

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 methoxy 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

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[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. KIRKPATRICK²

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 gossypol³ 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 ϵ 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

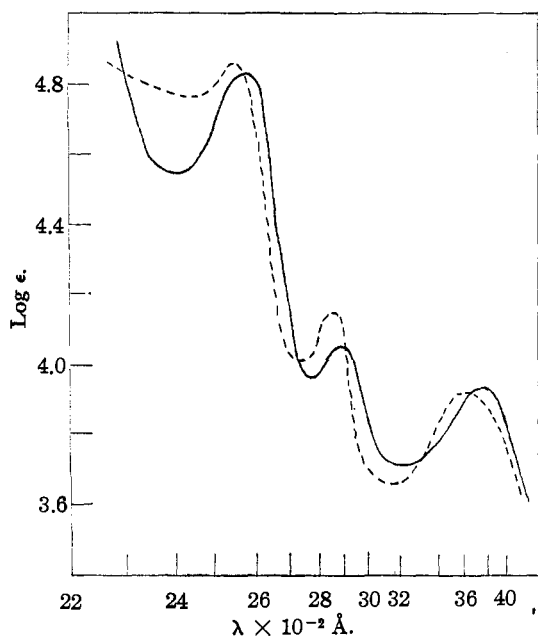
(1) For previous paper, see Adams and Butterbaugh, *THIS JOURNAL*, **60**, 2174 (1938).

(2) Portion of a thesis submitted in partial fulfillment 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.*, **266**, 295 (1936); see also Marchlewski, *J. prakt. Chem.*, **60**, 84 (1899); Tobler, Dissertation "Arbeiten über Gossypol," Zürich, 1932; Podolskaja, *Biochem. Z.*, **264**, 401 (1936); Zamyshlyayeva 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).

chromophores in the molecule, the peaks of the curves of the various derivatives of gossypol in which the functional groups have been modified or eliminated, should vary considerably from those of gossypol and in general should be less intense. This has been found not to be the case. Thus, for example, the absorption spectrum of apogossypol hexamethyl ether (Figs. 1 and 4) which has two aldehyde groups less than gossypol shows only minor differences in the two main peaks from that of gossypol, instead of approaching in resemblance more closely that of naphthalene.



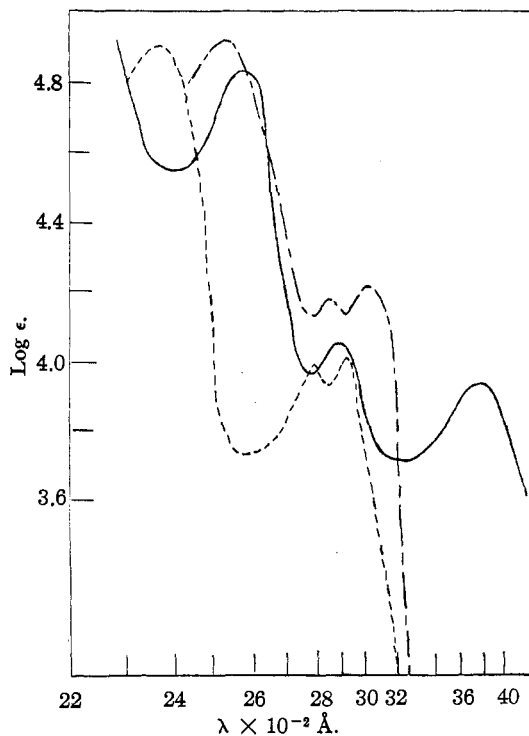
(1) —, Gossypol; (2) ---, gossypol dimethyl ether.

Fig. 1.

The spectra of compounds with more complex condensed ring systems such as phenanthrene, anthracene, chrysene, naphthacene, benzanthracene, etc., are quite different from those of naphthalene derivatives or of gossypol. Both absorption regions, $\log \epsilon = 4$ and $\log \epsilon = 5$, become much broader, contain many more peaks shifted toward the visible and in general show no sharp distinction between the two regions. Closely-packed ring systems such as pyrene and perylene enhance this effect so that they both have strong absorption in the visible. Fluorene, as illustrative of a molecule containing separated benzene rings, has much less intensity of absorption than gossypol. Oxygen-containing rings and

quinones also have distinctly different spectra.⁵

The difference between the two principal peaks in gossypol and in naphthalene derivatives is one of intensity. The enhancement of the intensity in the gossypol spectrum is by an amount which could be satisfactorily explained by assuming double the number of naphthalene rings per mole.



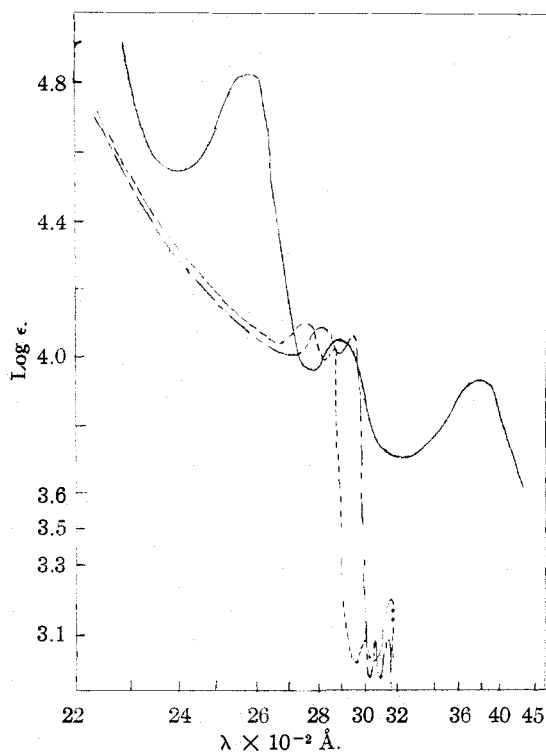
(1) —, Gossypol; (2) —, β,β -binaphthyl; (3) ---, α,α -binaphthyl.

Fig. 2.

Consequently, the absorption spectra have been determined of four dinaphthalene compounds: α,α -binaphthyl, β,β -binaphthyl, *sym*-di- α -naphthylethane, and *sym*-di- β -naphthylethane (Figs. 2 and 3). These all have absorption regions of the same character and intensity as gossypol but nearer the ultraviolet. Because of the many substituents in gossypol, it is impossible to identify its absorption spectrum with any one of the four. However, the $\log \epsilon = 5$ absorption region is closer to the visible in the two binaphthyls than in the two dinaphthylethanes and consequently the position and character of the peaks

(5) (a) Dadiou, *Z. physik. Chem.*, **135**, 347 (1928); (b) Mayneord and Roe, *Proc. Roy. Soc. (London)*, **152**, 299 (1935); **158**, 634 (1937); (c) Seshan, *Proc. Ind. Acad. Sci.*, **3A**, 148, 191 (1936); (d) Conrad-Billroth, *Z. physik. Chem.*, **B15**, 1 (1932); (e) Macbeth, Price and Winzor, *J. Chem. Soc.*, 325 (1935); (f) Shibata and Kimatsuki *J. Tokyo Chem. Soc.*, **39**, 771 (1918); Shibata and Nagai, *J. Chem. Soc. Japan*, **43**, 101 (1922); Tasaki, *Acta Phytochim.*, **2**, 119 (1925).

in the curves of the former two resemble more closely those of gossypol. The conclusion is that either an α,α -binaphthyl or a β,β -binaphthyl is probably the basic nucleus of gossypol.

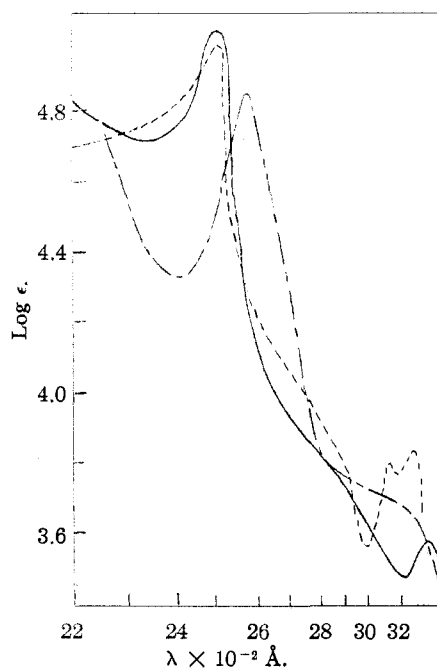


(1) —, Gossypol; (2) ---, *sym*-di- β -naphthylethane; (3) - · -, *sym*-di- α -naphthylethane.

Fig. 3.

Gossypol and gossypol dimethyl ether, which resemble each other chemically, have very similar absorption spectra, Fig. 1, with an absorption maximum at 3600 Å. This maximum was not found in the spectra of gossypol tetramethyl ether, hexamethyl ether or hexaacetate. All of these gossypol derivatives show none of the characteristic aldehyde reactions of gossypol and are in general less reactive. The fact that the region of strong absorption in or near the visible is a low energy excitation coupled with its complete disappearance in the more highly methylated or the acetylated derivatives points strongly to a tautomeric structure in gossypol and gossypol dimethyl ether. Tautomerism is also to be inferred from the fact that ordinary analogous hydroxy and methoxy compounds differ from each other in absorption spectra only slightly whereas a marked change occurs in these gossypol derivatives when methylation or acetylation has been effected.

The absorption spectra of gossypol hexamethyl ether, apogossypol hexamethyl ether and desapogossypol hexamethyl ether are shown in Fig. 4. The basic nuclei must be very similar or identical in all of these. Apogossypol hexamethyl ether is converted into desapogossypol hexamethyl ether by the action of cold sulfuric acid. The alteration which takes place in this transformation as deduced from absorption spectra comparisons must be in the substituents and not in the nucleus. This is confirmatory of the deductions from chemical information.

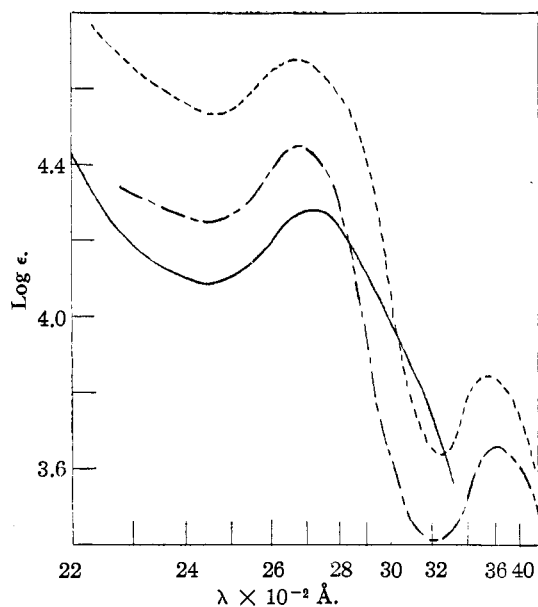


(1) —, Apogossypol hexamethylether; (2) ---, desapogossypol hexamethyl ether; (3) - · -, gossypol hexamethyl ether.

Fig. 4.

Gossypol hexamethyl ether is oxidized first to gossypolone tetramethyl ether, then to gossypolonic acid tetramethyl ether. Apogossypol hexamethyl ether is oxidized to apogossypolone tetramethyl ether. The molecular weights of all these oxidation products are not far different from the parent compounds. The absorption spectra, however, are materially different. In place of the sharp peak near 2500 Å. in the unoxidized substances, these products have less intense broad bands at a position nearer the visible, (Fig. 5). Solubility difficulties prevented the study of apogossypolone tetramethyl ether at higher concentrations which would allow

observation of the second less intense maximum. The curve obtained, however, is less intense though very similar to that of the gossypolone tetramethyl ether or gossypolonic acid tetramethyl ether. The difference in the absorption spectra between the unoxidized and oxidized products resembles the difference between the absorption spectrum of naphthalene and 1,4-naphthoquinone. Broad bands appear with decrease in intensity indicating a change in ring structure.^{5c,d} These facts substantiate the chemical experiments which gave evidence of quinone nuclei.



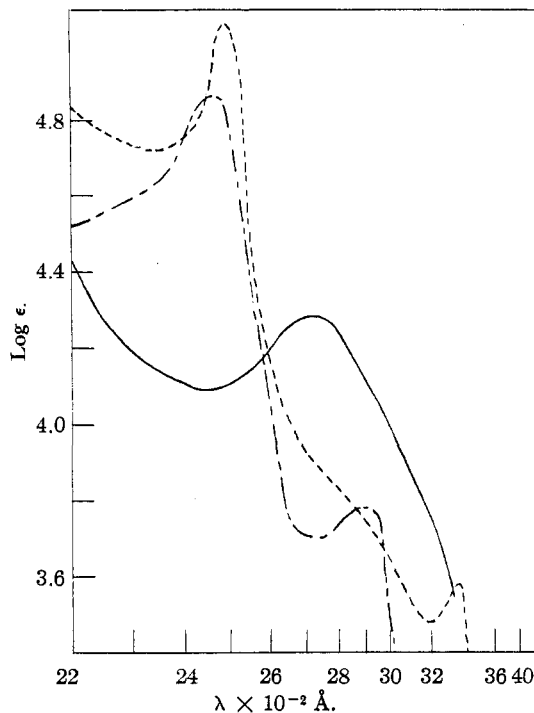
(1) —, Apogossypolone tetramethyl ether; (2) ---, gossypolone tetramethyl ether; (3) —·—, gossypolonic acid tetramethyl ether.

Fig. 5.

Reductive acetylation of apogossypolone tetramethyl ether results in hydroapogossypolone tetramethyl ether tetraacetate and the latter product⁶ has a spectrum resembling that of apogossypol tetramethyl ether, thus suggesting a return to the original ring structure. These changes are shown in Fig. 6. Conversion of a naphthalene to a naphthoquinone then reconversion to a naphthalene may be inferred from the absorption spectra of this series of compounds.

Anhydrogossypol (Fig. 7) has a spectrum en-

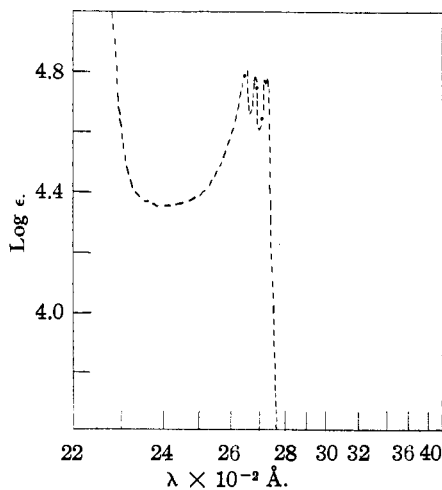
(6) In taking the absorption spectra, hydroapogossypolone octaacetate was the substance actually used. Since, however, the acetates and the ethers in the apogossypol series resemble each other very closely, the curve given in Fig. 6 must be essentially the same as that of the tetramethyl ether tetraacetate.



(1) ---, Apogossypol hexamethyl ether; (2) —, apogossypolone tetramethyl ether; (3) —·—, hydroapogossypolone octaacetate.

Fig. 6.

tirely different from those of other gossypol derivatives tested. It may be concluded that loss of water when gossypol is converted to anhydrogossypol involves a deep-seated change such as the formation of a new ring containing unsaturation and the alteration of the electronic configuration of the basic ring structure.



Anhydrogossypol.

Fig. 7.

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 H α , H β and H γ 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 $\times 10^{-4}$ molar were diluted to yield a series of seven concentrations between this and 5 $\times 10^{-6}$ 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 dioxane⁷ was used as a solvent for all four. That dioxane gives absorption spectra comparable to those taken in alcohol was shown by

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

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.

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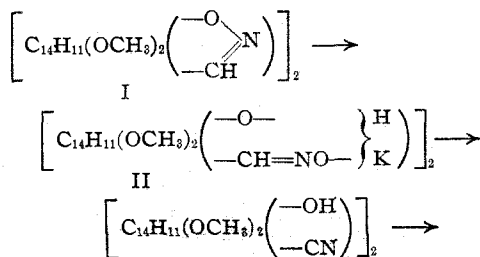
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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

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

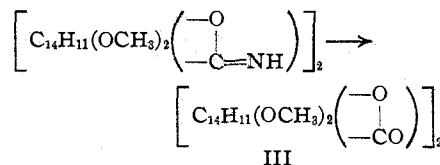
BY ROGER ADAMS AND T. A. GEISSMAN

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



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

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



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.

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