[Contribution from the Food Research Division, Chemical and Technological Research, Bureau of Chemistry and Soils, United States Department of Agriculture]

STUDIES ON GOSSYPOL. V. THE ACTION OF CHROMIC ACID UPON SOME GOSSYPOL DERIVATIVES

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In a former paper[2] experiments were reported in which attempts were made to oxidize gossypol. The experience gained in this work indicated that, before this approach to the structural nature of the substance could be successful, the hydroxyl groups in the molecules would have to be protected.

Several derivatives fulfilling this requirement have been prepared, and their behavior toward various oxidizing reagents has been studied. This report is concerned with the products obtained by the action of chromic acid upon hexa-acetylgossypol, hexa-acetylapogossypol and apogossypol hexamethyl ether.

When dissolved in acetic acid and oxidized with an aqueous solution of chromic acid, hexa-acetylgossypol yields a bright yellow neutral compound which begins to darken at 210° and becomes a black mass at 230° without definitely melting. This substance has the molecular formula $C_{33}H_{30}O_{12}$. It possesses no carboxyl or free hydroxyl groups but has four acetyl groups and is, therefore, a tetra-acetyl derivative of a substance, $C_{25}H_{22}O_8$, to which the name *gossypolone* has been given. The derivative obtained is, therefore, tetra-acetylgossypolone. From a consideration of the foregoing it follows that in the formation of tetra-acetylgossypolone from hexa-acetylgossypol two acetyl groups are replaced by two quinone groups, and carbon and hydrogen are lost in such proportions that gossypolone has a lower molecular weight than gossypol by C_5H_8.

Tetra-acetylgossypolone readily condenses with aniline in much the same manner as does gossypol. The condensation product is a chocolate-colored micro-crystalline substance which imparts a deep wine-red color to solvents capable of dissolving it. It would appear from this that the two carbonyl groups originally in gossypol have not been affected by the oxidation.

When hexa-acetylapogossypol is treated in the same manner as hexa-acetylgossypol, except that Kiliani's chromic acid mixture[3] is used, a substance similar in appearance to tetra-acetylgossypolone is obtained. This is a neutral material having a melting point of 230° and a molecular formula

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[1] This work was done under a research fellowship supported by the Interstate Cottonseed Crushers' Association.

[2] Clark, *J. Biol. Chem.*, **77**, 81 (1928).

[3] Kiliani, *Ber.*, **46**, 676 (1913).

of $C_{30}H_{28}O_{10}$. It has been shown to be a tetra-acetyl derivative of a compound $C_{22}H_{20}O_6$. The name assigned to this substance is *apogossypolone.* In the reaction by which this material is formed, as in the previous one, two quinone groups have been substituted for two acetyl groups and the new non-acetylated derivative has a lower molecular weight than apogossypol by C_6H_{10}. One property of this new acetyl derivative which distinguishes it from tetra-acetylgossypolone is its inability to condense with aniline.

The oxidation of apogossypol hexamethyl ether with chromic and sulfuric acids (Kiliani's mixture) gave a bright yellow neutral crystalline compound, $C_{32}H_{34}O_8$, having a melting point of 210°. It has been established that this material is a tetramethyl ether of a tetraquinone, $C_{28}H_{26}O_8$. The name *pseudogossypolone* has been assigned this substance, and hence the C_{32} compound isolated is *tetramethoxy-pseudogossypolone.* The process by which it is formed is analogous to the reactions involving the acetyl derivatives discussed before, in that two quinone groups have been substituted for two methoxyl groups. On the other hand, methoxy derivatives of gossypol in general are far more resistant to reagents than the corresponding acetyl derivatives. In this experiment chromic acid did not attack the apogossypol molecule in such a manner as to cause a loss of carbon. Two quinone groups, however, were substituted for two hydrogen atoms.

The observations recorded concerning these new substances cannot be interpreted at present, since the loss of the elements, C_5H_8 and C_6H_{10}, without the introduction of oxygen into the molecule is a condition which could be explained by several possible types of reaction. Furthermore, the substitution of quinone groups for hydrogen atoms, or acetyl or methoxyl groups, as the case may be, points at present to the existence in the molecule of only aromatic nuclei. This conclusion, of course, has already been arrived at from previous work.

Some interesting observations relative to Zeisel determinations were made upon several gossypol derivatives. In the estimation of the methoxyl content of tetramethoxy-pseudogossypolone and of certain related compounds to be reported upon at a future time, difficulties arose which at first seemed to render the determinations impossible. Later, results were obtained, but they failed to harmonize with other information available. Finally, however, successful determinations were made but the rather unusual and anomalous data leading thereto were such that it was deemed desirable to record them separately in the report which follows.

Experimental

Tetra-acetylgossypolone.—Two grams of hexa-acetylgossypol dissolved in 50 cc. of boiling acetic acid was treated with 10 cc. of a 10% aqueous solution of chromium trioxide. The mixture was kept boiling for one minute after the addition of the reagent and then the oxidation was stopped by the addition of about 100 g. of crushed ice. The dilution caused the reaction product to separate as a bright yellow amorphous precipitate. It was freed from the mother liquor, washed with water and dried. The yield was 1.6 g. When this material was digested with approximately 20 cc. of boiling methanol, it quickly dissolved, but almost immediately a crystalline fraction began to separate. After some time the crystals were removed from the mother liquor, washed with methanol and dried. The yield of this product was 0.6 g. Crude amorphous acetylgossypol obtained directly from acetylating gossypol in pyridine solution gave the same results as the crystalline material. In a series of experiments 28 g. of the crude acetyl derivative gave 23 g. of the amorphous oxidation product, which yielded 11 g. of crystalline substance.

This material was purified by dissolving one part of the substance in ten parts of acetic acid and adding five volumes of ether to this solution. Crystallization began immediately and was completed in a short time.

Thus purified, the substance consists of long bright yellow rods and needles which begin to darken at 210° and become a black mass at 230° without melting. Its indices of refraction are $n_\alpha = 1.559$; $n_\gamma = 1.676$ both ± 0.002. The elongation is negative and the extinction is essentially straight (the extinction angle is very small).[4] The analytical data relative to this and to the other compounds under consideration are presented in Table I.

TABLE I

ANALYSIS OF OXIDATION PRODUCTS

Substance	Formula	Mol. wt.[a]		Carbon		Hydrogen		Acetyl[b]	
		Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
Tetra-acetylgossypolone	$C_{33}H_{30}O_{12}$	618.4	622	64.06	64.10 64.03	4.89	4.80 4.79	4 groups, 27.83	24.02 24.00[c]
Tetra-acetylapogossypolone	$C_{30}H_{28}O_{10}$	548.4	555	65.67	65.49 65.76	5.15	5.18 5.18	4 groups, 31.38	29.03
								Methoxyl	
Tetramethoxypseudogossypolone	$C_{32}H_{34}O_8$	546.4	569	70.31	70.49 70.34	6.27	6.26 6.15	4 groups, 22.7	23.77 23.79

[a] Rast, *Ber.*, **55**, 1051 (1922).

[b] Perkin's N-Acyl method, *J. Chem. Soc.*, **87**, 107 (1905).

[c] Recrystallized a second time from benzene and ether.

Tetra-acetylgossypolone—Aniline Condensation Product, $C_{33}H_{30}O_{12} + 2\ C_6H_5NH_2 \longrightarrow C_{45}H_{40}N_2O_{10} + 2H_2O$.—Tetra-acetylgossypolone was suspended in methanol and an excess of aniline was added. The quinone dissolved with the formation of a deep wine-colored solution from which the condensation product soon began to crystallize. The process was completed after several hours. The material was purified by dissolving it in a large volume of boiling benzene, filtering the hot solution and allowing the substance to crystallize from the filtrate. Thus purified, it consisted of clusters of minute spindle-shaped chocolate-colored crystals which began to soften at 250° and melted at 255–256° (corr.).

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[4] The optical data reported in this communication were determined by Mr. George L. Keenan of the Food, Drug and Insecticide Administration of the Department of Agriculture.

Anal. Calcd. for $C_{45}H_{40}N_2O_{10}$: N, 3.64. Found: N, 3.61, 3.57.

Tetra-acetylapogossypolone.—Two g. of hexa-acetylapogossypol was dissolved in 100 cc. of boiling acetic acid and treated with 15 cc. of Kiliani's solution,[5] the reagent being added from a pipet during the course of half a minute. The reaction, which was vigorous, was terminated by adding about 300 g. of crushed ice. The yellow amorphous product was then treated with methanol as outlined in the foregoing experiment. The yield of the crude crystalline product was 0.45 g. or 29%. The material was recrystallized from boiling methanol. Thus purified, it consists of oblique prisms suggesting the common crystalline forms of gypsum. It begins to sinter at 220° and melts completely at 230° (corrected); $n_\alpha = 1.530$; $n_\gamma = 1.645$ (both ±0.003). The extinction on the elongated forms is straight and the sign of elongation is positive.

Tetramethoxypseudogossypolone.—This compound was prepared by essentially the same process that was used for the two substances just described. Two g. of apogossypol hexamethyl ether was dissolved in 100 cc. of boiling acetic acid and treated with 20 cc. of Kiliani's mixture in such a manner as to prevent too energetic boiling. After two minutes the reaction was stopped by adding about 200 g. of crushed ice. The yellow precipitate, which weighed 1.5 g., was dissolved in 20 cc. of boiling methanol and allowed to crystallize. The yield of the crystalline quinone was 0.4 g. It was recrystallized from its solution in 10 parts of acetic acid by the addition of 5 parts of methanol. Thus obtained, it consists of long yellow needles melting at 210° (corr.). Its indices of refraction are $n_\alpha = 1.585$; $n_\gamma = 1.700$ (both ±0.003). The extinction is inclined and the birefringence is strong.

Summary

Upon oxidation with chromic acid, hexa-acetylgossypol is converted into tetra-acetylgossypolone. In the reaction two acetyl groups are replaced by two quinone groups and the new non-acetylated substance has a lower molecular weight than gossypol by C_5H_8.

Hexa-acetylapogossypol yields by treatment with Kiliani's chromic acid mixture a new quinone, tetra-acetylapogossypolone. In this reaction two quinone groups are substituted for two acetyl groups and the apogossypol molecule loses the elements C_6H_{10}.

When oxidized with chromic and sulfuric acids, apogossypol hexamethyl ether loses two methoxyl groups, which are replaced by two quinone groups. Two hydrogen atoms are also oxidized to quinone groups. The new substance, $C_{32}H_{34}O_8$, is, therefore, a tetramethoxy derivative of a tetraquinone, $C_{28}H_{26}O_8$.

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[5] Four hundred g. of water, 80 g. of sulfuric acid and 53 g. of chromium trioxide.