b) From the point R on the diagonal line, draw another line CR through the 5% point on the impurity scale, extending it to cross the n axis. The value of the n-scale at this point estimates the number of plates needed for the desired 5% impurity separation.

The reverse of the above process may be used to determine the percentage of impurity of A in B, using a given number of plates, that is, the second line can be drawn from R to the number of plates used and the percentage of impurity read from the percentage scale. The error of the nomographic solution is usually less than the approximations introduced in the derivation of the equations and the use of the nomograph should greatly facilitate the application of the equations.

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Summary

Chlorophyll-a, chlorophyll-b, and carotene have been used to study the operation of countercurrent distribution apparatus. An incomplete separation of the chlorophylls was obtained by the use of the 25-tube Craig apparatus, but nearly complete separation of the chlorophyll and carotene pigments resulted. Degree of separation can be estimated by the modification of the equation of Martin and Synge which follows:

$$t = \sqrt{n}\ \frac{R - 1}{R + 1} = \sqrt{n}\ \frac{K_b - K_a}{K_a + K_b + 2K_a K_b}$$

Comparisons were made between the degree of separation predicted by these formulae and that calculated by the use of the binomial expansion. The utility of such predictions is illustrated by the problem of separating plant pigments. A nomographic solution of these equations is presented to facilitate their application.

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Determination of Total Gossypol Pigments in Cottonseed Materials

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THERE has been a recognized need for a reliable routine method for the determination of total gossypol pigments in cottonseed materials. Such a method must be fairly rapid and precise. Several methods have been suggested (4, 5). They are gravimetric methods, requiring hot aniline extractions, time-consuming precipitations, and individual attention. A method is proposed which involves an acid hydrolysis of the "bound" gossypol pigments in aqueous methyl ethyl ketone, and the colorimetric determination of the gossypol pigments by the method described recently (3) for free gossypol pigments.

The term "total gossypol pigments," as used in this paper, designates gossypol and closely related pigments, which after hydrolysis and reaction with *p*-anisidine give reaction products identical spectrophotometrically with that of pure gossypol. In this connection it must be pointed out that gossypol pigments such as gossyfulvin are convertible with ease to gossypol (1) and the gossypurpurin reacts with *p*-anisidine to give a product spectrophotometrically identical with that obtained from pure gossypol and the same reagent (3).

Reagents

a) Aqueous acetone: 700 ml. A.C.S.-grade acetone plus 300 ml. of distilled water.

b) Methyl ethyl ketone — water azeotrope: Mix 1106 ml. of reagent grade methyl ethyl ketone with 110 ml. of water and distill through a column such as a Vigreaux column. The azeotrope boils at 73.5°C. and contains 11.0% water by weight (2). Store in a brown bottle.

c) Oxalic acid solution: Dissolve 12.60 g. of A.C.S. grade oxalic acid dihydrate ($H_2C_2O_4 \cdot 2H_2O$) in the methyl ethyl ketone azeotrope and make up to 1 liter with the azeotrope. This solution is 0.1 molar in oxalic acid.

d) Barium acetate solution: Dissolve 136.73 g. of A.C.S.-grade barium acetate hydrate [$Ba(C_2H_3O_2)_2 \cdot H_2O$] in distilled water and dilute to 1 liter with water. This solution is 0.5 molar in barium acetate.

e) Isopropanol: Reagent-grade diluted to 80% by volume with distilled water.

f) Glacial acetic acid: A.C.S. reagent-grade.

TABLE I

Influence of Time of Hydrolysis on Total Gossypol Pigments Found

Time of Heating at 75°C.	Total Gossypol[1]			
	Raw Meats	Cooker Meats	Hydraulic-Pressed Meal	Screw-Pressed Meal
Hours	%	%	%	%
2	1.24	1.35	1.31-1.41	0.53
3	1.24	1.44	1.48	0.55
4	1.22	1.46	1.54	0.58
5	1.24	1.50	1.55	0.60
6	1.24	1.51	1.56	0.60
7	1.23	1.50	1.58	0.61
8	1.23	1.51	1.56	0.60
16	1.23	1.51	1.55	0.60

[1] Air-dry basis.

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TABLE II

Recovery of Added Gossypol

Gossypol Added to 100 ml. Volumetric Flasks	Time of Heating at 75°C.					
	0 Hr.		6 Hr.		16 Hr.	
	Gossypol Found	Recovery	Gossypol Found	Recovery	Gossypol Found	Recovery
	Mg.	%	*Mg.*	%	*Mg.*	%
2.038 mg. pure gossypol	2.110	103.5	2.090	102.6	1.920	94.2
4.075 mg. pure gossypol	4.093	100.4	4.150	101.8	3.878	95.2
4.098 mg. pure gossypol	4.090	99.8	4.080	99.6	3.920	95.7
0.250 g. meal[1] + 1.605 mg. gossypol	.....	.....	1.640	102.2	1.525	95.0
0.250 g. meal[1] + 2.038 mg. gossypol	.....	.....	2.045	100.3	1.965	96.4
0.250 g. meal[1] + 3.247 mg. gossypol	.....	.....	3.255	100.2	3.070	94.5
0.250 g. meal[1] + 4.075 mg. gossypol	.....	.....	4.010	98.4	3.955	97.1

[1]Acetone-extracted meats containing 0.02% total gossypol.

g) *p*-Anisdine: Prepare a saturated solution of technical grade *p*-anisidine in hot water and filter through paper. Upon cooling in a water bath with stirring at room temperature, the black oxidation products settle out on the sides of the beaker. Decant the slightly yellow supernatant into a clean beaker and keep overnight in a refrigerator. The crystalline product is usually pure. If slightly yellow, recrystallize. Dry in a desiccator over phosphorus pentoxide and store in a brown bottle. *p*-Anisidine in the solid state has proved to be stable for at least several months.

h) *p*-Anisidine solution: Dissolve 0.500 g. recrystallized *p*-anisidine in 80% isopropanol. Add 1 ml. of glacial acetic acid and make to 50 ml. with 80% isopropanol. Store in a brown bottle and prepare fresh daily.

i) Acetic acid solution: Dilute 1 ml. glacial acetic acid to 50 ml. with 80% isopropanol.

j) Standard gossypol solution: Dissolve 25 mg. of pure gossypol in and make to 200 ml. with the aqueous acetone. This stock solution contains 0.125 mg. gossypol per ml. Dilute 2, 5, 10, 15, 20, 25, 30, 35, and 40 ml. of the stock solution to 50 ml. with the aqueous acetone to provide standard solutions to prepare the standard curve.

Sample Preparation

a) Raw cottonseed: Dehull cottonseed in a Bauer mill and separate the meats from hulls and lint. Grind the meats to pass a 20-mesh sieve.

b) Cooker meats: These samples cannot usually be ground as such. Extract the oil from a portion of the sample by cold percolation with commercial pentane and then grind to pass a 20-mesh sieve. Determine moisture and oil on the original sample and the extracted sample. This allows calculation of results back to the original meats basis. The content of gossypol pigments of the extracted oil is negligible. If desired, the gossypol pigments can be determined on the extracted oil and correction made for it.

c) Hydraulic- screw-pressed, and solvent-extracted meals: Grind to pass through a 20-mesh sieve.

Analytical Procedure

Weigh sufficient sample material to contain from 2 to 10 mg. of total gossypol pigments into a glass-stoppered 100-ml. volumetric flask. This will require from 0.25-0.50 g. for most materials. By means of a pipette add 25 ml. of the oxalic acid solution, washing down any sample material adhering to the neck of the flask. Place in a water bath at 75°C., let come to temperature, then stopper the flask and heat for at least 6 hours. If more convenient, heat for 16 hours (overnight). Remove from the bath, cool to room temperature, and add about 30 ml. of the aqueous acetone followed by 5 ml. of the barium acetate solution. Mix well and make to volume with the aqueous acetone. Allow to stand for 10 minutes for complete precipitation of barium oxalate. Filter through dry 11-cm. paper of medium retentivity into a small glass-stoppered flask, discarding the first portion of the filtrate. Place a watch glass over the funnel to reduce evaporation. A reagent blank consisting of 25 ml. of the oxalic acid solution is processed at the same time and in the same manner.

Pipette duplicate 2-ml. aliquots of the sample filtrate into 25-ml. volumetric flasks. To one aliquot add 3 ml. of the acetic acid solution and make to volume with 80% isopropanol. This is the gossypol blank. To the other aliquot add 3 ml. of the *p*-anisidine solution and heat in a water bath (with the flask loosely stoppered) for one-half hour at 60°C., cool, and make to volume with 80% isopropanol. Treat the duplicate 2-ml. aliquots from the reagent blank as outlined for the sample aliquots. Determine the percentage transmission of the sample solution reacted with *p*-anisidine, designated as T_1, with a photoelectric colorimeter equipped with a filter having a maximum of transmission between 447 and 468 mμ., using the reagent blank as the reference solution. A spectrophotometer may be used instead of a colorimeter, making the measurement at 447 mμ. Determine the percentage transmission of the gossypol blank, designated as T_2, using the solvent as reference solution. Obtain the ratio T_1/T_2 and use the logarithm of this ratio (log. T_1/T_2) to find the concentration of gossypol pigments in the sample aliquot from the standard curve. As a 2/100-aliquot is used for analysis, multiply the milligrams of gossypol pigments found in the sample aliquot by 50 to obtain the milligrams of gossypol pigments in the original sample.

TABLE III

Total Gossypol Pigment Content of Specified Cottonseed Materials[1]

Cottonseed Material	Free Gossypol Pigments	Total Gossypol Pigments	
		6 Hr. Hydrolysis	16 Hr. Hydrolysis
	%	%	%
Raw meats	1.19	1.21	1.22
Raw meats	0.584	0.621	0.626
Cooker meats	0.699	0.937	0.933
Cooker meats	0.068	1.51	1.50
Hydraulic-pressed meal	0.078	1.20	1.23
Hydraulic-pressed meal	0.129	1.55	1.57
Screw-pressed meal	0.009	0.599	0.596
Screw-pressed meal	0.013	0.512	0.521
Hexane-extracted meal	1.21	1.17	1.20
De-pigmented and de-fatted meal	0.065	0.268	0.291

[1]Air-dry basis.

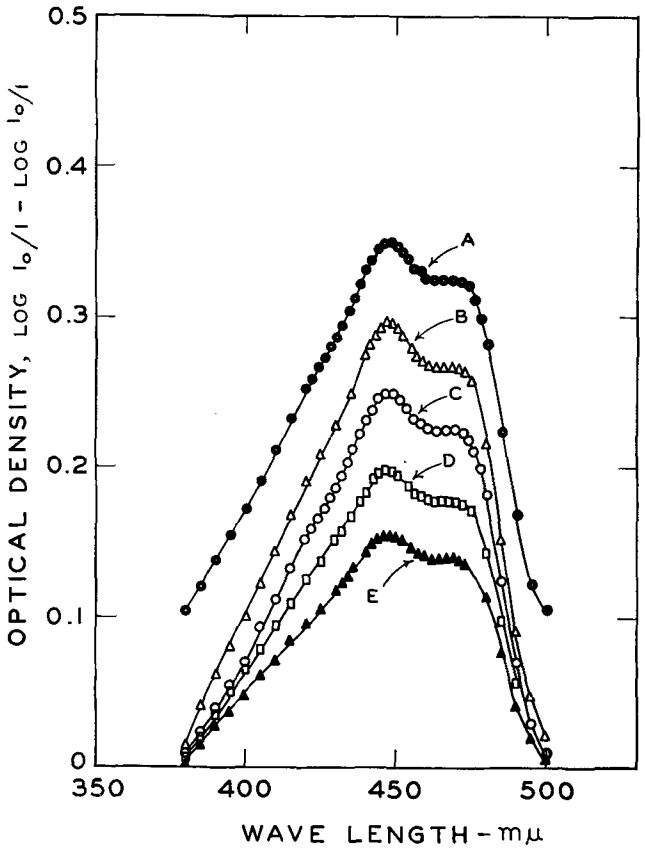

FIG. 1. Absorption spectra of hydrolyzates of typical cottonseed materials after reaction with *p-anisidine* as described under analytical procedure.
A. Hydraulic-pressed meal (set up 0.10 units in optical density).
B. Pure gossypol.
C. Cooker meats.
D. Raw meats.
E. Screw-pressed meal.

Prepare the standard curve by pipetting duplicate 2-ml. aliquots of each of the standard gossypol solutions into 25-ml. volumetric flasks and proceeding as directed for the sample solution, using the necessary blanks. Plot the logarithms of the T_1/T_2 values against milligrams of gossypol in the 25-ml. volume to obtain the standard curve.

Development of Optimum Hydrolysis Conditions

Analogy of "bound" gossypol with Schiff's bases indicated an acid hydrolysis of the bound gossypol pigments in an organic solvent containing water and use of elevated temperatures to increase the rate of hydrolysis. Because of the known stability of gossypol in aqueous acetone (3) it was used as the solvent in which to examine the hydrolysis of the bound gossypol pigments in commercial cottonseed meals. A number of organic and inorganic acids were employed for the acid hydrolysis. Of the acids tested, oxalic acid proved to be the best as it gave the most rapid rate of hydrolysis and did not produce extraneous colors. An oxalic acid concentration of 0.1 molar was found to give as rapid hydrolysis as more concentrated solutions. With oxalic acid in aqueous acetone 48 hours were required for complete hydrolysis. In order to shorten the period of hydrolysis a higher-boiling ketone which would form a stable compound with gossypol in solution similar to that formed between acetone and gossypol was indicated. The azeotropic mixture of methyl ethyl ketone and water boiling at 73.5°C. and containing 11.0% water by weight (2) was found satisfactory. Use of this solvent permitted the reaction to be conducted in stoppered volumetric flasks, in a water bath at 75°C., and eliminated the necessity of reflux condensers. Distillation of the azeotrope serves two purposes: first, the water content of the hydrolyzing solvent is always constant and, second, some purification of the ketone takes place.

The methyl ethyl ketone used for hydrolysis gives slightly higher reagent blanks than those obtained in the method for free gossypol pigments (3). In order to minimize the reagent blank only 25 ml. of this ketone was used and the solution is made to 100-ml. volume with aqueous acetone.

The calibration curve used for the *p*-anisidine free gossypol method (3) was found to be applicable to the present method. This is substantiated by the recovery experiments described in Table II and by the data in Table III, showing the identical values for free and total gossypol pigments obtained on raw meats and hexane-extracted meals.

Time Required for Hydrolysis

The time required for hydrolysis of bound gossypol pigments in typical cottonseed materials was established by heating them with the oxalic acid solution for various periods after which the filtered hydrolyzates were analyzed for total gossypol pigments. The results (Table I) indicate that the hydrolysis of bound gossypol pigments in cooker meats, hydraulic-, and screw-pressed meals is complete in 5 to 7 hours and that the hydrolysis may proceed for 16 hours (overnight) with no apparent loss of gossypol pigments. The sample of raw meats gave constant values for total gossypol pigments of 1.23 to 1.24% from 2 to 16 hours hydrolysis. The observation that the content of free gossypol pigments in this sample was 1.19% is further indication that the conditions of hydrolysis do not lead to any apparent destruction of gossypol pigments.

Recovery of Added Gossypol

Table II gives recovery values of known amounts of pure gossypol treated with the oxalic acid solution for 0, 6, and 16 hours at 75°C. under the conditions of the method. Results of similar recovery experiments are also given for pure gossypol added to acetone-extracted meats (0.02% gossypol) before hydrolysis. The data indicate that gossypol pigments are not seriously affected by the conditions of hydrolysis as specified in the method. The satisfactory recovery of pure gossypol added to the acetone-extracted meats is evidence that the gossypol pigments do not appear to recombine under the conditions of hydrolysis.

Specificity of the Method

Spectrophotometric curves of the *p*-anisidine reaction products, as prepared for final colorimetric evaluation are shown in Fig. 1 for (A) hydraulic-pressed meal, (B) pure gossypol, (C) cooker meats, (D) raw meats, and (E) screw-pressed meal. In obtaining these curves the respective hydrolyzates before reaction with *p*-anisidine were used as reference solutions. All of these curves are identical in shape with the curve for pure gossypol, and all show the same maxima at 447 and 468 mμ. To demonstrate the

identity of these curves appropriate factors to reduce them to a common optical density basis at 440 mμ. were obtained. When calculated by these factors, all of the curves are identical above 420 mμ. Further, the absorption bands at 447 and 468 mμ. are identical with those previously reported (3) for the reaction product of pure gossypol with *p*-anisidine.

Hydraulic- and screw-pressed meals, cooker meats, raw meats, and pure gossypol were treated with the oxalic acid solution for 16 hours as outlined in the proposed method. Spectrophotometric curves obtained for these solutions before reaction with *p*-anisidine all showed the principal absorption band at 373 mμ. for gossypol in the solvent used. All curves showed the same absorption as pure gossypol in the region of 370 to 450 mμ., indicating the absence of other materials absorbing characteristically in this region.

Application

The application of the method for following the state of gossypol during the processing of cottonseed is illustrated by the analysis of raw meats and press cake taken from the same stream of a hydraulic-press cottonseed oil mill. When calculated on a moisture- and oil-free basis the percentages of total gossypol pigments were 1.93 and 1.81 and of free gossypol pigments 1.87 and 0.146, respectively. Thus 95% of the gossypol pigments present in the raw meats was accounted for in hydraulic-pressed cake.

Table III shows the total gossypol pigment content of a number of typical cottonseed materials. Values for free gossypol pigments are included (3). It is apparent that in raw meats and hexane-extracted meal practically all of the gossypol pigments are present in the free form. Most of the gossypol in cooker meats, hydraulic-, and screw-pressed meals is present in the bound form. In general, the total gossypol values obtained for 6- and 16-hour hydrolysis periods are comparable.

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Some Physical and Chemical Properties of Certain Snake Oils

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Introduction

WELL-FED snakes in good physical condition have fat lobes deposited along both sides of the intestines in the area between the stomach and vent. This fat deposition involves about one-fourth of the total length of the snake. At the end of the hibernation period this fat supply is nearly or completely depleted. Although snake oil has been used and discussed for generations, very little information concerning its source or composition is available in the literature. Most of the publications which have dealt with physical and chemical properties of snake oil have neglected to state whether the oil was obtained from the whole snakes or from the lobes. For this reason the available data have little value for purposes of comparison with respect to species differences.

Experimental

Physical and chemical properties presented in this paper were determined from separate batches of cold-pressed oils from the fat lobes of boa constrictor *(Constrictor constrictor)*, prairie rattler *(Crotalus viridis viridis)*, and moccasin *(Agkistrodon piscivorus)*.

The cold-pressing method of extraction (Carver laboratory press) was employed for all samples since it eliminates changes in the lipoids which often are caused by heat and oxidation. From three to 20 snakes of each species were butchered soon after capture and the lobes of the respective species pooled. The fat lobes were dried on filter paper, and all connective tissues and blood vessels were carefully removed. The lobes were then wrapped in filter cloth and pressed. The expressed oils were centrifuged at 1400 r.p.m., and clear samples were siphoned off for analysis. The percentage yields of oil from lobes were: moccasin, 43.0; prairie rattler, 70.0; boa constrictor, 33.0. The percentages of unsaponifiable matter contained in these samples of oil were: moccasin, 0.46; prairie rattler, 0.25; boa constrictor, 0.55.

TABLE I

Characteristics of Cold-Pressed Oil From Fat Lobes[a] of Certain Snakes

	Moccasin *(Agkistrodon piscivorus)*	Prairie Rattler *(Crotalus viridis viridis)*	Boa Constrictor *(Constrictor constrictor)*
Specific gravity (25°/4)	0.9268	0.9323	0.9252
Refractive index (25°)	1.4690	1.4700	1.4670
Specific rotation[b]	−0.12°	−0.12°	−0.17°
Saponification number	192.6	193.5	194.7
Iodine number (Hanus)	104.4	114.0	89.6
Thiocyanogen value	77.2	84.0	70.1
Soluble acids (%)	0.13	0.20	0.04
Insoluble acids (%)	94.85	94.0	92.8
Reichert-Meissl value	0.07	0.13	0.13
Polenske value	0.04	0.12	0.00
Saturated acids (%)	22.7	16.80	25.35
Unsaturated acids (%)	72.7	77.44	67.71
Free fatty acids (%)	0.52	0.17	0.25
Acetyl value	4.1	6.0	7.0

[a] Pooled samples were used. [b] These values are in the range of experimental error.