

components smaller in size than the ligneous material.<sup>9</sup>

4. While the average integral diffusion coefficients of substances oxidized by periodic acid are higher than corresponding values determined on any other basis, it is obvious that a part of the ability to reduce periodic acid is associated with a macro-molecule. That is particularly apparent during the fourth and fifth intervals. This suggests that the lignin sulfonic acids contain structural features oxidizable by periodic acid. The deductions in footnote 7 indicate that the non-ligneous material which reacts with periodic acid is made up of relatively small molecules of low equivalent weight. These are doubtless carbohydrate materials resistant to fermentation and to the action of sulfite pulping chemicals.

By means of the Sutherland-Einstein equation<sup>8</sup> the average integral diffusion coefficients can be translated into average molecular weights. Thus determined<sup>10</sup> the sizes of the methoxyl-bearing

(9) A higher average integral coefficient for the diffusion of sulfur could arise from the presence of rapidly diffusing salts such as ammonium sulfate or ammonium sulfite. Since with cells of the dimensions used in this work only 10% of any ammonium sulfate originally present would remain after the third interval, while 60% of the total sulfur would not have migrated, inorganic salt transfer can account for only a small portion of the increased rate of diffusion.

(10) The equation used for the calculation is  $M = 18.3/D^2$ , where  $M$  = the average molecular weight,  $D$  = the average integral diffusion coefficient, and the constant 18.3 contains a density value = 1.35 g./cm.<sup>3</sup> obtained on the material described in footnote 7 and a viscosity value for five per cent. aqueous ammonium carbonate solution of  $\eta = 1.072 \times 10^{-2}$  poise. The remainder of the constants in the Sutherland-Einstein equation were obtained from "International Critical Tables."

Although the Sutherland-Einstein equation was derived from assumptions valid only in infinitely dilute solutions, it has been applied

components vary from an average molecular weight of 3,100 in the first interval to an average molecular weight of 10,000 in the last two intervals. It is apparent that on this basis well over 60% of the methoxyl-bearing material has an average molecular weight equal to 10,000 or more. If the assumptions made in footnote 7 are accepted, the molecular weights of the non-ligneous substances average about 250.

Diffusion analysis is being applied to the study of lignin sulfonic acid preparations, and to the examination of the products of their reactions.

### Summary

The heterodisperse character of the lignin sulfonic acids in sulfite waste liquor has been demonstrated by determination of a sequence of average-integral diffusion coefficients observed over successive intervals of diffusion.

Calculations made by application of the Sutherland-Einstein equation to the experimentally determined diffusion coefficients show the lignin sulfonic acids to vary in molecular weight from 3,000-10,000 or more.

The non-ligneous substances in sulfite waste liquor appear to be of low molecular weight, leaving the lignin sulfonic acids as probably the only macromolecules.

with success by Northrop and Anson, and by McBain to egg albumin at 1% concentration and by Friedman to several sugars [see Friedman and Carpenter, *THIS JOURNAL*, 61, 1745 (1939), also Dean and Loring, *J. Biol. Chem.*, 157, 717 (1945)]. In solutions as dilute as those used for this work (0.5%) the error introduced in the calculated molecular weights is certainly not greater than that corresponding to the precision claimed in the measurements.

RECEIVED AUGUST 17, 1946

[CONTRIBUTION FROM THE SOUTHERN REGIONAL RESEARCH LABORATORY OF THE BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY]

## The Pigments of Cottonseed. II. Gossyaerulin, a Blue Quinonoid Gossypol Derivative Occurring in Cooked Cottonseed

BY CHARLOTTE H. BOATNER, CAROLYN S. SAMUELS, CATHERINE M. HALL AND MAIZIE C. CURET

In the first recorded investigation of the pigments of cottonseed, Kuhlmann<sup>1</sup> reported the formation of a brilliant blue pigment when acidified cottonseed foots were subjected to steam distillation for the recovery of fatty acids. Following Kuhlmann's original partial characterization of "cottonseed blue" nothing further has been reported relative to this pigment.

During isolation of gossypol from ethereal extracts of non-defatted cottonseed by a modification<sup>2</sup> of a procedure recommended by Carruth,<sup>3</sup> a brilliant green precipitate was obtained upon heating the crude gossypol acetic acid suspended

in a mixture of diethyl ether and acetic acid. Prolonged heating (three to four days) of a suspension of pure gossypol in concentrated hydrochloric acid produced a similar green product. The absorption spectra of chloroform solutions of the green products exhibited a slight maximum at 610 m $\mu$  in addition to that at 365 m $\mu$  which is characteristic of gossypol.

A blue compound has now been prepared free of gossypol by heating pure gossypol in methanol acidified with sulfuric acid. It has been named gossyaerulin because of its color and its occurrence in cooked cotton seed. This blue pigment was probably a component of Kuhlmann's "cottonseed blue."

The reaction of gossypol in methyl alcohol solu-

(1) Kuhlmann, *Compt. rend.*, 53, 444-452 (1861).

(2) Boatner, Caravella and Samuels, *THIS JOURNAL*, 66, 838 (1944).

(3) Carruth, *ibid.*, 40, 647-663 (1918).

tions acidified with sulfuric acid was found to follow alternate paths under the influence of heat. When the reaction was carried out at low temperatures or in the presence of low concentrations of sulfuric acid, preponderant amounts of a yellow compound were formed. This compound was detected by means of the absorption spectrum of its antimony trichloride reaction product,<sup>4</sup> which exhibited an absorption band with maximum at 430  $m\mu$ , thus differentiating it sharply from the antimony trichloride reaction product of unreacted gossypol which has absorption maxima at 520 and 380  $m\mu$  and a minimum at 430  $m\mu$ ,<sup>4</sup> and from that of gossyaerulin which has an absorption maximum at 610  $m\mu$ . With the use of higher concentrations of sulfuric acid or upon more prolonged heating, the yield of gossyaerulin is reduced by the formation of a purple reaction product of gossyaerulin with sulfuric acid, which exhibits a broad but low absorption band with maximum at 570  $m\mu$ ,  $E_{1\%}^{1\text{cm}}$  98.7. Neither the yellow, nor the purple product could be converted into gossyaerulin. No reaction conditions resulting in the formation of gossyaerulin free of both the yellow and the purple gossypol conversion products could be found.

The absorption spectrum of the antimony trichloride reaction product of the best preparation of crude gossyaerulin (Fig. 1) indicated the presence of the yellow by-product. Two successive recrystallizations from a mixture of diethyl ether and petroleum naphtha (Skellysolve F) yielded a dark blue solid, m. p. 169° (dec.). The solution of this compound in chloroform acidified with hydrochloric acid exhibits a well-defined absorption band with maximum at 605  $m\mu$ ,  $E_{1\%}^{1\text{cm}}$  315.4, and an absorption minimum at 430  $m\mu$ ,  $E_{1\%}^{1\text{cm}}$  40.8 (Fig. 1).

Gossyaerulin was found to undergo a reversible color change from blue in acid to yellow in neutral or alkaline solution. The blue form can then be regenerated by acidification of the solution. The maximum blue color, measured spectrophotometrically, is exhibited in solutions containing very low concentrations of acid, and is independent of the kind of acid. The blue, acidic form of the pigment is more soluble in polar than in non-polar solvents, whereas the neutral or alkaline form is more soluble in non-polar solvents. On the basis of these observations, it seems probable that the two forms are tautomeric, and the blue is the more polar or ionic form.

Color reactions with boroacetic anhydride<sup>5</sup> and with stannic chloride in benzene or chloroform<sup>6</sup> indicate the presence of a carbonyl group which is attached in a position alpha or adjacent to a hydroxyl group. The ready and reversible reduction of gossyaerulin, coupled with the known

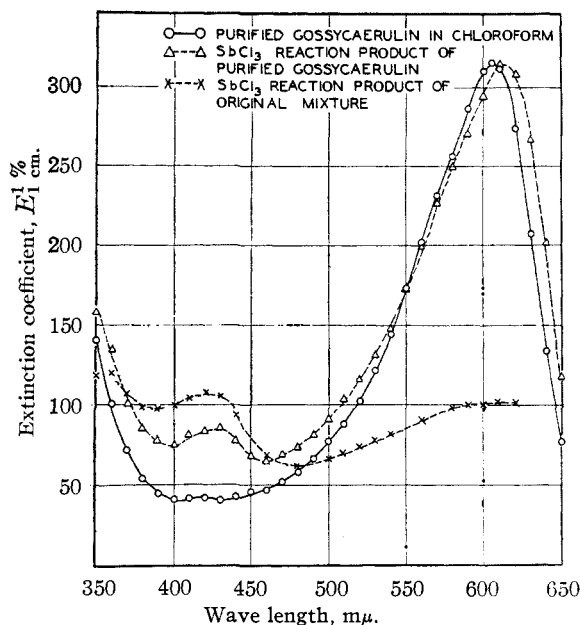


Fig. 1.

polyphenolic nature of gossypol, present fairly certain evidence that the blue pigment is a quinoid oxidation product of gossypol.

The quinoid nature of the purple reaction product of gossyaerulin with concentrated sulfuric acid can be deduced from its origin and its ready and reversible reduction.

The observation that the amount of gossyaerulin formed in cooked cottonseed increases as the gossypol content and the pH of the seed decrease upon continued heating suggests that the blue pigment or its neutral, yellow form may account for some of the color products obtained by processing of cottonseed. Since it has not been customary to control or determine the pH of seed under various processing conditions, additional investigation will be necessary to determine the effect of pH on the color of products obtained on a commercial scale.

### Experimental

**Estimation of Yields.**—Since the only known properties of gossyaerulin are its blue color and its characteristic absorption spectrum with an absorption band at 610  $m\mu$  (Fig. 1) these properties of the crude reaction mixtures were used to follow the formation of gossyaerulin from gossypol.

At the end of any particular reaction which was being investigated, the solvent was removed from the crude reaction mixture. When water immiscible solvents were used, they were removed under reduced pressure. When the reaction was carried out in water miscible solvents, the crude reaction mixture was poured into water, and the resultant precipitate was freed of the liquid mixture by centrifuging. When sulfuric acid was used in the reaction, it was found necessary to remove all traces of it before drying the precipitate in order to avoid the formation of the purple sulfuric acid reaction product of gossyaerulin. The crude precipitates were freed of sulfuric acid by repeatedly dissolving them in ethanol and reprecipitating them by the addition of an equal volume of dilute hydrochloric acid.

(4) Boatner, Caravella and Kyame, *Ind. Eng. Chem., Anal. Ed.*, **16**, 566-572 (1944).

(5) Dimroth, *Ann.*, **446**, 97-122 (1926).

(6) Pfeiffer, *Ber.*, **62**, 111-115 (1927).

TABLE I  
COMPARISON OF DIFFERENT ACIDS FOR PREPARATION OF  
GOSSYCAERULIN IN METHANOL<sup>a</sup>

Acid added— Kind	Amt.	Length of heating, hours	Color of product	$E_{1.27\text{ cm.}}^{1\%}$ at 610 $m\mu$ of $\text{CHCl}_3$ soln.
Glac. HOAc	0.5 ml.	240	Green	...
HCl (gas)	Satd.	48	Light blue	30.7
HCl (gas)	Satd.	72	Light blue	40.2
HCl (gas)	Satd.	120	Dark blue	111.6
$\text{H}_3\text{PO}_4$ (l.)	1 ml.	120	Dark blue	142.0
$\text{H}_2\text{SO}_4$	0.16 ml.	72	Dark blue	128.0
$\text{H}_2\text{SO}_4$	.16 ml.	96	Dark blue	122.0

<sup>a</sup> Solutions of 0.1 g. of gossypol in 5 ml. of methanol refluxed on a steam-bath.

(Table I) indicated that acidification of methanol solutions of gossypol with sulfuric acid yielded the largest amounts of gossycaerulin during a given period of heating, and that the maximum yield of gossycaerulin was obtained by heating these solutions for seventy-two hours.

Experiments in which the concentration of sulfuric acid and the period of heating were varied (Table II) showed that lower concentrations of sulfuric acid (0.3 and 1.6% by volume) resulted in the formation of preponderant amounts of the yellow component which forms an antimony trichloride reaction product with an absorption maximum at 430  $m\mu$ . Further heating of the mixtures containing large amounts of this compound and small amounts of gossycaerulin resulted in the formation of very little additional gossycaerulin. When the concentration of sulfuric acid was increased to 10% or higher, preparations 9 to 12, gossycaerulin was formed, but it reacted further with sulfuric acid to form a purple compound from which gossycaerulin could not be recovered.

TABLE II  
OPTIMUM CONCENTRATION OF SULFURIC ACID FOR PREPARATION OF GOSSYCAERULIN FROM GOSSYPOL<sup>a</sup>

Prepn.	Sulfuric acid % by volume	Period of heating, hours	Crude product 610 $m\mu$	Extinction coefficients: $E_{1.27\text{ cm.}}^{1\%}$				
				610 $m\mu$	Antimony trichloride 570 $m\mu$	reaction product 520 $m\mu$	430 $m\mu$	380 $m\mu$
1	0.3	24	43.2	73.4	...	...	369.6	257.4
2	.3	48	48.8	95.5	...	261.5	393.5	398.0
3 <sup>b</sup>	.3	48	48.5	86.5	...	239.5	378.5	384.2
4	.3	72	65.9	98.5	...	...	355.6	299.8
5	1.6	72	30.7	65.8	...	221.3	359.0	350.9
6	3.2	24	9.7	48.4	186.8	224.4	350.0	243.3
7	3.2	72	157.0	138.4	...	95.5	133.1	125.7
8	3.2	72	128.0	...	...	...	...	...
9	10.0	96	122.0	...	...	...	...	...
10	20.0	96	116.5 <sup>c</sup>	...	...	...	...	...
11	30.0	96	125.4 <sup>c</sup>	130.5	141.3	104.9	85.8	148.0
12 <sup>d</sup>	20.0	2	...	121.9	126.1	100.0	76.5	99.1

<sup>a</sup> Solutions of 0.1 g. gossypol in 5 ml. methanol refluxed on a steam-bath. <sup>b</sup> Crude product from preparation 2 heated further. <sup>c</sup> Value of  $E_{1.27\text{ cm.}}^{1\%}$  at maximum at 570  $m\mu$ . <sup>d</sup> Gossycaerulin used as reactant.

For the spectrophotometric analysis of the crude dried precipitates, weighed amounts were dissolved in U. S. P. chloroform saturated with gaseous hydrogen chloride. A Coleman monochromator spectrophotometer, equipped with cells having a light path of 1.27 cm., was used to determine the absorption at 10  $m\mu$  intervals from 650 to 350  $m\mu$ . The relative yields of gossycaerulin were estimated on the basis of the value of the  $E_{1.27\text{ cm.}}^{1\%}$  at 610  $m\mu$  of chloroform solutions of the crude precipitates.

The antimony trichloride reaction was carried out with the crude precipitates according to the procedure previously described,<sup>4</sup> and the values of the absorption at 380, 430, 520 and 610  $m\mu$  were used as a basis for estimating the relative concentrations of unreacted gossypol, yellow byproduct and gossycaerulin formed during the reaction.

The amount of purple gossycaerulin-sulfuric acid reaction product was estimated on the basis of the ratio of the extinction at 570  $m\mu$  to that at 610  $m\mu$  of an acidified chloroform solution of the crude precipitate or its antimony trichloride reaction product.

**Determination of Optimum Conditions for Preparation of Gossycaerulin.**—The color of the products formed by heating gossypol in acidified solutions of benzene, 1,4-dioxane, acetone, methanol, ethanol, *i*-propanol, and concentrated aqueous hydrochloric acid demonstrated that, of these solvents, only water and alcohols were suitable reaction media for the conversion of gossypol to gossycaerulin. The alcohols were more effective than water, and methanol was the most satisfactory of the alcohols tested.

Comparison of the results obtained with different acids

As shown by the data in Table III refluxing of gossycaerulin in the presence of sulfuric acid in concentrations

TABLE III  
REACTION OF GOSSYCAERULIN WITH SULFURIC ACID<sup>a</sup>

Sulfuric acid % by volume	Period of heating, hr.	Temperature of heating, °C.	Ratio of extinction coefficients <sup>b</sup>	
			610/570 <sup>c</sup>	610/450 <sup>d</sup>
20	20	105	1.22	2.58
18	20	105	1.25	3.24
13.5	24	50	1.27	3.07
12.5	24	105	1.27	4.22
12.5	48	105	1.10	2.74

<sup>a</sup> Nine-ml. portions of methanol solutions of different concentrations of sulfuric acid added to 1 ml. aliquots of 0.02% methanolic solution of gossycaerulin. <sup>b</sup> Determined on aliquots of reaction mixture. <sup>c</sup> Ratio of  $E$  at 610  $m\mu$  to  $E$  at 570  $m\mu$  of original gossycaerulin was 1.34. <sup>d</sup> Ratio of  $E$  at 610  $m\mu$  to  $E$  at 450  $m\mu$  of original gossycaerulin was 4.33.

as low as 12.5% result in the formation of the purple sulfuric acid reaction product of gossycaerulin upon prolonged heating. However, gossycaerulin is quite stable in methanol solutions containing as high as 25% sulfuric acid (by volume) if the solutions are maintained at room temperature.

When solutions of gossypol in acidified methanol were heated at temperatures below 100° (Table IV), pre-

ponderant amounts of the yellow by-product were formed and the yields of gossycaerulin were poor.

TABLE IV

EFFECT OF REACTION TEMPERATURE ON FORMATION OF GOSSYCAERULIN<sup>a</sup>

Sulfuric acid, % by vol.	Period of reaction, days	Temp., °C.	Color of product	Product 610 mμ	Extinction coefficients: $E_{1.27}^{1\%}$ cm.			
					Antimony trichloride reaction product			
				610 mμ	430 mμ	360 mμ	257.4 mμ	
2	99	25	Pale green	...	...	...	...	
4	1	50	Green	2.6	47.3	293.7	345.1	
4	1	65 <sup>b</sup>	Blue	23.4	71.5	425.2	445.4	
2	7	65 <sup>b</sup>	Blue	48.8	88.8	174.4	201.8	
4	7	65 <sup>b</sup>	Purple	(580) 52.4	...	...	...	
3	4	65 <sup>b</sup>	Blue	...	63.7	258.4	261.2	
3	3	65 <sup>b</sup>	Blue	30.8	69.3	257.2	265.8	
3.2	3	105 <sup>c</sup>	Blue	157.0	138.4	133.1	125.7	
0.3	1	105 <sup>c</sup>	Pale blue	43.2	73.4	369.6	257.4	
.3	3	105 <sup>c</sup>	Pale blue	65.9	98.5	355.6	299.8	

<sup>a</sup> Solutions of 0.1 g. of gossypol in 5 ml. of methanol containing different concentrations of sulfuric acid used as initial reaction mixtures. <sup>b</sup> Temperature of hot-plate regulated to 65° so that solution was just under boiling. <sup>c</sup> Heated on a steam funnel to obtain vigorous boiling.

**Preparation and Purification of Gossycaerulin.**—The best yield of gossycaerulin from gossypol was obtained by heating a solution of 0.1 g. of gossypol in 5 ml. of methanol containing 3.2% (by volume) sulfuric acid under reflux on a steam-bath for seventy-hours (prepn. 7, Table II).

Attempts to duplicate this preparation were not entirely successful; a similar acidified methanolic gossypol solution heated for the same period of time yielded less gossycaerulin (prepn. 8, Table II).

The crude reaction product is soluble in diethyl ether and insoluble in petroleum naphtha (Skellysolve F). Two successive recrystallizations from diethyl ether and petroleum naphtha produced a brilliant blue solid m. p. 169° (dec.), and a pale yellow solution. The absorption spectrum of an acidified chloroform solution of the purified gossycaerulin (Fig. 1) exhibited a broad band with a maximum at 605 mμ,  $E_{1.27}^{1\%}$  cm. 315.4, and a minimum at 430 mμ,  $E_{1.27}^{1\%}$  cm. 42.0. The absorption spectrum of the antimony trichloride reaction product was similar to that of the unreacted compound. A secondary absorption maximum at 420 mμ  $E_{1.27}^{1\%}$  cm. 85.7, indicated the presence of small amounts of the pale yellow by-product.

**Properties of Gossycaerulin.**—When gaseous ammonia was passed into acidified methanol solutions of measured gossycaerulin content, the color changed from blue to green and finally to a pale yellow as the solution became alkaline. The absorption spectrum of the alkaline solution exhibited no absorption band in the visible and near ultraviolet wave length regions, but exhibited slowly increasing absorption toward the near ultraviolet. When gaseous hydrogen chloride was passed into the methanol solution, the original blue color was restored, and the absorption spectrum was identical with that of the original gossycaerulin solution. The blue form is stabilized by concentrations of sulfuric acid in methanol as low as 0.01% (by volume).

The blue acidic form of gossycaerulin is soluble in methanol, ethanol, *i*-propanol, amyl alcohol, acetic acid, acetic anhydride and diethyl ether. It is slightly soluble in chloroform and very slightly soluble in petroleum naphtha (Skellysolve F), benzene, toluene, water, hot saturated aqueous sodium bisulfite and aqueous sodium hydroxide. The yellow neutral form is soluble in petroleum naphtha, toluene, and benzene; slightly soluble in chloroform; and very slightly soluble in methanol, ethanol and diethyl ether.

Gossycaerulin undergoes the following color reactions. In concentrated sulfuric acid, a purple solution is formed

having an absorption spectrum identical with that of the product obtained by refluxing a solution of gossypol in methanol containing high concentrations of sulfuric acid (prepn. 12, Table II).

When boroacetic acid in acetic anhydride is added to a solution of gossycaerulin in acetic anhydride,<sup>5</sup> a purple solution stable to heat is formed. The product formed by reaction of gossycaerulin with stannic chloride in benzene<sup>6</sup> is colored bluish purple.

Treatment of ethanol solutions of gossycaerulin with zinc and hydrochloric acid, sodium bisulfite or sodium dithionite (Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>) converts them from blue to yellow. The original color of the solutions is restored upon contact with air or by treatment with hydrogen peroxide.

Ethanol solutions of gossycaerulin are unaffected by treatment with hydrogen peroxide at room temperature. However, reaction of gossycaerulin at room temperature in benzene or chloroform solutions with lead tetraacetate produces a change in color from blue to yellow to brown; and similar color changes are observed upon reaction of periodic acid at 38°F. Corresponding treatments of gossypol solutions with the above-mentioned oxidizing agents result in a sequence of color changes from yellow to green, to yellow and finally to brown. The same color changes are observed upon treatment of ethanol solutions of gossypol with dilute ferric chloride.

Reaction of hydrogen peroxide with an acidified methyl alcoholic solution of gossypol at 50° for twenty-four to forty-eight hours produces a pale yellow product. The acidified chloroform solution of this product exhibits no absorption band in the visible and near ultraviolet wavelength regions. Similar results were obtained when air was aspirated through an acidified methanolic solution of gossypol maintained at 50° for twenty-four hours.

**Properties of the Purple Gossycaerulin-sulfuric Acid Reaction Product.**—Spectrophotometric analysis of solutions of the purple gossycaerulin-sulfuric acid reaction product in chloroform acidified with hydrogen chloride showed the presence of an absorption maximum at 570 mμ,  $E_{1.27}^{1\%}$  cm. 125.4, a minimum at 440 mμ,  $E_{1.27}^{1\%}$  cm. 49.4 and increasing absorption toward the near ultraviolet,  $E_{1.27}^{1\%}$  cm. at 360 mμ, 160.5. Reaction with antimony trichloride shifts the absorption maximum to 580 mμ,  $E_{1.27}^{1\%}$  cm. 142.4, and the minimum to 470 mμ,  $E_{1.27}^{1\%}$  cm. 81.2.

Upon treatment of a methanol solution of the purple compound with ammonia the color is changed to blue green and the solution exhibits no absorption bands in the visible wave length region. Acidification of this solution with hydrogen chloride restores the original purple color.

TABLE V

FORMATION OF GOSSYCAERULIN DURING HEATING OF COTTONSEED<sup>a</sup>

Length of heating at 115° minutes	Cooking of seed kernels			Cooking of undecorticated seed		
	Extractable gossypol, %	$E_{1.27}^{1\%}$ at 610 mμ × 100	pH of seed kernels	Extractable gossypol, %	$E_{1.27}^{1\%}$ at 610 mμ × 100	pH of seed kernels
0	0.807	1.01	6.78	1.18	1.88	6.88
5	.64	1.66	6.85	0.835	3.46	6.58
10	.914	1.88	6.68	.795	2.67	6.64
15	.939	2.57	6.62	.844	3.33	6.62
20	.902	2.52	6.63	.715	3.34	6.61
30	.906	4.48	6.89	.552	4.22	6.60
60	.574	4.16	6.46	.376	8.08	6.64
90	.458	5.97	6.40	.294	8.79	6.35
120	.329	6.32	6.36	.260	9.09	6.25

<sup>a</sup> Initial moisture content 7.6%. <sup>b</sup> Determined by application of the antimony trichloride spectrophotometric method to chloroform extracts prepared by equilibration at 38°F. for twenty-four hours. <sup>c</sup> Determined with aqueous extracts (10 ml./0.25 g. macerated kernels).

When ethanol solutions of the purple compound are treated with sodium bisulfite or sodium dithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) the purple color fades to yellow. Treatment of the yellow solution with a solution of hydrogen peroxide or shaking it in the presence of air restores the original purple color. Reaction of a chloroform solution of the purple compound with lead tetraacetate results in the formation of a yellow solution which cannot be restored to its original purple color by treatment with reducing agents.

**Formation of Gossyaerulin During Cooking of Cottonseed.**—As shown in Table V, the gossypol content of two lots of seed heated at  $115^\circ$  decreased sharply during the initial period of heating, increased slightly over a short period, and then decreased slowly as the period of heating was prolonged. On the other hand, the gossyaerulin contents of the cooked seed increased slowly throughout the entire period of cooking, and the pH decreased at approximately the same rate.

### Summary

A blue pigment, gossyaerulin, has been prepared by heating acidified solutions of gossypol, and reaction conditions which favor the formation of this pigment have been determined.

The properties of purified gossyaerulin have shown it to be a polar, alpha hydroxy, quinonoid, oxidation product of gossypol. It exhibits a maximum blue color having an absorption maximum at  $605 \text{ m}\mu$  in acid solution and is reversibly convertible into a non-polar, yellow, neutral form.

A yellow pigment has been detected as a by-product formed from gossypol during its conversion to gossyaerulin. A purple compound resulting from the reaction of gossyaerulin with sulfuric acid has been shown to exhibit the reactions of a quinonoid compound.

Gossyaerulin has been detected in cooked cottonseed, and the extent of its formation has been shown to be correlated with the disappearance of gossypol, the length of heating, and the pH developed during heating of the seed.

NEW ORLEANS, LOUISIANA

RECEIVED JULY 26, 1946

[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY<sup>1</sup>]

## The Preparation of Acetopropyl Alcohol and 1,4-Pentanediol from Methylfuran<sup>2</sup>

By L. E. SCHNIEPP, H. H. GELLER AND R. W. VON KORFF<sup>3</sup>

Development of practical methods<sup>4,5</sup> for the conversion of furfural to methylfuran makes it of interest to investigate this material as an intermediate for organic syntheses.

Although methylfuran can be catalytically reduced to tetrahydromethylfuran,<sup>5,6</sup> the dihydrofurans appear to be more attractive starting materials, since they hydrolyze to give hydroxycarbonyl derivatives. It has been shown by Wilson,<sup>7</sup> for example, that  $\delta$ -hydroxybutyraldehyde can be prepared from 2,3-dihydrofuran. Earlier work by Paul<sup>8</sup> had demonstrated that 4,5-dihydro-2-methylfuran hydrolyzes to acetopropyl alcohol which was later obtained by Topchiev<sup>9</sup> directly from methylfuran by a combination of hydrogenation and hydrolysis reactions.

Since Topchiev did not report the details of his experimental work, his method was reinvestigated to establish optimum conditions for this conversion. The complete hydrogenation of the reac-

tion mixture to produce 1,4-pentanediol was also studied.

Results obtained show that acetopropyl alcohol can be prepared in 25–35% yields or 1,4-pentanediol in 50–60% yields by hydrogenating methylfuran in the presence of water and a very small amount of acidic materials. Hydrogenation in the presence of water alone did not effect cleavage of the furan ring. Small amounts of acidic materials were necessary to produce the desired difunctional aliphatic derivatives. Best yields and most readily reproducible results were obtained by using formic acid in concentrations of 0.01–0.10% by weight of the reaction mixture.

Investigations were carried out in dioxane or alcohol solutions and with water alone. The conversion of methylfuran to 1,4-pentanediol was conveniently accomplished by conducting the reaction in a two-phase, methylfuran–water mixture. The products were readily separated by fractional distillation of the aqueous solution of tetrahydromethylfuran and 1,4-pentanediol produced by the reaction. Use of a blending solvent was advantageous when acetopropyl alcohol was the desired product. This hydrogenation was interrupted when approximately 1 mole of hydrogen/mole of methylfuran had been absorbed and less unreacted methylfuran was recovered than from the two-phase water system.

Results of this investigation substantiate the mechanism of acetopropyl alcohol formation proposed by Topchiev.<sup>9</sup> Available evidence is in accord with the sequence of reactions

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Presented under the title "The Hydrogenation of Methylfuran in Aqueous Media" before the Division of Organic Chemistry, 109th Meeting of the American Chemical Society, Atlantic City, New Jersey.

(3) Present address: 712 Lincoln Avenue West, Minneapolis, Minnesota.

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