

# CHEMISTRY OF GOSSYPOL

## Part I. Preparation and Properties

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Received April 17, 1942

GOSSYPOL was first isolated as its acetic acid compound by Marchlewski<sup>1</sup> with great difficulty from the 'foots' of cotton-seed oil purification. Later Carruth<sup>2</sup> effected considerable simplification by employing raw cotton-seed kernels. They were first percolated with gasoline to remove most of the oil and subsequently extracted with ethyl ether in order to dissolve the gossypol. By the addition of glacial acetic acid to the concentrated ether extract and allowing to stand for several weeks crude 'gossypol acetate' was obtained. It was purified by crystallization from ether-glacial acetic acid mixture. From this product free gossypol was generated by dissolution in ether, treatment with water and subsequent distillation of the solvent. This method of isolation of gossypol has been adopted by all the subsequent workers, Clark,<sup>3</sup> Karrer and Töbler<sup>4</sup> and Adams *et al.*<sup>5</sup>

Another possible method of preparing gossypol from cotton-seed kernels suggests itself from the work of Carruth.<sup>2</sup> It consists in employing aniline in the place of acetic acid, thus producing the sparingly soluble anilino compound of gossypol. He found that gossypol itself was produced from this compound, when he decomposed it with alcoholic potash, removed the aniline formed by steam distillation and finally acidified the alkaline liquid by treatment with mineral acid. The scope of this procedure as a preparative method does not seem to have been explored by Carruth or by other workers.

In the course of his work, Carruth reported the existence of another form of gossypol in cotton-seed and he called it D-gossypol. It could not be extracted with ether with the same facility as ordinary gossypol and could be obtained by employing aniline as the solvent. At first it was thought to be a derivative of gossypol due to oxidation or hydrolysis. But Clark<sup>6</sup> showed that the aniline compound of D-gossypol was identical with that

of the ordinary gossypol and hence concluded that the greater difficulty in the isolation of this fraction was due to the existence of a portion of the gossypol content of the seeds in a combined form (bound gossypol). Later Halverson and Smith<sup>7</sup> showed that by moistening the oil-free seeds the extraction of gossypol with ether could be rendered complete. By employing seed powder moistened with 22% of water both free and bound gossypol could be isolated. In their experiments they obtained gossypol as the aniline compound and since it is sparingly soluble, quantitative comparisons could be readily effected by weighing it. Though no attempt has been made in the past to explain the nature of the bound gossypol it may be suggested at this stage that it is probably a combination with free fatty acids present in the seed. In support of this may be mentioned the marked tendency exhibited by gossypol to combine with fatty acids and ketones to form sparingly soluble compounds which undergo decomposition in the presence of water.

In the course of our attempts to work out a convenient method of preparing gossypol in fairly large quantities, the methods of Carruth were first employed. His acetic acid method gave a poor yield of gossypol-acetic acid and consequently led to a low recovery of gossypol. With a view to improve the yield moistening the meal had to be carried out. But then no gossypol-acetic acid would separate from the extract even after allowing to stand for a considerable length of time. This is due to the fact that its separation is markedly prevented by the existence of moisture and also of any residual oil. Consequently precipitation with aniline had to be employed. The formation of the aniline compound is not adversely affected by the presence of moisture. In the course of our trials it was found inconvenient and unnecessary to use such a large quantity of water as suggested by Halverson and Smith and 10% of moisture on the weight of the oil-free kernels was found to be the most convenient.

Again, the need for large quantities of petroleum ether for removing the oil and of ether for extracting gossypol from cotton-seeds made this preparation very costly. The loss of these volatile solvents was particularly large. The possibility, therefore, of using other solvents was explored. Alcohol, in spite of the high solubility of the substance in it, was found to be unsuitable. It gave rise to very poor yields and seemed to cause considerable decomposition of the colouring matter, especially when attempts were made to recover the solvent. On the other hand, chloroform was found to be quite satisfactory. There was no need for the preliminary removal of the oil or for moistening the seeds. Complete extraction was effected by cold percolation of the fresh seed meal for a comparatively short

period. The anilino compound separated out quite readily and a good portion of the solvent could be recovered without any reduction in the yield of gossypol. This solvent seems to be the most satisfactory for extraction.

Though the precipitation of gossypol as its aniline compound is quick and quantitative, the liberation of the colouring matter from it is not so simple as from gossypol-acetic acid. Carruth's method<sup>2</sup> of employing alcoholic potash leads to considerable decomposition and the isolation of even a small yield of pure gossypol is very difficult. By the action of concentrated sulphuric acid Clark<sup>6</sup> and later Adams and co-workers<sup>8</sup> noted that the aniline compound gave rise to gossypol. The liberated gossypol was purified by conversion into the acetic acid compound and subsequent regeneration. Though this method works fairly satisfactorily when small quantities of the anil are employed, the yield is not very good with larger quantities. The reaction takes place in a complex manner, giving rise to various other products and thus considerably reducing the yield and purity of gossypol. A new method has, therefore, been worked out. This consists in heating the dry anilino compound with acetic anhydride for a few minutes in order to produce a clear solution. When it is subsequently poured into ice water, crude gossypol-acetic acid is obtained in almost quantitative yield. The aqueous solution is found to contain acetanilide. Under these conditions no acetylation of gossypol takes place to any appreciable extent. As a matter of fact Adams *et al.* have recorded that no acetylation of gossypol is effected by acetic anhydride in the absence of a catalyst even when heated for half an hour. The above method has, therefore, been adopted largely for the preparation of gossypol.

The gossypol-acetic acid obtained by the hydrolysis of the anilino compound was found to be identical with the sample obtained by direct precipitation from the ether extract of the seeds. For its recrystallization it is essential to avoid heating in the presence of water since under this condition the product contains high carbon obviously due to partial decomposition of the compound into gossypol. In the cold it is not markedly affected by water but on boiling for a few minutes with it, complete conversion into gossypol takes place. A remarkable feature about our samples of gossypol is that they all have the same melting point (189° decomp.) irrespective of the solvent employed for the crystallization and they also appear more or less in the same crystalline form (yellow rectangular plates). It may be useful to recount here the previous records regarding crystalline appearance and melting point. Carruth,<sup>2</sup> Clark,<sup>3</sup> Karrer and Töbler<sup>4</sup> and Schmid and Margulies<sup>10</sup> reported the melting point of gossypol as 188°,

214°, 199° and 184° respectively. An explanation of this variation was offered by Adams *et al.*<sup>5</sup> who claimed to have obtained three definite crystalline forms of gossypol, each having a characteristic melting point, using different solvents for crystallization. When ether was employed the substance was a yellow micro-crystalline powder melting at 184°; from chloroform, it was obtained as bright yellow needles melting at 199° whereas from ligroin it was produced as small bright yellow plates melting at 214°. The interconversion of the forms could be effected by recrystallization using the appropriate solvent. It may, however, be pointed out that this does not exactly agree with the results of previous workers since Karrer and Töbler obtained a melting point of 199° for their sample crystallized from ether-petroleum ether mixture and not from chloroform, and Clark obtained a melting point of 214° for samples crystallized from dilute alcohol or from a mixture of ether and light petroleum. A scrutiny of the photomicrographs of Adams *et al.*<sup>5</sup> discloses that there is no correspondence between them and the description of the crystals given. The differences between the crystal structures do not seem to be fundamental. Our experience with a number of batches of gossypol preparations is not in accordance with the statements of Adams *et al.* All our samples were bright yellow in colour, had more or less the same crystalline form and the same melting point (189° decomp.). Methyl and ethyl alcohols and acetone were not suitable for getting good crystals since the substance was highly soluble in them. Ligroin, chloroform, benzene and ether-petroleum ether mixture were suitable and the products appeared very much like those presented in the photomicrographs of Adams *et al.* for samples obtained from chloroform.

Previous workers have stated that gossypol does not give correct results in combustion analysis and that it even gives an appreciably high methoxyl value (Clark<sup>11</sup>; Adams *et al.*<sup>12</sup>). Employing pure samples crystallized from chloroform, ether and ligroin, we have obtained consistent values for carbon and hydrogen agreeing with the formula  $C_{30}H_{30}O_8$  and they do not give any methoxyl value when tested by the micro-Zeisel method. Our samples of gossypol were found to possess all the properties and exhibit all the colour reactions recorded in the literature. A few new observations are given in the experimental section.

#### Experimental

The seeds of the Cambodia cotton (*Gossypium hirsutum*) were the most suitable since analysis of different species indicated that these contain the largest amount of gossypol. Delinting of the seeds with concentrated sulphuric acid rendered the separation of kernels easy but this

treatment was not found to be convenient or quite necessary. Breaking up the raw seeds in a disintegrator and sieving yielded the kernels in a satisfactorily powdered form and could be used directly. Fresh material was employed as far as possible since storage caused considerable deterioration in yield.

*Extraction.*—The method of Carruth was first adopted. The dry meats were first percolated with petroleum ether in order to remove the oil and then with ethyl ether in order to extract the gossypol. When the concentrated extract was treated with glacial acetic acid and allowed to stand for 4 weeks, the yield of gossypol acetic acid was 0.12% of the kernels taken. This corresponds to a yield of 0.11% of gossypol. When, however, aniline was added in the place of acetic acid, the separation of the anil was complete in about 24 hours and the yield of the pure anil amounted to 0.3% corresponding to a yield of 0.22% of gossypol.

With a view to improve the extraction of gossypol the fat-free meal was impregnated with water as suggested by Halverson and Smith (*loc. cit.*). 10% of moisture was found to be convenient as more than this amount yielded a pasty mass difficult to handle and this did not increase the yield very much.

The seed meal (1 kg.) was percolated with petroleum ether in order to remove the oil and the fat-free residue, thoroughly mixed with 10% of water, was extracted with peroxide-free ether by cold percolation. When glacial acetic acid was added to the concentrated extract, the separation of gossypol-acetic acid did not take place to any extent in the course of one month. On the other hand, addition of excess of aniline gave a good yield of the anilino compound amounting to 0.8% of the weight of the raw seeds. This corresponded to a yield of about 0.6% of gossypol.

The preliminary removal of the oil is not absolutely essential for obtaining fairly good yields of the anilino compound though it may be said to be desirable. Nor is it essential to add ethylene glycol or pyridine to the ether extract in order to complete the precipitation of the anil as suggested by Halverson and Smith.

The fresh meal (1 kg.) ground to pass through a 10-mesh sieve was treated with 100 c.c. of water and thoroughly mixed. It was extracted by cold percolation six times with peroxide-free ether containing 3% of alcohol and 2% of water in the course of 72 hours. The combined ether extracts were filtered, if necessary, treated with 10 c.c. of aniline and the solvent distilled off as far as possible. On adding sufficient petroleum ether to dissolve the oily matter and allowing to stand overnight, a good yield of

bright yellow anilino compound was obtained. After filtering the solid the mother-liquor was treated with a little more aniline to make sure that the precipitation was complete. The yield frequently amounted to 0.75%.

When employing chloroform fresh cotton-seed meal (1 kg.) was directly extracted with it by cold percolation (4 times) in the course of 12 hours. The combined extracts were treated with aniline as before and the solution concentrated by distilling off the solvent. Further operations were just as given above and the yield of the anilino compound was 0.8%.

Hot extraction of the seeds always resulted in poor yields.

*Decomposition of the anilino compound to obtain gossypol.*—the anilino compound obtained as described above was purified by crystallization first from aniline and again from hot benzene. It was thus obtained as an orange yellow crystalline solid (rectangular plates) melting with decomposition at 303°. It was insoluble in most of the solvents and also in dilute acids and alkalis and was unaffected by boiling with glacial acetic acid for several hours. It was however, easily soluble in alcoholic potash, producing an orange red solution and undergoing slow decomposition.

The method of Carruth using alcoholic potash gave rise only to a very small yield of impure gossypol (0.2 g. from 2 g. of the anil) which was very difficult to purify. Treatment with concentrated sulphuric acid according to the procedure of Clark at 90° for 5 minutes gave better results when small quantities of the anil were employed. The crude gossypol obtained by pouring the sulphuric acid solution in ice water was converted into the acetic acid compound and recrystallized. Though the yield was good (0.6 g. from 1 gram of the anil) and the melting point (184°) satisfactory the sample was rather brown and the colour was difficult to remove by repeated recrystallization from ether-petroleum ether mixture. When the experiment was repeated with larger quantities of the anil (10 g.), the yield of gossypol-acetic acid was very small, the main product being a highly coloured (pink red) substance which did not melt below 340°. It was found to contain some nitrogen and sulphur also. All attempts to obtain gossypol or any of its derivatives by further treatment failed.

The most satisfactory method using acetic anhydride was carried out as below:—

The anil (5 g.) was treated with acetic anhydride (25 c.c.) and the mixture heated on a wire-gauze till a clear solution was obtained. After cooling it was poured into ice water. The product first separated as a liquid and when subjected to vigorous stirring, turned into a yellow solid. It was filtered

and washed with hot water. It was subsequently dissolved in glacial acetic acid by warming and the cold solution treated with water. By this treatment gossypol-acetic acid was obtained as a bright yellow crystalline substance melting at  $185^{\circ}$  with decomposition. The yield was about 4 g. The aqueous mother-liquor from which the crude product separated yielded acetanilide on concentration.

The sample of gossypol-acetic acid had all the properties mentioned in the literature. It gave no tests for nitrogen and yielded acetic acid vapours on heating and after a final recrystallisation from acetic acid petroleum ether or ether-petroleum ether, yielded correct results by combustion analysis. [Found : C, 66.3 ; H, 5.9 ;  $C_{30}H_{30}O_8$ ,  $CH_3COOH$  requires : C, 66.4 ; H, 5.9%.]

Care should be taken not to precipitate the acetic acid compound by the addition of water to a hot solution in acetic acid since this brings about partial decomposition into gossypol. Though the product gives nearly the same melting point and exhibits other properties similar to pure gossypol-acetic acid, its carbon value is markedly high indicating admixture with gossypol.

*Preparation of gossypol.*—The decomposition of gossypol-acetic acid was carried out by previous workers by dissolving it in ether, adding water to the solution and distilling off the ether. Gossypol separated out in the form of crusts. It has now been found that this could be effected by boiling gossypol-acetic acid with water for 15 minutes. The solid was filtered, dried (1 g.) and dissolved in the minimum quantity of peroxide-free ether (10 c.c.). The clear solution was then treated with excess of petroleum ether. Gossypol crystallised out slowly as yellow rectangular plates, melting at  $189^{\circ}$  with decomposition. In a similar way it could also be crystallised by dissolving in chloroform and adding petroleum ether. This sample also melted at the same temperature and had the same crystal form [Found : C, 69.3 ; H, 5.9 ;  $C_{30}H_{30}O_8$  requires : C, 69.4 ; H, 5.8%].

The pure substance is tasteless and odourless. It gives no methoxyl value when tested by micro-Zeisel and readily forms the anilino and acetic acid derivatives.

The following reactions of gossypol have been noted besides those already described by previous workers. It reduces Fehlings solution and ammoniacal silver nitrate rapidly. It is quite stable in the presence of cold hydrochloric and sulphuric acids, dilute or concentrated, but it is readily changed by nitric acid and sodium peroxide. It does not give the typical colour reactions of flavones forming pyrylium salts when subjected

to reduction in acid or alkaline medium. Though it contains large number of phenolic hydroxyl groups, it does not readily react with phenyl isocyanate in benzene solution. It does not combine with picric acid to form a picrate. In alkali it produces a pale yellow solution and no characteristic differences are noticed with alkaline buffer solutions with different  $p_H$ .

#### Summary

Various methods of extracting gossypol from cotton-seed meal have been examined: (1) removal of the oil with petroleum ether and then extraction of gossypol with ether, (2) same as (1) but moistening the seed meal before ether extraction, (3) precipitation of gossypol as the acetic acid compound, (4) precipitation of gossypol as the aniline compound and (5) use of other solvents particularly chloroform not so far investigated. It has been confirmed that moistening of the meal is necessary for complete extraction with ether and when this is done only aniline works satisfactorily as the precipitant. The preliminary removal of the oil is not absolutely essential though desirable. With chloroform as the solvent, neither removal of the oil nor addition of moisture is necessary. Hence the use of chloroform as the solvent and aniline as the precipitant is most satisfactory. For recovering gossypol from the anilino compound decomposition using potash and sulphuric acid are not satisfactory. A new method has been evolved using acetic anhydride. Pure gossypol thus obtained melts at  $189^\circ$  and the solvent employed makes no difference. It gives no methoxyl values. A few other properties are described.

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