of concentrated nitric acid and 40 cc. of water. The solid first became gummy, then gradually formed a hard pale yellow mass (with spots of red-brown gum). After cooling, the solution was neutralized and extracted with ether, the solid dissolved in this ether solution and the solvent removed and replaced with methanol. On standing, 0.48 g. of bright yellow needles separated. Recrystallized from benzene–ligroin (b. p. 60–110°), the compound formed soft yellow needles which gave no depression in melting point when mixed with the product obtained in a similar oxidation of gossypol hexamethyl ether, and gave identical color reactions in sulfuric acid (brownish-red) and methanolic alkali (blue-green).

Dinitrogossylic Acid Lactone Tetramethyl Ether (XIV).—A suspension of 0.95 g. of the lactone in a mixture of 20 cc. of glacial acetic acid and 3 cc. of concentrated nitric acid was heated to boiling on a hot-plate. The lactone dissolved in the course of several minutes to a clear brownish-yellow solution. Water was added carefully. The solution was kept at the boiling point until crystallization started, and the mixture was allowed to cool. The product separated in pale yellow, flat needles melting at 246–248°, and weighed 0.87 g.

Recrystallized from acetone–methanol, ether–petroleum ether or ethyl acetate–petroleum ether, the compound formed pale yellow prisms which melted at 238–239° (corr.), resolidified, and then remelted at 247–248° (corr.). Recrystallized from dilute acetic acid the compound formed flat, slender, pale yellow needles which melted sharply at 247–248°.

Anal. Calcd. for  $C_{28}H_{20}(OCH_3)_4\left(\begin{array}{l}—O\\ \ |\\ —CO\end{array}\right)_2(NO_2)_2$: C, 61.82; H, 4.85; N, 4.25. Found (methanol–acetone): C, 61.97; H, 5.03; N, 4.43. Found (dilute acetic acid): C, 62.16; H, 5.06; N, 4.48.

The compound gives a yellow color with concentrated sulfuric acid and no ferric chloride color. It is insoluble in aqueous alkali, but dissolves readily in cold methanolic alkali to a deep orange-red solution.

Diaminogossylic Acid Lactone Tetramethyl Ether.—Reduction of the nitro compound with zinc dust in acetic acid, sodium hydrosulfite in alkali or iron in acetic acid yields the same compound.

To a hot solution of 0.5 g. of the nitro compound (XIV) in 5 cc. of acetic acid was added 0.5 g. of iron powder. The pale yellow color of the solution changed quickly to a deep orange. After the addition of a drop of hydrochloric acid the solution was heated for thirty minutes on the steam bath. A heavy precipitate of orange needles formed after a few minutes.

At the end of thirty minutes some acetic acid was added to dissolve the product which had separated and the solution was filtered, several small portions of boiling acetic acid being used to extract the product which was admixed with the iron. The hot solution was diluted carefully until crystallization started. On cooling, there was obtained

0.38 g. of bright orange platelets, and on dilution of the mother liquors there was obtained an additional 80 mg. of what was largely unreduced nitro compound.

Recrystallization of the main portion several times from slightly diluted acetic acid (with the addition of a small amount of iron) resulted in the formation of bright orange platelets, m. p. 293–294° (corr.), with decomposition.

Anal. Calcd. for $C_{28}H_{20}(OCH_3)_4\left(\begin{matrix}-O\\ |\\ -CO\end{matrix}\right)_2(NH_2)_2$: C, 68.00; H, 6.00; N, 4.67. Found: C, 67.52; H, 6.09; N, 4.54.

The compound gives a pale yellow color with concentrated sulfuric acid and is readily soluble in methanolic hydrochloric acid (but not in methanol) or in a mixture of acetic acid and hydrochloric acid. Upon the addition of hydrochloric acid to the orange solution of the compound in acetic acid the color fades to pale yellow. It dissolves with some difficulty in warm alcoholic alkali with the formation of a yellow solution which rapidly turns deep blue in air.

Acetylation of Amino Derivative.—A solution of 0.27 g. of the diamine and 0.1 g. of anhydrous sodium acetate in 5 cc. of acetic anhydride was refluxed for one hour. The initially deep orange solution soon became a pale yellow. The excess acetic anhydride was decomposed with water and the product, which weighed 0.29 g., was recrystallized twice from methanol, from which it formed pearly-white leaflets, m. p. 252–253° (corr.).

Anal. Calcd. for $C_{28}H_{20}(OCH_3)_4\left(\begin{matrix}-O\\ |\\ -CO\end{matrix}\right)_2(NHCOCH_3)_2$: C, 66.66; H, 5.85; N, 4.10. Calcd. for $C_{28}H_{20}(OCH_3)_4\left(\begin{matrix}-O\\ |\\ -CO\end{matrix}\right)_2[N(COCH_3)_2]_2$: C, 65.63; H, 5.73; N, 3.65. Found: C, 66.03; H, 5.71; N, 3.71.

The compound is insoluble in alcoholic hydrochloric acid.

Summary

Gossylic acid lactone tetramethyl ether has been shown to be a *peri*-naphthalene lactone. It can be hydrolyzed to the corresponding hydroxy acid which can be methylated with diazomethane or alkali and dimethyl sulfate to the ether ester. The hydroxy acid is oxidized with dilute nitric acid to gossypolonic acid tetramethyl ether, the same water-insoluble quinone obtained by the oxidation of gossypol hexamethyl ether with nitric acid. The color indicates a *p*-quinone which demonstrates the probability of unsubstituted hydrogens para to the hydroxyls in the hydroxy acid.

The lactone is resistant to oxidation but is nitrated to a dinitro derivative. The nitro groups presumably occupy the unsubstituted positions para to the hydroxyls.

Gossypol can be considered as having two naphthalene nuclei each containing the following substitutions: 1-aldehydo, 5-hydrogen, 8-hydroxyl.

URBANA, ILLINOIS RECEIVED JUNE 27, 1938

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

Structure of Gossypol. XIII.[1] Conversion of Gossic Acid to Apogossypolic Acid

BY ROGER ADAMS AND R. C. MORRIS

Two products are obtained by oxidation of gossypol hexamethyl ether with nitric acid, a water-insoluble, gossypolonic acid tetramethyl ether, and a water-soluble, gossic acid. The former is converted into the latter by the action of potassium permanganate.[2] Gossic acid has approximately half the molecular weight of the original compound and consequently knowledge of its structure would be of unusual value in clarifying the structure of gossypol.

It was shown previously that gossic acid contains two methoxyls, one carboxyl, and one dibasic acid anhydride linkage formulated as I. These facts were deduced from a Zeisel determination, titration for a tribasic acid in aqueous solution, formation of a monomethyl ester (II) by the action of diazomethane and a molecular weight determination. Various derivatives and degradation products now have been prepared. Gossic acid is hydrolyzed by alkali to a tribasic acid (III) which could not be isolated in a pure state due to its tendency to revert to gossic acid. However, an ether solution of the hydrolyzed gossic acid was methylated with diazomethane and a stable crystalline trimethyl ester (IV) obtained. This confirms the presence of a carboxyl and a dibasic acid anhydride residue in gossic acid. By demethylation of gossic acid, the corresponding dihydroxy acid (V) was isolated readily. That no rearrangement was involved was indicated by the action of diazomethane on the demethylated product. The methyl ester

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[1] For previous paper in this series see Adams and Geissman, THIS JOURNAL, **60**, 2184 (1938).

[2] Adams, Morris and Kirkpatrick, *ibid.*, **60**, 2170 (1938).