13. Mankowich, A. M., Ind. Eng. Chem., *47*, 2175 (1955).
14. McBain, J. W., and Green, A. A., J. Am. Chem. Soc., *68*, 1731 (1946).
15. Merrill, R. C., and Getty, R., J. Phys. and Colloid Chem., *52*, 774 (1948).
16. Preston, W. C., J. Phys. and Colloid Chem., *52*, 84 (1948).
17. Rigg, M. W., and Liu, F. W. J., J. Am. Oil Chemists' Soc., *30*, 14 (1953).
18. Vitale, P. J., Ross, J., and Schwartz, A. M., Soap, *32*, No. 6, 41 (1956).

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Kinetic Study of Gossypol Fixation in Cottonseed Oil[1]

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THE DEVELOPMENT of red alkali-fast color bodies on the storage of certain crude cottonseed oils has been attributed to gossypol present in the oil (1, 2, 3). When gossypol is removed from the oil immediately on its removal from the seed, either by processing (1, 4) or by chemical treatment (5), color fixation does not occur on storage of the oil. While the evidence from the investigations cited is that reactions of gossypol with constituents of cottonseed oil are responsible for the development of color bodies in the oil, little is known of the chemistry involved.

Recent studies (6) show that fixation of gossypol occurs when gossypol is added to purified triglycerides, ethyl acetate, and crude cottonseed oil. An ester exchange reaction of gossypol and the glyceride esters of the oil has been suggested as a possible first step in a sequence of reactions that lead to the production of alkali-fast color bodies (7) although there is little evidence to support the suggestion.

The investigation reported herewith, which is concerned with the kinetics of the initial reaction in the development of orange and red alkali-fast color bodies in refined and bleached cottonseed oil when gossypol is added, was initiated in the hope that more information on the chemistry of color fixation might be obtained.

Experimental

Stock solutions containing 4 g./l. of gossypol were prepared by dissolving purified gossypol in refined, bleached, and deodorized cottonseed oil at 25°C. They were held at −18°C. until used. Aliquots of the stock solutions (*ca.* 10 ml.) were sealed in glass vials under nitrogen and brought up to temperatures for storage in the dark at 40°, 60°, and 80°C. The vials were removed from each storage at predetermined intervals. A portion of each vial was used for the determination of absorbance at 365 mμ with a Beckman Model B spectrophotometer, using cyclohexane as the dilution and reference solvent. Another portion was dissolved in 30 ml. of peroxide-free diethyl ether, and the unreacted gossypol was removed from solution by four successive extractions with 25-ml. portions of 4% aqueous sodium carbonate, which contained 0.1% sodium hydrosulfite as an antioxidant. The oil-ether solution was washed with distilled water, then dried over anhydrous sodium sulfate. The ether was removed by gently heating under a stream of nitrogen. The absorbance of the gossypol-free oil was determined at 365 mμ, as described above. The same procedure

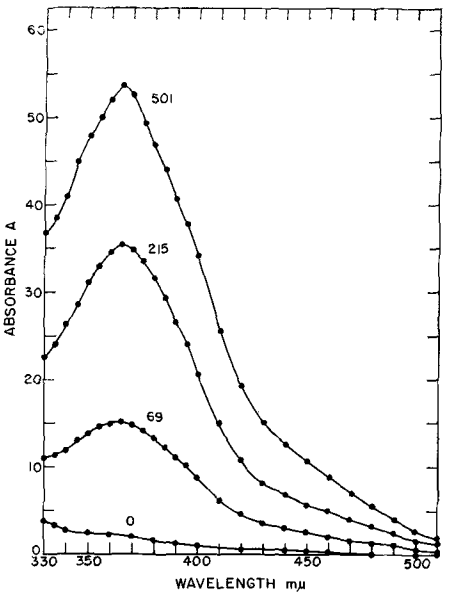

FIG. 1. Absorption spectra of carbonate insoluble gossypol reaction product for reaction times varying from 0 to 501 hrs. at 60°C.

was applied to the original stock solution to determine the initial absorbances at the time of preparing the vials for storage (zero time).

The absorptivity of gossypol in cottonseed oil was determined experimentally to be 37.5 at 365 mμ; the concentration of gossypol was expressed as g./l. of oil. This factor was used in all calculations. The initial concentration of gossypol in the stock solution (C_o), g./l., was determined from the relationship:

$$C_o = (A_1 - A_2)/37.5,$$

where A_1 and A_2 are the absorbances of the stock solution before and after alkali extraction. The concentration of the unreacted gossypol in the stored aliquots (C_l) was calculated similarly:

$$C_l = (A_3 - A_4)/37.5,$$

where A_3 and A_4 are the absorbances of the stored oil before and after alkali extraction. The concentration of the reaction product in the oil is $C_o - C_l$.

The absorptivity (a) of the reaction product of gossypol was calculated in terms of gossypol equivalents by use of the expression:

$$a = (A_4 - A_2)/(b \times c),$$

where b is the cell length in cm. and c the concentration of the reacted gossypol in g./l. of oil ($C_o - C_l$).

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Absorption spectra of gossypol and of the gossypol reaction product in the oil were determined in the region of 330–550 mμ, using cyclohexane as dilution and reference solvent.

Results

It was noted that there is a gradual decrease with time of storage in the absorbance at 360 mμ (the wavelength of maximum absorption for gossypol in cottonseed oil), and a gradual shift of the absorption maximum to 365 mμ. The absorption maximum for the solution is at 365 mμ after the unreacted gossypol is removed by the carbonate extraction, as may be seen from the data plotted in Figure 1, where the absorbance is plotted against the wavelength. The effect of time of storage is noted by the progressive increase in absorption at 365 mμ. The similarity of these spectra suggests the production of one major reaction product of gossypol. When the spectra in this figure were calculated in terms of absorptivity, the curves were superimposable, within experimental errors, giving further indication of only one reaction product being formed.

Differences in the absorptivity of gossypol and its reaction product are illustrated in Figure 2. In the region from 330–395 mμ the absorptivity of gossypol was the higher, while in the visible region from 400–550 mμ that of the reaction product was the greater. As a consequence the oils exhibited a progressive increase in red coloration with increase in time of storage.

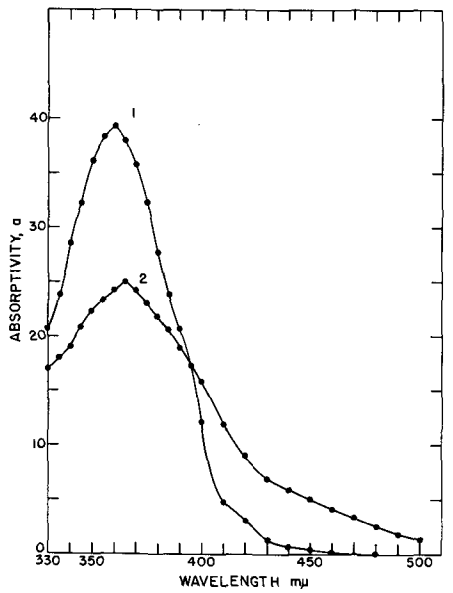

FIG. 2. Absorptivity of gossypol and of the gossypol reaction product in cottonseed oil. Curve 1—gossypol. Curve 2—gossypol reaction product.

Data accumulated in the kinetic study of the fixation of gossypol at three different temperatures are recorded in Tables I, II, and III.

These data very strongly suggest that the initial reaction in the fixation of color in cottonseed oils is of the second order with respect to gossypol. Thus the reciprocal of the gossypol concentration is proportional to the time of storage, as shown by the data plotted in Figure 3. This means that two molecules of gossypol enter into the reaction simultaneously.

It may be noted from the data plotted in Figure 3 that the reaction is strongly temperature dependent; the specific reaction rates are 2.4×10^{-4} for the reaction at 40°, 6.8×10^{-4} for the reaction at 60°, and 52×10^{-4} for the reaction at 80°. These reaction rate constants, expressed in terms of the reciprocal of the product of grams per liter times hours, were calculated by the method of least squares, using the data recorded in Tables I, II, and III.

TABLE I

Rate of Gossypol Fixation in Cottonseed Oil at 40°C.

Elapsed time	Gossypol in solution	Gossypol—reacted	Absorptivity of reaction product
hrs.	*g./l.*	%	*a*
0	3.688	....	.....
68	3.549	3.8	20.9
166	3.467	6.0	22.9
234	3.344	9.3	27.9
402	3.221	12.7	25.5
596	2.912	21.0	22.9
933	1.973	46.5	26.7
1,457	1.848	49.9	27.0
1,745	1.472	60.1	25.5

TABLE II

Rate of Gossypol Fixation in Cottonseed Oil at 60°C.

Elapsed time	Gossypol in solution	Gossypol—reacted	Absorptivity of reaction product
hrs.	*g./l.*	%	*a*
0	3.688	....	....
4	3.637	1.4	15.2
21	3.597	2.5	20.6
45	3.333	9.6	18.3
69	3.133	15.1	22.0
93	2.963	19.7	26.4
165	2.443	33.8	25.9
215	2.363	35.9	24.3
261	2.161	41.4	24.6
339	1.819	50.7	24.7
501	1.538	58.3	23.4
837	1.227	66.7	21.6

TABLE III

Rate of Gossypol Fixation in Cottonseed Oil at 80°C.

Elapsed time	Gossypol in solution	Gossypol—reacted	Absorptivity of reaction product
hrs.	*g./l.*	%	*a*
0	3.773	....	....
6	3.509	7.0	....
24	2.992	20.7	23.5
48	2.380	37.0	23.4
72	1.824	51.7	23.7
96	1.493	60.4	23.8
118	1.235	67.3	23.1
147	0.947	74.9	22.7
171	0.893	76.3	23.1
194	0.869	77.0	21.4
219	0.835	77.9	21.9
313	0.448	88.1	21.1
405	0.400	89.4	19.1
574	0.333	91.2	19.6

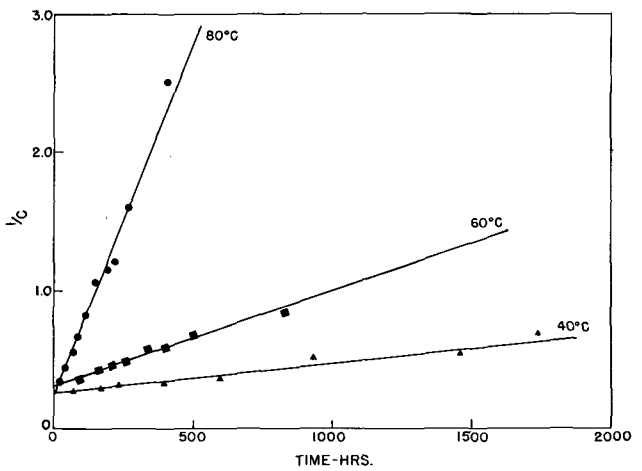

FIG. 3. Reciprocal of gossypol concentration plotted against time of storage at 40°, 60°, and 80°C.

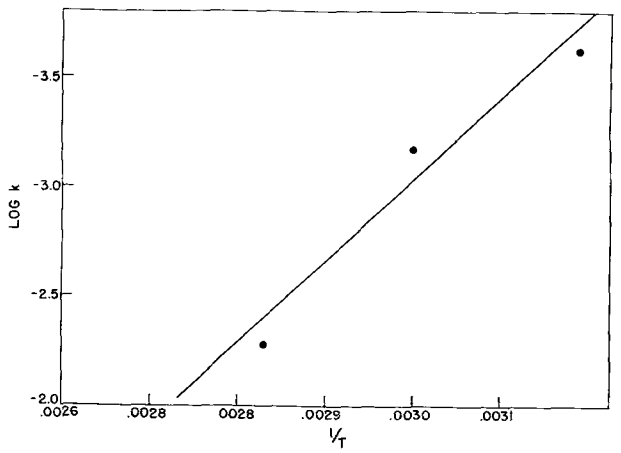

FIG. 4. Relationship between the reciprocal of the absolute temperature of storage and the specific reaction rate constant for the reaction of gossypol in cottonseed oil.

The reaction product is the same at each storage temperature, and there is no evidence of secondary or of consecutive reactions under the anaerobic conditions used. This suggestion is supported by the fact that the rate studies show the second-order equation to hold even when a large proportion of the gossypol has reacted. Further there is an absence of a drift with time or with temperature in the absorptivity of the reaction product, as may be seen from the data recorded in Tables I, II, and III. In addition, the van't Hoff relationship, $\log k = K/T + R$, where k is the specific reaction rate constant, T is the absolute temperature, and K and R are constants, holds reasonably well, as shown by the data plotted in Figure 4.

The temperature dependence of the specific reaction rate constant for the fixation of gossypol in cottonseed oil is indicated by the data plotted in Figure 4, where the logarithm to the base 10 of the specific reaction rate constant is plotted against the reciprocal of the absolute temperature. The energy of activation for this reaction was calculated to be 17,000 calories per mol.

Summary

It was shown in experiments carried out under anaerobic conditions that the fixation of gossypol in cottonseed oil is a reaction of the second order with respect to gossypol. In other words, the rate of fixation is proportional to the square of the gossypol concentration in the oil. The rate of fixation is temperature-dependent and increases 22-fold with an increase in temperature from 40° to 80°C.

REFERENCES

1. Thurber, F. H., Vix, H. L. E., Pons, W. A. Jr., Crovetto, A. J., and Knoepfler, N. B., J. Am. Oil Chemists' Soc., *31*, 384–388 (1954).
2. Williams, P. A., Boatner, C. H., Hall, C. M., O'Connor, R. T., and Castillon, L. E., J. Am. Oil Chemists' Soc., *24*, 362–369 (1947).
3. Pons, W. A. Jr., Thurber, F. H., and Hoffpauir, C. L., J. Am. Oil Chemists' Soc., *32*, 98–103 (1955).
4. King, W. H., Wolford, L. T., Thurber, F. H., Altschul, A. M., Watts, A. B., Pope, C. W., and Conly, J., J. Am. Oil Chemists' Soc., *33*, 71–74 (1956).
5. Dechary, J. M., Kupperman, R. P., Thurber, F. H., and O'Connor, R. T., J. Am. Oil Chemists' Soc., *31*, 420–424 (1954).
6. Berardi, L. C., and Frampton, V. L., J. Am. Oil Chemists' Soc., *34*, 399–401 (1957).
7. Frampton, V. L., Kuck, J. C., Dechary, J. M., and Altschul, A. M., J. Am. Oil Chemists' Soc., *35*, No. 8, 18, 20 (1958).

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The Separation of Glycerides by Crystallization in a Thermal Gradient[1]

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FRACTIONAL CRYSTALLIZATION from solvents has been used for a long time to separate natural mixtures of glycerides and other lipides (1); however the separation achieved is generally much poorer than would be predicted on the basis of the relative solubilities of the lipides under the conditions of the separation. This has been attributed to mixed crystal formation, the mutual effects of the solutes on the other's solubility, and the difficulty of completely separating the crystals and mother liquor. These effects can be minimized and the separation improved by repeated crystallizations from dilute solutions, but this is a laborious and time-consuming operation. Recently Baker and Williams (2) designed an apparatus which automatically subjects a solute to repeated recrystallizations as the solution moves through a thermal gradient. They demonstrated the effectiveness of the apparatus in the separation of polystyrene into fractions of different molecular weights. Since the use of this apparatus appeared promising as a technique for separating glycerides and many other lipide mixtures, an apparatus similar to that of Baker and Williams was constructed. The present paper presents the results achieved in the separation of some synthetic triglycerides by using this apparatus.

Experimental

The apparatus is essentially a copy of that of Baker and Williams (2). The only major modification was the use of mechanical refrigeration instead of cold water to cool the bottom of the column. In these experiments acetone was the starting solvent, and the solvent reservoir was filled with 200 ml. of acetone. Skellysolve B was the eluting solvent, which was added continuously to the solvent reservoir as fractions were collected. The sample was 0.5 g. and was made up of equal weights of the two glycerides to be separated. Fractions of 10.5 ml. were collected by a fraction collector. The temperature at the bottom of

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