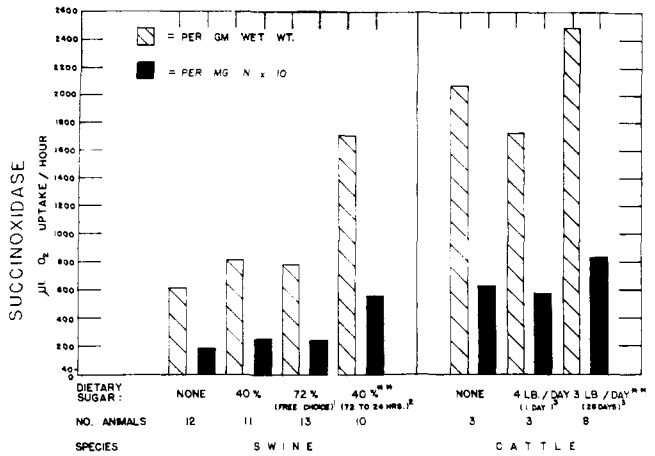

Figure 1. Effect of various sugar diets on the succinoxidase of the liver of swine and cattle

** $P < 0.01$

1. Sugar and protein supplement free choice (see Table I). 2. Basal diet, then 40% sugar diet from 72 to 24 hours before slaughter. 3. Sugar, 4 pounds per day, given as saturated aqueous drench. 3 pounds per day fed in dry diet (see Table I) during 28-day period

plain the lack of response of the succinoxidase to the sugar after 24 hours, but not the increase observed at the 28-day period. It may be that continuous stimulation of the enzyme over the 28-day period by the sugar results in a more active enzyme in the liver of cattle. The increase in size of the liver observed in animals fed sucrose may be related to increased metabolic activity in the liver in general, of which succinoxidase activity is only a part. It may be that the liver has the capacity to keep the dextrose at a near normal level by means of greater enzyme activity. The present observations demonstrate that cattle and swine have a succinoxidase system in the liver that is sensitive to dietary sugar in a manner similar to that observed in rats by Shipley *et al.* (*5*) and Bargoni (*2*).

Succinoxidase was also determined in the heart of the heifers, but was not affected by the dietary treatments. All values ranged from 900 to 1000 μl. of oxygen uptake per mg. of nitrogen per hour in the left ventricle. Lactic dehydrogenase was determined in both the liver and heart of the heifers, but did not vary among the dietary groups. The heart had approximately 9 times as much lactic dehydrogenase activity as the liver—i.e., approximately 180 as compared to 20 μl. of oxygen uptake per mg. of nitrogen per hour.

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GRAIN WAX COMPONENTS

Fractionation of Sorghum Grain Wax

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Considerable study has been given to the problem of finding a wax to replace carnauba wax in some of its many uses. Sorghum grain wax is somewhat similar, but there are some differences in their physical properties (*2*). If the compounds present in sorghum grain wax could be determined, perhaps means could be devised to alter their proportions to produce a material with more of the characteristics of carnauba wax. Column chromatography was used to study the isolation and identification of sorghum grain wax components.

Experimental

Extraction of Wax. Sorghum grain (Midland variety) was extracted, with Skellysolve B, in 1200-gram batches in a large Soxhlet extractor. The extract was evaporated to 100 ml. and 500 ml. of hot acetone were added. Upon cooling to 4° C. a white, flocculent precipitate was obtained. The precipitate was collected on a Büchner funnel and washed with small portions of cold acetone. Two grams of crude wax (melting point 80–4° C.) were obtained from each batch of grain.

Attempts to obtain a reaction between the crude wax and 2,4-dinitrophenylhydrazine were unsuccessful, and it was concluded that the wax did not contain a ketone. Absence of unsaturation was shown by failure of the wax to decolorize a bromine–carbon tetrachloride solution.

Chromatography of Known Compounds. The physical constants of certain components of saponified sorghum wax (*2*) were similar to those of some of the components of alfalfa wax (*1*), indicating that the components of the two waxes may be similar. Therefore, a preliminary study was made of the chromatographic behavior of purified alfalfa wax components to establish conditions that might be used to separate the components of sorghum grain wax.

Samples of pure paraffin, ester, and alcohol from alfalfa wax were available from the work of Blair and others (*1*). Adsorbents selected for the study were: tricalcium phosphate, Supercel, 1 to 1 by weight; magnesia (Westvaco 2641), Supercel, 1 to 1 by weight; and silicic acid (Mallinckrodt AR, 100 mesh), Supercel, 1 to 1 by weight. Adsorption tubes (1 × 18 inches) were attached to suction flasks. The adsorbent was added, under vacuum, to the tubes in small portions and was tamped firmly with a cork mounted on a glass rod. The final length of the adsorbent column was 15 inches. One-tenth gram of a single wax component was dissolved in 50 ml. of warm

Crude sorghum grain wax was isolated by extracting the grain with Skellysolve B and precipitating the wax with acetone. The crude wax was fractionated by adsorption on columns of tricalcium phosphate and silicic acid. A weakly adsorbed paraffin fraction was eluted from the silicic acid column with a small quantity of Skellysolve B. A more strongly adsorbed fraction, obtained by additional elution with Skellysolve B, was identified as esters. A third fraction was eluted with 2% acetone in Skellysolve B and shown to consist of alcohols. Melting points with x-ray diffraction studies indicated that each fraction was a mixture of homologs, rather than a single compound. Of the material recovered, approximately 5% was paraffins, 49% was esters, and 46% was free alcohols.

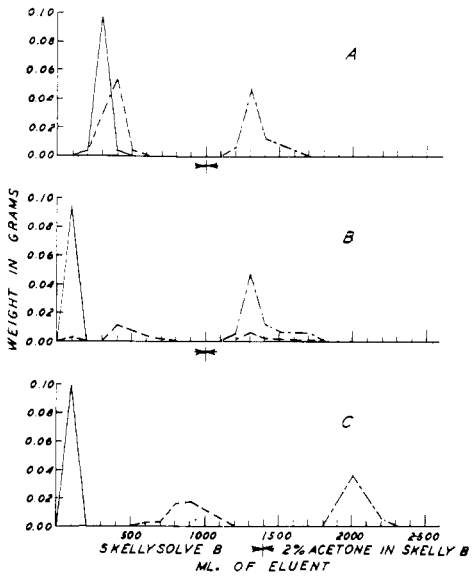

Figure 1. Elution behavior of alfalfa wax components on three adsorbents

A. Tricalcium phosphate ——— Paraffin
B. Magnesia — - — Alcohol
C. Silica acid — — — Ester

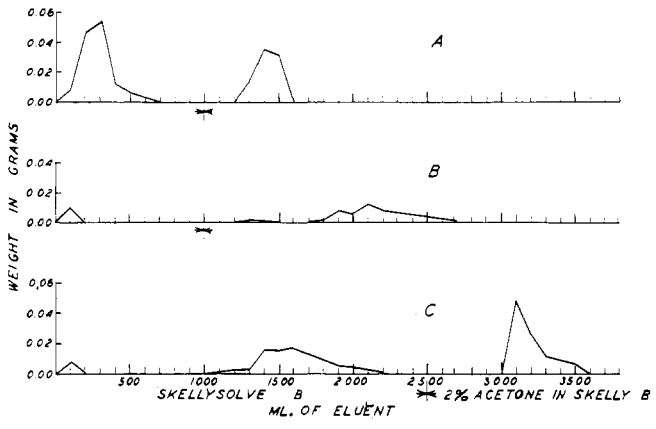

Figure 2. Elution behavior of sorghum grain wax on three adsorbents

A. Tricalcium phosphate
B. Magnesia
C. Silicic acid

Skellysolve B, and the solution was poured onto the column. When all of the solution had been drawn into the adsorbent, enough additional Skellysolve B was added just to moisten the entire column of adsorbent. The appropriate eluting agent then was added in 100-ml. portions, and when the last of each portion had been drawn into the adsorbent, the eluate from the column was transferred to a weighed beaker and evaporated to dryness. The residue was weighed.

Figure 1 indicates that tricalcium phosphate should be an effective adsorbent for separating an alcohol from an ester or a paraffin. The latter two compounds were eluted with Skellysolve B alone, while 2% of acetone in Skellysolve B was needed to elute the alcohol. As the ester and the paraffin were eluted with about the same quantity of eluting agent, they would not be separated effectively by this adsorbent, if they were present in a single solution.

The behavior of the ester on magnesia caused doubt as to the ability of this adsorbent to separate a mixture of these components. A greater amount of Skellysolve B was needed to remove the ester than was needed for the paraffin, indicating that these two possibly could be separated with magnesia. However, some of the ester was not removed until 2% of acetone in Skellysolve B was added. As the alcohol also was removed with this eluting agent, it is apparent that ester and alcohol would not be separated sharply if both were present in a wax.

Silicic acid was the most effective of the three adsorbents. The paraffin was eluted easily by Skellysolve B, while the ester was eluted only after a considerable quantity of Skellysolve B was used. The alcohol was removed only after 2% of acetone in Skellysolve B was added. Hence, good resolution of a mixture containing these three components should be possible with silicic acid columns.

Chromatography of Sorghum Grain Wax. Three-tenth-gram portions of the crude sorghum grain wax were chromatographed on columns of tricalcium phosphate, magnesia, or silicic acid (Figure 2).

The tricalcium phosphate column separated the wax into two main fractions. From the elution behavior previously obtained with alfalfa wax components, it was concluded that one fraction contained a mixture of paraffin and ester, and the other fraction contained alcohol.

Sharp resolution was not obtained with magnesia columns. A fraction corresponding to the alfalfa paraffin was obtained, but there was no sharp elution of a fraction corresponding to the alfalfa ester. Also, some material remained on the column after elution with 2% of acetone in Skellysolve B and was not eluted until 10% of isopropyl alcohol in Skellysolve B was used. It was concluded, therefore, that this adsorbent was not suitable for separating the sorghum wax components, except perhaps as a means of purifying the paraffin.

Silicic acid was the best of the three adsorbents for separation of the sorghum wax into its components. Fractions having elution behaviors very similar to the alfalfa wax components were obtained. From these data, it was concluded that sorghum grain wax contains paraffin, alcohol, and ester.

Isolation of Sorghum Wax Components. Four-tenth-gram portions of crude sorghum grain wax were dissolved in 100 ml. of Skellysolve B, and the solutions were drawn through tricalcium phosphate columns by vacuum. Each column was developed with two successive 500-ml. portions of Skellysolve B, followed by two 500-ml. portions of 2% of acetone in Skellysolve B. These eluates were collected separately and were evaporated to dryness. The residues were dissolved in hot acetone, and the precipitates which formed on cooling were filtered. The yield and melting points of the fractions obtained from a typical adsorption are shown in Table I.

Based on chromatographic behavior (Figures 1 and 2), it was assumed that most of the paraffin and ester were eluted by the first 500 ml. of eluent (fraction A), and that most of the alcohol was eluted by the first 500 ml. of 2% of acetone in Skellysolve B (fraction C). Fraction A thereafter was used as a source of paraffin and ester, and fraction C was used as a source of alcohol. The minor quantities of material present in fractions B and D were discarded.

PARAFFIN. Tricalcium phosphate did not appear to be capable of separating paraffin and ester, while silicic acid seemed suitable. To resolve fraction A into its components, 0.2 gram of the

Table I. Resolution of Crude Wax and Its Fractions by Chromatography

Fraction	Eluting Agent	Yield, Grams	Melting Point, ° C.
Crude Wax on Tricalcium Phosphate			
A	[a]	0.1610	67–76
B	[a]	0.0116	72–78
C	[b]	0.1358	82–83
D	[b]	0.0158	83–84
Fraction A on Silicic Acid			
A-1	[c]	0.0120	59–60
A-2	[a]	0.0000	...
A-3	[a]	0.0260	77–84
A-4	[a]	0.0900	72–76
A-5	[a]	0.0680	67–68
A-6	[b]	0.0000	...
Fraction C on Silicic Acid			
C-1	[c]	0.0000	...
C-2	[a]	0.0000	...
C-3	[a]	trace	72–74
C-4	[a]	0.0000	...
C-5	[a]	0.0000	...
C-6	[b]	0.0230	81–82
C-7	[b]	0.1540	82.5–83.2
C-8	[b]	0.0000	...

Eluting agents. [a] 500 ml. Skellysolve B. [b] 500 ml. 2% acetone in Skellysolve B. [c] 100 ml. Skellysolve B.

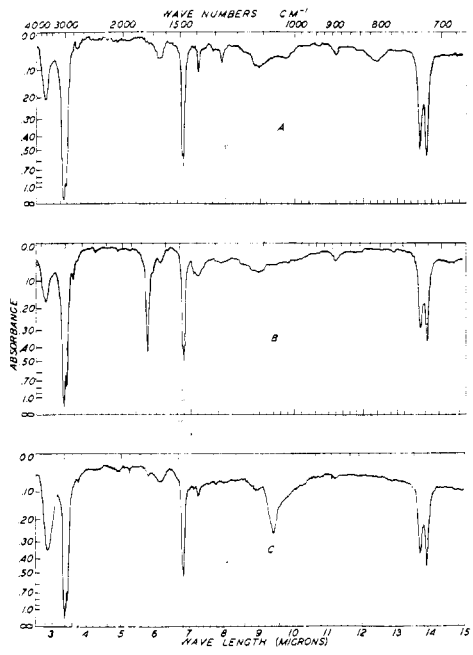

Figure 3. Infrared absorption spectra of sorghum grain wax components

A. Paraffin
B. Ester
C. Alcohol

Table II. Physical Data on Paraffins

Samples	Melting Point, ° C.	Transition Point, ° C.	Long Spacing, A.
Isolated paraffin treated with H_2SO_4	62.5–63.0	52.7–53.5	37.76
n-Nonacosane	63.4–63.6	57.3	38.68
Equimolar mixture of C_{27}, C_{29}, C_{31} paraffins	63.5–63.6	62.3	37.75

Table III. Physical Properties of Various Alcohols and Their Derivatives

Sample	Melting Point, ° C.	Acetate Melting Point, ° C.	Acid Melting Point, ° C.	Long Spacing, A.
Isolated material	82.5–83.2	66.7–67.5	85.5–86.5	77.97
n-Octacosanol	83.2	64.6	90.8	75.5
Mixed alcohols (20% C_{26}, 40% C_{28}, 40% C_{30})	82.3	65.5	84.8	78.9

combined fractions A was dissolved in 100 ml. of Skellysolve B and was chromatographed on a silicic acid column as described (Table I). From the preliminary studies (Figures 1 and 2) it was concluded that fraction A-1 was predominantly paraffin, and fractions A-3, A-4, and A-5 were ester.

Additional adsorptions of fraction A-1 on silicic acid columns did not change the melting point. However, after chromatography on magnesia, it melted at 62.5–3.0° C. This material was heated with concentrated sulfuric acid at 100° C. for 4 days. Although this treatment caused some charring, indicating the presence of compounds other than paraffins, the melting point was unchanged. The infrared absorption spectrum of the acid-treated material was obtained by the potassium bromide pellet technique (Figure 3). With the exception of slight absorption at 2.9 and 6.1 microns, the spectrum contained only peaks which are characteristic of hydrocarbons. Absorption at these wave lengths is considered to be due to a trace of water remaining in the pellet. Carbon and hydrogen analyses of the acid-treated paraffin yielded the following results: C, 84.74%; H, 14.70%. The theoretical values for *n*-nonacosane are C, 85.29%; and H, 14.71%. The long spacing was obtained from the x-ray diffraction pattern of the acid-treated paraffin. The melting point, transition point, and long spacing of the acid-treated paraffin are compared in Table II with those of *n*-nonacosane, and with those of a mixture of synthetic paraffins prepared by Piper *et al.* (*6*). The constants of the acid-treated paraffin are similar to those of the mixture. The paraffin of sorghum wax probably is a mixture of *n*-heptacosane, *n*-nonacosane, and *n*-hentriacontane.

ALCOHOL. Fraction C (Table I) was subjected to additional chromatography in an effort to increase its purity. Two-tenths gram of the fraction was chromatographed on a 1 × 15 inch column of silicic acid. Its chromatographic behavior is shown in Table I. The major portion of the material was not eluted until 2% of acetone in Skellysolve B was added. The principal fraction had a melting point of 82.5–3.2° C. The infrared absorption spectrum of this material (Figure 3) contained peaks at 2.9 and 9.4 microns, which are characteristic of alcohols. Carbon and hydrogen values on this fraction were: C, 81.70%; H, 14.21%. Theoretical values for octacosanol are: C, 81.95%; H, 14.15%.

The acetate of the alcohol (fraction C-7) was prepared by the method of Koonce and Brown (*4*). A portion of the alcohol was oxidized to a fatty acid by the method of Pollard *et al.* (*8*). The long spacing of the alcohol was obtained from its x-ray diffraction pattern. The physical properties of the alcohol and of its derivatives are compared in Table III with the data of Piper *et al.* (*7*) for *n*-octacosanol and for a mixture of normal primary alcohols. These show that the free alcohol of sorghum grain wax is probably a mixture of *n*-hexacosanol, *n*-octacosanol, and *n*-triacontanol.

ESTER. From the data of Table I it appears that silicic acid columns not only separated the paraffin and ester components, but caused some resolution of the ester fraction as well. In an attempt to resolve the ester fraction further, 0.2 gram of fraction A-4 (Table I) was adsorbed again on a 1 × 15 inch column of silicic acid and the column was developed with successive 100-ml. portions of Skellysolve B. Twelve fractions having different melting points were obtained, indicating that the wax contained a mixture of esters rather than a single compound. However, the quantities of these fractions were too small to attempt further purification, or to use for identification. Subsequent work, therefore, was carried out on the total ester by combining fractions A-3, A-4, and A-5 (Table I). This combination seemed justified, because the infrared spectra of these fractions were essentially identical. Each contained a peak at 5.75 microns, indicating an ester carbonyl group, but showed no other functional group peaks. The absorption spectrum of fraction A-3 is shown in Figure 3.

Attempts to obtain a good saponification number for the combined fraction failed, due to the extreme difficulty of accomplishing complete saponification of wax esters (*5*). However, a modification of the method of Chibnall *et al.* (*3*) gave sufficient saponification for isolation and identification of the products. Five-tenths gram of the combined ester (melting point 68 to 70° C.) was placed in 100 ml. of ethyl alcohol to which had been added 3.25 grams of sodium. The mixture was refluxed for 8 hours and then was extracted with 3 portions of Skellysolve B. These extracts were combined and concentrated to 50 ml., and the resulting solution was poured into 50 ml. of a 10% ethanolic potassium hydroxide solution. After refluxing for one hour, 3 grams of calcium chloride were added and refluxing was continued for 2 hours. Water was added and the mixture was extracted with hot benzene. The benzene extract was evaporated to dryness and the residue recrystallized from acetone.

The alcohol thus isolated had a melting point of 81.5–2.5° C. Its acetate was prepared and had a melting point of 65.5–6.0° C. The alcohol was converted by chromic acid oxidation to a fatty acid melting at 85–7° C. From these data, it was concluded that the alcohol component of the wax ester probably is a mixture similar to the free alcohol mixture (Table III).

The saponification mixture, from which the alcohols had been removed by benzene extraction, contained a precipitate of the calcium salts of the fatty acids liberated from the ester. This precipitate was removed by filtration and was washed twice with boiling benzene. The precipitate was acidified with 10% hydrochloric acid, the fatty acids were extracted with ether, and the ether solution was evaporated to dryness. After recrystallization from acetone, the fatty acids melted at 79 to 84° C. Further purification was accomplished by agitating the fatty acids with ethanolic sodium hydroxide to form the insoluble sodium soaps. The precipitate was washed with ether and acidified. The fatty acids then were extracted with ether and the ether solution was evaporated to dryness. The residue had a melting point of 84–6° C. (compare with data of Table III). Repetition of this technique did not change the melting point.

The neutralization equivalent of the purified fatty acid was 430. The neutralization equivalent of octacosanoic acid is 424, and that of triacontanoic acid is 452. The melting point and neutralization equivalent indicate that the fatty acid component of the wax ester probably is a mixture of *n*-hexacosanoic, *n*-octacosanoic, and *n*-triacontanoic acids.

Discussion

Warth (*9*) has reported the following composition for carnauba wax: esters, 80%; free alcohol, 12%; paraffins, 1%; lactone, 3%; and resins, 4%. From the present study, the composition of sorghum grain wax appears to be: esters, 49%; free alcohols, 46%; and paraffins, 5%. Sorghum grain wax may contain other components, because minor constituents might have been lost during isolation and resolution.

The components of the two waxes which perhaps cause the principal differences in their physical properties would seem to be esters and free alcohols. If the percentage of free alcohols in sorghum grain wax could be reduced, the properties of the modified wax might be more similar to those of carnauba wax. This study indicates that the free alcohols can be removed with little difficulty. Removal of either of the other major components from the wax would be more difficult.

Acknowledgment

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VEGETABLE OIL EXTRACTION

Toxicity of Amine-Extracted Soybean Meal

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EXPERIMENTS designed to identify a possible factor in soybean meal that could reverse the antimalarial activity of *m*-chloridine (*14*) led to the discovery that soybean-meal residues after extraction with organic amines were highly toxic for chicks. Hexane-extracted soybean meal is widely used as feed for domestic animals with no ill effect, but soybean meal extracted with trichloroethylene is toxic for cattle (*7, 9, 11, 13, 15*), sheep (*5*), chickens (*2, 4, 8*), and guinea pigs (*3, 10*). The toxic substance produced by trichloroethylene extraction of soybean meal may be *S*-(dichlorovinyl)-L-cysteine (*6*). Commercial preparations of trichloroethylene contain an inhibitor, sometimes an organic amine, to prevent the corrosive action of degradation products. It is therefore of interest that residues from the extraction of soybean meal with organic amines were far more toxic for the chick than residues from extraction with trichloroethylene.

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Materials and Methods

Except for experiments described in Tables IV and V, day-old New Hampshire Red chicks were used throughout. They were fed variants of a purified diet designed by Briggs *et al.* (*1*), which is adequate for the growth of chicks. This diet is composed of casein, gelatin, methionine, and glucose, and is supplemented with adequate vitamins and minerals. Test substances were added to the diet by replacing an equal weight of glucose (Cerelose). Unless otherwise stated, chicks were weighed on days 0, 6, and 10, the last being the final day of the experiment.

The soybean meal used was a com-