undesired increases in viscosity. On the other hand, where a low pour-point could be obtained by introduction of small substituents, the viscosity index was excessively low.

Although none of these diesters turned out to have characteristics that would justify their use as replacements for bis (2-ethylhexyl) sebacate, they represent considerable improvements over earlier materials based on higher fatty acids.

REFERENCES

1. Adams, H. E., and Powers, P. O., J. Applied Phys., *17*, 325 (1946).
2. Anderson, L. V., and Porter, J. V., Ind. Eng. Chem., *41*, 741 (1949).
2a. ASTM Standards, Part 4, 1952, pp. 167, 237.
3. Bartlett, J. H., U. S. Patent 2,637,697 (May 5, 1953); Chem. Abs., *47*, 7206 (1953).
4. Bonhorst, C. W., Althouse, P. M., and Triebold, H. O., Ind. Eng. Chem., *40*, 2379 (1948).
5. Bowden, F. P., Gregory, J. N., and Tabor, D., Nature, *156*, 97 (1945).
6. Bridgman, P. W., Proc. Natl. Acad. Sci. U. S., *11*, 603 (1925); Proc. Am. Acad. Arts Sci., *61*, 57 (1926).
7. Bried, E. M., Kidder, H. F., Murphy, C. M., and Zisman, W. A., Ind. Eng. Chem., *39*, 484 (1947).
8. Cannegeiter, D., Paint Oil Chem. Rev., *110*, No. 4, 17 (1947).
9. Cowan, J. C., *et al.*, Ind. Eng. Chem., *41*, 1647 (1949).
10. Cowan, J. C., and Teeter, H. M., Ind. Eng. Chem., *36*, 148 (1944).
11. Cowan, J. C., and Wheeler, D. H., J. Am. Chem. Soc., *66*, 84 (1944).
12. Dean, E. W., Bauer, A. D., and Berglund, J. H., Ind. Eng. Chem., *32*, 102 (1940).
13. Doolittle, A. K., and Peterson, R. H., J. Am. Chem. Soc., *73*, 2145 (1951).
14. Frenkel, J., "Kinetic Theory of Liquids," Oxford, pp. 191–208, 1946.
15. Gast, L. E., Croston, C. B., Schneider, W. J., and Teeter, H. M., Ind. Eng. Chem., *46*, 2205 (1954).
16. Georgievskii, R. G., and Shakhkeldyan, B. N., Zhur. Priklad. Khim., *24*, 593, 572 (1951); Chem. Abs., *46*, 2485, 4247 (1952).
17. Giammaria, J. J., U. S. Patent 2,616,852 (Nov. 4, 1952); Chem. Abs., *47*, 1925 (1953).
18. Glasstone, S., Laidler, K. J., and Eyring, H., "The Theory of Rate Processes," McGraw-Hill, New York, pp. 480–516, 1941.
19. Glavis, F. J., Ind. Eng. Chem., *42*, 2441 (1950).
20. Gregory, J. N., and Spink, J. A., Nature, *159*, 403 (1947).
21. Gros, A. T., and Feuge, R. O., J. Am. Oil Chemists' Soc., *29*, 313 (1952).
22. Joglekar, R. B., and Watson, H. E., J. Soc. Chem. Ind., *47*, 365T (1928).
23. Kaufmann, H. P., and Funke, S., Fette u. Seifen, *45*, 255 (1938).
24. Kemp, A. R., and Peters, H., Ind. Eng. Chem., *35*, 1108 (1943).
25. Kern, D. Q., and Van Nostrand, W., Ind. Eng. Chem., *41*, 2209 (1949).
26. Kimura, O., J. Chem. Soc. Japan, *64*, 895 (1943); Chem. Abs., *41*, 3336 (1947).
27. Kuhn, W. E., Chem. Eng. News, *30*, 979 (1952).
28. Lieber, E., and Cashman, A. F., U. S. Patent 2,633,471 (Mar. 31, 1953); Chem. Abs., *47*, 6132 (1953).
29. Meier, K., Farbe u. Lack, *58*, 55 (1952).
30. Menter, J. W., and Tabor, D., Proc. Roy. Soc. (London) *A204*, 514 (1951).
31. Menu, F. E., Fr. Patent 885,865 (Spt. 28, 1943); Chem. Abs., *47*, 4599 (1953).
32. Morgan, J. D., U. S. Patents 2,379,850 (July 3, 1945); 2,383,147 (August 21, 1945).
33. Mumford, S. A., J. Chem. Soc., *1952*, 4897.
34. Neher, H. T., Van Horne, W. L., and Bauer, L. N., U. S. Patent 2,623,036 (Dec. 23, 1952); Chem. Abs., *47*, 4134 (1953).
35. Paschke, R. F., and Wheeler, D. H., J. Am. Oil Chmists' Soc. *31*, 208 (1954).
36. Port, W. S., O'Brien, J. W., Hansen, J. E., and Swern, Daniel, Ind. Eng. Chem., *43*, 2105 (1951).
37. Ravich, G. B., Akad Nauk SSSR (Conference on viscosity of liquids and colloidal solutions), *1*, 427 (1941); Chem. Abs., *40*, 6271 (1946).
38. Sanders, G. V., and Tabor, D., Proc. Roy. Soc. (London), *A204*, 514 (1951).
39. Sims, R. P. A., Ind. Eng. Chem., *47*, 1049 (1955).
40. Tabor, D., Proc. Roy. Soc. (London), *A212*, 498 (1952).
41. Teeter, H. M., Gast, L. E., Bell, E. W., and Cowan, J. C., Ind. Eng. Chem., *45*, 1777 (1953).
42. Treibs, W., Ber. *75B*, 331 (1942).
43. Von Mikusch, J. D., Ind. Eng. Chem., *32*, 1061 (1940).
44. Winkler, W. B., U. S. Patent 2,663,649 (Dec. 22, 1953).
45. Young, D. W., and Lieber, E., U. S. Patent 2,411,178 (Nov. 19, 1946); Chem. Abs., *41*, 856 (1947).

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Conversion of Dianilinogossypol to Pure Gossypol

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Aniline reacts readily with gossypol, under certain conditions giving quantitative yields of dianilinogossypol. This is a condensation reaction in which water is split out from the amino hydrogen atoms of two molecules of aniline and the oxygen atoms of the two carbonyl groups of gossypol. Dianilinogossypol is a relatively stable compound and is only sparingly soluble in most organic solvents at ordinary temperatures. Because of these properties aniline has been used to recover gossypol from extracts of cotton seed (1, 2, 3).

Unlike gossypol-acetic acid, which is readily dissociated to gossypol and acetic acid, dianilinogossypol is relatively resistant to splitting into its component parts. The literature describes three reagents by which its decomposition has been effected: a) with alcoholic potash, b) concentrated sulfuric acid, and c) with acetic anhydride. With all of these reagents the objective was to remove the liberated aniline by distillation or to change the aniline into chemical compounds which are no longer capable of recombining with the gossypol formed. None of these procedures has been found entirely suitable for the recovery of larger quantities of pure gossypol.

In the present work it was desired to develop a procedure involving a mild treatment in order to protect the reactive gossypol molecule from decomposition so as to improve the yield of gossypol. Considerable difficulty was experienced in the search for a solvent which would separate gossypol and aniline following alkaline hydrolysis, because of the rapidity with which gossypol and aniline recombined. Excellent results were obtained however by rapidly hydrolyzing dianilinogossypol with alcoholic potash diluted with water, followed immediately by cooling and acidification with dilute, aqueous mineral acid which precipitated the gossypol and left the aniline in the aqueous solution as a salt. Following are the details of this procedure.

Hydrolysis of Dianilinogossypol

To 25 g. of powdered dianilinogossypol in a 1-liter Florence flask were added 300 ml. of hot (50°C.) half normal ethanolic potassium hydroxide (in 95% ethanol). Next 0.2 g. of sodium hydrosulfite ($Na_2S_2O_4$) was added, and the mixture was swirled for ½ min. to dissolve essentially all of the solid material. Then 240 ml. of water (80–90°C.), to which 0.25 g. of $Na_2S_2O_4$ had just been added, were poured into the flask, which was then heated by immersion in a steam bath for 8 to 10 min. (4 to 5 min. to heat to the boiling point followed by 4 to 5 min. of boiling). One tenth gram of $Na_2S_2O_4$ was added, the flask was closed, and the contents were cooled rapidly to 50°C. The mixture was acidified with 210 ml. of normal hydrochloric

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acid, and the precipitated gossypol was poured into 600 ml. of cold water containing 0.1 g. of $Na_2S_2O_4$. The mass was stirred in the water until the solid gossypol became granular.

Spectrophotometric Determination of Gossypol and Dianilinogossypol in Mixtures

In order to determine quantitatively the composition of crude hydrolysis products prepared by the method described the following analytical method was devised. This method was used to evaluate variations of the hydrolysis procedure to determine optimum conditions of hydrolysis.

Approximately 0.1000 g. of the mixture (accurately weighed) was dissolved in 100.0 ml. of benzene (warming on the steam bath if necessary). A suitable dilution to give an accurate absorbance (A) reading (*e.g.,* 1.00 ml. to 100.0 ml.) was made in the same solvent. The absorbance was determined at the dianilinogossypol peak near 440 millimicrons wavelength on a Beckman model B spectrophotometer against the solvent in a 1-cm. cell. The absorptivity *a* was calculated, using the following equation:

$$a = E^{g./l.}_{1\,cm.} = \frac{A}{\text{Concentration in g./l.}}$$

The dianilinogossypol content of the crude hydrolysis product was calculated, using the following equation:

$$\%\ \text{dianilinogossypol} = \frac{a(100)}{65.7}$$

The value 65.7 in the above equation was carefully determined to be the consistent absorptivity value, in benzene, for specimens of dianilinogossypol of the highest purity, prepared by the writers after multiple recrystallizations from various solvents followed by washing and drying. (Benzene, chloroform, aniline, toluene, and chlorobenzene[2] were used as solvents.)

For the gossypol determinations 12.0 ml. of the above diluted sample solution were transferred to a 25-ml. glass-stoppered graduated cylinder, and 10 drops of redistilled aniline were added. The cylinder was tightly stoppered, and, after the contents were mixed, was placed in a water bath at 60–70°C. for 1 hr. This quantitatively converts the gossypol to dianilinogossypol. The cylinder was removed from the bath, cooled to room temperature, and, when necessary, benzene was added to bring the volume back to the original mark to replace any evaporation loss. The 4% increase in volume of the sample solution, resulting from the addition of aniline is accounted for in the correction shown below upon calculating the corrected absorbance. An aniline-benzene blank was prepared in the same way. After determination of the absorbance of both as described above, the corrected absorbance of the treated sample solution was calculated as follows:

$$A_g = A_{sa} + 0.04A_{sa} - A_a$$

A_g = corrected absorbance
A_{sa} = absorbance of the treated sample solution
A_a = absorbance of the aniline blank.

Calculation of *a* for the treated solution was then made as above, using the corrected absorbance and % of gossypol in the original sample was calculated from the following equation:

$$\%\ \text{gossypol} = \frac{[a\ (\text{treated sample sol.}) - a\ (\text{original sample soln.})]\ 77.5}{65.7}$$

The value 77.5 is the percentage of gossypol theoretically obtainable from dianilinogossypol. The spectrophotometric data in Figure 1, which are typical of the data for the crude hydrolysis product prepared from dianilinogossypol from the various sources used, confirm the validity of the above methods of analysis and, together with recovery data, show that no interfering substances are produced by the hydrolysis procedure described. A very slight absorptivity due to gossypol at the wavelength of 440 mμ was found not to interfere significantly with the precision of the method for the purpose used.

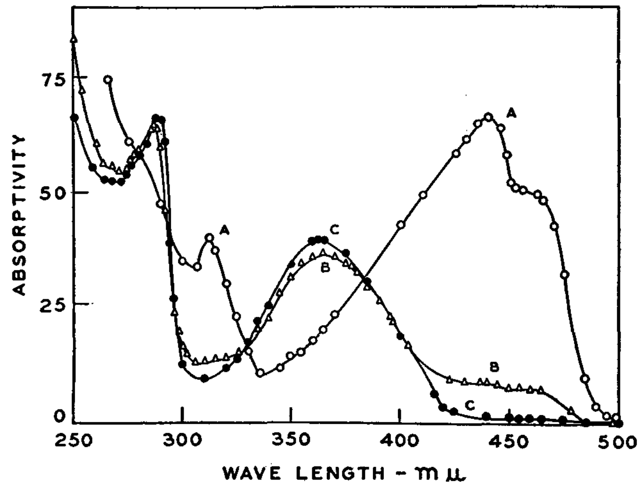

FIG. 1. Absorption Spectra of Dianilinogossypol and Gossypol.
A—○ Dianilinogossypol prepared from pure gossypol.
B—△ Gossypol prepared by hydrolysis of dianilinogossypol—crude hydrolysis product.
C—● Gossypol prepared by hydrolysis of dianilinogossypol—obtained by purifying the crude hydrolysis product.
(in chloroform)

When purified dianilinogossypol is used as the starting material, the crude hydrolysis product was found to contain approximately 13% of unchanged dianilinogossypol. Any water-insoluble impurities present in the starting material will also be found in the crude hydrolysis product. Table I gives typical analyses of crude hydrolysis products, prepared from starting material obtained from different sources.

Separation of Gossypol and Dianilinogossypol

Since dianilinogossypol is slightly soluble in ether, it was found that two recrystallizations of the crude hydrolysis product are necessary to obtain pure gos-

TABLE I

Analyses of Crude Hydrolysis Products

Source	% Gossypol	% dianilinogossypol
Unrecrystallized precipitate obtained from ether solution of purified gossypol by addition of aniline	86	13
Recrystallized dianilinogossypol obtained by extraction of raw, oil-free cottonseed flakes with a mixture of chloroform and aniline	87	13
Crude dianilinogossypol of 83% purity[3]	71	13
Crude dianilinogossypol[3] recrystallized from chlorobenzene	85	13

[3] Supplied by Buckeye Cottonoil Company, Cincinnati, O.

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[2] Chlorobenzene is an excellent solvent for recrystallizing dianilinogossypol, giving much better yields than the other solvents mentioned.

sypol. This reduces the yield. In order to increase the yield as well as to shorten the purification operation a new procedure was developed, which consists of dissolving the crude hydrolysis product in chloroform (both gossypol and dianilinogossypol are quite soluble in chloroform) and selectively extracting the gossypol from the chloroform solution with aqueous sodium hydroxide solution. This procedure produces gossypol of the highest purity in a single crystallization. The details of this procedure follow.

Purification of Crude Gossypol

The aqueous slurry of the crude hydrolysis product was transferred to a separatory funnel, and the solid material was extracted with one 400-ml. portion of chloroform. The chloroform layer was filtered through cotton into an open-top cylindrical special separatory funnel. This funnel is approximately 3.5 in. in diameter and of sufficient length to provide adequate capacity for the extraction operation. Then 200 ml. of normal NaOH solution, containing 1 g. of $Na_2S_2O_4$, were carefully poured down the side of the funnel so as to form a layer above the chloroform solution. Immediately 50 ml. of xylene were carefully poured down the side to form a third layer above the NaOH solution to protect the alkaline solution of gossypol from oxidation by air. The chloroform layer was carefully stirred for 30 min. in such a way as to keep the three layers separate and dissolve the gossypol in the NaOH solution. The chloroform layer was then carefully withdrawn. (In some cases where it was difficult to distinguish the aqueous and chloroform layers, the chloroform was drawn off slowly so that the interface could be seen which it approached the stopcock. A clean separation is necessary for the best quality of product.)

The NaOH layer was next drawn off into a separatory funnel containing 250 ml. of normal HCl solution and 300 ml. of peroxide-free ether.[4] This was shaken well to dissolve the gossypol in the ether. The aqueous layer was drawn off, and the ether layer was decanted through a folded filter paper (S & S No. 588 or its equivalent)[5] which had previously been half-filled with pulverized anhydrous sodium sulfate, into a 1-liter filtering flask containing 125 ml. of xylene. Boiling aids were added, and the flask was closed with a rubber stopper through which a thermometer had been inserted, extending nearly to the bottom of the flask. The ether was boiled off under reduced pressure (not exceeding 25 mm.) on a covered steam bath. The flask was shaken occasionally during the distillation and frequently toward the end. The temperature of the liquid in the flask was not allowed to rise above 25°C. When crystals began to form, the flask was removed from the steam bath and shaken continuously during the crystallization. The flask and contents were allowed to stand in an ice water bath for about 30 min. after releasing the vacuum. The crystals were filtered by suction on a fritted glass filter funnel (Buchner type). As soon as the mother liquor was drawn off, the flask and crystals were washed with petroleum ether. The crystals were washed several times more by slurrying with petroleum ether in the funnel followed by filtering each time. The crystals were finally dried in a vacuum oven to constant weight at 70°C. (Overnight is usually sufficient.)

Filtrates and residues may be treated with aniline and the dianilinogossypol may be reworked, as above.

Purified gossypol was obtained by the procedure with 63% yield based on the amount present in the original dianilinogossypol starting material, and 72% yield based on the actual amount of dianilinogossypol converted.

Discussion

The procedure was found to be applicable to the following: dianilinogossypol prepared from pure gossypol (by precipitation from ether with aniline followed by washing and drying), dianilinogossypol prepared by cold extraction (25°C.) of raw, defatted cottonseed flakes with a mixture of aniline and chloroform, and from a specimen of dianilinogossypl prepared by the Buckeye Cottonoil Company, Cincinnati, O.[3] The crude hydrolysis products were obtained in essentially quantitative yield. Further gossypol can be recovered by reworking the filtrates from the purification process, either as described previously (4) or by adding aniline to reprecipitate dianilinogossypol.

Spectral data show that the purified gossypol meets the standards of purity suggested by the writers elsewhere (4) and the absence of nitrogen was confirmed by the microkjeldahl method. Figure 1 shows typical qualitative and quantitative spectral characteristics of dianilinogossypol prepared from purified gossypol, the crude hydrolysis product prepared by the procedure outlined, and the purified gossypol obtained. These data show that approximately 13% of the dianilinogossypol remains unhydrolyzed in the crude hydrolysis product.

Gravimetric determination of aniline as $[Cu\text{-}An_2(SCN)_2]$ by the method of Pacu and Dima (5) in the aqueous washings from the hydrolysis product and from the vapors condensed during the heating period in a specially conducted hydrolysis demonstrate that only 3% of the aniline formed by hydrolysis passes out with the vapors while the remainder resides in the acidified aqueous solution from which the gossypol was precipitated.

Summary

A procedure for the isolation of gossypol (in 86% yield) from dianilinogossypol, together with a procedure for the purification of gossypol, is recorded. Dianilinogossypol is hydrolyzed in alcoholic potassium hydroxide, and the hydrolysis mixture is rapidly cooled and acidified, whereupon the gossypol is precipitated while the aniline remains in solution as the salt. Directions are given for the separation of gossypol from unhydrolyzed dianilinogossypol through partition between aqueous alkali and chloroform.

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REFERENCES

1. Carruth, F. E., J. Am. Chem. Soc., *40*, 647–663 (1918).
2. Clark, E. P., J. Biol. Chem., *76*, 229–235 (1928).
3. Murty, V. K., Murty K. S., and Seshadri, T. R., Proc. Indian Acad. Sci., *A 16*, 54–61 (1942).
4. King, W. H., and Thurber, F. H., J. Am. Oil Chemists' Soc., *30*, 70–74 (1953).
5. Spacu, G., and Dima, L., Z. anal. Chem., *110*, 25–29 (1937).

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[4] Wash the ether just before using with water which contains a small amount of $Na_2S_2O_4$ to remove peroxides.

[5] Trade names have been used only for the purpose of identifying equipment or materials actually used in conducting the work, and such use does not imply endorsement or recommendation by the U. S. Department of Agriculture over other firms or similar products not mentioned.