DENSITY MEASUREMENTS
OF TUNG OIL—ACETYLENE TETRABROMIDE SOLUTIONS

% Tung Oil	DENSITY AT 20° C.	25° C.	30° C.	35° C.
0	2.9445	2.9335	2.9242	2.9124
14	2.2600	2.2520	2.2439	2.2359
15	2.2235	2.2154	2.2074	2.1996
16	2.1878	2.1800	2.1721	2.1543
17	2.1536	2.1456	2.1388	2.1305
18	2.1197	2.1123	2.1049	2.0973
19	2.0872	2.0799	2.0725	2.0651
20	2.0560	2.0489	2.0417	2.0343
100	0.9380	0.9349	0.9315	0.9282

EXAMPLE

Assuming that the density of the tung oil solution is 2.104 at 25° C., it is found, by reference to the density-per cent tung oil curve for 25° C., that this solution contains 18.40 per cent tung oil, and X (per cent tung oil in kernels) =

$$\frac{0.1840 \times 14.753^*}{1 - 0.1840} \times \frac{100}{5} = 66.5\%.$$

If there were 30% kernels in the tung fruit, then the % oil in tung fruit = 66.5% × .30 = 19.95%.

COMPARISON OF RESULTS FOR OIL CONTENT OF OIL FRUIT

COMPARISON OF RESULTS FOR OIL CONTENT
OF TUNG CONTENT
by
Solvent Extraction and Proposed Rapid Method

Sample No.	Per cent Oil by Solvent Extraction**	Per cent Oil by Rapid Method
1	17.47	17.43
2	17.07	17.23
3	17.15	17.15
4	20.81	20.75
5	18.21	18.27
6	18.29	18.36
7	20.05	20.21
8	17.71	17.80
9	20.50	20.90
10	12.32	12.21
Average	17.96	18.03

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* This number represents the weight in grams of the acetylene tetrabromide delivered at 25° C. by the five-milliliter pipette used. If the particular pipette used delivers a different weight of this solvent at the temperature used, then this number should be replaced by the weight of solvent delivered by the pipette used.

** Oil and Soap, *16*, 151 (1939).

Cotton-Root Bark as a Source of Gossypol

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IN VIEW of the fact that the recent extensive researches of Adams and associates (1-20, 23, 24, 25) on the structure of gossypol have aroused wide interest in this compound, a brief description of a new and practical method for the preparation of pure gossypol is offered at this time. The proposed method is essentially a modification of that of Carruth (21), in which the precipitation of gossypol-acetic acid is applied to a solvent extract of cotton-root bark, rather than to an extract of the cotton seed. The advantages of cotton-root bark over the seed as a source of gossypol are chiefly the higher gossypol content and lower oil content of the root bark. The gossypol content of air-dried cotton-root bark has been found to range as high as 1.8 per cent, compared to about 1.0 per cent maximum for decorticated cottonseed meats, and the virtual absence of oil in the bark facilitates the separation and purification of the gossypol. Thus, the proposed method will yield about 0.9 per cent of purified gossypol, based on the air-dried bark, whereas, a good yield from seed is about 0.3 per cent (20).

Cotton-root bark, as a recognized drug (22), is obtainable from wholesale pharmacists, particularly in the South, although in this form its cost as a source of gossypol may be rather high. In the present investigation, it was found to be more economical to hire the labor to harvest, strip, and dry the cotton-root bark in the field, storing and grinding it in the laboratory as required. The most comprehensive available reference on the active medicinal principles in cotton-root bark, by Power and Browning (26), does not identify gossypol as one of the active ingredients, but a perusal of their experimental data leaves the impression that gossypol, or related phenolic substances, probably constitute a considerable part of the active material.

DISTRIBUTION OF GOSSYPOL IN THE COTTON PLANT

As early as 1935 short staple cotton plants, gathered at various stages of maturity, were collected in this Laboratory, and the various parts of the plants were separated, air-dried, and analyzed for gossypol content by the pyridine-aniline method (27). All of the cotton plants analyzed in this work were upland short staple (1 inch), Delta and Pineland 11 A strain. The results in Table I are based on plants grown in 1936 near Meldrim, Georgia, on a sandy loam soil, in a season of average rainfall.

For the gossypol determinations, 10 grams of the dried, ground, plant structures were extracted exhaustively with cold ethyl ether, the extract evaporated at reduced pressure below 35° almost to dryness, and then taken up with 50 ml. petroleum ether (Skellysolve F). Any precipitate that formed was filtered off, washing the filter with petroleum ether, and 3 ml. of pyridine-aniline mixture (4:1) was added to the filtrate. The samples were then shaken, loosely stoppered, and allowed 3 to 7 days to precipitate, depending upon

TABLE I
Distribution of Gossypol in the Upland Variety Short Staple Cotton Plant at Various Stages of Maturity

Plant Structure	Percentage Gossypol, air-dry basis A Immature Plant bolls just starting to form. July 15	B Mature green plant, most of bolls open. Sept. 15	C Dead Plant Jan. 15	D Dead Plant Feb. 15
Root Bark	0.70	1.53	1.80	1.82
Root (Whole)	0.25	—	—	—
Root (bark-free)	0.08	0.03	Trace	Trace
Stalk	0.05	0.0013	Trace	—
Leaf	Trace	Trace	—	—
Square	0.12	—	—	—
Boll	Trace	0.18 (Immature boll)	—	—
Seed (Mature)	—	0.52	—	—

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the gossypol content. With less than 0.01 per cent gossypol present, more than 3 days are necessary for completion of the reaction. Owing to the tendency of the precipitate to adhere to the walls of the precipitation vessel, the use of a wide mouth extraction flask is recommended. The brick-red precipitate of dianilinogossypol, containing pyridine of crystallization, is transferred to a tared gooch crucible, washed with 50-75 ml. petroleum ether containing about 2 per cent pyridine, and dried to constant weight at 160° C. (about one hour). The pyridine of crystallization is removed quantitatively by this procedure. The factor for converting the precipitate (dianilinogossypol, $C_{42}H_{40}N_2O_6$), to gossypol ($C_{30}H_{30}O_8$) is 0.775.

It will be noted from the analyses in Table I that the root bark is much richer in gossypol than the other portions of the plant at all stages of maturity. The cotton-root bark suffers no decomposition or loss of gossypol by remaining in the ground during the winter, and since it is common practice in some areas to plow up the plants in the winter, raking into piles and burning to clear the field for another crop, the only extra labor incident to harvesting the bark is that of stripping and drying.

ESTIMATED AVERAGE YIELD OF COTTON-ROOT BARK GOSSYPOL

In the winter of 1937, one thousand roots were gathered from a field of upland short staple cotton (Delta and Pineland, 11 A), and the following data were obtained on the air-dried bark (Table II). It should be pointed out that these figures, based as they are on the root from one variety, for a single season, and grown on a single type of soil, make no allowances for these variables, and the reported results may not be representative of a general average for the state or section. Ultimately, it is intended to collect samples of several varieties grown under various soil and climatic conditions to obtain general averages for the gossypol content of cotton-root bark, but the present figures will serve to show the value of the bark as a source of gossypol.

TABLE II

ESTIMATED AVERAGE YIELD OF ROOT-BARK GOSSYPOL PER ACRE OF COTTON

Average number of roots per acre	18,000
Yield of air-dried bark per root	8.5 grams
Yield of air-dried bark per acre	153 kg.
Gossypol content of air-dried cotton-root bark	1.5 per cent
Yield of crude gossypol per kg. of root-bark	13.0 grams
Yield of purified gossypol (mol. wt. 518) per kg. of root-bark	8.8 grams
Estimated yield of purified root-bark gossypol per acre of cotton	1346.4 grams
Percentage recovery of purified gossypol (based on weight of dry bark)	0.88

The purified gossypol was prepared from the air-dried root bark in the following manner. One kilogram of dry, coarsely ground bark was percolated with 4 liters of cold, peroxide-free, ethyl ether, and the ether extract was evaporated at reduced pressure, below 35°, to a volume of 150 ml. Fifty ml. of glacial acetic acid were added, and after stirring and allowing to stand loosely covered overnight, the first crop of greenish-yellow gossypol-acetic acid crystals (11.2 grams) was filtered off and washed with a small volume of a mixture of 3 parts ethyl ether and 1 part acetic acid. The combined filtrate and washing, upon evaporation at room temperature to about one-half of its original volume, yielded a second crop of brownish yellow crystals (4.8 grams). The combined yield of 16 grams of crude gossypol-acetic acid was redissolved in 400 ml. of ethyl ether, 50 ml. of acetic acid added, and allowed to stand 24 hours, yielding 13.3 grams of purified gossypol-acetic acid in the form of yellow crystals. This product was converted to gossypol by the method of Campbell, Morris, and Adams (20), dissolving it in a small volume of peroxide-free ether and pouring this solution into an equal volume of water containing about 0.4 per cent of sodium hydrosulfite. The ether was then evaporated below 60° to leave a crust of free gossypol floating on the water. The above treatment was repeated to insure the complete removal of acetic acid, and the yellow crust of free gossypol resulting from the second treatment was air-dried and powdered, giving a yield of 10.8 grams of fairly pure gossypol. Final purification was accomplished by dissolving it in 100 ml. of peroxide-free ether, adding an equal volume of petroleum ether (Skellysolve F), and concentrating the solution under diminished pressure until crystals started to form. An equal volume of petroleum ether was then added, and the solution was allowed to evaporate slowly at room temperature for 3 hours. The yield was 8.8 grams of small bright yellow crystals, melting at 180°, and having a molecular weight of 518.5, based on the nitrogen content (4.19 per cent) of the dianilino derivative (calc. mol. wt. 518.2 for $C_{30}H_{30}O_8$). A check determination of molecular weight by titration of the gossypol with 0.25N sodium hydroxide yielded a value of 518.0. High purity of this preparation is also indicated by the formation of perfect symmetrical crystals, illustrated in Plate 1. In this Laboratory, seed gossypol has rarely been obtained in a sufficient state of purity to form large perfect crystals, although the melting point and molecular weight of such preparations indicated a high degree of purity.

EXTRACTION OF COTTON-ROOT BARK WITH VARIOUS SOLVENTS

While ethyl ether was used in all large-scale extractions of gossypol from cotton-root bark, and proved to be quite satisfactory for this purpose, small samples of the powdered bark were subsequently extracted with other organic solvents and analyzed for gossypol con-

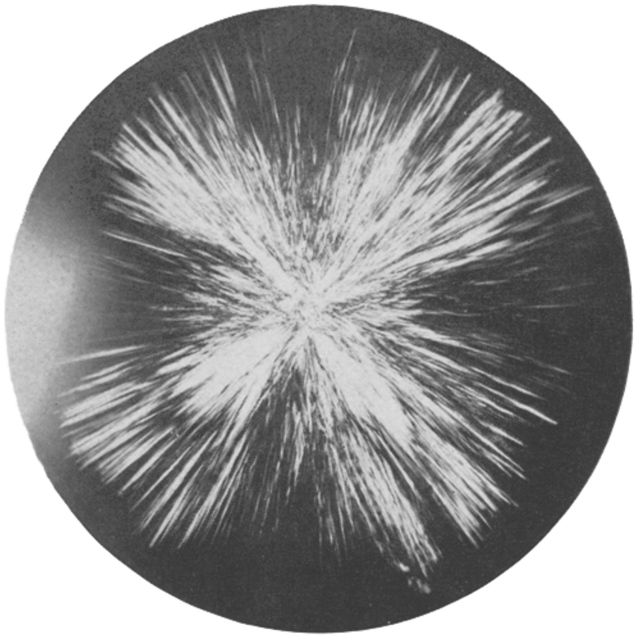

Plate 1.—Purified Gossypol (450X), m.p. 184°, crystallized from a mixture of ether and petroleum ether

tent by the pyridine-aniline method. These extracts were obtained by percolating the air-dried, powdered bark with the warm solvent, followed by evaporation of the solvent under diminished pressure, below 35°. The residues were finally heated to constant weight in vacuo at 50°, and, with the exception of the acetone extract, were free from solvent odor when weighed. The acetone extract appeared to retain some solvent under these conditions, probably combined with the gossypol as gossypol acetonate.

TABLE III

GOSSYPOL CONTENT OF VARIOUS ORGANIC SOLVENT EXTRACTS OF COTTON-ROOT BARK

Dried bark from upland short staple, Delta and Pineland 11 A variety.

Solvent	Crude Extract percent of air-dried bark	Gossypol* percent of air-dried bark
Petroleum ether (Skellysolve F)	1.81	0.62
Ethyl ether	3.40	0.97
Alcohol, 95%	13.46	0.58
Acetone	4.83	1.30
Chloroform	3.35	0.91

* Pyridine-aniline method.

It will be noted from Table III that while the bark yields much more extractive matter with alcohol than with ether, the latter extract is richer in gossypol and gives a higher percentage yield of gossypol, based on the weight of bark. The best yield, however, is shown by acetone, and preliminary tests on the precipitation of gossypol-acetic acid from the acetone extract show promise of giving better yields of crude gossypol than the described ether extraction method. Whether or not the crude gossypol-acetic acid obtained from the acetone extract lends itself to purification as readily as that from the ether extract has not yet been determined. Chloroform extracts a major portion of the gossypol from cotton-root bark in readily purifiable form, but petroleum ether and alcohol do not appear to be satisfactorily solvents for this purpose. The failure of petroleum ether to extract all of the gossypol is accounted for by the insolubility of gossypol in this solvent. In the case of 95% alcohol, it is thought that gossypol extraction is fairly complete, but that the large amount of alcohol-soluble extractive matter which accompanies it prevents complete separation of the gossypol as the dianilino derivative.

SUMMARY

Cotton-root bark from upland short staple cotton contains up to 1.8 per cent gossypol (air-dried basis). Analyses of other parts (stalk, bark-free root, leaves, squares, immature bolls) of the cotton plant showed little or no gossypol in these structures. The gossypol content of the root bark increases with maturity of the plant, and is highest in roots gathered in the winter after the stalk has dried.

Owing to the absence of oil in the root bark, the gossypol obtained therefrom by ether extraction is more easily purified than that from cottonseed. The experimental recovery of pure gossypol from one sample of root bark was 0.88 per cent.

It is estimated that one acre of cotton will yield 150 kilograms of dry root bark, from which about 1.4 kilograms of pure gossypol can be recovered.

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A Study of Dienometry*

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A CHEMICAL method for the quantitative determination of conjugation in fats and oils was first suggested by Kaufmann and Baltes (1), who found that the conjugated double-bonds present in fatty acids or fats underwent a Diels-Alder reaction when heated with an excess of maleic anhydride solution. The addition product formed was separated from the reaction mixture, and the unreacted maleic anhydride determined by titration. Shortly afterward Ellis and Jones (2) published a rather similar method, claiming increased accuracy and decreased reaction time. This was followed by Kaufmann's iodometric method (3) which differed from the earlier method only in the manner of determination of the excess maleic anhydride. Under the conditions of Kaufmann's methods it is claimed that one mole of the anhydride will react with one double-bond of a conjugated system; so the extent of reaction may be expressed in terms of iodine equivalents. The *diene number* is thus used to express the parts of maleic anhydride, calculated in equivalent amounts of iodine, taken up by 100 grams of fat. Beta-eleostearic acid, for example, is said to have a *diene number* of 91.3. Later work, however, has revealed that maleic anhydride will react not only with conjugated double-bonds but also with certain oxidation products, notably hydroxy compounds (4, 5, 6, 7, 8, 9). Furthermore, McKinney and Jamieson (10) were unable to secure quantitative addition of maleic anhydride to alpha- and beta-eleostearic acids, but instead obtained for both acids a diene number of 78.4, corresponding to 86.6 per cent of the theoretical value.

It is obviously of some practical importance to know the true diene numbers of pure conjugated fatty acids and glycerides, and in this connection the following preliminary study was made in order to ascertain the diene number of beta-eleostearic acid. Since Pelikan and von Mikusch (4) have already shown that

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