ISOLATION, PURIFICATION AND PARTIAL CHARACTERIZATION OF A GOSSYPOL RELATED BROWN PIGMENT FROM COTTONSEED PIGMENT GLANDS

By

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CHAPTER I

INTRODUCTION

The gossypol content of the cotton plant is of importance chiefly in relation to the use of cottonseed meal as an animal feed; the use of the oil in food products; and in more recent years the use of cottonseed flour for human consumption. Cottonseed pigments, particularly gossypol and closely related compounds, have therefore become of economic importance to the cotton industry. These coloring materials in cottonseed oil are of tremendous importance to processors of cottonseed oil. Cottonseed oil processors have been forced into great expenditures to produce the desired color in their finished products, since consumers want light colored oils. As far as the crude cottonseed processor is concerned, highly colored oils are a source of concern and expense.

Cottonseed pigments are of importance to the feed industry, primarily because of their toxicity to monogastric animals, thus preventing the free use of cottonseed meals as a feed for the animals. The major pigment, gossypol, is found in cottonseed pigment glands and constitutes 20-40 per cent of their weight. Gossypol is associated in the seeds by a small number of other pigments in limited amounts.

CHAPTER II

REVIEW OF LITERATURE

Longman first isolated gossypol in 1886. However the name "gossypol" inspired by the chemical nature and origin of the compound "Gossyp (ium phen) ol" appeared first in 1899 in the more complete study by Marchlewski (1, 2).

The structure of gossypol determined by Adams and co-workers as 2,2'-bi-8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methyl naphtyl and was authenticated by synthesis of gossypol by Edwards in 1958 (3, 4).

O'Connor and co-workers studied the infrared spectra of gossypol (5) and Dollear investigated the IR Spectra of gossypol, 13 derivatives, degradation products, and 18 model compounds in the region from 2 to 12 and provided supporting evidence for the structure proposed by Adams (3, 6), Figure 1. Shirley and co-workers (7) have contributed to the study of the chemical reactions of gossypol. Desapogossypol hexamethyl ether was synthesized by Shirley and co-workers (7).

It has been known for many years that free gossypol is toxic to many species of animals (8, 9, 10, 11, 12). The earliest statement on the toxic effect of cottonseed was attributed to Voelker in 1859 (13). It has been possible to evaluate this toxicity with the availability of pure gossypol (16, 17) and of cottonseed pigment glands separated from cottonseed kernels (14, 15) by a flotation method. Eagle, however, (18) found that the toxicity of cottonseed pigment glands

could be attributed to some components of the glands in addition to gossypol. This observation led to further examination of the composition of the pigment glands. It was found that the different samples of untreated cottonseed pigment glands containing 40.0, 37.6 and 33.7 per cent gossypol, respectively, were more toxic to the rat than pure gossypol (19).

Eagle and co-workers (20) in attempts to find components of cottonseed pigment glands which were more toxic than the original glands conducted various fractionating procedures. It was found that the acetone-soluble, water-insoluble fraction had an LD-50 value of 700 mg/Kg, making it the most toxic material extracted from cottonseed pigment gland material. The acetone-soluble, water-insoluble fraction was one-half as toxic as the original glands even though the gossypol content had increased from 40 per cent in the original glands to 90 per cent in this less toxic fraction.

Eagle and Davis (21) found that cottonseed pigment glands were slightly less toxic when administered in oil, as compared to the administration in water. When gossypol is administered in oil, it is more toxic than when administered in water. Groschke and co-workers (27) found a definite weight suppression when chicks were fed a ration containing 0.79 per cent cottonseed pigment glands. These pigment glands were reported by Eagle (28) as having LD-50 value of 925 mg/Kg, which is the most toxic sample of all the intact untreated pigment glands. Boatner et al. (22) observed relatively little retardation of chick growth when 0.13 per cent gossypol was added to a ration containing screw-pressed soybean meal, whereas 0.65 per cent cottonseed pigment glands caused a greater weight retardation.

Eagle and Bailek et al. (23) studied the effect of gossypol on the body weight of rats with sixteen different doses and found that body weight losses were proportional to the amount of pure gossypol administered. They also reported four experiments in which they studied the effects of feeding various levels of pure gossypol in the diets of rats and concluded that the body-weight depression caused by gossypol was proportional to the amount of gossypol added to the diet. Clark et al. (25, 26) studied the toxicity of crude gossypol acetate, pure gossypol and apogossypol. Apogossypol was found to be the least toxic among these. However, he found that there was no apparent difference between the toxicity of the crude gossypol acetate and analytically pure gossypol. Cottonseed pigments are unique in chemical nature and also in the manner of occurrence in the seed. Most of cottonseed pigments are contained in distinct morphological structures, which are relatively large ovoid or spherical bodies 100 to 400 microns on the long axis (29). Brelfeld et al. (30) noted the occurrence of pigment glands in cottonseed in 1887 and other investigators reported the existence of a gland membrane made up of two layers (31, 33). A complicated internal structure of the cottonseed pigment gland in which discrete particles ranging in size from one micron to less than 0.2 microns in diameter are held within a membranous mesh-like network has been demonstrated with the electron microscope.

This structure is very sensitive to water; and it may be the reason explosive release of pigment particles from the gland on exposure to moisture was observed. The outside walls of the gland appear to consist of platelets which exist as flattened compartments each of which has a complex internal structure (34). It has been suggested

that the gossypurpurin may be concentrated in the outer wall of the gland and the gossypol deposited in the interior (35). The density of the pigment glands varies from 1.26 to 1.37; that of the hulls is greater than 1.45 while the density of the remaining cottonseed tissues varies from 1.40 to 1.45 (14). This has been used as a basis for the separation of the pigment glands by using a solvent mixture with a specific gravity of 1.378 (15).

Chemical and Physical Characteristics of of Cottonseed Pigments

Gossypol is a bright yellow pigment which occurs in the genus Gossypium. It has been isolated from the seed and root bark and detected in other parts of the upland cotton plant (36, 37). It has been found to occur in amounts as much as six per cent of the weight of the kernels. Gossypol has a molecular weight of 518 (9) and the molecular formula is $C_{30}H_{30}O_8$ (25, 26, 28-41). Adams (3) showed that gossypol is a polyhydroxy phenolic compound having two carbonyl groups and thus reacts with both acids and bases. It has been demonstrated (9) that gossypol reacts as a strong dibasic acid to form neutral salts of sodium, potassium, lead and iron. Gossypol also reacts with one mole of acetic acid. It is soluble in methanol, 2-propanol, n-butanol, diethyl ether, diethylene glycol, cold dioxane, ethyl acetate, acetone, chloroform, carbon tetrachloride and pyridine; it is slightly soluble in glycerol and cyclohexane; and insoluble in low-boiling petroleum ether and water (42). Absorption spectrum of gossypol in ether solution shows that it is similar to alpha, alpha-binaphthyl and beta, beta-binaphthyl (26). This typical curve is also demonstrated

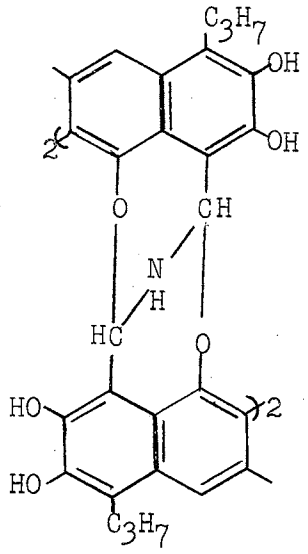
in chloroform solution with two absorbancy maxima at 288 to 289 mμ and 363.5 mμ (43). Gossypol has three crystalline forms each with different melting points; gossypol crystallized from ether, from chloroform and from ligroin with melting points of 184° C, 199° C, and 214° C, respectively (25, 42). It is possible to interconvert these three crystalline forms by using appropriate solvents for recrystallization. The most biologically important reactions are the formation of bound gossypol (38, 44) and the antioxidant properties (45-48).

The minor pigments, so far reported from cottonseed, which are related to gossypol are gossypurpurin (49-52), gossyfulvin (53-57), gossycaerulin (58), and gossyverdurin (59, 60).

Gossypurpurin, a naturally occurring purple-colored pigment of cottonseed, was first isolated by Boatner (49) from the red crystals, so-called "red gossypol," obtained from chloroform extracts of cottonseed kernels (50, 51). Pominski et al. (52) in 1951 isolated gossypurpurin from cottonseed pigment glands and prepared it from gossypol via diamino-gossypol and proposed $C_{30}H_{32}O_7N$ as a molecular formula. Gossypurpurin is very soluble in dioxane, acetone, pyridine, and slightly soluble in chloroform, ethyl acetate, diethyl ether and benzene; very soluble in methanol and ethanol and it is insoluble in water and in concentrated sulfuric acid.

Several qualitative tests were performed with gossypurpurin in order to determine this compound's relationship to gossypol. Tollen's test, Fehling's test, hydroxylamine hydrochloride, and phenylhydrazine hydrochloride tests were positive for gossypurpurin and for gossypol indicating the presence of one or more carbonyl groups. Both pigments also gave positive results with ferric chloride, pyroboroacetate and

stannic chloride indicating the presence of two ortho-phenolic hydroxyls and/or a hydroxyl para or ortho to a carbonyl group (52). Gossypurpurin produced a yellow-green color with concentrated sulfuric acid which later turned to orange at room temperature, whereas gossypol gave a dark red color immediately when treated with concentrated sulfuric acid. Gossypurpurin produced a green precipitate with glacial acetic acid, but gossypol gave yellow precipitate. Gossypurpurin showed two absorbancy maxima at 530 mμ and 565-566 mμ in chloroform solution, whereas gossypol does not show a peak in this region. The absorbancy maxima of gossypurpurin at 326-327 mμ and 370-371 mμ indicated gossypurpurin may be structurally related to gossypol which also has two peaks at 288-289 mμ and at 363.5 mμ as previously mentioned. 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 also indicate that the basic structure of the two pigments are similar (52). The structure of gossypurpurin has not been established but a proposed structure is shown below (6):



Proposed Structure of Gossypurpurin

Gossyfulvin, an orange colored pigment, has been detected in cottonseed by Boatner et al. (53) in low concentrations. Gossyfulvin forms rather large orange-colored rhombohedra crystals which melt at 212° C, changing to a more deeply colored form which melts with decomposition at 238-239° C (54). The crystalline form of gossyfulvin is very different from gossypol. Gossypol, upon recrystallization from diethyl ether and petroleum naphtha, forms clusters and melts at 182.5-183.5° C. Gossyfulvin has a molecular weight of 598 and the molecular formula is $C_{34}H_{34}N_2O_4$ (54).

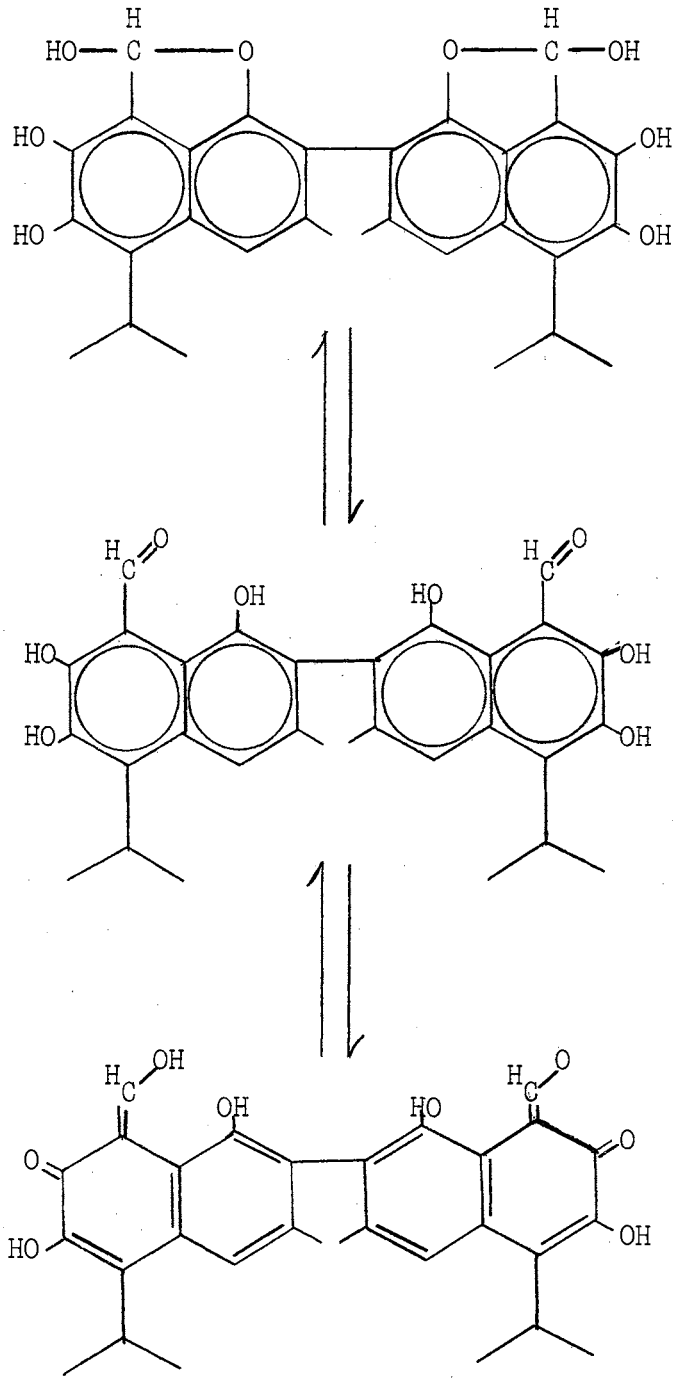
Comparison of gossyfulvin with nitrogen derivatives of gossypol, such as diamino and dianilinogossypol, reveals several significant differences, as shown in Table I (54).

Diaminogossypol is reported to evolve ammonia and revert to gossypol when it is dissolved in diethyl ether or warmed in acetic acid (55). On the other hand, dianilinogossypol is one of the more stable compounds formed from gossypol and only concentrated sulfuric acid (56), alcoholic potassium hydroxide (9), or hot acetic anhydride (57) can hydrolyze it. Gossyfulvin is very unstable in solution, and it decomposes without loss of nitrogen in the absence of strong mineral acids. The absorption spectrum of gossyfulvin is identical with that of dianilinogossypol within the limits of experimental error, but very different from that of gossypol. The exact correspondence of the absorption spectra of gossyfulvin and dianilinogossypol has been assumed as evidence that they are structurally similar. The dissimilarity with that of gossypol is assumed to be different from the preponderant tautomeric form of gossypol (54) as shown in Figure 1. Although gossyfulvin has been shown to differ from gossypol in many of its properties, it can be

TABLE I

COMPOSITION AND MELTING POINTS OF OXIMES FORMED FROM GOSSYPOL, GOSSYFULVIN AND DIANILINOGOSSYPOL

Compound	M.P. °C	Formula	Elementary Composition		
			C	H	N
			per cent		
Dioxime of gossypol	312	$C_{30}H_{32}O_8N_2$	65.70	5.72	5.04
Dioxime formed by gossyfulvin	204.5	$C_{30}H_{32}O_8N_2$	65.35	6.02	5.00
Dioxime formed by dianilino-gossypol	221-221.5	$C_{30}H_{32}O_8N_2$	65.25	6.03	5.33



II

I

III

Figure 1. Structural Formula of the Tautomers of Gossypol According to R. Adams and Co-Workers

I. Hydroxy-aldehyde tautomer
II. Lactal tautomer
III. Keto-enol tautomer

readily converted into gossypol by acid hydrolysis.

Gossycaerulin, a blue pigment, has not been reported as a naturally occurring pigment but has only been detected in cooked cottonseed when treated with heat and mineral acids (58). Color reactions with boro-acetic anhydride and with stannic chloride in benzene or chloroform indicate the presence of a carbonyl group which is attached in a position alpha or adjacent to a hydroxyl group and the ready and reversible reduction of gossycaerulin, coupled with the known poly-phenolic nature of gossypol provided evidence that the gossycaerulin is a quinonoid oxidation product of gossypol. Thus, the structure of gossycaerulin has been suggested as an alpha-hydroxy quinonoid, oxidized gossypol, by Boatner and co-workers (58). Gossycaerulin exhibits a maximum blue color having absorption maxima at 605 mμ and 315 mμ in acid solution and is reversibly convertible into a non-polar, yellow, neutral form. This yellow pigment has been detected as a by-product formed from gossypol during its conversion to gossycaerulin.

Gossyverdurin, a newly isolated green pigment from cottonseed pigment glands, has been shown to be more toxic than gossypol by Lyman and El-Nockrashy (59). These workers isolated gossyverdurin by the use of column chromatography with DEAE cellulose ion exchanger and silicic acid. This bright green-colored pigment from cottonseed pigment glands, which is more toxic than gossypol, has been reported to be structurally related to gossypol (59, 60). Gossyverdurin is very soluble in chloroform, methanol, acetone, diethyl ether and ethanol and insoluble in petroleum ether. It shows absorption maxima at 250, 370 and 560 mμ. In addition a second peak at 342 mu appears on reaction with para-anisidine. This indicates important structural

differences between gossypol and gossyverdurin (59). Gossyverdurin was found to contain nitrogen and an ash content. Fractionation of the pigment extracts on silicic acid columns led to the observation that other pigments were naturally occurring components of the cottonseed pigment gland.

Statement of Problem

The present study was designed to isolate, purify and characterize one of the minor pigments related to gossypol from cottonseed pigment gland material which was observed as a brown band on the silicic acid column. This band was observed to follow gossypol and precede gossyverdurin in the chromatographic separation of gossypol related pigments.

CHAPTER III

EXPERIMENTAL PROCEDURES

Isolation of Pigment Glands from Cottonseed Meal

The cottonseed meal used for this experiment was supplied by the Texas A and M University Cottonseed Products Laboratory. The meal was prepared by decortication, screw-press treatment and cold hexane extraction of the oil. The defatted cottonseed meal was then air-dried and ground to pass a 60 mesh screen. Isolation of the pigment glands from the cottonseed meal was effected by a modified semi-pilot plant process as reported by Spadaro et al. (61, 62). Instead of using a disintegrator as described by Spadaro et al. (61) for the disintegration of cottonseed meal and the liberation of the glands, a Wiley mill with a one mm screen was used in this study.

In the preparation of cottonseed pigment gland material, approximately ten pounds of the hexane-extracted cottonseed meals were ground in a Wiley mill, then added to a dispersing solvent mixture. Commercial hexane and tetrachloroethylene, mixture of specific gravity 1.378, was used as the dispersing solvent. About ten gallons of solvent mixture were required for ten pounds of cottonseed meal. The dispersed mixture of cottonseed meal was allowed to settle for several hours and the pigment glands were carefully collected from the top of the solvent mixture by siphoning and skimming. Excess

solvent was removed by placing the glands in a Buchner funnel and then in a hood to dry overnight.

The dried pigment glands were subjected to a second flotation process using the same type solvent mixture and the same conditions as above in order to obtain a higher concentration of pigment glands in the preparation. Air-dried pigment glands obtained from the second flotation process were sieved using a U.S. Standard Sieve Series: Sieve No. 40, 60, 100, with openings of 420, 250, 140 microns, respectively. Extremely fine, light yellowish green pigment glands were obtained after they had passed through 100 mesh sieve. This material was covered with aluminum foil for protection from light and then stored at 4° C. Three ten-pound batches of meal were processed to obtain enough pigment gland material for this study.

Isolation of the Brown Pigment

Twenty grams of the pigment glands were extracted for five minutes with 200 ml of five per cent water in acetone in a Waring blendor. The mixture was filtered through a Büchner funnel with a water aspirator suction on Whatman filter paper No. 1. The residue of gland walls was re-extracted with 200 ml of five per cent water in acetone, filtered and then washed with 100 ml of acetone. The filtrates were combined and reduced in volume with a rotary evaporator placed in a water bath; the temperature was held below 40° C. When the acetone was removed, the pigment residue was mixed with 50 ml of chloroform. The chloroform phase was retained for chromatography and the water phase was washed twice with 20 ml of chloroform. The chloroform phases were combined, dried with anhydrous sodium sulfate and placed on a

2.2 x 23.5 inch silicic acid column. The column was prepared as follows. Five hundred grams of silicic acid was activated by drying in an oven overnight at 150° C. A 25 per cent hexane in chloroform solvent was mixed with the silicic acid until a uniform slurry was obtained. This slurry was poured into a 2.2 x 23.5 inch column and the column was packed by gravity and used for separation of pigments from extracts of pigment gland material.

The chloroform dissolved pigments were loaded on the silicic acid column and the column was developed with chloroform -25 per cent hexane until all pigments were eluted that could be removed with this solvent. The first solvent of the schedule was 1500 ml of 25 per cent hexane in chloroform, followed by one liter of ten per cent hexane in chloroform; then 100 per cent chloroform was used for further elution. Colored bands showed distinctly as the column developed and thus were collected as fractions with rate of flow of four milliliters per minute. The dark brown band which followed the reddish yellow gossypol band was eluted with ten per cent hexane in chloroform. The solvent was removed by evaporation and this fraction was subjected to further purification. A silicic acid elution pattern is shown in Figure 8.

Purification and Crystallization of the Brown Pigment

Purification of the brown pigment fraction was conducted by four silicic acid column chromatographic separations with recrystallization of the brown pigment fraction from each purification. The brown pigment fraction was further purified on a silicic acid column with a different solvent schedule as follows: 50 ml of the brown pigment fraction was placed on the silicic acid column and 3.5 liters of

75 per cent hexane in chloroform, four liters of 70 per cent hexane in chloroform, three liters of 65 per cent hexane in chloroform, 60 per cent hexane in chloroform and then chloroform were used as eluents in the order shown.

The reddish yellow gossypol band and the brown band were separated with this solvent system. The brown band was collected as one fraction and evaporated to dryness in a rotary evaporator. An oil was obtained which was dissolved in a minimum amount of chloroform, then n-hexane was added until a yellowish brown pigment was precipitated. The precipitate was collected by centrifugation and dried under vacuum in a desiccator containing phosphorous pentoxide. After determining the melting point of the brown pigment, the remainder was dissolved in a small amount of chloroform and chromatographed a third time using the first solvent schedule. A fourth silicic acid column chromatography of the brown pigment was effected and the purified brown pigment fraction was crystallized as the acetic acid derivative. This brown pigment was then used for chemical tests, absorption spectra measurements and other reactions.

All ultraviolet and visible absorption on spectra of pigments and pigment derivatives were obtained with a Cary 14 Spectrophotometer. The infrared absorption spectra of gossypol and the brown pigment were obtained with a Beckman IR5 Infrared Spectrophotometer using a potassium bromide pellet. A Rast molecular weight was attempted as described in Morrison and Boyd (67). All melting points were determined using a Kofler micro melting point apparatus. The same microscope used for micro melting points was used to prepare color photographs of the pigment crystals. Color tests were conducted to check the behavior of

the purified brown pigment with gossypol. A nuclear magnetic resonance spectra was attempted with the Varian A-60 Analytical NMR Spectrometer.

CHAPTER IV

RESULTS AND DISCUSSION

The proximate composition was determined of hexane extracted cottonseed meal, cottonseed pigment gland material and pigment gland residue. This work, shown in Table II, was the same as that analyzed by Lyman's group. Further, the morphology of the cottonseed pigment gland has been thoroughly studied (64) so the gland material was simply examined under a light microscope to verify that a high concentration of pigment glands was present. Twenty-six grams of yellowish green pigment gland material were obtained from approximately ten pounds of defatted cottonseed meal.

Chromatographic separation of the pigments present in the pigment gland material on a silicic acid column showed the second fraction was a mixture of gossypol and the brown pigment. This mixture was further treated as described under methods. Table III shows the melting points of hexane precipitated and acetic acid crystalline products.

The first brown fraction was not crystallized, as it contained a large amount of gossypol. The gossypol fraction from the second chromatography was fairly pure and so only the brown fraction was subjected to further chromatographic purification and recrystallization.

Figure 2 shows color photographs of pigment glands, gossypol acetic acid and brown pigment acetic acid crystals.

Seventy-eight mg of brown pigment were obtained from 26 grams

TABLE II

COMPOSITION OF AIR-DRY, HEXANE EXTRACTED COTTONSEED MEAL, COTTONSEED PIGMENT GLAND MATERIAL AND GOSSYPOL FREE PIGMENT GLAND MATERIAL[a]

Sample	Moisture %	Ash %	Ether Soluble[b] Solids %	Protein (N x 6.25)[c] %
Cottonseed meal (hexane extracted)	10.5	6.1	0.49	47.5
Pigment gland material	12.7	3.1	14.75	27.5
Pigment gland[a] residue	8.3	5.4	1.46	43.8

[a]Water-acetone (5:95 v/v) extracted five times.

[b]Goldfinch extraction with anhydrous ether.

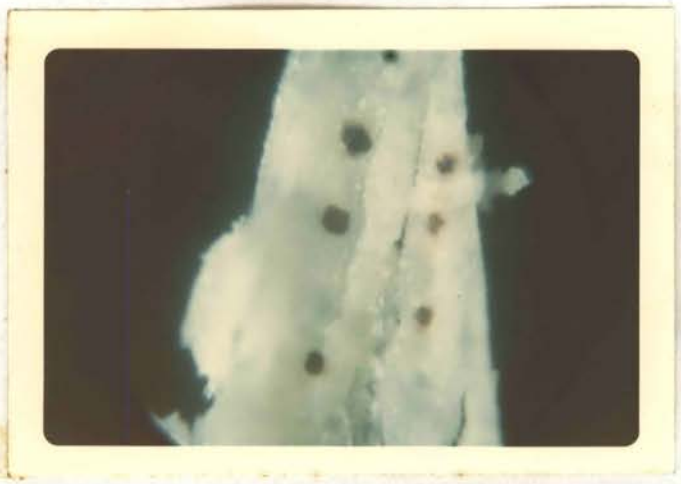
[c]Kjeldahl nitrogen procedure.

TABLE III

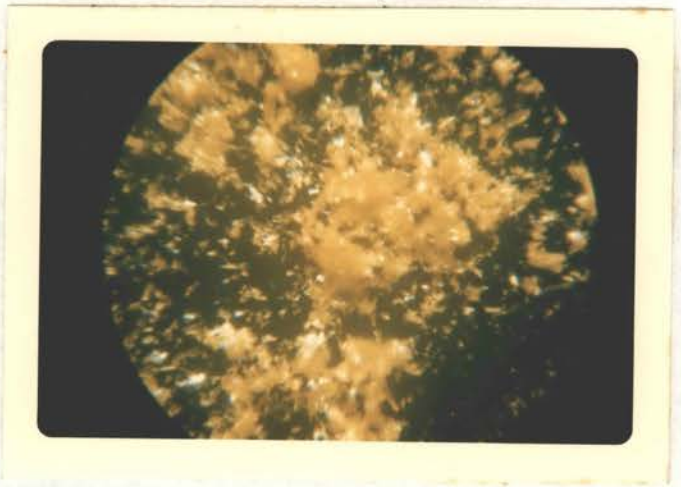
MELTING POINTS OF PRODUCTS OBTAINED IN THE PURIFICATION SCHEME

Chromatographic Separation	Pigment Fraction	Melting Point (°C)		Precipitation[a] Solvent
		First Crystallization	Second Crystallization	
1	mixture of gossypol and brown band	--	--	--
2	gossypol brown brown	89 84 94-96	185-190 95-102 95-100	Hexane Hexane glacial acetic acid
3	brown	150-158 95-98	160-168 96-100	Hexane glacial acetic acid
4	brown	98-102	98-100	glacial acetic acid

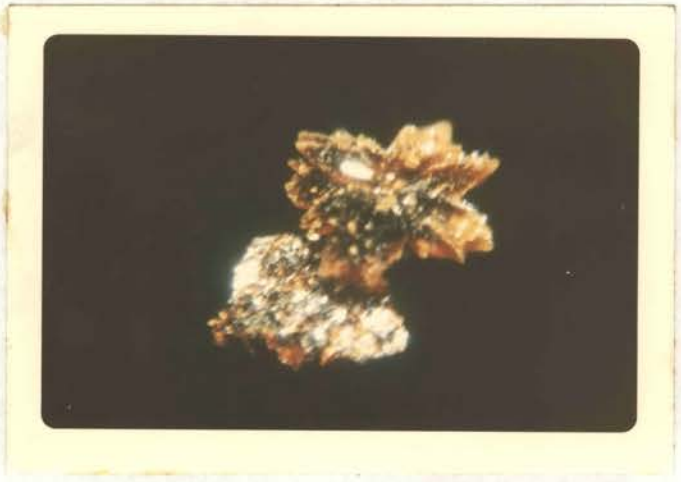
[a]Compound was first dissolved in a minimum amount of $CHCl_3$.



1. Pigment glands



2. Gossypol acetic acid



3. Brown pigment acetic acid crystals

Figure 2. Color Prints of the Pigment Glands, Gossypol Acetic Acid and Brown Pigment Acetic Acid Crystals

of cottonseed pigment gland material as an acetic acid adduct form.

The pattern of pigment separation on a silicic acid column is shown in Figure 3.

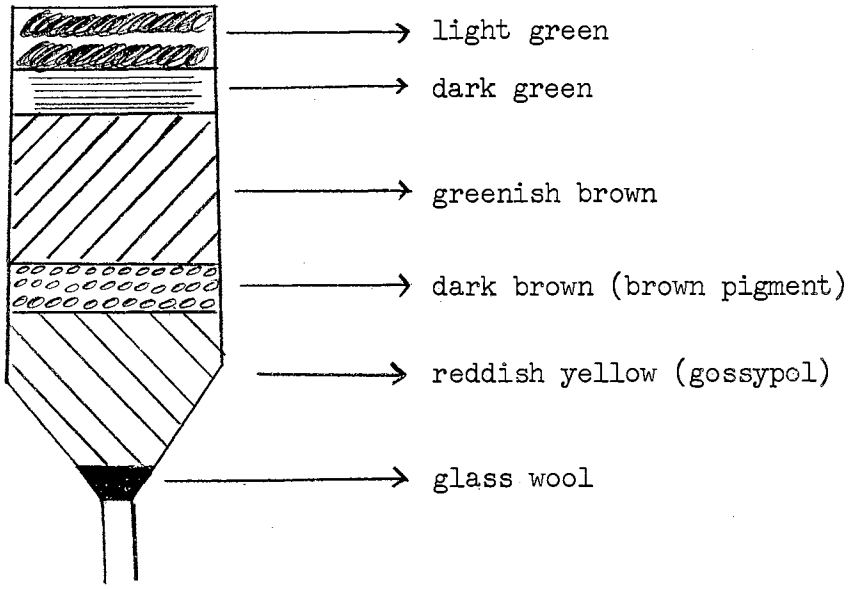


Figure 3. Separation of Cottonseed Pigment Gland Material on Silicic Acid Column (2.2 x 23.5 inches)

A high percentage of hexane in chloroform in the second solvent schedule separated reddish yellow gossypol from the brown compound. The brown pigment was slightly soluble in hexane whereas gossypol was precipitated.

The recrystallized gossypol obtained from the second chromatography was pure with a melting point of 185-190° C. The reported value for the purest gossypol isolated from cottonseed pigment gland material is 214° C (42). The dark brown band, following right after the reddish yellow gossypol band in the silicic acid column, was difficult to collect as a single fraction. Much brown pigment was lost in this fraction collection procedure. However, we were more interested in separating

the pure compound than obtaining a large quantity.

The brown band, eluted with 60 per cent hexane in chloroform with the second solvent schedule, was precipitated from hexane and glacial acetic acid.

The brown pigment precipitated from different solvents showed different melting points while acetic acid derivative of brown pigments was somewhat lower. The brown pigment fraction after the fourth chromatography was chromatographically pure and no precipitate was formed with hexane. It was a gummy, sticky brown material; however, it formed a crystalline precipitate with glacial acetic acid.

As shown in Figure 4, the ultraviolet and visible spectra of the brown pigment and gossypol are the same. No structural information, as to differences, may be obtained from this data but similarities are indicated. The concentrations of the solutions for all spectra studies were adjusted so that the optical densities lay within the most sensitive range of the spectrophotometer, i.e., between 0.1 and 1.0.

The values of $E_{1\,cm}^{1\%}$ for gossypol and brown compound at critical points throughout the wavelength region from 240 to 370 mμ are reported in Table IV.

The infrared spectra of gossypol and the isolated brown pigment were obtained with a Beckman IR5 Infrared Spectrophotometer using a potassium bromide pellet and are shown in Figure 5. The brown pigment spectrum shows a pattern similar to the spectrum of gossypol with an added strong peak in the region of 5.75 to 5.90 μ wavelength. This strong peak indicates that the brown pigment may be related to a naphthoquinone type structure. The IR spectra of quinones exhibit

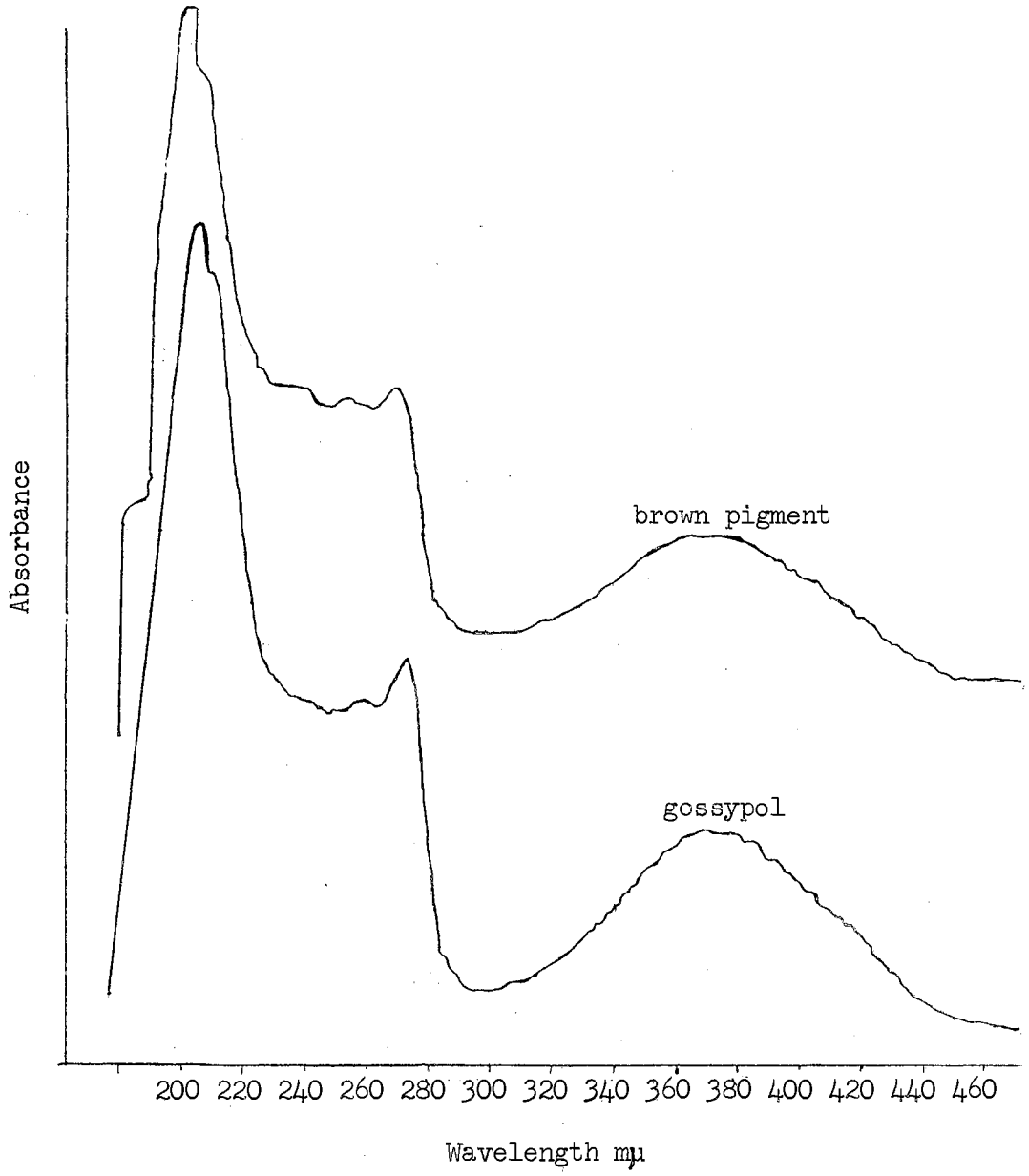


Figure 4. Ultraviolet and Visible Absorption Spectra of the Brown Pigment and Gossypol Isolated from Cottonseed Meal.

TABLE IV

SPECIFIC EXTINCTION COEFFICIENTS ($E^{1\%}_{1cm}$) OF BROWN PIGMENT AND GOSSYPOL IN CHLOROFORM AT POINTS OF CHARACTERISTIC ABSORPTION

Compound	Wavelength, millimicrons	Description	$E^{1\%}_{1cm}$	Log $E^{1\%}_{1cm}$
Brown pigment	240	maximum	1160	3.064
	274	maximum	540	2.732
	282	minimum		
	286	maximum	5517	2.746
	305	minimum		
	364	maximum	279	2.446
Gossypol	240	maximum	1190	3.076
	277	maximum	520	2.716
	287	maximum	581	2.765
	367	maximum	315	2.498

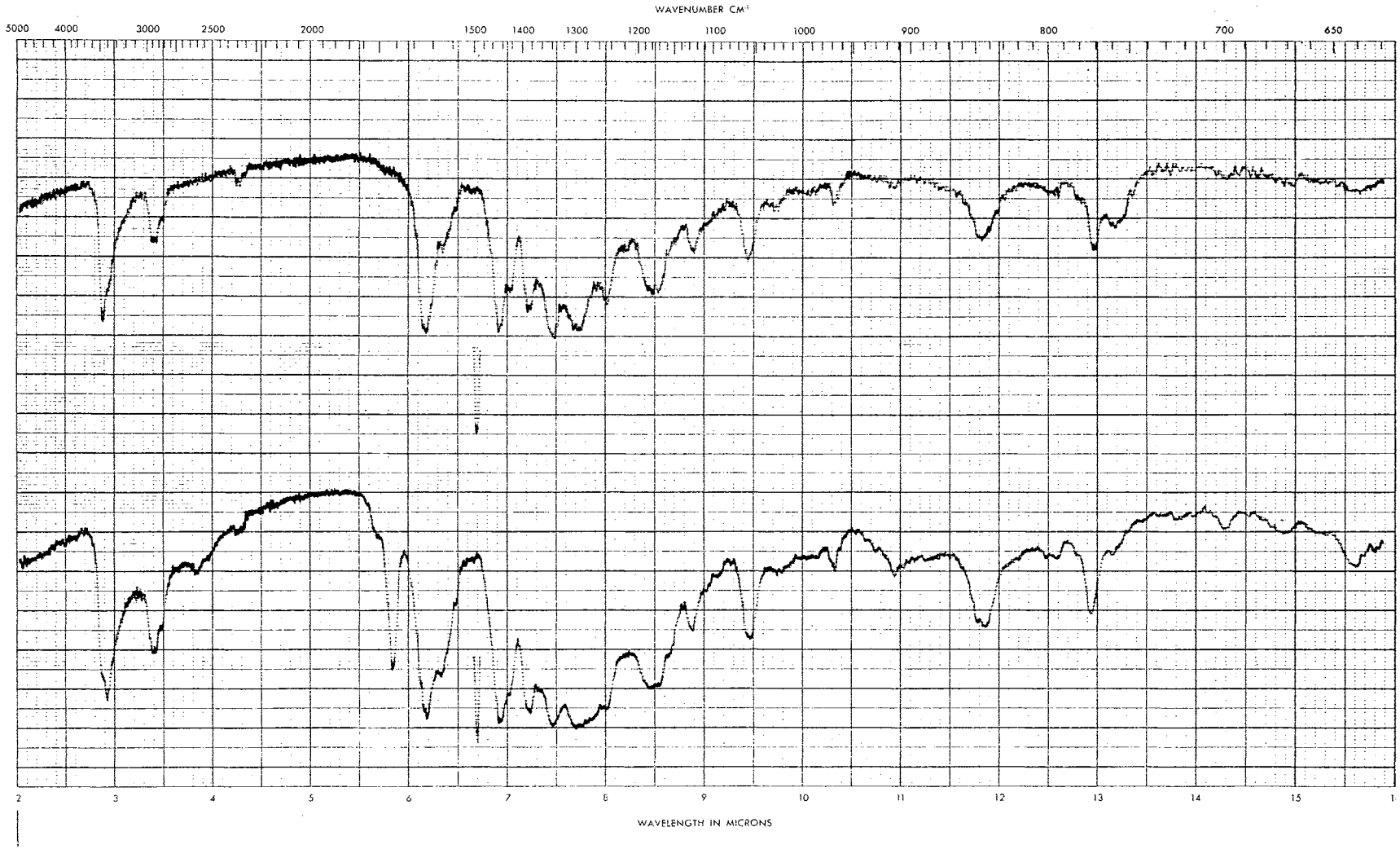


Figure 5. Infrared Spectra of the Brown Pigment and Gossypol Isolated from Cottonseed Pigment Glands.

bands with maxima at 5.98 μ. This C=O stretching vibration is shifted above 6.0 μ, gossypol and the brown compound showed a peak at 6.18 μ. The position of the maximum dependent on the degree of hydrogen bonding varies from about 2.7 μ to above 3.0 μ with increasing bonding (65). The region 2.70 to 3.5 μ (Figure 5) indicates more hydrogen bonding present in the brown pigment than in gossypol. The 2.90 to 2.95 μ peak indicates the presence of phenolic hydroxyl groups in the brown compound. The infrared spectrum of the brown pigment revealed the structure of the brown compound to be similar to gossypol with an indication that this pigment may be more highly oxidized.

Because of the low sensitivity of the instrument, attempts to measure the nuclear magnetic resonance spectrum of the brown pigment failed.

A ferric chloride test on the brown pigment was positive when conducted in chloroform solution. Both the brown pigment and the yellow gossypol formed a dark green color with one drop of ferric chloride test solution. This indicates that gossypol and the brown compound are structurally similar in having two ortho phenolic hydroxyls or a hydroxy para or ortho to a carbonyl.

The visible spectrum of the aniline derivative of the brown compound shows the disappearance of the 364 mμ peak and the appearance of a single new peak at 450 mμ. This spectra is compared to the spectra of dianilino gossypol (Figure 6).

The visible spectra of the brown compound and gossypol in antimony trichloride solution gave peaks at 500 and 370 mμ. The test was positive for both compounds and the spectra shown in Figure 7 are similar. This reaction was carried out with chloroform solutions

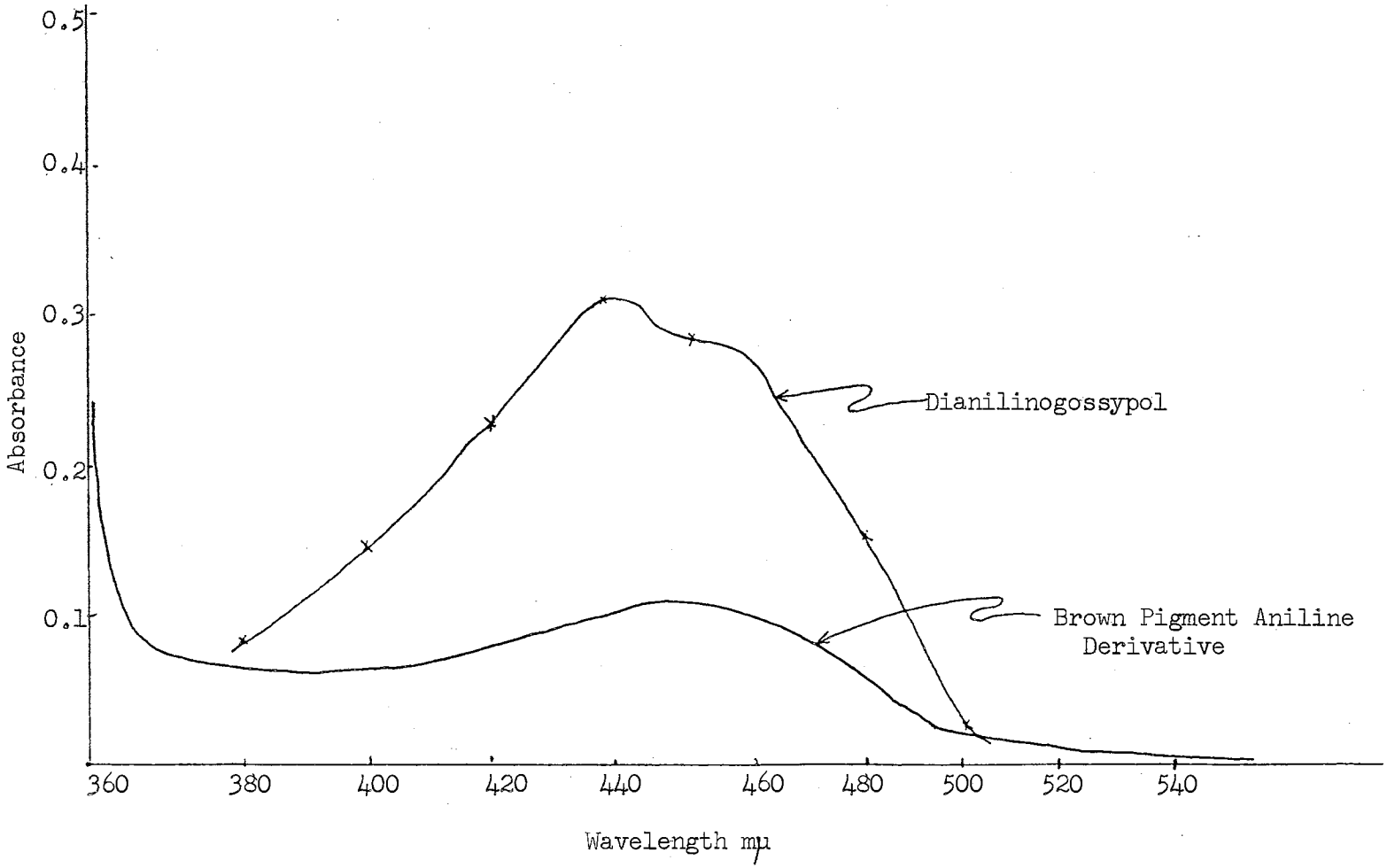


Figure 6. The Visible Spectrum of the Aniline Derivative of the Brown Compound and the Dianilinogossypol.

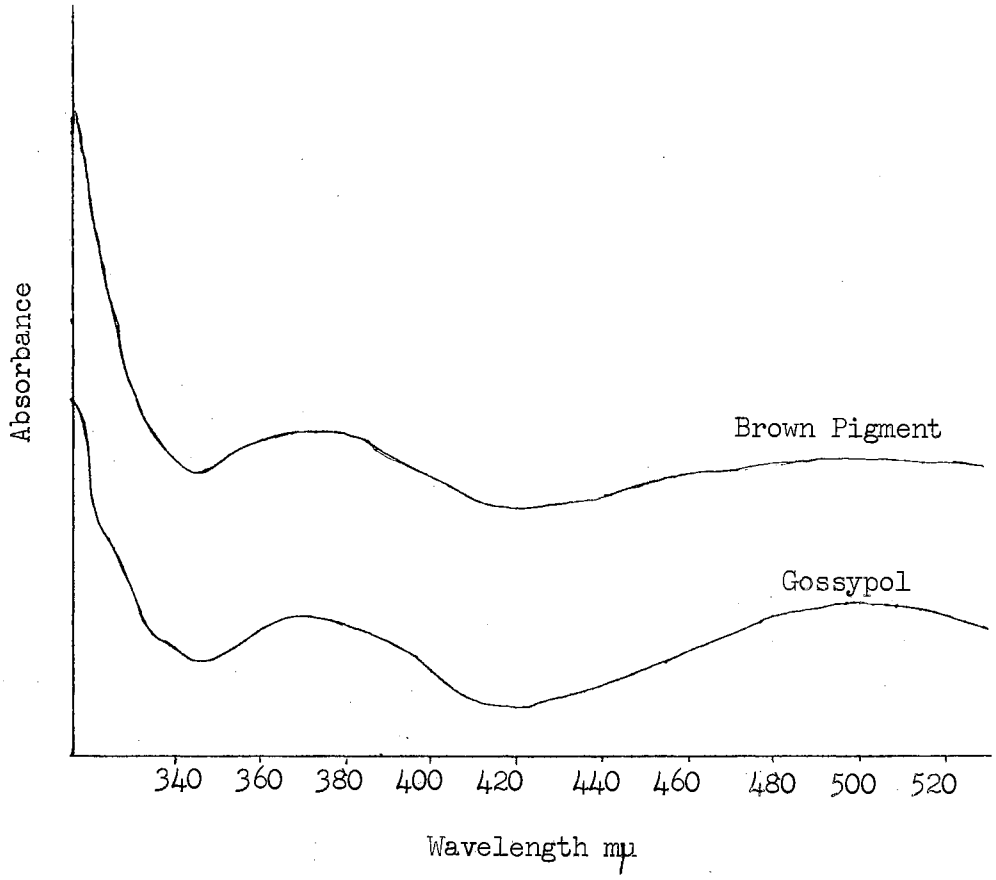


Figure 7. The Visible Spectrum of the Brown Compound and Gossypol in Antimony Trichloride Solution.

of the brown compound and gossypol according to the procedure described for the antimony trichloride-spectrophotometric method for the determination of gossypol in cottonseed (66). A higher concentration of the brown pigment was apparently required for this test.

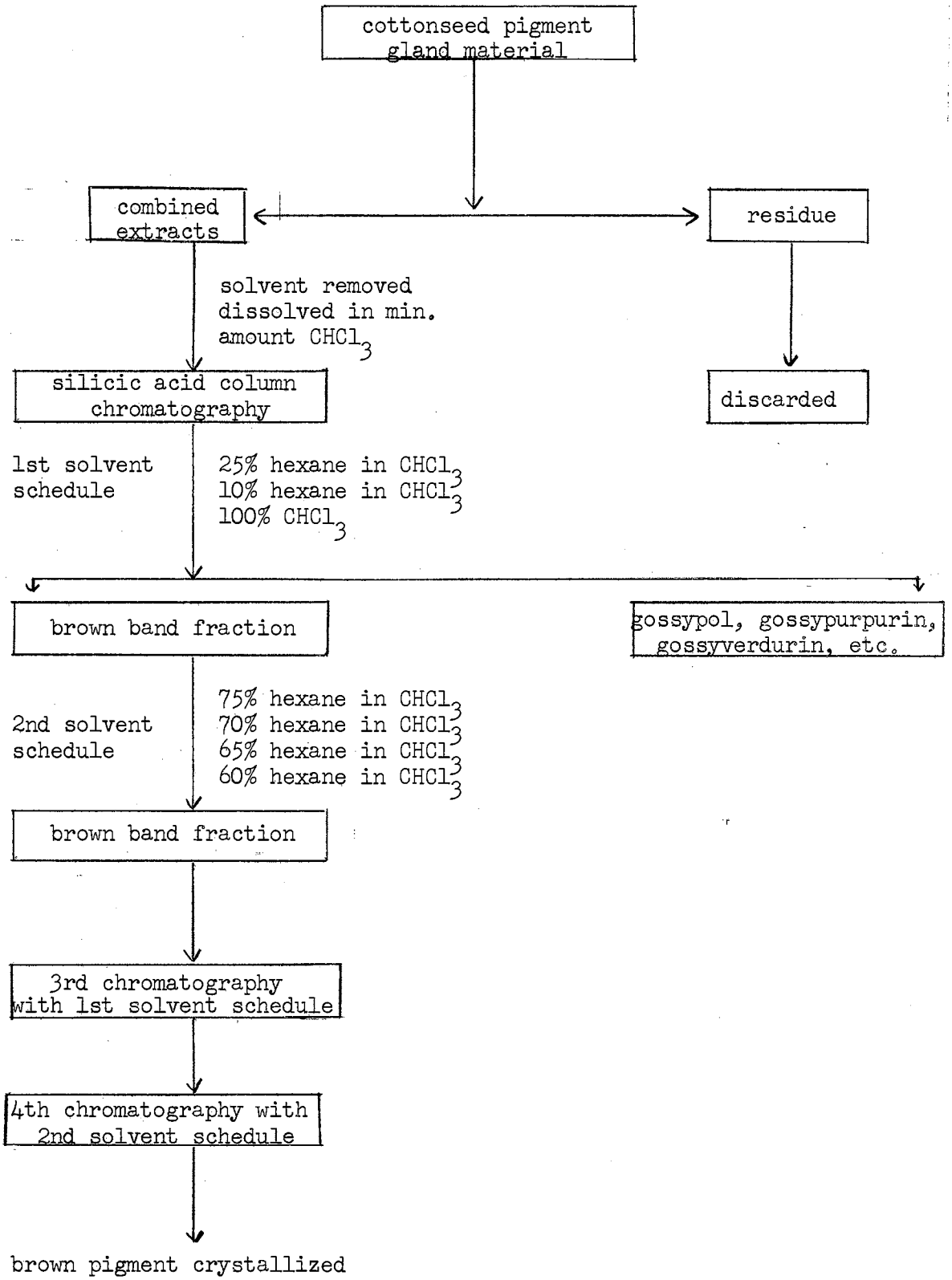


Figure 8. Fractionation of Constituents of Cottonseed Pigment Gland Material.

CHAPTER V

SUMMARY

A brown pigment structurally similar to gossypol has been isolated from cottonseed pigment gland material. It was purified and crystallized as the acetic acid adduct and certain chemical and physical characteristics of this compound have been determined.

Further work is needed to prove the structure of this compound but data from this study indicate the compound may be a more highly oxidized form of gossypol. A naphthoquinone or naphthydroquinone type structure has been indicated by the infrared spectra. The structure of this compound is proposed as 6,6'-dihydroxy-5,5'-diisopropyl-3,3'-dimethyl-(2,2'-binaphthalene)-1,1',7,7'-tetrone-8,8'-dicarboxaldehyde.

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