in an autoclave and pressed under the same conditions used in Tests Nos. 4, 5, and 6, a high proportion of the glands was completely disintegrated by hydraulic pressing. Further studies on the relationship between source of seed, time elapsed after harvesting, and the susceptibility of the pigment glands to mechanical rupture are contemplated.

In the laboratory experiments with the hydraulic press, even at pressures of 20,000 pounds per sq. in., it was found that some glands still remained intact while in the commercial screw-press meal substantially all of the glands were ruptured. Evidently the screw press develops forces other than direct pressure, perhaps shearing forces, that bring about the immediate disintegration of the glands and spreading of the contents onto the meal and into the oil.

Summary

A procedure is given for estimating the amount of intact pigment glands in cottonseed kernels and meal.

The amounts of intact pigment glands, free gossypol, and total gossypol in uncooked meats, cooked meats, and press cake samples from commercial hydraulic-press mills, a screw-press mill, and laboratory-scale tests were determined. It was observed that when meats were cooked with adequate moisture present, as is common in hydraulic-press mills, most of the free gossypol was converted to bound gossypol during the cooking operation and further conversion during the pressing stage was of slight magnitude. The trend of the change in percentage of recoverable glands was roughly parallel to that of the free gossypol. By contrast, in the screw-press mill where cooking was carried out without the addition of moisture, little change in either component occurred during cooking, but both were reduced to very low levels during passage of the cooked meats through the screw press.

In laboratory tests cooked cottonseed meats were subjected to hydraulic pressures at levels of 2,000 and 20,000 pounds per sq. in. of cake surface. When meats were cooked at low moisture content, no significant change in either free gossypol or recoverable glands occurred during cooking, but hydraulic pressing at both 2,000 and 20,000 pounds per sq. in. reduced the percentage of recoverable glands. No corresponding decrease in free gossypol during pressing could be found. Wet cooking of meats decreased the percentages of free gossypol and intact glands and, although hydraulic pressing failed to further reduce the free gossypol, the percentage of recoverable glands was sharply reduced by pressing at both levels of pressure.

It is suggested that the high degree of effectiveness of the screw press in rupturing and disintegrating pigment glands in cottonseed meats is due to the development of shearing forces in combination with direct or compressive type pressure. It is believed that a shearing action is more effective for this purpose than compressive force of similar magnitude.

REFERENCES

1. Bailey, Alton, E., "Industrial Oil and Fat Products," Interscience Publishers Inc., New York, N. Y., pp. 473-476.
2. Boatner, Charlotte H., and Hall, Catherine M., Oil and Soap, *23*, 123-128 (1946).
3. Boatner, C. H., Altschul, A. M., Irving, G. W. Jr., Pollard, E. F., and Schaefer, H. C., Poultry Science *27*, 315-328 (1948).
4. Boatner, C. H., Hall, C. M., O'Connor, R. T., Castillon, L. E., and Curet, C. M., J. Am. Oil Chem. Soc., *24*, 97-106 (1947).
5. Clark, E. P., Jour. Biol. Chem., *76*, 229-235 (1928).
6. Gallup, W. D., J. Biol. Chem., *77*, 437 (1928).
7. Haddon, R., *et al.*; and Thurber, F. H., *et al.*, Cotton Gin and Oil Mill Press, April 29, 1950.
8. Lyman, Carl M., Holland, Bryant R., and Hale, Fred, Ind. and Eng. Chem., *36*, 188-190 (1944).
9. Olcott, H. S., J. Am. Oil Chem. Soc., *25*, 125-127 (1948).
10. Pons, W. A., and Guthrie, J. D., J. Am. Oil Chem. Soc., *26*, 671-676 (1949).
11. Pons, W. A., Hoffpauir, C. L., and O'Connor, R. T., J. Am. Oil Chem. Soc., *27*, 390-393 (1950).
12. Williams, P. A., *et al.*; and Hall, C. A., *et al.*, J. Am. Oil Chem. Soc., *26*, 28-34 (1949).
13. Withers, W. A., and Carruth, F. E., J. Agr. Res., *5*, 261-288 (1915).
14. Withers, W. A., and Carruth, F. E., J. Agr. Res., *12*, 83-102 (1918).

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Pigments of Cottonseed. IV. Gossypurpurin, a Purple Pigment Related to Gossypol[1]

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GOSSYPURPURIN, a naturally occurring, purple-colored pigment of cottonseed, exhibits a characteristic absorption spectrum in chloroform, with maxima in the visible wave length region at 565-568 mμ and 530-532 mμ. This pigment was first isolated from the red crystals (so-called "red gossypol") obtained from chloroform extracts of cottonseed kernels (2, 3, 8). It has also been obtained by treating an ethereal extract of cottonseed kernels with dilute ammonium hydroxide (2, 6). In this procedure the treatment was believed to have effected a conversion of gossypol in the ethereal solution to gossypurpurin. Gossypurpurin was not obtained readily or in large amount in either of the above cases. Except for its absorption spectrum in chloroform solution (6), no information concerning this pigment has heretofore been available. There follows a report of the conversion of gossypol to gossypurpurin via diaminogossypol and of the isolation of the native pigment from cottonseed pigment glands (5, 10). The properties of the isolated gossypurpurin are compared with those of the artificial product, and some observations are reported on the structure and relationship of this pigment to gossypol.

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Experimental

Preparation of Diaminogossypol. Gaseous ammonia was passed into a warm solution containing 1.5 g. pure gossypol in 75 ml. chloroform. The solution was refluxed 45 minutes, during which time the addition of ammonia was continued. At the end of this period heating was discontinued and the chloroform solution

was allowed to cool slowly. A bright yellow, crystalline precipitate separated from the solution. Recrystallization from chloroform yielded bright yellow plates, which, after drying in a vacuum desiccator, melted at 219-221°C. Yield, 0.75 g.

Anal. calc'd for $C_{30}H_{34}O_7N_2$: C, 67.4; H, 6.37; N, 5.24; Found: C, 67.3; H, 6.37; N, 5.02.

The absorption spectrum of a chloroform solution of diaminogossypol has maxima at 378 mμ, $E^{1\%}_{1cm.} = 344$, and at 250 mμ, $E^{1\%}_{1cm.} = 1015$ (Figure 1). The spectrum was determined with a Beckman quartz spectrophotometer, equipped with absorption cells having an optical path of 1 cm.

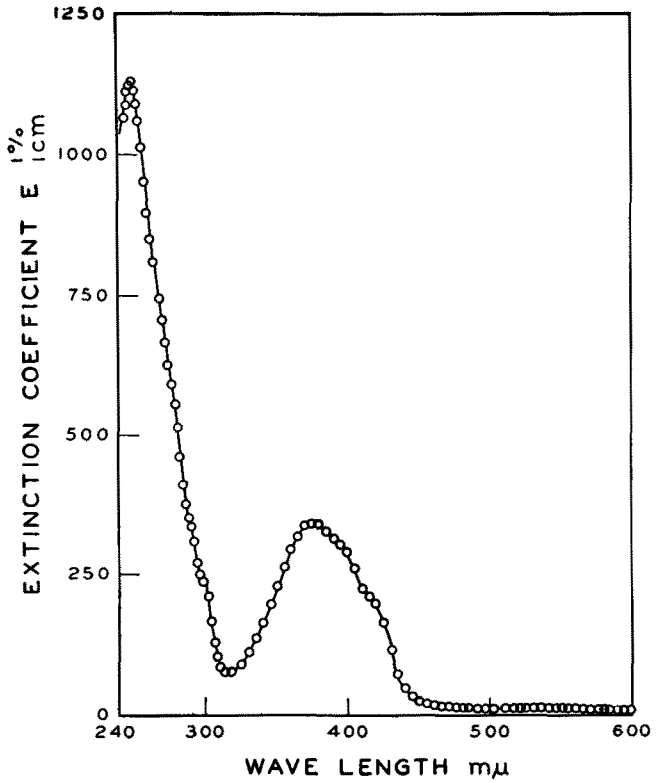

FIG. 1. Absorption spectrum of diaminogossypol in chloroform solution.

Diaminogossypol in chloroform solution gives a yellow reaction product with antimony trichloride, which exhibits an absorption maximum at 410-416 mμ, as shown in Figure 2, in contrast to the red product obtained with gossypol (4), which has absorption maxima at 375 and 516 mμ. This test was applied to diaminogossypol as a test of the purity of this intermediate.

Preparation of Gossypurpurin. Recrystallized diaminogossypol (0.59 g.) was heated *in vacuo* at 150-160°C. for one hour. The resulting purple-red product (approx. 0.3 g.) had the same crystal form as the original diaminogossypol. Chloroform (24 ml.) was added to this product in a low-actinic flask. The suspension was allowed to stand for six days at 3°C. A dark-purple precipitate was obtained upon the addition of light petroleum naphtha to the suspension. The precipitate was washed with 75 ml. light petroleum naphtha by centrifugation and decantation, followed by drying in a vacuum desiccator. Yield, 0.26 g., m.p. 200-204°C. (dec.).

Anal. calc'd for $C_{30}H_{32}O_7N$: C, 69.6; H, 6.28; N, 2.70; Found: C, 70.0; H, 5.97; N, 2.34.

Spectral Characteristics of Synthetic 'Gossypurpurin. The absorption spectrum of synthetic gossypurpurin in chloroform solution has maxima at 566-568 mμ, at 530-532 mμ, and at 370 mμ and a minimum at 440 mμ. The specific extinction coefficients at these points, $E^{1\%}_{1cm.}$, are 348, 279, 226, and 104, respectively, with ratios 566-568 mμ/530-532 mμ = 1.24; 566-568 mμ/370 mμ = 1.42; and 566-568 mμ/440 mμ = 3.34.

Synthetic gossypurpurin, in chloroform solution, forms a characteristic, but unstable blue-green reaction product with antimony trichloride. The absorption spectrum of this product in chloroform exhibits maxima at 665 and 375 mμ. Because of the instability of this reaction product a freshly prepared reaction mixture was used for each range of 10 mμ in determining the absorption spectrum, and a composite curve was drawn (Figure 3).

Reversion of Gossypurpurin to Gossypol in Presence of Acid. A chloroform solution of gossypurpurin was treated with a drop of concentrated hydrochloric acid and the resulting solution treated with antimony trichloride. Spectral examination of the reaction product indicated that the gossypurpurin was completely converted to gossypol.

When the reaction product of gossypurpurin with antimony trichloride was allowed to stand for one hour at room temperature, a slight maximum developed at 510-520 mμ, where the gossypol-antimony trichloride reaction product exhibits its principal absorption. Simultaneously, the maxima at 665 and 375 mμ decreased (Figure 3). After 24 hours the reaction product was identical with that of gossypol and antimony trichloride, thus indicating the com-

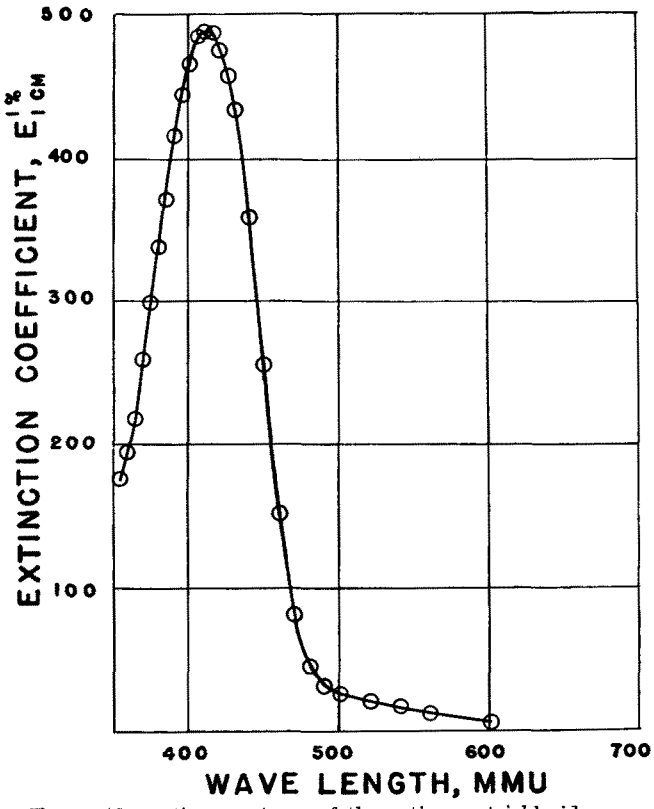

FIG. 2. Absorption spectrum of the antimony trichloride reaction product of diaminogossypol.

plete conversion of gossypurpurin to gossypol by the acidic antimony trichloride.

Examination of Chemical Properties of Gossypurpurin. The synthetic gossypurpurin is unstable to varying degrees in all the organic solvents (principally polar) in which it is soluble. It decomposes to give a yellow pigment which has not yet been characterized, but which does not yield the typical gossypol-antimony trichloride reaction product.

Several qualitative tests (9) were applied to gossypurpurin in an effort to determine to what extent it was related to gossypol. Positive reactions were obtained with the same reagents which were found to give positive results with gossypol, namely those indicating the presence of one or more carbonyl groups, such as Tollen's reagent, Fehling's solution, hydroxylamine hydrochloride, phenylhydrazine hydrochloride, and those indicating the presence of two ortho-phenolic hydroxyls and a hydroxyl peri or ortho to a carbonyl (1), such as ferric chloride, pyroboroacetate, and stannic chloride. Concentrated sulfuric acid reacts with gossypurpurin to produce a yellow-green color which becomes orange after ten minutes, in contrast to the dark red color developed immediately with gossypol. A green precipitate is obtained when gossypurpurin is treated with glacial acetic acid, in contrast to the yellow precipitate obtained with gossypol. The reactions of gossypurpurin with benzene sulfonyl chloride and nitrous acid were inconclusive but would seem to indicate the absence of an aromatic or aliphatic primary amine group.

Preparation of the Reaction Product of Synthetic Gossypurpurin with Aniline. To a solution of 0.2 g. gossypurpurin in 12 ml. diethyl ether was added 12 ml. freshly distilled aniline. After heating the resulting reddish-brown solution for 25 minutes on a steam bath, 20 ml. 95% ethanol was added and the mixture maintained overnight at 3°C. The bright-orange crystals, which separated after washing with 95% ethanol by centrifugation and decantation and drying in a vacuum desiccator, weighed 0.14 g. After two recrystallizations from hot chloroform and diethyl ether, 0.03 g. bright-orange plates were obtained, m.p., 298-300°C.

Anal. calc'd for $C_{42}H_{40}N_2O_6$: C, 75.4; H, 6.03; N, 4.19; Found: C, 74.8; H, 6.12; N, 4.42.

The absorption spectra for the reaction product of gossypurpurin and aniline and dianilinogossypol (7) were identical. When the antimony trichloride test was applied to the reaction product of gossypurpurin and aniline and dianilinogossypol, the results were identical.

Isolation of Native Gossypurpurin. Separated pigment glands (200 g.) were mixed with approximately 150 ml. water to give a thick sludge, which was allowed to stand four hours at 3°C. to assure complete rupture of the glands. The sludge was then mixed in approximately 50 g. portions with 500 ml. 95% ethanol in a Waring Blendor. The total mixture was centrifuged, and the supernatant ethanol extract, which contained most of the gossypol in the glands, was discarded. The residue was washed several times with ethanol, discarding the wash solutions each time. The residue was allowed to dry in the dark. The gland walls and residual contents were extracted with chloroform in a Soxhlet extractor. The first few extracts, which were brown in color, contained

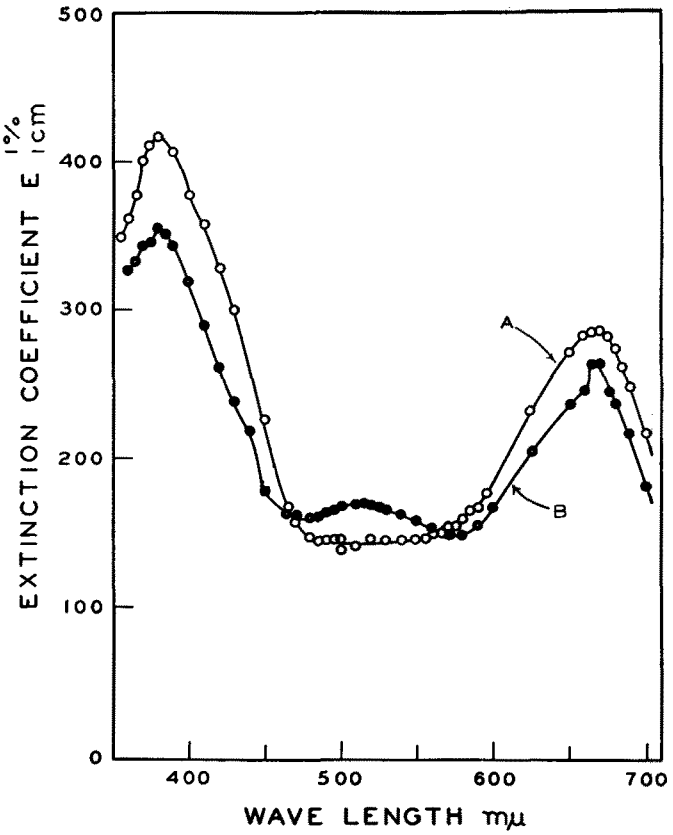

FIG. 3. Absorption spectra of (A) the antimony trichloride reaction product of gossypurpurin and (B) the same reaction product after standing for one hour.

the remaining gossypol left in the glands and were discarded. Extraction with chloroform was continued, and when the extract became purple, it was removed. The purple extract was filtered, mixed with dilute (approx. 10%) acetic acid in a separatory funnel, and allowed to settle. The water-washed purple chloroform layer was removed and dried for one hour over sodium sulfate in a low-actinic flask. To the decanted chloroform solution approximately four times the volume of light petroleum naphtha was added. The purple precipitate which separated from the solution was filtered off, and the dilute purple filtrate was allowed to stand at 3°C. overnight. A small amount (30 mg.) of dark purple, amorphous, but finely divided, native gossypurpurin separated from the filtrate on standing. After drying in a vacuum desiccator, it melted with decomposition at 200-204°C.

Spectral Characteristics of Native Gossypurpurin. The absorption spectrum of native gossypurpurin in chloroform solution has maxima at 566-568 mμ, at 530-532 mμ, and at 370 mμ and a minimum at 440 mμ. The specific extinction coefficients at these points, $E^{1\%}_{1cm.}$, are 315, 250, 233, and 85.0, respectively, with ratios 566-568 mμ/530-532 mμ = 1.26; 566-568 mμ/370 mμ = 1.35; 566-568 mμ/440 mμ = 3.70.

Native gossypurpurin in chloroform solution gave the same blue-green reaction product with antimony trichloride as the artificially prepared gossypurpurin.

Native gossypurpurin, like the synthetic product, was completely converted to gossypol upon treatment with hydrochloric acid.

Discussion

A tentative molecular formula for gossypurpurin, $C_{30}H_{32}O_7N$, is proposed on the basis of its quantitative elemental composition. The relative ease of conversion of gossypurpurin to gossypol by mineral acid and the identity of the reaction products of both gossypurpurin and gossypol with aniline would seem to indicate that the basic structures of the two pigments are similar. Qualitative tests indicate that gossypurpurin possesses reactive groups similar to those of gossypol, i.e., one or more carbonyl groups, two or more phenolic hydroxyls, and one or more hydroxyls peri or ortho to a carbonyl.

Purification of the isolated native pigment proved rather difficult, owing to the simultaneous extraction from pigment glands by chloroform of materials other than gossypurpurin. Removal of these impurities from the isolated pigment by treatment with dilute acetic acid apparently was not complete since it was not possible to obtain consistent analytical data for the isolated gossypurpurin, and they did not agree exactly with those for the synthetic product. Therefore it cannot be stated with absolute certainty that the two pigments are completely identical. However the native gossypurpurin exhibited an absorption spectrum in chloroform solution with maxima in the same positions as that of the artificial product, and the ratios of the extinction coefficients at 566-568 mμ to those at 530-532 mμ were identical. The similarity of the absorption spectra of the two products indicates that the chromophoric groups are very similar, if not identical, in both native and synthetic gossypurpurin. Native gossypurpurin also gave the same melting point, characteristic blue-green antimony trichloride test, and qualitative tests as the gossypurpurin prepared from gossypol and the conversion to gossypol by acid was identical in both cases. The similarity or identity of the chemical and physical properties of the two compounds would seem to indicate that they possess the same reactive groups and basic nuclei.

Summary

Gossypurpurin was prepared from gossypol via diaminogossypol, and its properties compared with gossypurpurin isolated from cottonseed pigment glands. A tentative molecular formula for synthetic gossypurpurin, $C_{30}H_{32}O_7N$, has been proposed on the basis of its elementary composition. The native pigment could not be obtained in the same degree of purity as the synthetic product and the analytical data could therefore not be brought into exact agreement for the two products. However solutions of both pigments in chloroform exhibit almost identical absorption spectra and identical antimony trichloride tests. Qualitative reactions seem to indicate that the functional groups of both native and synthetic gossypurpurin are identical, and the ready conversion of both products to gossypol upon contact with acid seems to indicate that their basic structures are similar.

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REFERENCES

1. Adams, R., Morris, R. C., Geissman, T. A., Butterbaugh, D. J., and Kirkpatrick, E. C., J. Am. Chem. Soc., *60*, 2193-2204 (1938).
2. Boatner, C. H., Chapter VI, in "Cottonseed and Cottonseed Products," ed. A. E. Bailey, Interscience Publishers, New York, 1948.
3. Boatner, C. H., Oil & Soap, *21*, 10-15 (1944).
4. Boatner, C. H., Caravella, M., and Kyame, L., Ind. Eng. Chem., Anal. Ed., *16*, 566-572 (1944).
5. Boatner, C. H., and Hall, C. M., Oil & Soap *23*, 123-128 (1946).
6. Boatner, C. H., Hall, C. M., O'Connor, R. T., Castillon, L. E., and Curet, M. C., J. Am. Oil Chem. Soc., *24*, 97-106 (1947).
7. Boatner, C. H., O'Connor, R. T., Curet, M. C., and Samuels, C. S., J. Am. Chem. Soc., *69*, 1268-1273 (1947).
8. Podol'skaya, M., Biochem. Z., *284*, 401-411 (1936).
9. Shriner, R. L., and Fuson, R. C., "The Systematic Identification of Organic Compounds," Ed. 3, Wiley, New York, 1948.
10. Vix, H. L. E., Spadaro, J. J., Westbrook, R. D., Crovetto, A. J., Pollard, E. F., and Gastrock, E. A., J. Am. Oil Chem. Soc., *24*, 228-236 (1947).

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Antioxidants in Aqueous Fat Systems[1]

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WITHIN the past few years several highly effective antioxidants have been developed for the stabilization of fats and oils. Standardized, accelerated tests for the evaluation of antioxidants are widely used, and the results of such tests seem to be generally applicable to the practical problem of protecting substantially pure dry fats during handling and storage.

The situation with respect to the protection of foods in which fat is one component of a more complex, polyphasic system is less encouraging. Antioxidants which have been successful in dry fats have in many instances proved unsuitable for the stabilization of meats, fish, dairy products, baked and fried foods, etc. Precise methods for the evaluation of antioxidants in aqueous fat systems are lacking.

The present study is an evaluation of a number of antioxidants in an artificial aqueous fat system. The study includes phenolic as well as synergistic antioxidants. The phenolic inhibitors included are those now being used in edible fats, i.e., tocopherol, the gallates, nordihydroguaiaretic acid (NDGA), and butylated hydroxyanisole (BHA).

Of a number of synergistic antioxidants previously tested in aqueous fat systems, a group of polyphosphates was most effective in contact with lard (plain or containing added tocopherol) (4). Hence the activity of this group has been further investigated in contact with lard containing the other phenolic inhibitors listed above. A few experiments have also been included on ammonium and potassium polyphosphates, not previously investigated. Orthophosphates, citric acid, and ascorbic acid, the three synergists which have probably been most widely studied in food fats, were also compared with the polyphosphates.

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