Analyses for sulfur were either too low or too high for cyclic polymers. Also, a cyclic polymer would not possess a variable melting point since it would represent one molecular species. These facts indicate rather clearly that the above polymers are linear. The length of the chain and consequently the physical properties were determined by the conditions under which they were formed. If the terminal valence were satisfied by non-sulfur groups, the sulfur content was found to run low, and *vice versa*.

Vacuum distillations of the polymers in the anthracene series led to results entirely different from those obtained in the naphthalene and benzene series. The polymers, m. p. 266 and 263°, upon being heated above their melting points, gave tars from which nothing definite was isolated except 9-methylanthracene. Much hydrogen sulfide was evolved.

Summary

1. The 2,4-dinitrophenylhydrazones of anthracene-aldehyde-9 and β-ethoxynaphthaldehyde were characterized.

2. A monomeric thioaldehyde was isolated and its tendency to polymerize was studied.

3. An excellent synthesis for naphthyl substituted ethylenes was obtained.

4. Several new polymerized thioaldehydes were prepared.

5. The effect of group size upon ring closure was discussed.

CHAPEL HILL, N. C. RECEIVED JUNE 23, 1937

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

The Structure of Gossypol. I

BY K. N. CAMPBELL, R. C. MORRIS[1] AND ROGER ADAMS

Gossypol,[2] the yellow pigment present in cottonseed, was first isolated by Marchlewski.[3] Although it has been investigated in several laboratories,[4] no structural formula has as yet been suggested for it.

The principal difficulty thus far encountered in the study of gossypol has been due, primarily, to the lack of methods of preparation of more than a very limited number of pure crystalline derivatives or degradation products suitable for structural deductions. Gossypol is highly reactive chemically but is sensitive to oxidation, particularly in alkaline solution, and exhibits an especial tendency under the usual experimental conditions to yield amorphous or ill-defined crystalline compounds or mixtures extraordinarily resistant to purification.

The preliminary results in this investigation were similar and led to the conclusion that only by limiting the study to the preparation of those derivatives of definite, unquestioned purity, could important contributions to the knowledge of the chemistry of gossypol be accomplished. The extraction of gossypol, its purification, the explanation of its various melting points and its color reactions are described in this preliminary paper.

Many researches have been reported on the methods of isolation. The procedure found most satisfactory is that described by Carruth,[4d] who extracted the oil from the ground seed with petroleum ether and then extracted the gossypol with ether. The gossypol was isolated as gossypol-acetic acid.

A more rapid method of extraction described by Clark[5] also was investigated, but due to the large amount of oil present the separation of gossypol-acetic acid was found to be extremely slow. The method of Carruth was adopted in preference.

The gossypol-acetic acid was converted into gossypol by dissolving in ether, adding water and evaporating the ether from the mixture. A brownish product is thus obtained which is difficult to get perfectly pure by crystallization and which, even after purificaton, gives a reddish solution when dissolved in ether. The recorded observations that gossypol is oxidized readily and is not reduced by sulfur dioxide led us to a slight modification of the procedure which proved

---

(1) A portion of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

(2) The authors are indebted to the Buckeye Cotton Oil Company, subsidiary of The Proctor and Gamble Company, for a gift of the cottonseed meats from which the gossypol was extracted.

(3) Marchlewski, *J. prakt. Chem.*, **60**, 84 (1899).

(4) (a) Carruth and Winters, *J. Biol. Chem.*, **32**, 245 (1917); Schwartz and Alsberg, *J. Agr. Research*, **25**, 285 (1923); (b) Carruth and Winters, *ibid.*, **12**, 83 (1918); (c) Gallup, *J. Biol. Chem.*, **77**, 437 (1928); (d) Carruth, THIS JOURNAL, **40**, 647 (1918); (e) Clark, *J. Biol. Chem.*, **75**, 725 (1927); (f) *ibid.*, **76**, 229 (1928); (g) *ibid.*, **77**, 81 (1928); (h) *ibid.*, **78**, 159 (1928); (i) THIS JOURNAL, **51**, 1475, 1479 (1929); *Oil & Fat Industries*, **6**, July (1929); (j) Karrer and Tobler, *Helv. Chim. Acta*, **15**, 1204 (1932); (k) Schmid and Margulies, *Monatsh.*, **65**, 391 (1934); (l) Grünbaumówna and Marchlewski, *Biochem. Z.*, **286**, 295 (1936); (m) Podolskaja, *ibid.*, **284**, 401 (1936); *Fettchemische Umschau.*, **42**, 96 (1935).

(5) Clark, *Oil & Fat Industries*, **6**, 15 (1929).

to be highly advantageous. A small amount of sodium hydrosulfite was merely dissolved in the water used for decomposing the gossypol-acetic acid. The product thus obtained is crystallized readily in the form of brilliant yellow needles, m. p. 184°, which dissolve in ether to give a yellow solution.

Several melting points have been reported for gossypol, 184°,[4k] 199°,[4j] and 214°,[4e] but no explanation of the wide variance has been offered. A careful study has revealed that three crystalline forms of this substance exist, each with a characteristic melting point, and that interconversion of the forms may be accomplished by use of the appropriate solvents.

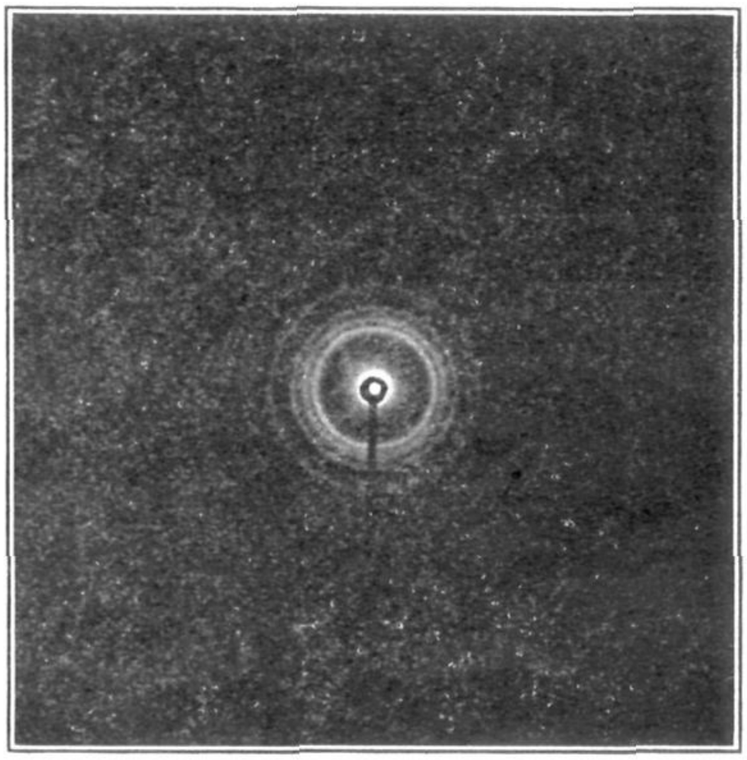

Plate 1.—Gossypol, m. p. 184°.

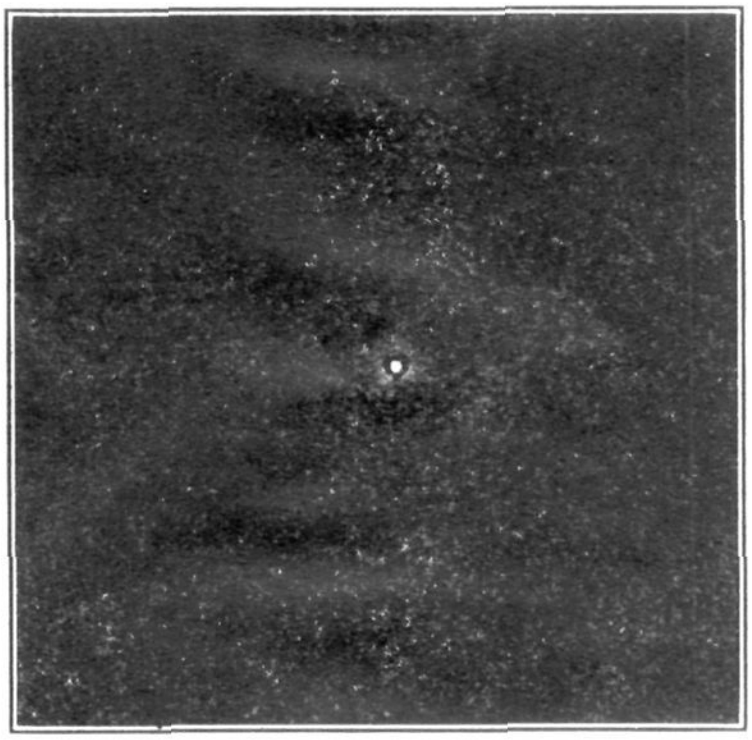

Plate 2.—Gossypol, m. p. 199°.

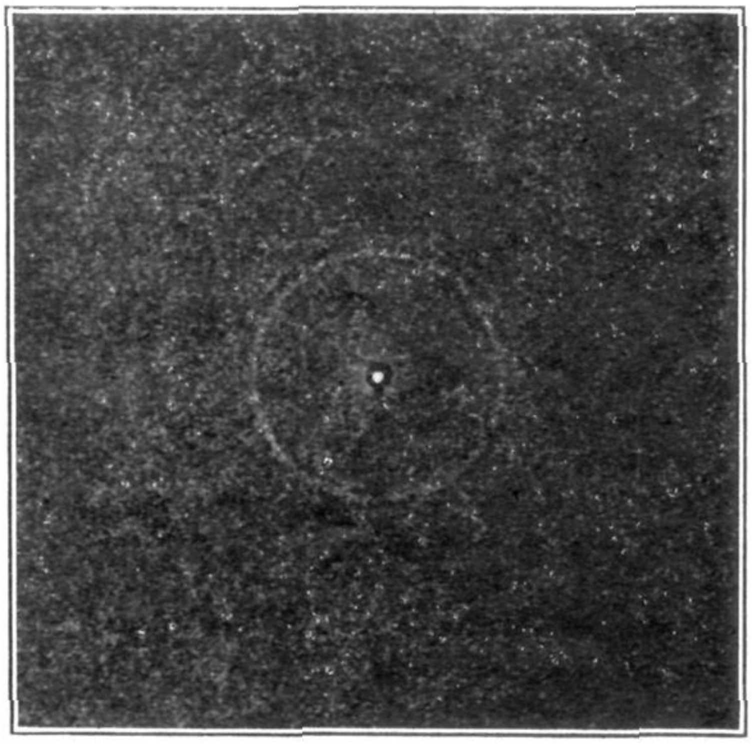

Plate 3.—Gossypol, m. p. 214°.

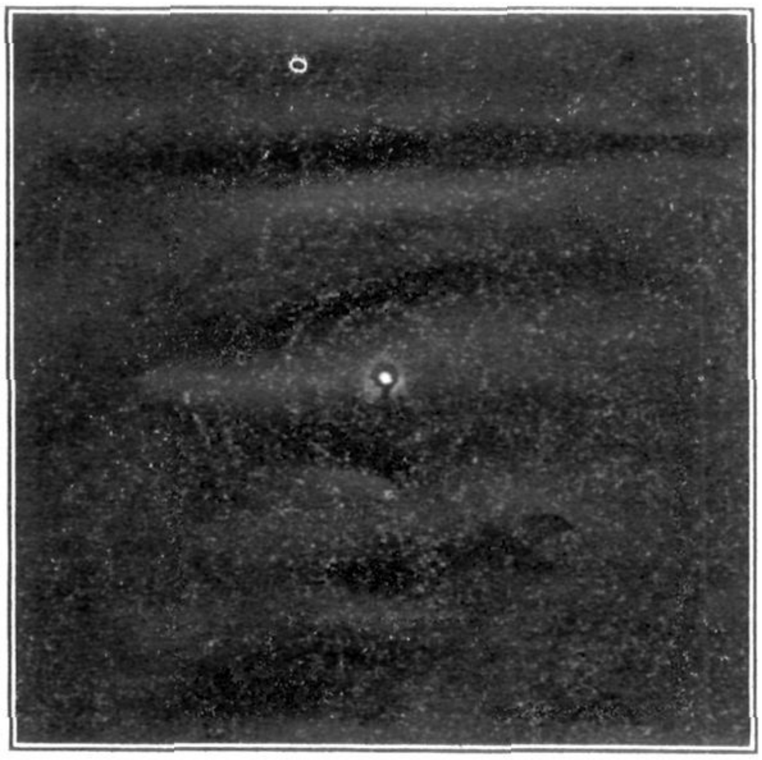

Plate 4.—Gossypol-acetic acid.

Plates 1–5.—X-Ray diffraction patterns of crystals. The X-Ray diffraction patterns were registered using the monochromatic pinhole technique, the metalix iron target tube operating at 15 milliamperes under a potential of 24 kilovolts, the sample thickness being adjusted to absorb approximately two-thirds of the incident intensity. The plate to specimen distance was 5.0 cm. and the exposure time three hours.

(See opposite page for Plate 5).

When pure gossypol, m. p. 184°, is recrystallized from chloroform, it undergoes a change in crystalline form and the product melts at 199°; crystallization from ether causes a reversion of the 199° to the 184°. The form melting at 199° may be converted into that melting at 214° by recrystallization from high-boiling ligroin (b. p. 60–110°). The reverse change is effected by recrystallization from chloroform. The form melting at 184° may be changed into the one melting at 214° by the use of high-boiling ligroin (b. p. 60–110°). Ether reverses this change. These experiments are summarized in the following diagram:

$$\begin{tikzcd}
& \text{Gossypol, m. p. } 184^\circ \arrow[dl, shift left, "\text{CHCl}_3"] \arrow[dr, shift left, "\text{Ligroin}"] & \\
\text{M. p. } 199^\circ \arrow[ur, shift left, "\text{Ether}"] \arrow[rr, shift left, "\text{Ligroin}"] & & \text{M. p. } 214^\circ \arrow[ul, shift left, "\text{Ether}"] \arrow[ll, shift left, "\text{CHCl}_3"]
\end{tikzcd}$$

X-Ray diffraction patterns were made of these various forms and showed unequivocally that the products are crystalline (Figs. 1, 2, 3, 4, 5). Examination under a polarizing microscope showed that the various modifications have different optical properties and different crystalline forms (Figs. 6–10).[6]

A red isomeric form of gossypol has been isolated recently by Podolskaja.[4m] This material melts at 184–185° and gives the reactions of ordinary yellow gossypol to which it is readily converted by means of solvents such as alcohol or ether. Yellow gossypol in chloroform gives a red solution when treated with dry hydrogen chloride, but this color is not due to red gossypol, since yellow gossypol can be recovered readily by precipitation with ligroin. Merely grinding the red form in a mortar converts it to the yellow.

Gossypol reacts readily with acetic acid to give gossypol-acetic acid, which is a very convenient intermediate in the extraction and purification process. Analogous derivatives are formed with other organic acids. Crystalline compounds composed of one molecule of gossypol and one molecule of the acids, formic, propionic, and butyric, have been prepared. It is not impossible that the highly-colored products formed when gossypol is treated with mineral acids are of a similar character.

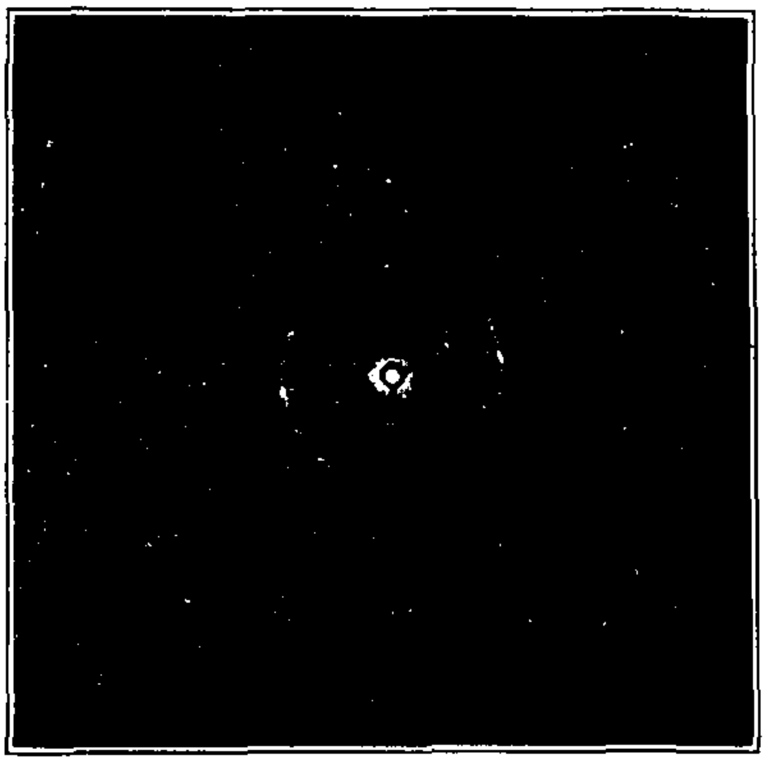

Plate 5.—Red gossypol.

Gossypol has been reported by several investigators to have the empirical formula $C_{30}H_{30}O_8$, and certainly the derivatives indicate this to be correct. Many samples of pure gossypol, extracted and purified in different ways, were analyzed during the present investigation. The results gave values for hydrogen somewhat too high for the above-mentioned formula. It is possible that this discrepancy can be explained by the fact that the usual precautions in the microanalytical technique are not adequate in the case of gossypol.

The previously described reactions of gossypol which have resulted in pure derivatives of significance for structural deductions may be reviewed briefly.

(1) A hexaacetate indicates six hydroxyl groups. Titration of gossypol or quantitative deacetylation of the hexaacetate demonstrates that two of the six hydroxyls are much more acidic than the other four. The hexaacetate on oxidation undergoes partial degradation to give a substance, tetraacetylgossypolone, reported as a quinone.[4j]

(2) Elimination of two molecules of water from gossypol takes place by heating and crystalline anhydrogossypol is obtained.[4e]

(3) Aniline and hydroxylamine give dianilino and dihydroxylamino derivatives with the loss in each case of two molecules of water.[4e]

(4) One molecule of o-phenylenediamine and one of gossypol react to form a red derivative with the loss of two molecules of water.[4j]

(5) Deep-seated degradation of gossypol by

---

(6) The diffraction patterns and photographs were kindly made for us by Dr. J. N. Mrgudich of the Division of Analytical Chemistry of the University of Illinois.

means of potassium permanganate results in the isolation of isobutyric acid.[4g]

(6) Catalytic reduction and subsequent distillation with zinc dust is reported to give β-isoamylnaphthalene.[4k]

(7) Strong caustic soda converts gossypol to a derivative designated as apogossypol ($C_{28}H_{30}O_6$), which has two carbon and two oxygen atoms less than gossypol.[4h] Although apogossypol is relatively unstable, it forms a stable hexamethyl ether and a hexaacetate. These derivatives do not react with ketone reagents and can be partially degraded by oxidation to crystalline compounds which are reported as quinones.[4i]

The color reactions, such as have been applied to naphthoquinones, anthraquinones and their derivatives have been tried in this investigation with gossypol and are summarized in the following table.

Color Tests—Gossypol

Concentrated H_2SO_4	Scarlet
Ferric chloride[a]	Dark olive green
Thiophene-H_2SO_4[b]	Negative
Active methylene[c]	Negative
Nickel acetate[d]	Violet
Pyroboroacetate[e]	Deep red
Stannic chloride[f]	Purple-red

[a] Perkin, *J. Chem. Soc.*, **103**, 209, 655 (1913). [b] Paternò, *Gazz. chim. ital.*, **21**, 377 (1891); Volhard, *Ann.*, **296**, 19 (1897); Hooker, This Journal, **58**, 1178 (1936). [c] Ionescu, *Bull. soc. chim.*, **41**, 1094 (1927); Kesting, *Z. angew. Chem.*, **41**, 358, 745 (1928); Winzor, *J. Chem. Soc.*, 326, 334 (1935). [d] Brissemoret and Coombes, *J. pharm. chim.*, **25**, 53 (1907); Winzor, *J. Chem. Soc.*, 326, 334 (1935). [e] Dimroth, *Ber.*, **54**, 3022 (1921); *Ann.*, **446**, 108 (1926); Fieser, This Journal, **51**, 2475 (1929). [f] Winzor, *J. Chem. Soc.*, 326, 334 (1935).

It is improbable that these tests are very specific or very reliable, and without other chemical evidence could not be used as a trustworthy indication of the presence of certain structural groups.

The violet color with nickel acetate might indicate a naphthalene nucleus; the green with ferric chloride, two phenolic hydroxyls *ortho* to each other; the deep red with pyroboroacetate, or the purple-red with stannic chloride, an hydroxyl *peri* or *ortho* to a carbonyl group.

The gossypol–stannic chloride compound was isolated and analyzed. It was shown to have a Sn:Cl ratio of 1:2. In the naphthalene series, this appears to indicate two *peri* hydroxyls and the presence of a carbonyl group; not an hydroxyl *ortho* or *peri* to a quinone.

A pure crystalline dipyridine salt was prepared from gossypol. This may indicate that two hydroxyls at least are not *peri* to carbonyl groups since these are reported not to give pyridine salts.

From all the results thus far described it has been established definitely, merely that gossypol contains six hydroxyls, two much more acidic than the other four. The structure of gossypol must account for the loss of two molecules of water and reactivity with aniline and hydroxylamine. It cannot be concluded that two ketone or quinone carbonyls are present, since other groupings are known to form derivatives with these reagents. Neither does *o*-phenylenediamine assure the presence of a 1,2-diketone or an *o*-quinone for the same reason. It is probable that gossypol contains a naphthalene nucleus and another grouping which gives on degradation isobutyric acid, but further confirmation is desirable.

Gossypol is not reduced with sulfur dioxide nor does it show any of the other usual characteristics of a true quinone. Various alkaline and acid reagents cause remarkable color changes upon reaction with gossypol. Every result indicates, as suggested by Podolskaja from his work on red gossypol, that gossypol is a sensitive, tautomeric compound.

On the basis of this assumption the investigation of simple stable derivatives of fixed structures rather than of gossypol itself appeared to be the most promising approach to the solution of the problem. Thus far, the number of such derivatives has been limited merely to the hexaacetate, the anhydrogossypol and the dianilino derivative, all of which have been described and are unquestionably crystalline entities. Unfortunately, however, only the last of the three is readily prepared. Subsequent papers to appear shortly will describe improved methods for the preparation of these and many other crystalline derivatives of gossypol.

Experimental

Extraction.—The directions of Carruth[4d] were used for the general procedure. The oil from 22.5 kg. of ground North Carolina cottonseed meats was removed by extraction with 20 gal. (77 liters) of petroleum ether (b. p. 30–60°) in a continuous Stokes basket extractor. The oil-free meats were then extracted with 20 gal. of peroxide-free ether. The ether solution of gossypol was concentrated to 4 liters under diminished pressure at 35°, filtered, and 400 cc. of glacial acetic acid was added. After standing with intermittent stirring, gossypol acetic acid crystallized. The first crystals appeared in about fifteen minutes and precipitation was complete after standing overnight (yield 80 g.). The mother liquors were allowed to stand at room temperature until the ether had evaporated and a further yield of gossypol-acetic acid resulted (about 33 g.). The 113 g. (0.5% yield) of gossypol-acetic acid was recrystallized by dissolving in 3 liters of peroxide-free ether and adding 300 cc. of glacial acetic acid and proceeding as described above. The total yield of product was 100 g., which was in the form of bright yellow crystals, m. p. 180–185°.

By the petroleum ether extraction described it is impossible to remove all of the oil from the meats. As a consequence the ether solution of gossypol from 22.5 kg. of meats contains about 0.5–1 liter of oil which may delay the precipitation of gossypol-acetic acid for a longer time than that cited. It was observed that if the ether solution of gossypol containing oil is overheated,[7] the crystallization of the gossypol-acetic acid is always very slow and often requires several days.

The gossypol-acetic acid was converted to gossypol by dissolving in 3 liters of peroxide-free ether and adding 1 liter of water containing 4 g. of sodium hydrosulfite. The mixture was warmed on a warm water-bath, without allowing the temperature of the mixture to exceed 60°, until all the ether had distilled. The gossypol which separated in the form of a yellow crust on the water, was filtered and washed. This material was then purified further by repeating the process, using 1 liter of peroxide-free ether and 1 liter of water containing 4 g. of sodium hydrosulfite. The product after air drying was crystallized by dissolving in 1 liter of peroxide-free ether, adding an equal volume of petroleum ether (b. p. 30–60°), and concentrating at room temperature under diminished pressure until gossypol started to crystallize. Two volumes of petroleum ether (b. p. 30–60°) was then added and the mixture allowed to stand for one hour. The product was filtered, washed with petroleum ether, and recrystallized as just described. The gossypol thus obtained was in the form of brilliant yellow micro crystals, m. p. 184°; yield, 54 g. (60%, based on gossypol-acetic acid).

If large crystals were desired, a solution of 2 g. of gossypol in 100 cc. of benzene was treated with 500 cc. of petroleum ether (b. p. 60–110°) and the mixture boiled until 75 cc. had evaporated. After standing for twelve hours at room temperature, bright yellow rosets, m. p. 184°, separated. Gossypol, after it has been purified by the general procedure described, is also crystallized readily from toluene (1 g. in 25 cc.), m. p. 184°.

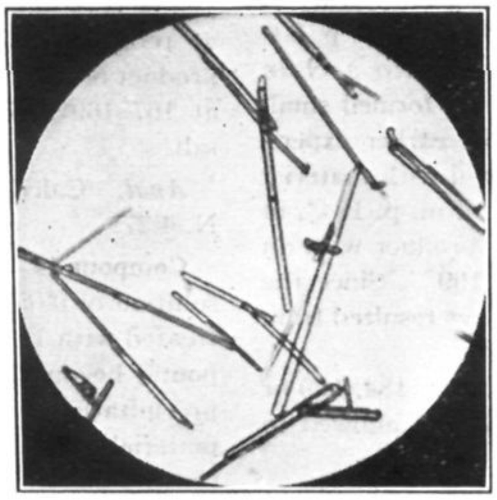

Plate 6.—Gossypol, m. p. 184°.

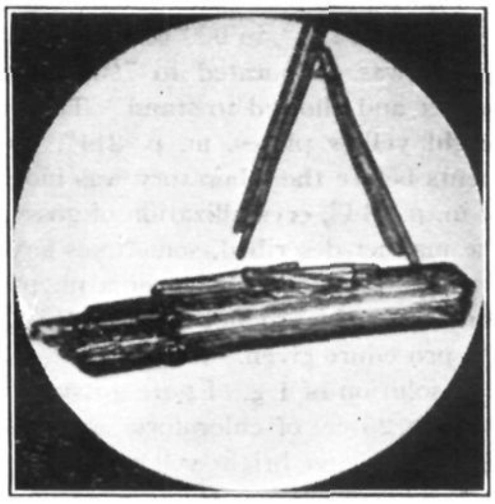

Plate 7.—Gossypol, m. p. 199°.

Plate 8.—Gossypol, m. p. 214°.

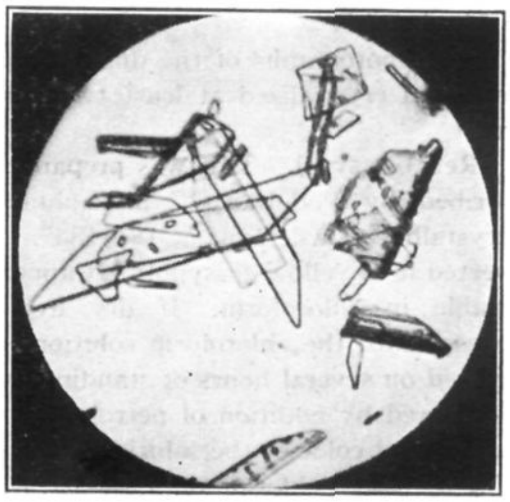

Plate 9.—Gossypol-acetic acid.

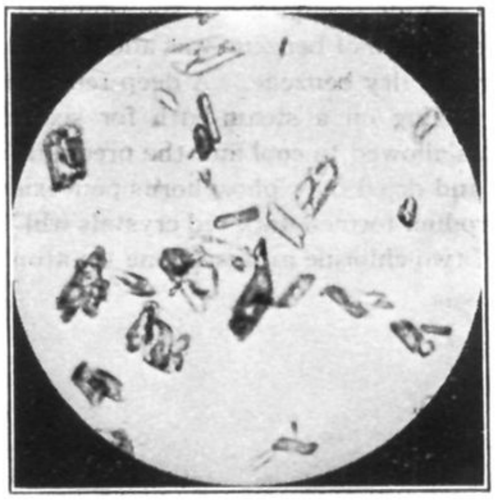

Plate 10.—Red gossypol.

Plates 6–10.—Microphotographs of crystals (× 1000).

All of the crystalline forms of gossypol gave essentially the same carbon and hydrogen analyses.

Gossypol is very soluble in cold dioxane, diethylene glycol, methyl-, ethyl-, isopropyl- and butyl alcohols,

---

[7] Private communication from Dr. J. H. Halverson.

ether, ethyl acetate, acetone, chloroform, carbon tetrachloride, and pyridine; it is slightly soluble in glycerol, cyclohexane, and petroleum ether (b. p. 60–110°); and insoluble in petroleum ether (b. p. 30–60°) and in water.

Change in Melting Points by Crystallization from Various Solvents.—A solution of 2 g. of pure gossypol, m. p. 184, 199 or 214°, in 900 cc. of petroleum ether (b. p. 60–110°), was evaporated to 700 cc., filtered into a clean beaker and allowed to stand. The product formed small bright yellow plates, m. p. 214°. In the earlier experiments before the laboratory was inoculated with material of m. p. 214°, crystallization of gossypol of m. p. 184°, in the manner described, sometimes gave a product with an unchanged m. p. of 184° or a m. p. of 199°. Since the early work the product of m. p. 214° always resulted from the procedure given.

A solution of 1 g. of pure gossypol of m. p. 184, 199 or 214° in 25 cc. of chloroform was filtered and allowed to stand. It gave bright yellow needles, m. p. 199°.

A solution of 1 g. of pure gossypol, m. p. 184, 199 or 214° in 25 cc. of peroxide-free ether was filtered and petroleum ether (b. p. 30–60°) was added as described in the extraction process. The yellow micro-crystalline product melted at 184°.

The photographs of the different forms were taken on material crystallized at least twice from the proper solvent.

Red Gossypol.—This was prepared by the method described by Podolskaja[4m] and obtained as a brick-red crystalline powder, m. p. 184–185°. Most solvents converted it to yellow gossypol. It appeared to be relatively stable in chloroform. If dry hydrogen chloride was passed into the chloroform solution, no color change occurred on several hours of standing and red gossypol was recovered by addition of petroleum ether (b. p. 30–60°).

The red color of the solution obtained by the action of dry hydrogen chloride on a chloroform solution of yellow gossypol was not due to red gossypol as yellow gossypol precipitated when the solution was treated with petroleum ether.

Gossypol–Stannic Chloride Complex.—A solution of 0.3 cc. of stannic chloride in 7 cc. of benzene was added to 0.1 g. of gossypol in 13 cc. of dry benzene. A deep red color developed. After refluxing on a steam-bath for sixteen hours, the mixture was allowed to cool and the precipitate filtered and washed, and dried over phosphorus pentoxide and paraffin. The product formed dark red crystals which contained the ratio of two chlorine atoms to one tin atom.

Anal. Found: Sn, 39.22; Cl, 24.15. Ratio 1:2.

Pyridine Salt of Gossypol.—A solution of 0.1 g. of gossypol in 10 cc. of warm dry pyridine was kept warm for a few minutes and then petroleum ether (b. p. 30–60°) was added until precipitation was complete. The dark yellow crystalline material was filtered, washed, and dried at room temperature over phosphorus pentoxide. The product begins to lose pyridine at 122–124° and then melts at 167–169°. It proved by analysis to be a dipyridine salt.

Anal. Calcd. for $C_{30}H_{30}O_8 \cdot 2C_5H_5N$: N, 4.14. Found: N, 4.27.

Compounds of Gossypol and Organic Acids.—A solution of 0.050 g. of gossypol in 10 cc. of dry ether was treated with 1 cc. of the organic acid. The addition compound began to crystallize in ten to fifteen minutes and precipitation was complete at the end of one hour. The material was filtered and washed with petroleum ether (b. p. 30–60°). This process was repeated and the bright yellow product was dried *in vacuo* at room temperature. (The formic acid derivative loses formic acid rather readily at higher temperatures but the compounds with the other acids appear to be relatively stable.)

		Analyses, %			
		Calcd.		Found	
	M. p., °C.	C	H	C	H
Gossypol-formic acid	197–198	65.9	5.67	65.91	6.08
Gossypol acetic acid	187	66.4	5.88	66.75	5.73
Gossypol-propionic acid	177–178	66.8	6.08	67.03	5.95
Gossypol-*n*-butyric acid	159–160	67.4	6.27	67.66	6.50

Summary

1. An improved method of purifying gossypol is described.

2. An explanation of the various melting points and methods for interconversion of the forms is given.

3. New color reactions of gossypol are listed.

4. The products from the reaction of gossypol and various organic acids are described.

Urbana, Illinois Received June 14, 1937