GOSSYPOL, A PIGMENT OF COTTONSEED[1]

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I. INTRODUCTION

The importance of the fiber obtained from the cotton plant is generally appreciated, but few realize that one of the major industries in the United States is the processing of cottonseed. The oil of cottonseed is used almost exclusively for human consumption and the meal as animal feed or fertilizer.

The coloring matter in cottonseed, because of its toxicity and property of imparting color to the oil, poses major problems to the processing. The principal pigment is gossypol. The large acreage in cotton and the enormous quantities of cottonseed produced, 16 million tons annually containing approximately 50,000 tons of gossypol (128), have stimulated extensive investigations of this pigment.

II. OCCURRENCE OF GOSSYPOL

Gossypol is a yellow coloring matter occurring, so far as is known, only in the genus *Gossypium* (sub-

tribe *Hibisceae*, natural order *Malvaceae*). The name "gossypol" was coined by Marchlewski (141), who derived it from "gossyp(ium phen)ol" to indicate its origin and chemical nature. It has been isolated (87, 192) from the seed and root bark and detected in other parts of the upland cotton plant by means of the characteristic red color test with concentrated sulfuric acid.

Gossypol, as it exists in the seed, is contained in pigment glands and constitutes 20–40 per cent of their weight. A cross-section of the seed kernel discloses the pigment glands as dark spots which are relatively large, ovoid spherical bodies, 100–400 microns in length. Thorough studies of these glands have been made (31, 33, 34, 36, 147, 221).

The seed kernel of the cotton plant is a pointed ovoid body approximately 8–12 mm. in length. Oil is embedded in the tissue in minute droplets. Cottonseed contains on the average 45 per cent hulls and linters and 55 per cent meats (kernels). An average ton (2000 lb.) of cottonseed gives 320–350 lb. of oil and 930–960 lb. of meal. An excellent review article on cottonseed has been published by Altschul (24).

The gossypol content of the cotton plant is of importance chiefly in relation to the use of cottonseed meal

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as an animal feed, the use of the oil in food products, and in more recent years the use of cottonseed flour for human consumption. Cottonseed meal, unlike the raw seed, has had the oil removed by processing and most of the gossypol has been converted to a "bound form" by cooking at some stage. Some gossypol passes into the oil (61, 175, 176, 183, 193, 228); the undesirable color that results has been the subject of investigation (28, 59, 169). The amount of gossypol in raw cottonseed kernels varies considerably and depends on the species, the environment, and specific varieties (24, 79, 91, 96, 173, 201, 222). Within several varieties of *G. hirsutum* the gossypol content is from 0.39 to 1.70 per cent. The seeds of different species are reported (79) to contain gossypol in unexpectedly variable quantities; on a moisture-free basis *G. stocksii* contains 0.13 per cent and *G. klatzshianium var. davidsonii* 6.64 per cent. The wide ranges in content cited above represent extremes greater than those commonly observed.

Gallup, in studying changes in the gossypol content of cottonseed during different stages of maturity of the bolls (47, 82, 85, 91, 214), reported that the greatest increase in gossypol occurs between the time of maturity of the boll and the time it is about to open. Such variations as early or late maturity have little effect.

III. ISOLATION OF GOSSYPOL

The first published account of a substance considered by later investigators to be a very impure form of gossypol was by Kuhlmann (129), who, in 1861, obtained a substance called by him "cottonseed blue" during attempts to recover fatty acids from the "foots" of cottonseed oil refining. Longmore (132), in 1886, observed that crude cottonseed oil contains a coloring matter and isolated from the "foots" a pungent, brown, alkali-soluble solid, which was undoubtedly impure gossypol.

The first isolation of a pure, crystalline substance from cottonseed oil "foots" was accomplished in 1899 by Marchlewski (139), who named the product gossypol. It was, however, a complex of gossypol with acetic acid.

The extraction (25) and isolation of gossypol from the seed kernels of the cotton plant were accomplished by Withers and Carruth (226) in 1915; since that time their basic procedure has generally been used. Refinements in the method have been described by Withers and Carruth (225), Carruth (45), Clark (49), and Campbell, Morris, and Adams (43), the last workers having been aided by suggestions made by Halverson (103). The procedure consists in extracting ground cottonseed kernels with low-boiling petroleum ether to remove the bulk of the oil, followed by extracting with diethyl ether (peroxide-free) to remove the gossypol along with the residual oil. The addition of acetic acid to the carefully concentrated ether solution results in the separation of gossypol-acetic acid as brownish crystals. Purification is carried out by reprecipitation from a clarified ether solution by means of acetic acid.

It is best to utilize cottonseed gums (177) or the "foots" from the alkali refining of solvent-extracted cottonseed oil (128) for the preparation of large quantities of gossypol. Extraction of intact pigment glands (36) or alkaline extraction of ethereal raw cottonseed extracts (35) are processes that may be used for the same purpose.

Chander and Seshadri (49a) have found that treatment of cottonseed oil with aqueous sodium borate, with or without alkali, prior to the alkali refining process will remove gossypol. However, this has not yet been developed into a commercial operation (private communication from Seshadri).

Gossypol–acetic acid, called by earlier workers (46, 232) "gossypol acetate," is undoubtedly the compound isolated by Marchlewski (141). It is a loosely bound complex of one molecule of gossypol and one molecule of acetic acid and can be used in place of gossypol in most transformations; analogous complexes have been formed (44) with formic, propionic, and butyric acids. The acetic acid can be removed by merely allowing an ether solution of gossypol–acetic acid to evaporate in contact with an aqueous layer; the gossypol separates as a crystalline solid (44). Other procedures have been described in which sodium hydroxide (127) and sodium carbonate (180) are used to remove the acetic acid. Kozhenikova and Gil'tburg (129) reported obtaining free gossypol from the acetic acid complex by steam distillation *in vacuo*.

Gossypol readily forms a highly insoluble crystalline compound with two molecules of aniline (45), a fact that has formed the basis of methods for estimating the gossypol content (both free and "bound") of cottonseed and cottonseed meal. The gravimetric procedures of Carruth (46) and Withers (230) for the conversion of gossypol into its dianiline derivative were employed by Sherwood (206) and Clark (51) and refined by Halverson and Smith (104, 105, 106, 107, 218, 219). The methods employed by Royce and Kibler (190, 193) for the determination of the gossypol content of crude cottonseed oils likewise depend upon the gravimetric determination of gossypol through the dianiline derivative. Determination of traces of gossypol in fats, oils, and fatty acids by paper chromatography has been studied by Schramm and Benedict (199). A titrimetric procedure for gossypol, based on the reduction of the cupric ion, has been reported by Podol'skaya (167). Numerous

colorimetric methods (196) are now available for the determination of gossypol. Several of these utilize the reaction of gossypol with aniline or p-anisidine (108, 116, 117, 136, 170, 171, 174, 175, 176, 181, 190, 191, 215, 216, 217, 219), and one employs phloroglucinol (224) for the color development. Antimony trichloride (32, 102) forms a stable red complex with gossypol; this reaction is the basis of another procedure for the determination of gossypol but cannot be applied directly to a crude extract. 3-Amino-1-propanol has been utilized as the complexing agent to remove gossypol from the meats, meal, oil, crude oil, or soapstocks prior to reaction with aniline (172, 179).

In the processing the fate of the gossypol that is present in the pigment glands is of importance from both the standpoint of the quality and refining characteristics of the oil and the use of the meal as a feed. Several procedures are currently used for the processing of cottonseed. After separation from the hulls the meats are cracked or rolled to expose as much surface as possible during the cooking (conditioning) and/or solvent extraction. Water is generally added to the rolled meats prior to cooking and increases their moisture content from 12 to 17 per cent. Cooking is then carried out for 20 to 90 min. in tailored steam-heaters during which time a temperature of 200–270°F. is reached. Moisture content is thereby reduced to approximately 4 per cent.

Hydraulic presses were used extensively in the original processing methods but are now being replaced by screw presses (expellers) or by a combination of screw presses and solvent extraction. A few plants use direct solvent extraction. The cottonseed meal, which is the oil-free (0.4 to 5 per cent residual oil) cake resulting from the above operation, is a high-protein material with valuable nutritive properties and has found widespread application as an animal feed.

Cottonseed meal and oil (90, 223) contain little of the original gossypol. Withers and Carruth (235) attribute this "disappearance" of the gossypol to oxidation in the cooking or pressing, while Sherwood (206) suggested that "hydrolysis" occurs at some stage. Cottonseed meal does, however, contain a substance that is nonextractable by ether; it was called "D" gossypol by Carruth (46). Extraction of the meal with aniline results in the separation of a dianiline derivative of this product. The samples of meal examined by Sherwood (206) contained from 0.335 to 1.076 per cent of "D" gossypol.

"D" gossypol itself has never been isolated. On the basis of the observation that gossypol in ethanolic solution forms precipitates with the alcohol-soluble proteins of wheat flour and cottonseed meal, Withers

suggested (230) (and later abandoned) the hypothesis that the reduction in gossypol content during processing is due to its combination with free amino groups of the seed proteins. This hypothesis was later reaffirmed by Clark (51), who showed the identity of the product from aniline and "D" gossypol with dianilinogossypol formed from aniline and gossypol from raw seeds. Clark suggested abandoning the term "D" gossypol and using the term "bound gossypol" to denote the nonextractable substance present in cottonseed meal. The term "free gossypol" is defined as the gossypol that can be extracted from cottonseed or cottonseed meal with aqueous acetone; "bound gossypol" is that which can be extracted only after acid treatment; "total gossypol" is obtained when the meal is first heated with oxalic acid and then extracted.

The pigments gossypurpurin, gossycaerulin, and gossyfulvin are related to gossypol and have been studied by Boatner (37, 38, 168).

IV. PHYSIOLOGICAL ACTION OF GOSSYPOL

Cottonseed was utilized as a feed for livestock in the early part of the 19th century. The first published account of injury to livestock resulting from its use appeared in 1859; since that time, with increasing production and more widespread feeding of the meal, other reports of injury to domestic animals have been recorded. Theories concerning the poisonous principle in cottonseed meal have occupied the attention of many workers; résumés of earlier studies on the subject have been written by Macy (138) and by Schwartze (200). More recently the relationship of gossypol to the feed value of cottonseed meal has been discussed by Hale and Lyman (100) and the role of gossypol as a toxic agent by Altschul, Lyman, and Thurber (24). Cottonseed poisoning was attributed by earlier workers to the presence in the meal of choline, betaine, or nitrogenous bases of the ptomaine type, and to pyrophosphates. By 1915, after the isolation of gossypol from cottonseed by Withers and Carruth (232), either gossypol or dietary deficiencies were considered to be the cause of the trouble. Gossypol is definitely toxic to guinea pigs, rabbits, and swine, but it is now known that the early reports of injuries to cattle from feeding cottonseed meal were due not to gossypol but rather to the low vitamin A content of the meal. Currently cottonseed meal is widely used as a protein supplement in the diets of cattle and sheep but not in that of young calves, since they are more sensitive to the harmful effects of gossypol until the rumen is fully functioning.

Gallup (88) found that rats on low vitamin A diets are more susceptible to gossypol poisoning than those fed on complete diets or on diets deficient in other

vitamins. The administration of gossypol to rats causes a depression of appetite, and in nonfasting experiments the depression of body weight is proportional to the quantity administered (25, 69). Schwartze and Alsberg (202, 203) concluded from their studies that the effects produced in rats fed extracted "gossypol-free" cottonseed to which known amounts of pure gossypol had been added correspond closely to effects observed on feeding raw cottonseed containing a known amount of gossypol.

When gossypol was fed to rabbits and pigs, Harms and Holley (109) found that hypoprothombinemia developed. When dogs were fed gossypol (15–200 mg. per kilogram of body weight per day for 5–12 days) by stomach tube, Eagle (67) reported the incidence of diarrhea, anorexia, weight loss, and finally death. When the dose was lowered to 1–5 mg. only slight decreases in food intake and body weight occurred. From their studies on ciphids and Mexican bean beetles, Breakey and Olcott (41) concluded that gossypol is ineffective as either a contact or a stomach poison for insects.

The inhibition of growth in chicks by gossypol has been reported (58, 132). Rigdon, Crass, Ferguson, and Couch (186) found that hemolytic anemia develops in chicks fed gossypol and a ceroid-like pigment appears in the duodenal villi and the sinusoids of the liver and spleen. Eggs from hens fed cottonseed meal are generally normal in appearance, but after several months of storage a discoloration may be found in the yolks and albumin which makes them unsalable (97, 111, 115, 135, 144, 187, 197, 205, 207, 208, 225, 226). Gossypol also has been shown to have an appreciable adverse effect on hatchability and egg weight (112, 113). It is reported that water-soluble condensation products of gossypol with carbohydrates, amino acids, and proteins are harmful to aquarium fish (48) and that the gossypol–dextrose condensation product is toxic to mice (49) but the gossypol–glycine product is not. Since none of these condensation products have been isolated or characterized, there is some question whether they actually exist.

Eagle (70, 72, 73) concluded that the free gossypol content of cottonseed meal is not a true measure of the toxicity of the meal; thus his opinion differs from that deduced from the results of numerous investigations (23, 50, 57, 71, 83, 84, 86, 88, 89, 92, 93, 94, 119, 122, 140, 147, 148, 152, 200, 202, 203, 232, 233, 235) that point to gossypol as the primary toxic agent.

The published results on the physiological activity of gossypol must be evaluated and interpreted with care, since many workers have not considered the effect on toxicity (93, 101) of the protein level of the diet, the physical state of the gossypol administered, the possibility of variance in concentration of other toxic materials, and the degree of inactivation of admixed gossypol before and/or after administration by reaction with components of the diet (92, 93, 94, 114). The latest review article on the physiology of gossypol has been published by Eagle (68) and contains many references to other work.

The most common of the toxic effects of gossypol is cardiac irregularity; death of an animal is generally caused by circulatory failure (23). Menaul (147, 148) found that gossypol prevents the liberation of oxygen from oxyhemoglobin and has a hemolytic effect on erythrocytes. In gossypol poisoning then, an extreme burden is placed upon the respiratory and circulatory organs owing to the reduced oxygen-carrying capacity of the blood.

Subacute cases of gossypol poisoning result in death from pulmonary edema, while chronic cases produce pronounced cachexia and inanition (23). The toxicity of gossypol is greatly increased when it is introduced (147) directly into the bloodstream, 0.05 g. being fatal to a rabbit in 4 min. The intraperitoneal administration of 25–35 mg. per kilogram of body weight to rats causes edema and intestinal inflammation.

Jones and Waterman (122) demonstrated that gossypol interferes markedly with the digestion of cottonseed globulin in vitro by pepsin and trypsin, and attributed the incomplete (83 per cent) digestion by animals of the protein in cottonseed meal to the inhibitory action of gossypol.

One publication (43) reports no ill effects after one year as the result of human feeding experiments of bread prepared in part from cottonseed meal, and another article (42) the incorporation of cottonseed flour into an all vegetable protein mixture for feeding to children. Because of the high nutritive value and availability of cottonseed meal, its use in many food products is likely to increase.

The cooking or moist heat treatment (31) during the processing of cottonseed causes changes that largely inactivate the gossypol. Studies (35, 56, 64, 65, 66, 80, 81, 83, 84, 87, 88, 110, 126, 139, 147, 149, 160, 177, 185, 204) on the autoclaving of moist meal under pressure for short periods show that this operation effectively renders the meal safe when its level of the total diet is no greater than 25 per cent, as shown by experiments on pigs (99, 133, 137, 138), which are the animals most susceptible to poisoning. Clark (51) postulated that this inactivation involves reaction of free amino groups of the protein molecules with gossypol to form "bound gossypol," and the results of many investigations (26, 57, 59, 84, 230) substantiate this conclusion. Other methods that have been suggested for the detoxification of cottonseed meal include treatment with mixtures of steam and alkali (161), steam and sulfur dioxide (40), steam

and ammonia (39), steam and methanol (120, 185), and phloroglucinol (98). The addition of amines to cottonseed is reported (184) to produce a meal harmless to single-stomached animals, but the information on this subject is very meager. Studies extending and evaluating this procedure have been made (63). The feasibility of removing gossypol by solvent extraction also has been investigated (35, 62, 125).

The addition of water-soluble iron salts to diets containing cottonseed meal reduces the danger of injury (77, 86, 88, 146, 188, 225, 231, 234), and the inclusion of sodium bicarbonate and calcium carbonate in the diet of experimental animals has been reported (92, 93, 94) to lower the toxicity of the meal. For this reason, Gallup and Reder (93) suggested that in the biological assay of gossypol-containing substances, the diet should contain only the amount of calcium and sodium necessary for maintenance and moderate growth. The salutary effect of iron salts is probably due to the formation of insoluble iron complexes of gossypol; experimental evidence supports this point of view (225). The inclusion of calcium and sodium salts probably results in formation of an alkaline medium in which gossypol is unstable.

The preservative action (182) of gossypol on crude cottonseed oil indicates that it may possess useful antioxidant properties. Royce (189) reported that a cottonseed oil containing 0.02 per cent of gossypol develops a rancid flavor after 41 hr., while a gossypol-free control under similar conditions develops rancidity after 14 hr. Vitamin A-containing materials are stabilized against oxidation by gossypol (124, 154). Gossypol is also an effective stabilizer (162) for vinyl compounds against polymerization. Other antioxidant (118, 145) and antipolymerization studies utilizing gossypol have been reported (29).

V. PROPERTIES OF GOSSYPOL

Campbell, Morris, and Adams (44) found gossypol to be polymorphic. When crystallized from ether, it melts at 184°C., from chloroform at 199°C., and from ligroin (b.p. 60–110°C.) at 214°C. All of these forms of gossypol give the same reactions. The red form of gossypol reported by Podol'skaya (165, 166) has been shown to be a mixture (30). Other investigators (155) have not observed these differences in melting point brought about by changes in the solvent for crystallization, but variable melting points have been reported (45, 46, 50, 123, 198).

Gossypol is soluble in the ordinary organic solvents and crystallizes readily. The addition of acetic acid to its solution in many solvents causes the separation of gossypol–acetic acid, furnishing a convenient and effective means of purification. Gossypol–acetic acid

has been reported to exist only in one crystalline modification, bright yellow plates melting at 187°C.

Polarographic investigations of gossypol under various conditions have been made (142, 143). Reports of the complexes of gossypol with iron (121), tin (163), antimony (229), and molybdenum (164) indicate that gossypol may have use as an analytical reagent.

VI. CHEMISTRY OF GOSSYPOL

A. Early studies

Marchlewski, who first isolated crystalline gossypol–acetic acid (141), showed that gossypol is not a glycoside, is soluble in alkali, is readily oxidized by air in alkaline solution, and gives a brilliant red color with concentrated sulfuric acid and a dark green color with ferric chloride. He was unable to isolate crystalline benzoyl or acetyl derivatives, but his experiments demonstrated the presence of free hydroxyl groups in the molecule. Carruth (46) was also unable to prepare crystalline acetyl or benzoyl derivatives but found that the amorphous products he obtained could be hydrolyzed to gossypol. Three derivatives that proved to be important in later work on the structure were described by Carruth: (a) a compound formed by the elimination of two molecules of water on heating, (b) a compound having two carbon atoms and two oxygen atoms less than gossypol, formed by the action of strong alkali on gossypol, and (c) a compound from aniline and gossypol.

Clark (50, 51, 52, 53, 54, 55) proposed for gossypol the molecular formula $C_{30}H_{30}O_8$. The product formed from gossypol by the loss of two molecules of water he named *anhydrogossypol*, $C_{30}H_{26}O_6$; the product resulting from the action of strong alkali, and accompanied by two molecules of formic acid, he designated as apogossypol, $C_{28}H_{30}O_6$; and the condensation product of gossypol with two equivalents of aniline with the loss of two molecules of water he called dianilinogossypol, $C_{42}H_{40}N_2O_6$.

Clark prepared a crystalline hexaacetate and a dioxime of gossypol and concluded that of the eight oxygen atoms in gossypol, two are present in carbonyl groups and six in hydroxyl groups. Titration of gossypol or quantitative deacetylation of the hexaacetate showed that of the six hydroxyl groups, two are much more acidic than the four others. Gossypol also forms a dipyridine salt (44).

Deep-seated degradation of gossypol by treatment with potassium permanganate results in the isolation of isobutyric and acetic acids (52). Catalytic reduction of gossypol and subsequent distillation of the resulting "hydrogossypol" was reported by Schmid and Margulies (198) to yield β-isoamylnaphthalene, but proof of the presence of such a product was by

no means unequivocal. Karrer and Tobler (123) reported that equimolar amounts of o-phenylenediamine and gossypol condense with the loss of two molecules of water, and concluded that an α-diketone (or o-quinone) function is present in the gossypol molecule. Adams, Price, and Dial (21) later showed that the compound described by Karrer and Tobler was a mixture, and that two molecules of o-phenylenediamine condense with one molecule of gossypol, with one amino group of each molecule of the diamine reacting in a manner exactly analogous to the amino group in aniline.

On the basis of these results and with the aid of a variety of color tests and characterization reactions (44), gossypol was accepted at the start of the intensive investigation to be described as having the following structural characteristics:

1. Empirical formula $C_{30}H_{30}O_8$.
2. Six hydroxyl groups, two carbonyl groups.
3. An aliphatic side chain, probably isopropyl, isobutyl, or isoamyl.
4. Possibly a naphthalene nucleus.
5. Two ortho phenolic hydroxyl groups.
6. A hydroxyl group peri or ortho to a carbonyl group.

B. Acetates of gossypol

The sensitivity of gossypol to various reagents led Clark (50) to study the preparation of acetates which might be more stable. The hexaacetate was not easily obtained in a pure state and then only in poor yields. Other investigators (151) were not much more successful. An entirely satisfactory synthesis was discovered later (158), but not until after the structural studies on gossypol had been completed through the study of other derivatives.
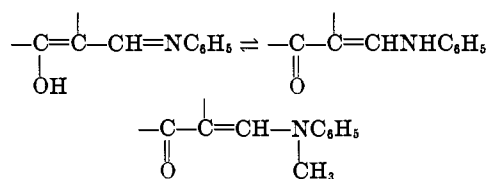
Gossypol hexaacetate, when subjected to reagents, is degraded along more than one path; consequently it is not particularly well suited for investigation of structure. Effort was directed instead to the ethers of gossypol.

The acetates and analogous compounds of gossypol are discussed in some detail in Section VIII.

C. Aniline, amine, and ammonia derivatives of gossypol

Gossypol reacts with two moles of aniline and similarly with a number of other primary aromatic amines to yield condensation products with the loss of two molecules of water. In the case of aniline, the name dianilinogossypol, although not particularly appropriate, was suggested by the first investigator and has been adopted by all later investigators. Although the analytical results were not always acceptable for these aniline derivatives all could be hydro-
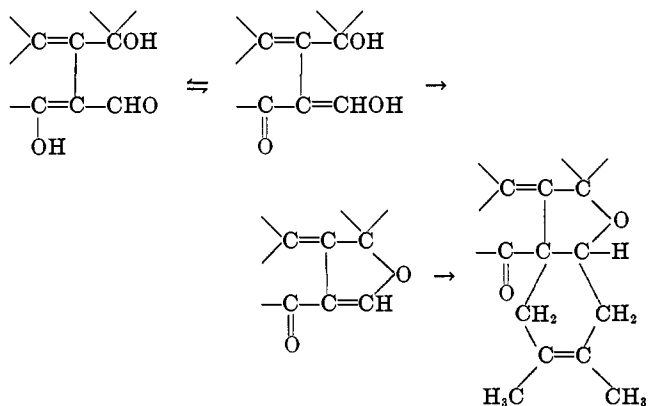
lyzed by acid to gossypol. Liquid ammonia reacts similarly to give diaminogossypol (150). Of importance from the structural standpoint were the methylation and acetylation products of the aniline derivative. Under proper conditions an N,N'-dimethylated compound was isolated, as shown by the fact that the methyl groups could not be removed by hydriodic acid nor could the substance be hydrolyzed to gossypol under the conditions used for the hydrolysis of dianilinogossypol. The dianilino compound is therefore not a simple Schiff base, and a phenylaminomethylene structure was postulated, analogous to the hydroxymethylene tautomeric form of an o-hydroxyaldehyde (21).

$$-C=C-CH=NC_6H_5 \rightleftharpoons -C-C=CHNHC_6H_5$$
$$\ \ \ \ | \|$$
$$\ \ OH \ O$$

$$-C-C=CH-NC_6H_5$$
$$\ \ \| \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ |$$
$$\ \ O \ \ \ \ \ \ \ \ \ \ \ \ \ \ CH_3$$

D. Anhydrogossypol

Anhydrogossypol, formed by the loss of two molecules of water, was obtained by Carruth (46) and Clark (50) by heating gossypol above its melting point, and by Miller and Adams (150) by treating gossypol with pyridine hydrochloride in toluene solution. It is rapidly hydrolyzed to gossypol in the presence of acids; acetic anhydride converts it to gossypol hexaacetate. In fact, in almost all its reactions it yields derivatives of gossypol. With butadiene and dimethylbutadiene (5), addition products result which are stable to hydrolysis. Gossypol also reacts with these dienes with the loss of two molecules of water to yield the same compounds as are obtained from anhydrogossypol. The diene addition products are readily acetylated and methylated to yield crystalline dimethyl and diacetyl derivatives, showing that of the six hydroxyl groups present in gossypol, four are affected in these transformations.

The previous partial structural postulations were extended (5) in the following manner to explain these reactions:

$$\begin{aligned}
&>C=C-COH \ \ \ \ \ \ \ >C=C-COH \\
&\ \ \ \ \ \ \ | \ \ \ \ \rightleftharpoons \ \ \ \ \ \ \ \ | \ \ \ \ \rightarrow \\
&-C=C-CHO \ \ \ \ -C-C=CHOH \\
&\ \ OH \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ O
\end{aligned}$$

$$\begin{aligned}
&\ >C=C-C \\
&>C=C-C \ >O \\
&\ \ \ \ \ \ | \ \ \ \ >O \ \rightarrow \ -C-C-C-H \\
&-C-C=CH \ \ \ \ \ \ \ \ \ \ \ \| \\
&\ \ \| \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ O \\
&\ \ O \ CH_2 \ \ CH_2 \\
&\ C=C \\
&\ H_3C \ \ \ \ CH_3
\end{aligned}$$

E. Ethers of gossypol

Gossypol is very sensitive to reagents and gossypol hexaacetate is not much less so. Since it seemed likely that the ethers would be more stable and suitable for stepwise degradation, gossypol tetramethyl and hexamethyl ethers were prepared. They were first reported by Morris and Adams (12, 153) and served as the starting point for the preparation of a series of mixed ethers (4, 6, 7), ether derivatives, and oxidation products from which important conclusions about the structure of gossypol were deduced.
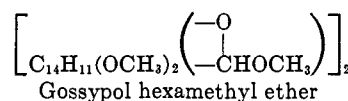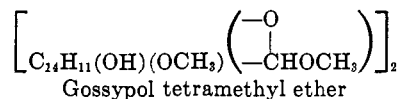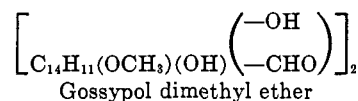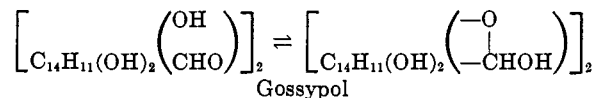
The tetramethyl ether, $C_{30}H_{26}O_4(OCH_3)_4$, and the hexamethyl ether, $C_{30}H_{24}O_2(OCH_3)_6$, are stable compounds. The hexamethyl ether exists in several forms which, however, react in the same way chemically. (They will be discussed later in this review.) The ethers have to be prepared under conditions which prevent decomposition of gossypol by the alkali used. The white tetramethyl ether is obtained by the addition of methanolic alkali to a solution of gossypol in methanol and dimethyl sulfate. Further methylation by the addition of methanolic alkali to this compound dissolved in benzene and dimethyl sulfate affords an excellent yield of a hexamethyl ether; it crystallizes as white needles from ligroin and melts at 175°C. The material made in this way (12) was used in most of the degradation reactions to be described. The tetramethyl ether is acetylated (153) to a diacetyl tetramethyl ether. In contrast to gossypol itself the tetramethyl and hexamethyl ethers are relatively difficult to oxidize and are stable in neutral or alkaline solution.

The tetramethyl and hexamethyl ethers do not condense with aldehyde reagents in neutral solvents but in acetic acid solution react rapidly, each with the loss of two methoxyl groups, to give products analogous to those obtained from gossypol. Oxidation with chromic acid, periodic acid, or dilute nitric acid occurs readily (20) to give a variety of products of significance in the structure proof. In the presence of traces of strong acids in glacial acetic acid, four methoxyl groups of gossypol hexamethyl ether are hydrolyzed with formation of a dimethyl ether (6) that resembles gossypol in many of its reactions. Catalytic reduction of gossypol hexamethyl ether does not occur in neutral solutions but proceeds in acetic acid to give a product in which two methoxyl groups have been lost (4).

The phenylhydrazine derivative of gossypol hexamethyl ether is a phenylhydrazone of a gossypol tetramethyl ether (7), while the phenylhydrazine derivatives of gossypol tetramethyl and gossypol dimethyl ether are, respectively, phenylhydrazones of two different gossypol dimethyl ethers.

These studies permitted the conclusion that two of the methoxyl groups in gossypol hexamethyl ether

differ from the other four, and their reactivity in acid media suggests that they are of the acetal type. From these results and the apparent symmetry of the gossypol molecule, the following formulas were constructed:
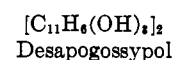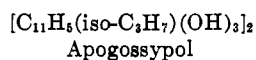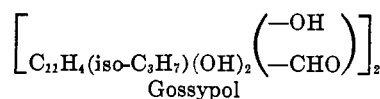
$$\left[C_{14}H_{11}(OH)_2\left(\begin{matrix} OH \\ CHO \end{matrix}\right)\right]_2 \rightleftharpoons \left[C_{14}H_{11}(OH)_2\left(\begin{matrix} -O \\ -CHOH \end{matrix}\right)\right]_2$$
Gossypol

$$\left[C_{14}H_{11}(OCH_3)(OH)\left(\begin{matrix} -OH \\ -CHO \end{matrix}\right)\right]_2$$
Gossypol dimethyl ether

$$\left[C_{14}H_{11}(OH)(OCH_3)\left(\begin{matrix} -O \\ -CHOCH_3 \end{matrix}\right)\right]_2$$
Gossypol tetramethyl ether

$$\left[C_{14}H_{11}(OCH_3)_2\left(\begin{matrix} -O \\ -CHOCH_3 \end{matrix}\right)\right]_2$$
Gossypol hexamethyl ether

F. Ethers of apogossypol, desapogossypol, and their oxidation products

The action of hot concentrated aqueous alkali (3, 46, 53) on gossypol removes two carbon atoms and two oxygen atoms, with the formation of two moles of alkali formate and one mole of a white unstable compound, apogossypol, $[C_{14}H_{12}(OH)_3]_2$. Methylation and acetylation afford stable hexasubstituted derivatives; the presence of six hydroxyl groups in this compound is thus demonstrated. Although gossypol reacts in different tautomeric forms, apogossypol always reacts as though it has a single structure. Neither apogossypol nor its derivatives were found to react with carbonyl reagents. Since gossypol is a hexahydroxy dialdehyde, it was assumed that apogossypol is formed by the loss of two aldehyde groups by the action of alkali, a type of elimination reaction characteristic of aromatic ortho-hydroxyaldehydes.

Apogossypol hexamethyl ether, $[C_{14}H_{12}(OCH_3)_3]_2$, undergoes an unusual reaction with concentrated sulfuric acid at room temperature (3). A white stable crystalline substance results that still retains six methoxyl groups; this compound has the formula $[C_{11}H_6(OCH_3)_3]_2$ and was named desapogossypol hexamethyl ether. The loss of C_6H_{12} in this reaction was postulated as the removal of two isopropyl residues, since distillation of the diluted sulfuric acid filtrate yielded a distillate that on oxidation gave a test for acetone.
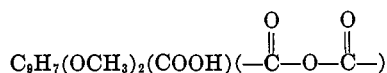
From the above results the relationship of gossypol, apogossypol, and desapogossypol was formulated as follows:

$$\left[C_{11}H_4(\text{iso-}C_3H_7)(OH)_2\left(\begin{matrix} -OH \\ -CHO \end{matrix}\right)\right]_2$$
Gossypol

$[C_{11}H_6(\text{iso-}C_3H_7)(OH)_3]_2$ $[C_{11}H_6(OH)_4]_2$
Apogossypol   Desapogossypol

Oxidation of desapogossypol hexamethyl ether with periodic acid leads to $[C_{11}H_5O_2(OCH_3)_2]_2$, desapogossypolone tetramethyl ether. Since reductive acetylation of this oxidation product results in a tetraacetate that can be reoxidized to desapogossypolone tetramethyl ether, the oxidation of desapogossypol hexamethyl ether was assumed to involve first the hydrolysis of two methoxyl groups and then conversion of the two phenolic residues to quinone nuclei (3). Potassium permanganate oxidizes desapogossypolone tetramethyl ether to a water-soluble dibasic acid, which was identified as m-hemipinic acid or 4,5-dimethoxyphthalic acid (3).

$$
\begin{array}{l}
CH_3O \\
CH_3O
\end{array}
\bigcirc
\begin{array}{l}
COOH \\
COOH
\end{array}
$$

m-Hemipinic acid

The chromic acid oxidation of apogossypol hexamethyl ether affords a product with the formula

mulated as 3-isopropyl-4,5-dimethoxyphthalic acid and this structure was confirmed by synthesis (1).

When apogossypolic acid (IX) is treated with nitric acid, the substitution of a carboxyl group by a nitro group (18) is effected. Reduction of this nitro compound results in an unstable amino acid that loses carbon dioxide to yield an amine. The structure of this amine was established by synthesis (15) as 3-isopropyl-4,5-dimethoxyaniline.

Hydrobromic acid degrades apogossypolic acid to a monobasic acid (18) formulated as 4,5-dihydroxy-3-isopropylbenzoic acid. This structure was established as correct by two unambiguous syntheses (14): the first from 3-isopropyl-1,2-dimethoxybenzene or 2-hydroxy-3-isopropyl-1-methoxybenzene and the second from the known 5-bromo-1-hydroxy-3-isopropyl-2-methoxybenzene.

IX
Apogossypolic acid

$[C_{14}H_{11}O_2(OCH_3)_2]_2$, apogossypolone tetramethyl ether; reductive acetylation followed by reoxidation to the original compound indicates the presence of two quinone groupings (3). Oxidation of apogossypolone tetramethyl ether by permanganate gives $C_9H_8(OCH_3)_2(COOH)_2$, apogossypolic acid (IX) (3).

The relationship in structure of the degradation products of apogossypol and desapogossypol hexamethyl ethers (18), just described, reveals that apogossypolic acid, $C_{13}H_{16}O_6$, and m-hemipinic acid, $C_{10}H_{10}O_6$, differ from each other merely by an isopropyl residue. Thus apogossypolic acid (IX) was for-

By controlled oxidation with chromic acid (20) gossypol hexamethyl ether is converted chiefly to gossypolone tetramethyl ether, $[C_{14}H_{10}O_2(CHO)-(OCH_3)_2]_2$, which contains aldehyde and quinone groupings. As with apogossypol and desapogossypol hexamethyl ethers, this oxidation was assumed to involve the hydrolysis of two methoxyl groups and oxidation of the resulting phenolic residues to quinone rings. Upon treatment with permanganate, gossypolone tetramethyl ether gives a water-soluble product, $C_{14}H_{14}O_7$, gossic acid. It contains two methoxyl groups. It forms a monomethyl ester with diazo-

methane but titrates as a tribasic acid in aqueous solution. From these reactions and the analyses, the structure of gossic acid was postulated as

$$C_9H_7(OCH_3)_2(COOH)(-\overset{\overset{\text{O}}{\|}}{C}-O-\overset{\overset{\text{O}}{\|}}{C}-)$$

Gossic acid, derived from gossypol hexamethyl ether, and the anhydride of apogossypolic acid, derived from apogossypol hexamethyl ether, differ structurally by a single carboxyl group. Since the oxidation products are obtained by similar reactions, and since aldehyde groups are present in gossypol and not in apogossypol, it was postulated that the aldehyde groups in the gossypol hexamethyl ether accounted for the carboxyl group found in gossic acid that was not present in apogossypolic acid.

This structural relationship was verified (17). Demethylation of gossic acid with hydrobromic acid affords a dihydroxy acid. That this reaction was uncomplicated was demonstrated by treatment of the hydroxy acid and gossic acid with diazomethane; both gave the same product. The demethylated gossic acid, when heated with quinoline and copper bronze, loses carbon dioxide and gives a dihydroxy dibasic acid anhydride, $C_{11}H_{10}O_5$. This in turn upon methylation with diazomethane affords a dibasic acid anhydride, which on hydrolysis yields a product identical with apogossypolic acid (IX).

Since the structure of apogossypolic acid was shown by synthesis to be 3-isopropyl-4,5-dimethoxyphthalic acid, the structure of gossic acid is established as 4-isopropyl-5,6-dimethoxy-2,3-hemimellitic anhydride.

IX
Apogossypolic acid
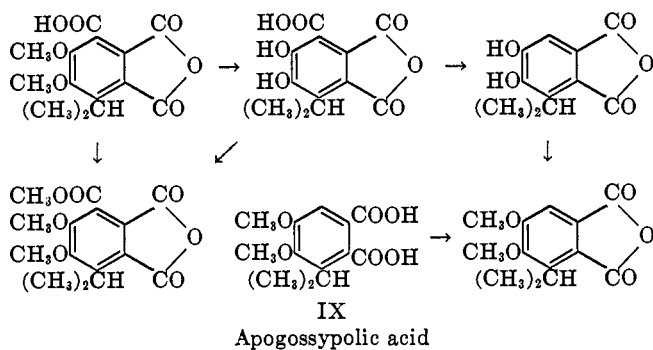
m-Hemipinic, apogossypolic, and gossic acids may all be formed by the permanganate oxidation of quinones; hence the two carboxyl groups in these compounds probably originate from the quinoid moieties. On the basis of the relationship among these acids the following formulations of partial structures of gossypol, apogossypol, and desapogossypol were possible.
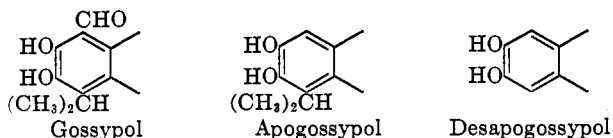
The ultraviolet absorption spectra of gossypol and many of its derivatives were next studied and compared with those of various compounds with known aromatic nuclei (16). It was concluded that gossypol was derived from either a 1,1'- or a 2,2'-binaphthyl.

Of the possible binaphthyl structures, that containing the carbon skeleton
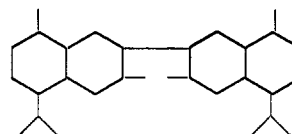
was selected as the most likely (19), because (a) it could be constructed from isoprene units, (b) one half of the above structure is a nucleus found in numerous naturally occurring compounds such as cadinene, copaene, α-, β-, and γ-cadinols, and at the time of this work on gossypol, no naturally occurring compounds were known containing half of the alternative structure,[4] and (c) the limited permanganate oxidation of gossypol results in considerable acetic acid along with formic and isobutyric acids. The aldehyde groups already established as present in gossypol would explain the formation of formic acid, and the isopropyl group together with one ring carbon atom, the formation of isobutyric acid. The acetic acid might conceivably be attributed to further degradation of the isobutyric acid but more likely, because of the mild conditions of oxidation, to degradation of some other part of the molecule. This makes unlikely a dinaphthylethane type of nucleus for gossypol.

The assembled experimental evidence permits formulation of structures for gossypol and its key derivatives. Gossypol was postulated as 2,2'-bi[8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthyl][5] (I).

Apogossypol, formed from gossypol by the loss of two aldehyde groups, was assigned the structure 2,2'-bi[1,6,7-trihydroxy-5-isopropyl-3-methylnaphthyl] and its hexamethyl ether would consequently have structure III. Apogossypolone tetramethyl ether,
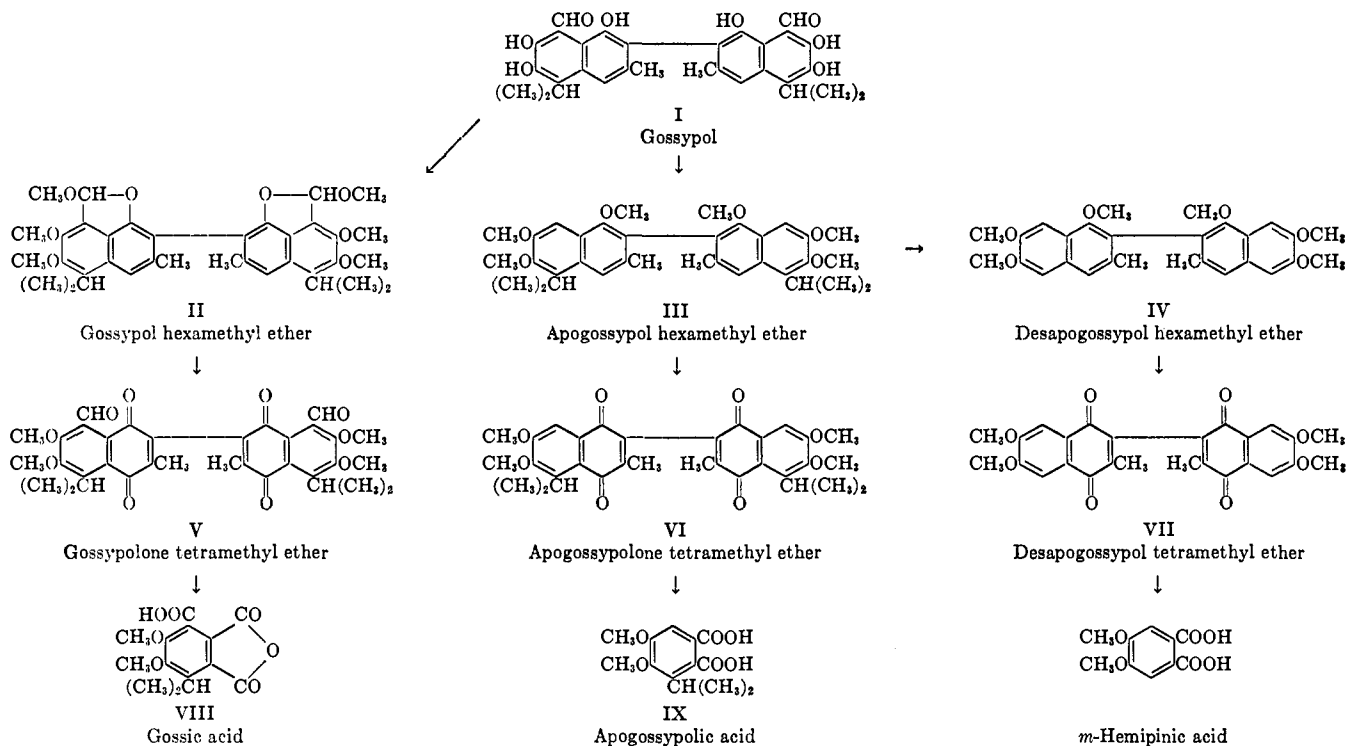
[4] Later carotol
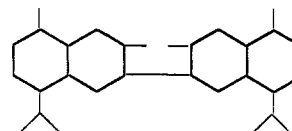
was reported (220).

[5] Names used by *Chemical Abstracts* for some of the compounds included in this article are listed below:

Gossypol = 1,1',6,6',7,7'-hexahydroxy-5,5'-diisopropyl-3,3'-dimethyl[2,2'-binaphthalene]-8,8'-dicarboxaldehyde.

Apogossypol = 5,5'-diisopropyl-3,3'-dimethyl[2,2'-binaphthalene]-1,1',6,6',7,7'-hexol.

2,2'-Bi[6,7-dimethoxy-3-methyl-1,4-naphthoquinone] = 6,6',7,7'-tetramethoxy-3,3'-dimethyl[2,2'-binaphthalene]-1,1',4,4'-tetrone.

I
Gossypol

II
Gossypol hexamethyl ether

III
Apogossypol hexamethyl ether

IV
Desapogossypol hexamethyl ether

V
Gossypolone tetramethyl ether

VI
Apogossypolone tetramethyl ether

VII
Desapogossypol tetramethyl ether

VIII
Gossic acid

IX
Apogossypolic acid

m-Hemipinic acid

obtained by the oxidation of apogossypol hexamethyl ether (VII), must be a diquinone (VI) and differs from gossypolone tetramethyl ether (V) by two aldehyde groups. The hexaacetate of apogossypol also undergoes oxidation to apogossypolone tetraacetate (54) and probably has an analogous structure.

Desapogossypol hexamethyl ether was assigned the structure 2,2'-bi[1,6,7-trimethoxy-3-methylnaphthyl] (IV) because it was formed from apogossypol hexamethyl ether by the loss of two isopropyl residues. By oxidation of desapogossypol hexamethyl ether (IV) the resulting diquinone was assumed to be 2,2'-bi[6,7-dimethoxy-3-methyl-1,4-naphthoquinone][5] (VII), which differs from apogossypolone tetramethyl ether (VI) by two isopropyl groups.

The following scheme depicts the relationship be-

tween gossypol and its important derivatives and degradation products (19).

G. Tautomerism of gossypol

The positions of the 4-isopropyl, the 6,7-dihydroxy, and the 8-formyl groups in the postulated formula of gossypol were deduced from the structural relationship of gossic, apogossypolic, and m-hemipinic acids. The other hydroxyl groups were placed in the 1,1'-positions in order to explain many of the characteristic reactions of gossypol, especially the tautomeric forms from which stem anhydrogossypol and its diene adducts, and the amino and anilino derivatives.

The stability of the tetra- and hexamethyl ethers of gossypol to alkali and alkaline oxidizing agents, and the unreactivity with carbonyl reagents in neutral

Ia

Anhydrogossypol

Ib

Ic
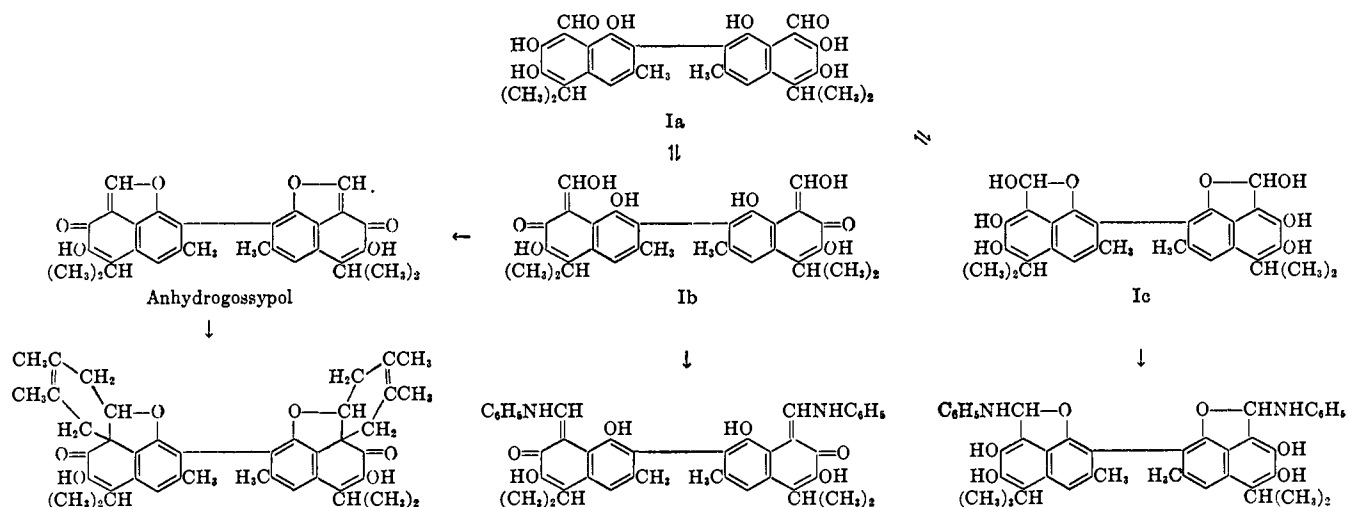
solvents are in contrast to the oxidation that takes place in acid solutions or the condensation with carbonyl reagents in acetic acid. In the latter reaction hydrolysis of two methoxyl groups occurs first and the reagents then react in the normal way with the resulting aldehyde groups. Such reactions suggest that two of the methoxyl groups are of the acetal type; hence the lactol tautomeric form of gossypol (Ic) was selected as the nucleus of gossypol hexamethyl ether (IIa), gossypol tetramethyl ether (IIb), and gossypol dimethyl ether (IIc).
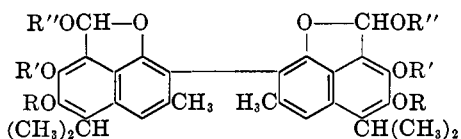
IIa: gossypol hexamethyl ether, R = R' = R'' = CH₃

IIb: gossypol tetramethyl ether, R = H; R' = R'' = CH₃

IIc: gossypol dimethyl ether, R = CH₃; R' = R'' = H

The presence of two asymmetric centers in such molecules explains the possible existence of stereoisomers (153) of the white gossypol hexamethyl ether.

Gossypol hexamethyl ether is partially demethylated to a dimethyl ether by means of concentrated sulfuric acid. This dimethyl ether, unlike the tetra- and hexamethyl ethers, exhibits many of the characteristic reactions of gossypol. It condenses with aniline, undergoes the apo reaction, forms an acetic acid complex, and is unstable in alkaline solution (6).

Gossypol tetramethyl ether, on the other hand, is converted to gossypol when treated with concentrated sulfuric acid. The two free hydroxyl groups in gossypol tetramethyl ether are obviously those that are methylated in gossypol dimethyl ether. Gossypol dimethyl ether was accordingly assigned structure IIc.

On the basis of structures IIb and IIc for gossypol tetramethyl and dimethyl ether, respectively, the formation therefrom of isomeric and not identical phenylhydrazones (7) becomes clear.
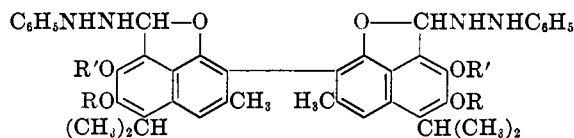
Phenylhydrazones of ethers

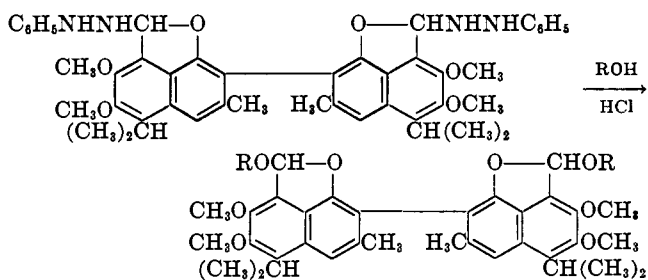Gossypol hexamethyl ether, R = R' = CH₃

Gossypol tetramethyl ether, R = H; R' = CH₃

Gossypol dimethyl ether, R = CH₃; R' = H

The reaction of hydroxylamine hydrochloride (7) in acetic solution with gossypol tetra- and hexamethyl ethers results in derivatives analogous to the phenylhydrazones. With gossypol hexamethyl ether, the oxime first formed loses two molecules of water to give orthoxazine rings (7, 8). Methanolic potassium hydroxide opens the rings in this product to form a crystalline potassium salt which on heating in acetic acid is converted, presumably through nitrile and imido ether intermediates, to gossylic acid lactone tetramethyl ether (XI).
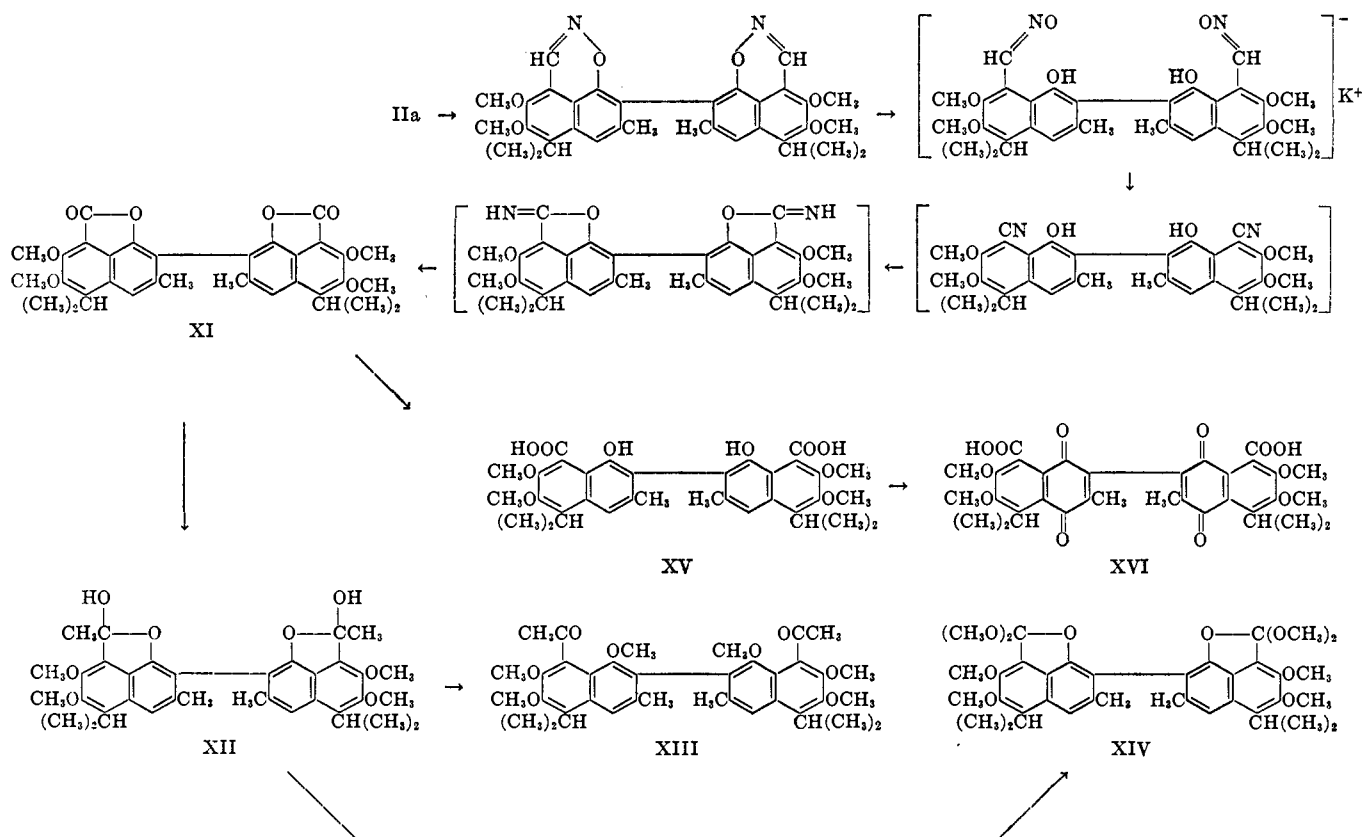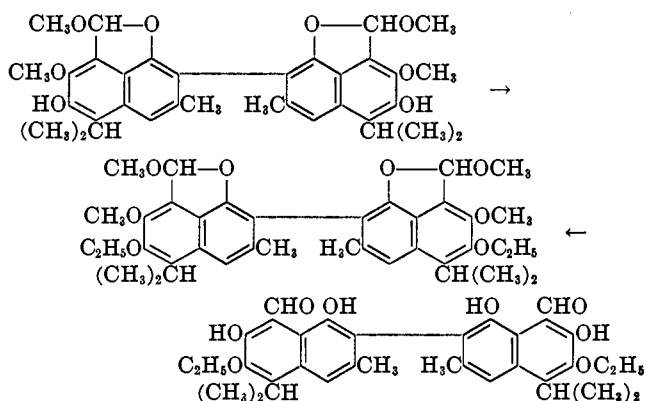
The lactone (XI) may be hydrolyzed to the corresponding hydroxy acid (XV), and this acid can be converted by means of diazomethane or by dimethyl sulfate in methanolic alkali to the dimethyl ester of gossylic acid hexamethyl ether. One molecule only of methylmagnesium iodide reacts with each lactone linkage of compound XI and the resulting hemiacetal (XII) may be methylated either with dimethyl sulfate to give a methoxy methyl ketone (XIII) or with methanol acidified with a drop of sulfuric acid, to give the acetal (XIV). These reactions provide further confirmation that a hydroxyl group is located peri to an aldehyde group and that the carbonyl group of the lactone XI is hindered. The lactone (XI) is surprisingly stable to oxidizing agents but the corresponding hydroxy acid (XV) is converted by dilute nitric acid to gossypolonic acid tetramethyl ether (XVI), the same product that is obtained by the oxidation of gossypol hexamethyl ether with the same reagent. The ring containing the peri hydroxyl group is obviously converted to a quinone, and since just two hydrogen atoms are lost, the para position to the hydroxyl group must be unsubstituted. The yellow color of the product is indicative of a p-quinone rather than an o-quinone. The para position to the hydroxyl group in the hydroxy acid (XV) may be readily substituted by a nitro group.

The reactions of certain mixed ethers of gossypol (4) were of significance in the structural studies. When the phenylhydrazone of gossypol tetramethyl ether, derived from gossypol hexamethyl ether and phenylhydrazine, is treated with alcoholic hydrochloric acid, a hexaalkyl ether is re-formed. The new alkyl groups are derived from the alcohol used in the alcoholysis reaction.

C₆H₅NHNHCH—O   O——CHNHNHC₆H₅

CH₃O   OCH₃   ROH
CH₃O CH₃ H₃C OCH₃   ⟶
(CH₃)₂CH   CH(CH₃)₂   HCl

ROCH—O   O——CHOR
CH₃O   OCH₃
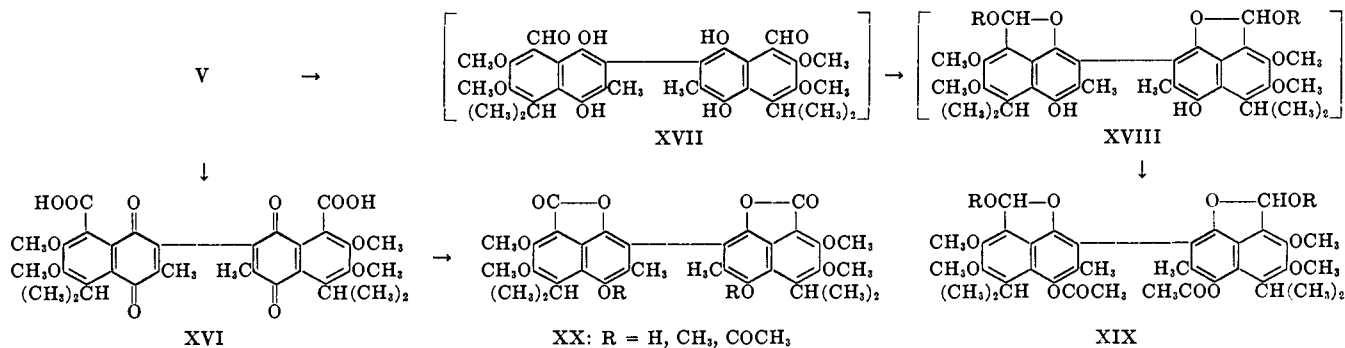CH₃O CH₃ H₃C OCH₃
(CH₃)₂CH   CH(CH₃)₂

A variety of mixed ethers was also prepared by direct alkylation, as illustrated by the formation of the same gossypol diethyl tetramethyl ether either by the methylation of gossypol diethyl ether or by the ethylation of gossypol tetramethyl ether.

CH₃OCH—O   O——CHOCH₃
CH₃O   OCH₃
HO CH₃ H₃C OH   ⟶
(CH₃)₂CH   CH(CH₃)₂

CH₃OCH—O   O——CHOCH₃
CH₃O   OCH₃
C₂H₅O CH₃ H₃C OC₂H₅   ⟵
(CH₃)₂CH   CH(CH₃)₂

CHO OH   HO CHO
HO   OH
C₂H₅O CH₃ H₃C OC₂H₅
(CH₃)₂CH   CH(CH₃)₂

The products from the oxidation of ethers of gossypol provided evidence that the initial attack occurs at the lactol linkage. Gossypol hexamethyl ether (II), upon oxidation with chromic acid (4), yields gossypolone tetramethyl ether (V) and a small amount of a by-product (20), gossylic acid lactone tetramethyl ether (XI). On the other hand, gossypol hexamethyl ether upon oxidation with nitric acid affords gossic acid (VIII) and gossypolonic acid tetramethyl ether, $C_{30}H_{22}O_8(OCH_3)_4$ (XVI). Gossypolone tetramethyl ether (V) is converted by dilute nitric acid into gossypolonic acid tetramethyl ether (XVI) and both gossypolone tetramethyl ether and gossypolonic acid tetramethyl ether are oxidized by permanganate (20) to gossic acid (VIII). The acid (XV) formed from gossylic acid lactone tetramethyl ether (XI) by hydrolysis is oxidized by dilute nitric acid (8) to gossypolonic acid tetramethyl ether (XVI).

The quinone structures of gossypolone tetramethyl ether (V) and gossypolonic acid tetramethyl ether (XVI) were established by the preparation of reduction products and their derivatives (12). The reduction of gossypolone tetramethyl ether (V) gives an unstable hydroquinone aldehyde (XVII) which, by the alcohol used as solvent, is converted to the lactol ether (XVIII); this last product was isolated as the acetate (XIX). The structure XIX is analogous to that proposed for gossypol hexamethyl ether. The reduction of gossypolonic acid tetramethyl ether (XVI) results in a lactone (XX) that can be readily

V   ⟶

CHO OH   HO CHO
CH₃O   OCH₃
CH₃O CH₃ H₃C OCH₃
(CH₃)₂CH OH   HO CH(CH₃)₂
XVII

⟶

ROCH—O   O——CHOR
CH₃O   OCH₃
CH₃O CH₃ H₃C OCH₃
(CH₃)₂CH OH   HO CH(CH₃)₂
XVIII

↓

HOOC O   O COOH
CH₃O   OCH₃
CH₃O CH₃ H₃C OCH₃
(CH₃)₂CH O   O CH(CH₃)₂
XVI

⟶

OC——O   O——CO
CH₃O   OCH₃
CH₃O CH₃ H₃C OCH₃
(CH₃)₂CH OR   RO CH(CH₃)₂
XX: R = H, CH₃, COCH₃

↓

ROCH—O   O——CHOR
CH₃O   OCH₃
CH₃O CH₃ H₃C OCH₃
(CH₃)₂CH OCOCH₃ CH₃COO CH(CH₃)₂
XIX

methylated and acetylated. The structure XX is analogous to that proposed for gossylic acid lactone tetramethyl ether (XI).

Additional light was thrown on the structures of the ethers of gossypol, gossypolone, and gossypolonic acid by studying their nitration products (13). The isopropyl group is replaced by a nitro group by the action of fuming nitric acid.

The reduction (4) of gossypol hexamethyl ether proceeds in acetic acid solution in the presence of Adams' platinum catalyst to give a compound $C_{34}H_{38}O_6$ (XXI), designated as desoxygossypol tetramethyl ether. Since the reduction occurs in acetic acid but not in neutral media, the overall reaction was assumed to involve hydrolysis of the two acetal methoxyl groups, reduction of the two resulting aldehyde groups to carbinols, and subsequent loss of water between the peri hydroxymethyl and hydroxyl groups, rather than direct hydrogenolysis of the acetal methoxyl groups.

XXI
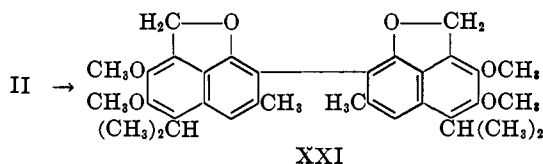
Mixed ethers were also subjected to reduction; in all instances the acetal alkoxy groups were the ones removed. The resulting desoxygossypol tetraalkyl ethers are relatively inert compounds, a fact that supports their postulated structures.

H. Ozonolysis of gossypol

Karrer and Tobler (123) ozonized gossypol in acetic acid solution and obtained a very low yield of a compound called by them gossypolic acid, along with large amounts of oxalic acid. Gossypolic acid yields a methyl ether ester when treated with diazomethane; on saponification the ether ester affords an ether acid. Further degradation of this ether acid as described by Adams, Geissman, Dial, and Fitzpatrick (11) gives, on the basis of the proposed structure for gossypol, a product formulated as a biphenyl. This is the only reaction of gossypol encountered which ruptures the outer rings of the binaphthyl nucleus to give a biphenyl.
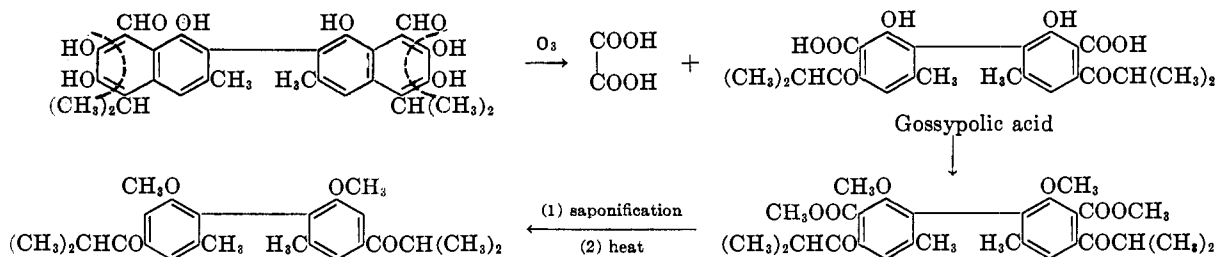
VII. SYNTHESIS OF GOSSYPOL

A. Desapogossypolone tetramethyl ether

The structure of desapogossypolone tetramethyl ether was formulated (19) as 2,2'-bi[6,7-dimethoxy-3-methyl-1,4-naphthoquinone][5] (VII). After two unsuccessful attempts (9, 10), the synthesis of this compound was realized by Adams and Baker (2) through the condensation of 2,3-dimethoxy-1,3-butadiene and 6,6'-dimethyl-2,2',5,5'-biphenyldiquinone,[6] with subsequent oxidation of the adduct by chloranil. The product obtained was identical with desapogossypolone tetramethyl ether. The synthetic product on reductive acetylation also gave a compound identical in all respects with that resulting from a similar treatment of desapogossypolone tetramethyl ether.
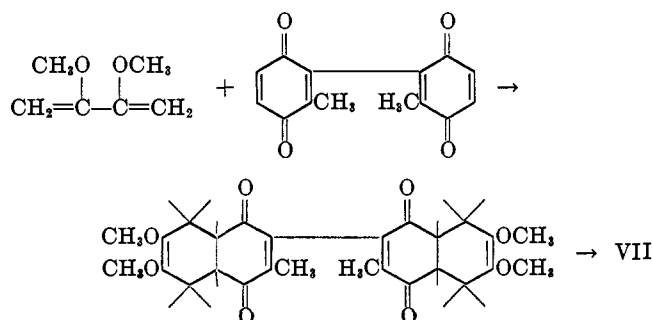

These results provided the first direct evidence for a 2,2'-binaphthyl nucleus in gossypol.

B. Desapogossypol hexamethyl ether

The synthesis of desapogossypol hexamethyl ether, the precursor of desapogossypolone tetramethyl ether, formulated (19) as 2,2'-bi[1,6,7-trimethoxy-3-methylnaphthyl] (IV) was reported by Shirley and Dean (211). Bromination of 6,7-dimethoxy-3-methyl-1-tetralone gave 2,2'-dibromo-6,7-dimethoxy-3-methyl-1-tetralone, which was dehydrobrominated and methylated. The product, 2-bromo-1,5,7-trimethoxy-3-methylnaphthalene, was converted to the lithium derivative and treated with cobalt(II) bromide to obtain a compound identical with desapogossypol hexamethyl ether.

[6] The name used by *Chemical Abstracts* for this compound is 3,3'-dimethyl-2,2'-dibenzoquinone.

Gossypolic acid
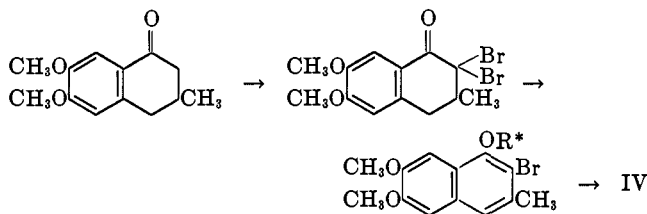
*R = H, CH₃.

### C. Apogossypol hexamethyl ether, apogossypol, and gossypol

The total synthesis of gossypol was achieved by Edwards (76) by the utilization of 2-isopropyl-3,4-dimethoxybenzaldehyde as the starting material. A Stobbe condensation of this aldehyde with diethyl succinate provided a half-ester which yields ethyl 1-acetoxy-5-isopropyl-6,7-dimethoxy-3-naphthoate on cyclodehydration with acetic anhydride. Reduction of this product with lithium aluminum hydride afforded the corresponding hydroxymethyl compound which was hydrogenolyzed to the key intermediate, 5-isopropyl-6,7-dimethoxy-3-methyl-1-naphthol. When this compound was heated above its melting point coupling to 2,2′-bi[1-hydroxy-5-isopropyl-6,7-dimethoxy-3-methylnaphthyl] occurred in quantitative yield. Methylation of this binaphthyl gave 2,2′-bi[5-isopropyl-1,6,7-trimethoxy-3-methylnaphthyl] identical with apogossypol hexamethyl ether (III), thus establishing conclusively the structure for this product proposed by Adams, Morris, Geissman, Butterbaugh, and Kirkpatrick (19). The coupling of the naphthol to a binaphthyl must have occurred in the 2-position, since desapogossypolone tetramethyl ether (VII), which is obtained by the oxidation of apogossypol hexamethyl ether, has been shown by synthesis (2) to possess the 2,2′-linkage.
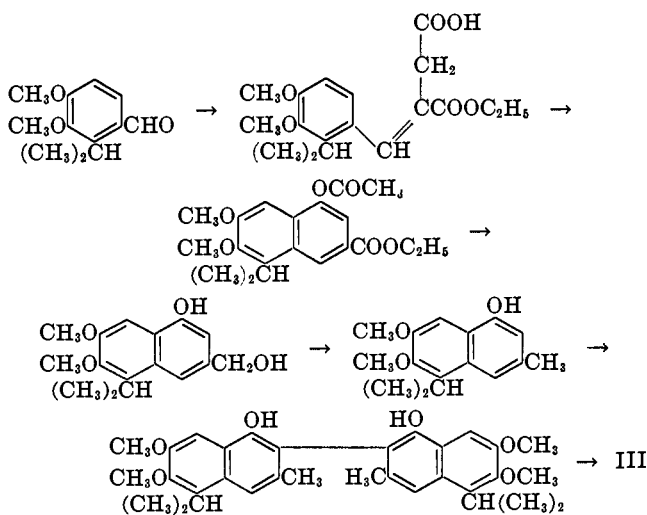

Treatment of apogossypol hexamethyl ether with boron tribromide (75) brings about demethylation and affords a product identical in all respects with apo-

gossypol (XXII), thus confirming the structure, 2,2′-bi[1,6,7-trihydroxy-5-isopropyl-3-methylnaphthyl] (19).

Reaction of apogossypol with N,N′-diphenylformamidine results in a product (XXIII) identical with dianilinogossypol (75). Since (a) the reaction of N,N′-diphenylformamidine with phenolic compounds is reported always to introduce the entering group (—CH=NC₆H₅) ortho to a hydroxyl group, which in this case then tautomerizes to a group of type Ic, (b) the hydrolysis of dianilinogossypol yields gossypol (46, 51, 155), and (c) the structure of apogossypol is unambiguous, the structure of gossypol is established as 2,2′-bi[8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthyl] (I) as formulated by Adams, Morris, Geissman, Butterbaugh, and Kirkpatrick (19).

XXII

Apogossypol

XXIII
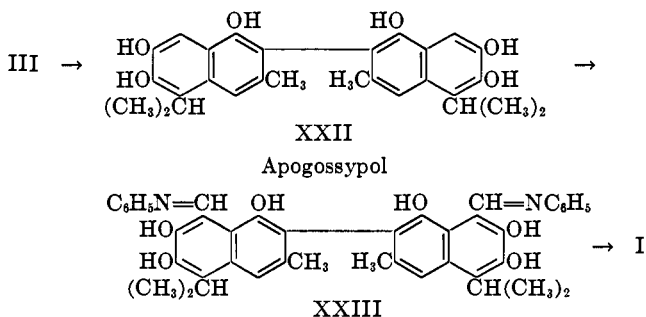
Barton and deMayo (27) have called attention to the cadalene dimer structure of gossypol. The biogenesis of such sesquiterpenes is envisioned by Ruzicka (194, 195) to proceed via isoprene units. Farnesol, which contains three isoprene units in regular head-to-tail arrangement, may be looked upon as a precursor of gossypol. The initial formation of a bisabolene is followed by cyclization and dehydrogenation to give a cadalene. Oxidative coupling of this cadalene would then result in formation of the gossypol structure.
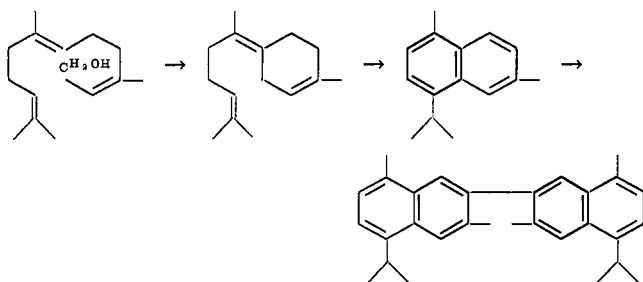
VIII. OTHER STUDIES OF GOSSYPOL

After completion of the investigations that led to the proposal of a structure for gossypol and before the total synthesis confirming this structure had been described, numerous papers on the chemistry of gossypol appeared. Some investigators raised questions about the postulated structures; readers are referred to the chapter "Pigments of Cottonseed" by Boatner (31) for review of some of these.

The esterification of gossypol has occupied the attention of many workers (31, 37, 158), but the esters have not been utilized in structural studies. Carruth (46) was the first to acetylate gossypol but was unsuccessful in obtaining a crystalline derivative, probably because impure gossypol was used in the experiments. Clark (50) selected as a reagent an excess of acetic anhydride and sodium acetate and, with relatively pure gossypol after 2 hr. boiling, obtained a 10 per cent yield of the white crystalline hexaacetate, m.p. 276–277°C. By the use of acetic anhydride in pyridine a somewhat higher yield was realized. Further improvement in the synthesis was reported by Miller, Butterbaugh, and Adams (151) by employment of less vigorous conditions—sodium acetate and acetic anhydride warmed for 15 min. on a steam bath. By this procedure, however, two compounds were isolated, a white hexaacetate, m.p. 276–279°C., and a yellow microcrystalline product, m.p. 184–186°C., that could not be purified completely.

Boatner, O'Connor, Curet, and Samuels (37) repeated this last method of acetylation and reported the same melting point (276–279°C.), but their analytical data were found to agree more closely with those for a tetraacetate. Since two other investigators have more recently studied the same acetylation reaction and have reported the formation of a hexaacetate, the results of Boatner, O'Connor, Curet, and Samuels are inexplicable.

Murty and Seshadri (156) described the white hexaacetate as melting at 278–279°C. and the yellow companion product at 180–185°C. Under more vigorous conditions, a third product appeared which is probably a pyrone formed by a Perkin condensation. More importantly, they discovered that gossypol when acetylated with acetyl chloride and pyridine in the cold gives an excellent yield of the pure white hexaacetate.

O'Connor, Von der Haar, DuPre, Brown, and Pominski (159) also acetylated gossypol by the Miller, Butterbaugh, and Adams procedure and separated the white and yellow products by the difference in their solubility in cold methanol. They considered that they obtained the yellow product in a pure state. The melting point of the white hexaacetate was reported as 283–286°C. and of the yellow compound as 188–192°C. No analytical data were recorded. It is logical to assume that gossypol hexaacetate has a formula analogous to that of the hexamethyl ether. The only degradation reaction of the hexaacetate was that described by Clark (54), who oxidized it with chromic acid and obtained a low yield of a yellow product that he called tetraacetylgossypolone. The properties classify it as a quinone that undoubtedly has a struc-

ture corresponding to that of gossypolone tetramethyl ether.

Hexabenzoates have been described (50, 151) and also a hexapalmitate (95).

Krishnaswamy, Murty, and Seshadri (130) have corroborated the o-hydroxyaldehyde function in gossypol by the preparation of α-pyrones from the reaction of gossypol with ethyl acetoacetate (XXIVa) or malonic acid ester (XXIVb),
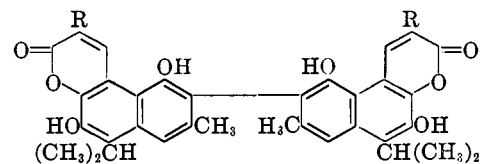
XXIVa: R = COCH₃

XXIVb: R = COOC₂H₅

and by the formation of flavylium salts (XXV) from the reaction of gossypol or dianilinogossypol with acetophenones.
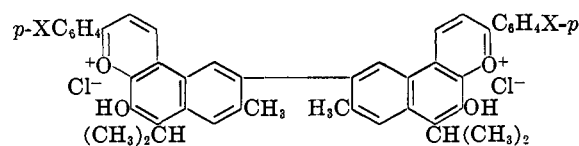
XXV: X = H or OH

Many amine derivatives of gossypol have been synthesized (22, 60, 213), because it was found that treatment with aniline served to decrease the toxicity of cottonseed meal. Although gossypol generally reacts with two moles of primary aromatic amines, Dechary and Brown (60) reported that it reacts with either one or two moles of o-nitroaniline and with only one mole of p,p'-diaminodiphenylmethane. It is noteworthy that mono Diels–Alder adducts are formed by the reaction of gossypol with isoprene (159) and of anhydrogossypol with cyclopentadiene (unpublished work of D. A. Shirley and P. W. Alley). From the reaction of aniline and gossypol Murty and Seshadri (156) reported the isolation of a product first formulated as a tetraanilinogossypol; later (130) this product was shown to be dianilinogossypol with adhering aniline. These authors (155) reported that dianilinogossypol reacts with acetic anhydride to form gossypol–acetic acid when no catalyst is used, but to form yellow gossypol hexaacetate with acetic anhydride and pyridine (151). Other investigators (31, 37) have reported tetraacetyldianilinogossypol as the reaction product of dianilinogossypol and acetic anhydride. The difference between these results and those of Adams, Price, and Dial (21), who reported the product as hexaacetyldianilinogossypol, may be attributed to different reaction conditions. The products isolated from the reaction of dianilinogossypol with hydroxylamine (37) and dinitrophenylhydrazine (31) were

reported as differing in melting point from those obtained with these reagents directly from gossypol; they were postulated (37) as isomers, but the discrepancy in the melting points may have been due to the presence of impurities.

Dianilinogossypol and pyridine react (191, 219) to form a thermally unstable salt that is probably dipyridyldianilinogossypol.

The ethers of gossypol have been studied by several investigators, who report a variety of results. The original method for the preparation of gossypol hexamethyl ether (153) consisted in adding aqueous sodium hydroxide to a solution of gossypol in dimethyl sulfate and 98 per cent sulfuric acid. The result was a red hexamethyl ether. A white tetramethyl ether was prepared by adding methanolic potassium hydroxide to a solution of gossypol in dimethyl sulfate and methanol. This was converted to a white hexamethyl ether by addition of dimethyl sulfate to its solution in methanolic potassium hydroxide. The hexamethyl ether was isolated in two isomorphic forms: prisms from a mixture of acetone and methanol, m.p. 235–237°C., and white needles from petroleum ether, m.p. 221°C. Successive preparations by the same investigators, however, sometimes gave products with melting points at variance with those first found.

By methylation with dimethyl sulfate in methanol at 10–15°C. (6) a tetramethyl ether was formed which was methylated to a hexamethyl ether, m.p. 239–240°C., whether it was crystallized from acetone and methanol or ligroin, but if the original methylation was conducted at 20–25°C. the final hexamethyl ether was reported to melt at 224–225°C. and to give a depression in melting point upon admixture with the form melting at 239–240°C. These appear to be stereoisomers. From ligroin the product melting at 224–225°C. was converted into white needles, melting at 179°C., which resolidified and melted at 223–225°C.

By still further study a detailed procedure was found (12) that was reported to give consistent results, yielding a hexamethyl ether, which melted at 175°C. after crystallization from ligroin.

Murty and Seshadri (156, 157) studied a variety of methylation procedures, all of which were reported to yield a yellow gossypol hexamethyl ether, m.p. 130°C., which was stable to dilute sulfuric acid. These methods included the methylation of gossypol, gossypol hexaacetate, and dianilinogossypol with dimethyl sulfate and alkali and the methylation of gossypol with diazomethane in absolute methanol and with methyl iodide and potassium carbonate in acetone.

Von der Haar and Pominski (227) conducted the methylation of gossypol with diazomethane in ether and with methyl iodide and potassium carbonate in acetone.

By both methods very low yields of the same product resulted, a yellow hexamethyl ether, m.p. 160–162°C. Finally O'Connor, Von der Haar, DuPre, Brown, and Pominski (159) repeated the detailed procedures of Von der Haar and Pominski but obtained thereby after crystallization from a mixture of acetone and methanol, a less soluble white hexamethyl ether, m.p. 220–222°C., a more soluble pale yellow ether, m.p. 160–162°C., and a violet form, m.p. 146–148°C.

These results are most confusing and appear to be explicable only on the basis of differences in reaction conditions which cause a shift in the amounts of different tautomeric forms. The methylation products then reflect varying compositions of methylated tautomers.

Shirley (209, 212) reduced gossypol with lithium aluminum hydride and isolated two compounds in the form of derivatives. One was the tetraacetate of desoxygossypol with a structure corresponding to desoxygossypol tetramethyl ether (IV) obtained by Adams and Dial (4) from the catalytic reduction of gossypol hexamethyl ether. The other was methylapogossypol, isolated as the hexaacetate (XXVIa) and the hexamethyl ether (XXVIb).

XXVIa: R = COCH₃

XXVIb: R = CH₃
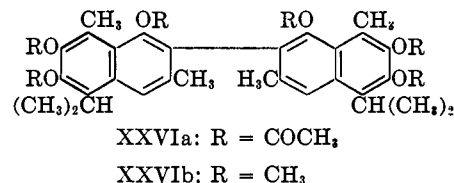
Shirley and Dean in 1955 (210) synthesized 1,6,7-trimethoxy-3-methylnaphthalene and compared its ultraviolet absorption spectrum with that of desapogossypol hexamethyl ether. Because of the dissimilarities, they concluded that there was reason to question the structure postulated for desapogossypol hexamethyl ether (19). Later, however, Shirley (211) synthesized the compound having the structure proposed by Adams, Morris, Geissman, Butterbaugh, and Kirkpatrick (19) and found it to be identical with the compound prepared from gossypol.

Infrared spectrophotometry had not been adequately developed at the time of the original studies of gossypol, so it was not utilized to the extent that it would have been used today. As early as 1938 Zamyshlyaeva (236, 237, 238) determined the infrared spectrum of gossypol and deduced that the molecule contains a carbonyl group, a hydroxyl group, and a hydrocarbon side chain. In the spectra of gossypol hexaacetate and hexaacetyl dianilinogossypol, the hydroxyl band is no longer present and the carbonyl band has changed. It was not until 1954 that O'Connor, Von der Haar, DuPre, Brown, and Pominski (159) presented the results of a detailed infrared

spectral study of gossypol and many of its derivatives. The spectra confirm the tautomeric structure of gossypol and the presence of numerous groups but do not reveal the binaphthyl nucleus or the isopropyl groups. These authors concluded that the white and yellow gossypol hexaacetates are derived from gossypol structure Ic, and the dianilino and diaminogossypol and gossypol diphenylhydrazones from the gossypol tautomers Ia and Ib.

At the time when the structures of gossypol, its derivatives, and its degradation products were formulated by Adams, Morris, Geissman, Butterbaugh, and Kirkpatrick (19), the most direct evidence for the 2,2'-binaphthyl nucleus came from the ultraviolet absorption spectra studies (16). Later Adams and Baker (2) established the presence of a 2,2'-binaphthyl nucleus by the synthesis of desapogossypolone tetramethyl ether. Although many of the derivatives of gossypol have maxima in the 2,2'-binaphthyl absorption region (250–260 mμ), gossypol exhibits a maximum at 236–237 mμ (78, 178); this value is different from that originally reported (16). The displacement of the maximum might be expected from the presence of chromophoric formyl substituents. Ethanolic solutions of gossypol on prolonged standing or heating exhibit a shift in the 237 mμ maximum to 260 mμ (74). Since only unchanged gossypol could be isolated from these solutions, it may be assumed that an unstable hemiacetal type derivative with a typical 2,2'-binaphthyl absorption is formed in solution.

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