terial processed if heptane solvent is used. Higher maximum costs may be expected from the chlorinated solvents. However the actual solvent loss will vary appreciably with the solvent used, design of equipment and of solvent recovery unit, and care in maintenance and operation of the plant. In normal operations this solvent cost should be appreciably lower than the maximum costs indicated.

As a further adjustment in the evaluation of the two rendering methods, the additional fat yield of about 2 pounds per 100 pounds raw material should be credited to the azeotropic process in comparing with costs for dry rendering and pressing without subsequent solvent extraction.

For continuous plant operations, worthwhile savings can be obtained over batch operations. The total labor costs for a high capacity continuous plant will be no more than for a small batch plant, the steam and power will be more efficiently used, and the solvent loss will be less than one-third per unit of raw material processed.

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Reactions of Some Gossypol-Like Pigments With Aniline and p-Anisidine

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GOSSYPOL, the yellow pigment of cottonseed, reacts with aniline to give a stable, relatively insoluble product with a characteristic absorption spectrum (5, 8, 10). This product, known as dianilinogossypol, has been used as the basis of numerous methods for determining gossypol in cottonseed and cottonseed products (1).

Recently there was described (12) a new method for the determination of gossypol in cottonseed and cottonseed products in which *p*-anisidine was used in place of aniline. This method is simple and rapid and yields readily duplicable results. The authors (12) stated that pigments other than gossypol are simultaneously measured by the method but gave no further details concerning the nature or extent of the concomitant reactions. The following report deals with the reactions of other gossypol-like pigments with aniline and with *p*-anisidine.

In addition to gossypol, cottonseed contains a dark purple pigment which has been named gossypurpurin (2) and which has been assigned the empirical formula, $C_{30}H_{32}O_7N$, on the basis of some of its reactions and its elementary composition (11). Gossypurpurin has also been isolated from cottonseed pigment glands and has been prepared in the laboratory from diaminogossypol by a procedure involving the treatment of gossypol, $C_{30}H_{30}O_8$, with gaseous ammonia to form diaminogossypol, $C_{30}H_{34}O_7N_2$, which is then converted to gossypurpurin. It has been postulated that diaminogossypol also exists in cottonseed, particularly in seed which has been stored for long periods of time (7). It was found that the absorption spectra of non-alkali-extractable portions of chloroform extracts of stored cottonseed and that of diaminogossypol are quite similar. Their antimony trichloride reaction products also possess similar absorption spectra. On the basis of these similarities it was postulated that diaminogossypol occurs in cottonseed and is formed by the influence of metabolic changes on gossypol in the living seed.

These pigments, gossypurpurin and diaminogossypol, are known to be closely related to gossypol but are separate and distinct compounds. Gossypol, when treated with antimony trichloride in chloroform, has been shown to give a bright red reaction product with a characteristic absorption spectrum (3, 9). By contrast, the reaction product of diaminogossypol with antimony trichloride is yellow and that of gossypurpurin is blue-green (11). However both of these reaction products are unstable, and, if allowed to stand for prolonged periods of time, are converted to the characteristic red reaction product of gossypol and antimony trichloride.

The apparent close structural relationship of diaminogossypol and gossypurpurin to gossypol is sufficient evidence to justify application of the term "gossypol-like" pigments to these two pigments. The reactions of these two "gossypol-like" pigments with aniline and *p*-anisidine have been investigated with the results reported herein.

Experimental

Gossypol was isolated from cottonseed pigment glands by the method described by Castillon *et al.* (6). Its chemical and physical properties agreed with those previously reported for this product. The preparation of diaminogossypol from gossypol using gaseous ammonia and of gossypurpurin from diaminogossypol was carried out by the method of Pominski *et al.* (11). These reaction products agreed in melting point, absorption spectra in chloroform, antimony trichloride reaction product, and elementary composition with those previously reported for diaminogossypol and gossypurpurin.

Treatment with Aniline. A 0.3-g. sample of diaminogossypol was dissolved in 18 ml. diethyl ether, and

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TABLE I

Extinction Coefficients at Points of Characteristic Absorption for the Reaction Products of Gossypol and Gossypol-like Pigments with Aniline and *p*-Anisidine

Points of characteristic absorption	Specific extinction coefficients[a]		
mμ	Gossypol	Diaminogossypol	Gossypurpurin
	p-Anisidine reaction product[b]		
447 (maximum)	816[c]	721	768
462-464 (minimum)	733[c]	642	645[d]
468 (maximum)	768[c]	689	691[d]
	Aniline reaction product[e]		
440 (maximum)	634.4	636	634.4
456-462 (shoulder)	555	552	552.2

[a] Expressed as $E^{1\%}_{1cm}$.
[b] Specific extinction coefficients were calculated on the basis of the weight of the pigments before reaction with *p*-anisidine. The reaction products with *p*-anisidine were not isolated.
[c] See reference 3.
[d] See reference 11.
[e] Specific extinction coefficients were calculated on the basis of the weight of the isolated reaction product dissolved in chloroform.

18 ml. freshly distilled aniline was added. The mixture was heated about 10 minutes on the steam bath and was then cooled overnight at about 3°C. The orange-colored crystalline precipitate which was obtained was recrystallized four times from chloroform, and the orange-colored plates thus obtained melted at 298-300°C. The absorption spectrum of the product in chloroform solution was identical with that of dianilinogossypol as seen from the data in Table I. The absorption spectrum of its reaction product with antimony trichloride was also identical with that of dianilinogossypol. The product gave on analysis: carbon, 74.7%; hydrogen, 6.09%; nitrogen, 4.10%. As may be seen from Table II, these values agree with those calculated and found for dianilinogossypol.

Gossypurpurin has been shown previously to react with aniline to give a product which is identical with dianilinogossypol (11).

Treatment with p-Anisidine. A solution of diaminogossypol in 70% aqueous acetone was treated in the same manner as the gossypol solution used as a standard in the *p*-anisidine method for determining gossypol (12). The absorption spectrum, in the visible and near ultraviolet region, of the reaction product of diaminogossypol with *p*-anisidine was determined spectrophotometrically and was found to be identical with that of gossypol and *p*-anisidine as far as positions of absorption maxima and minima are concerned, as may be seen in Figure 1. Specific extinction coefficients at points of characteristic absorption are shown in Table I.

Treatment of a solution of gossypurpurin in 70% aqueous acetone with *p*-anisidine in the same manner gave a reaction product which was also identical with that of gossypol and *p*-anisidine in the positions of the absorption maxima and minima. The specific extinction coefficients at points of characteristic absorption are also recorded in Table I.

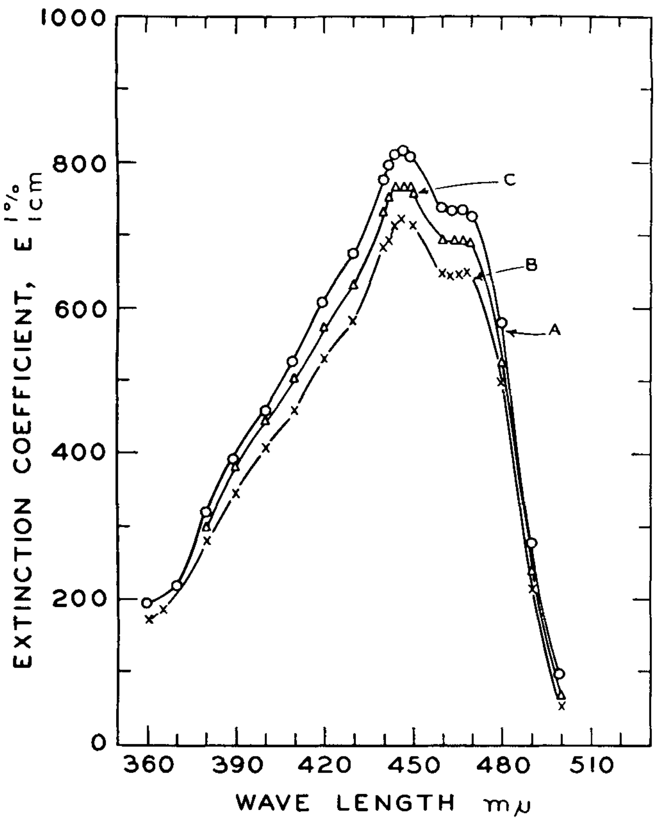

FIG. 1. Absorption spectra of reaction products with *p*-anisidine of (A) gossypol, (B) diaminogossypol, and (C) gossypurpurin.

Discussion

Diaminogossypol and gossypurpurin have both been shown to react with aniline to give a product which is identical with dianilinogossypol. The reaction products of these gossypol-like pigments with *p*-anisidine have absorption spectra which are identical in positions of absorption maxima and minima with that of gossypol and *p*-anisidine.

These observations indicate that any analytical method for the determination of gossypol, using either aniline or *p*-anisidine, will include any diaminogossypol or gossypurpurin which is present in addition to gossypol.

Consequently the rapid *p*-anisidine method may be used with adequacy for determining the gossypol and gossypol-like pigments in cottonseed and cottonseed products. If however it is desired to determine unmodified gossypol only, a reagent which is specific for gossypol must be used.

TABLE II

Composition of Gossypol-like Pigments and Their Reaction Products with Aniline

Compound	Empirical formula	Calculated			Found		
		C %	H %	N %	C %	H %	N %
Gossypol	$C_{30}H_{30}O_8$	69.5	5.79	......	69.46	6.08	......
Diaminogossypol	$C_{30}H_{34}O_7N_2$	67.4	6.37	5.24	67.3	6.37	5.02
Gossypurpurin	$C_{30}H_{32}O_7N$	69.6	6.28	2.70	70.0	5.97	2.34
Dianilinogossypol	$C_{42}H_{40}O_6N_2$	75.4	6.03	4.19	75.26	6.08	4.12
Dianilinogossypol from diaminogossypol	$C_{42}H_{40}O_6N_2$	75.4	6.03	4.19	74.7	6.09	4.10
Dianilinogossypol from gossypurpurin	$C_{42}H_{40}O_6N_2$	75.4	6.03	4.19	74.8	6.12	4.42

Summary

The gossypol-like pigments, gossypurpurin and diaminogossypol, have been shown to react with aniline to give the same reaction product as does gossypol. These pigments also react with *p*-anisidine to give products whose absorption spectra exhibit maxima in the same positions as that of the reaction product of gossypol and *p*-anisidine.

Acknowledgment

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ABSTRACTS . . . . Don Whyte, Editor

• Oils and Fats

R. A. Reiners, Abstractor

Color in margarine. I. Evaluation of color using the Lovibond tintometer. Audrey M. K. Brabant-Smith (Ontario Res. Found., Toronto). *Can. J. Tech.* 29, 296(1951). Readings of duplicate samples of each of 4 margarines was made by a panel of 12 members, using a B.D.H. pattern Lovibond tintometer. Statistical analysis of the results indicated that the accuracy of the method was ± 0.5 units.

II. An indirect method for measurement of color in terms of the Lovibond color system. *Ibid.* 303. A three-filter photoelectric reflectometer has been adapted to measure the color of margarine. From the results chromaticity co-ordinates can be calculated and these co-ordinates converted to Lovibond units.

Solvent extraction research. W. D. Harris (Texas A. and M. College, College Station). *Oil Mill Gazetteer* 56(1), 49(1951). The extraction of cottonseed with isopropyl alcohol is discussed. A new type solvent extractor is also mentioned.

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