

FRACTIONATION OF SORGHUM  
GRAIN WAX

by

JACK LEE DALTON

B. S., Nebraska State Teachers College,  
Chadron, Nebraska, 1953

---

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE  
OF AGRICULTURE AND APPLIED SCIENCE

1958

LD  
2668  
T4  
1958  
D39  
c.2  
Documents

TABLE OF CONTENTS

10-21-56 B.L.

INTRODUCTION . . . . .	1
REVIEW OF LITERATURE . . . . .	1
EXPERIMENTAL . . . . .	4
Chromatography of Alfalfa Wax Components. . . . .	4
Isolation and Preliminary Study of Sorghum Grain Wax. . . . .	8
Isolation and Identification of Sorghum Grain Wax Components. . . . .	9
Paraffin. . . . .	13
Alcohol . . . . .	17
Ester . . . . .	19
DISCUSSION . . . . .	24
SUMMARY. . . . .	25
ACKNOWLEDGEMENTS . . . . .	26
LITERATURE CITED . . . . .	27

## INTRODUCTION

Considerable study has been given to the problem of finding a wax which could be used to replace carnauba wax in at least some of its many uses. Due to the similarity of sorghum grain wax and carnauba wax (2), it was thought that perhaps this was a desirable substitute. However, there are some differences in the properties of these two waxes. If the compounds which are present in sorghum wax could be determined, perhaps some means could be devised to alter their proportions to produce a material with more of the characteristics of carnauba wax.

The research reported below is concerned with the isolation, purification and identification of the compounds of sorghum grain wax.

## REVIEW OF LITERATURE

Although waxes frequently are defined as esters of long chain monohydroxy alcohols and fatty acids, it has been found that the wax fraction of many plants also contains free alcohols, long chain hydrocarbons, and ketones.

It has been known for centuries that plants contain waxes, but only in recent times has work been done to elucidate the chemical nature of the components which are present in these waxes. One of the difficulties encountered in a study of plant waxes is the similarity of the physical properties of the various components, making it difficult to isolate and characterize the individual compounds. Another handicap to investigators in this

field is the small amount of wax in plants. For example, alfalfa contains about 0.12 per cent (1), ryegrass 0.38 per cent (10), and wheat grass 0.44 per cent (11).

Three general methods have been used for obtaining plant waxes. When the wax occurs as a heavy leaf coating, the leaves are dried and the wax is shattered off by beating the leaves. Some waxes can be obtained by placing the plant in boiling water and allowing the melted wax to rise to the surface. When the wax occurs within the plant tissue, or in such small amounts that the first two methods are impractical, it is extracted with fat solvents.

The usual method for investigating waxes involves precipitating them from a hot acetone solution to eliminate other lipid materials. The wax then is dried and saponified. The unsaponifiable fraction is removed from the saponification mixture with a fat solvent. The fatty acids then are removed by precipitating them as the calcium salts. The sodium and potassium salts of the longer chain fatty acids are appreciably soluble in the usual fat solvents. Hence, it is desirable to precipitate them as the calcium salts to insure complete separation of the soaps and the unsaponifiable material (7).

Several methods of separating the compounds present in the unsaponifiable fraction have been used, most of which depend upon differing solubilities in various solvents. Chibnall, et. al. (4) separated primary and secondary alcohols by differences in ether solubility of the alcohol phthalate sodium salts.

Channon and Chibnal (5) separated paraffins and ketones by taking advantage of the greater solubility of ketoximes in a mixture of light petroleum ether and acetone. Zwieg and Taub (14) were able to separate alcohols and paraffins by dissolving the alcohols in a mixture of amyl alcohol and hydrochloric acid. The paraffins do not dissolve, and can be removed by filtration. A more recent technique, chromatography was first used by Schuette and Baldinus (12), who separated the paraffins of candelilla wax by means of activated alumina columns. Chromatography has become very useful in the difficult task of separation and purification of wax components.

Bunger and Kummerow (2) compared the physical constants of sorghum grain wax and carnauba wax. They also reported the presence of paraffin and ester in sorghum grain wax, but their technique did not demonstrate the presence of free alcohol. Their method consisted of saponifying the wax and using differing solubilities to separate the resulting paraffin, alcohol, and fatty acid mixture.

Blair, et al. (1) used chromatography to show that alfalfa wax contains paraffin, ester, and alcohol.

## EXPERIMENTAL

Chromatography of  
Alfalfa Wax Components

The physical constants of certain of the components of sorghum wax, as reported by Bunger and Kummerow (2), were similar to the physical constants of the components of alfalfa wax as reported by Blair, Mitchell, and Silker (1), indicating that the components of the two waxes are similar. Therefore, a preliminary study was made of the chromatographic behavior of isolated alfalfa wax components to establish conditions which might be used in separating the components of sorghum grain wax.

Samples of pure paraffin, ester, and alcohol from alfalfa wax were available from the prior work of Blair, et al. (1). Adsorbents selected for study were: 1) tricalcium phosphate: Supercel, 1:1 by weight, 2) magnesia (Westvaco 2641):Supercel, 1:1 by weight, and 3) silicic acid (Mallinckrodt AR, 100 mesh): Supercel, 1:1 by weight. Adsorption tubes, 1 x 18 inches, were attached to suction flasks. With vacuum applied, the adsorbent was added 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 Skellysolve B, and the solution was poured on the column. Enough additional Skellysolve B was added to just moisten the entire column of adsorbent. Skellysolve B then was added in 100 ml. portions,

and when each portion had been just drawn into the adsorbent, the eluate from the column was transferred to a weighed beaker and was evaporated to dryness. The weight of the residue was obtained by the increase in weight of the beaker.

The results of these studies are shown in Plate I. They indicate that tricalcium phosphate should be an effective adsorbent for removing an alcohol from an ester and a paraffin, since 2 per cent acetone in Skellysolve B was necessary to remove the alcohol from the column, while the ester and the paraffin were eluted with about the same quantities of eluting agent, and therefore would not be separated effectively by tricalcium phosphate, if they both were present in a single solution.

The behavior of the ester on magnesia caused doubt of the ability of this adsorbent to separate a mixture of these components. Greater amounts of Skellysolve B were needed to remove the ester than were needed for the paraffin, indicating that these two possibly could be separated with magnesia. However, some of the ester was not removed until 2 per cent acetone in Skellysolve B was added. Since 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 quite 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 per cent acetone in Skellysolve B was added. Hence, sharp

EXPLANATION OF PLATE I

Elution behavior of alfalfa wax components

Fig. 1. Tricalcium Phosphate: Supercell Column

Fig. 2. Magnesia: Supercell Column

Fig. 3. Silicic Acid: Supercell Column

———— Paraffin

- - - - Ester

- - - - Alcohol

## PLATE I

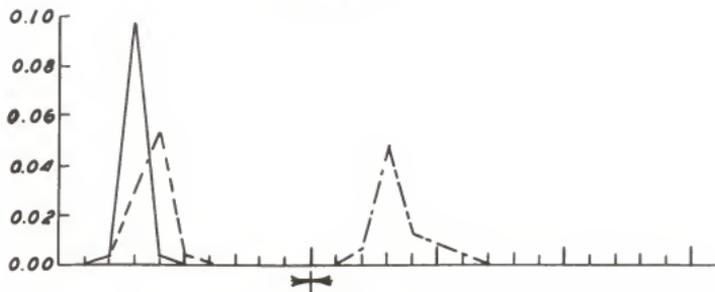


Fig. 1

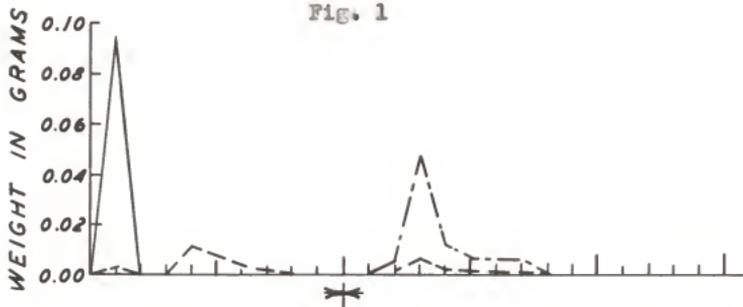


Fig. 2

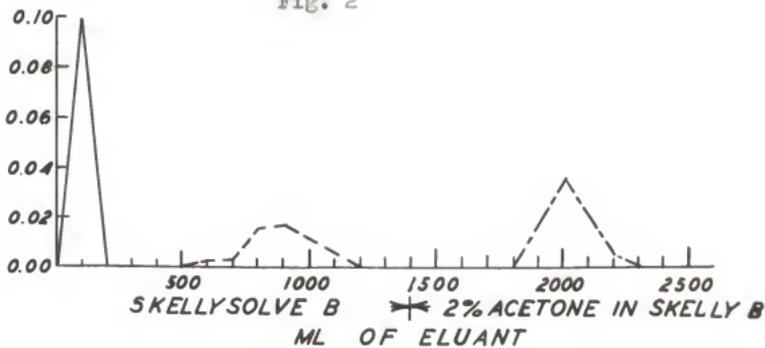


Fig. 3

resolution of a mixture containing these three components should be possible with silicic acid columns.

#### Isolation and Preliminary Study of Sorghum Grain Wax

The Midland variety of sorghum grain was selected as the source of wax for study. The grain was ground in a Wiley mill equipped with a 40-mesh sieve. The ground grain (1200 grams) was placed in a large Soxhlet extractor and was extracted with Skellysolve B for eight hours. The extract was evaporated to a volume of 75-100 ml. and 500 ml. of acetone were added. The mixture was boiled for five minutes and then was cooled to 4° C. The mixture was filtered on a Buchner filter and the precipitate was washed with small portions of cold acetone. After drying at room temperature, the crude wax was a hard, white material, m.p. 80-84° C. The yield of crude wax was three grams, or 0.25 per cent of the weight of the grain. No further crystallizations were attempted, since it was thought that some of the components of the wax might be slightly soluble in cold acetone, causing some loss. Attempts to obtain a reaction between the 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  $\text{Br}_2/\text{CCl}_4$  solution.

Three-tenth gram portions of the crude wax were chromatographed on columns of tricalcium phosphate, magnesia, and silicic acid as described earlier. The results are shown in

Plate II.

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.

The results obtained with the magnesia column were not conclusive. A fraction corresponding to the alfalfa paraffin was found, but there was no sharp elution of a fraction corresponding to the alfalfa ester. Also, the material remaining after elution with 2 per cent acetone in Skellysolve B was not eluted until 10 per cent isopropyl alcohol in Skellysolve B was used as the eluting agent. It was concluded therefore, that this adsorbent was not suitable for use in 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 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 and Identification of  
Sorghum Grain Wax Components

Due to the length of time and the amount of eluting agent required to separate the wax components on silicic acid columns, it was decided to use tricalcium phosphate to separate the wax into two fractions, a paraffin-ester fraction and an alcohol

EXPLANATION OF PLATE II

Elution behavior of sorghum grain wax

Fig. 1. Tricalcium Phosphate: Supercel Column

Fig. 2. Magnesia: Supercel Column

Fig. 3. Silicic Acid: Supercel Column

PLATE II

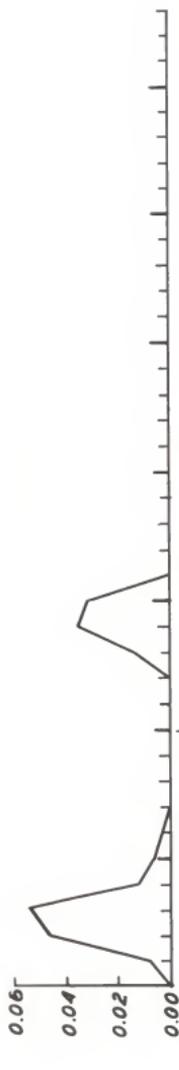


Fig. 1.  $\text{Ca}_3\text{PO}_4$

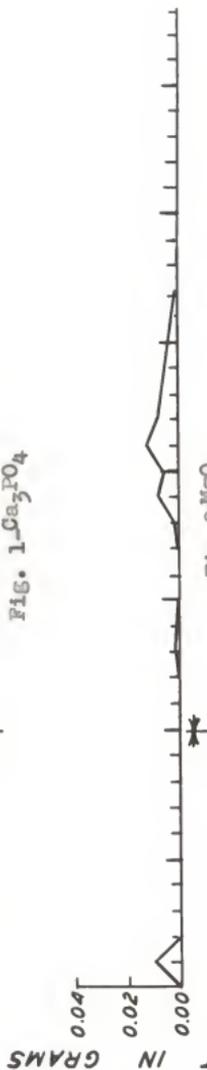


Fig. 2.  $\text{MgO}$



Fig. 3. Silicic Acid

fraction. Silicic acid columns then were used to achieve additional purification of these two fractions.

A 1 x 15 inch column of tricalcium phosphate:Supercel was prepared. Four-tenths of a gram of crude wax was dissolved in 100 ml. of Skellysolve B, and the solution was drawn through the adsorbent by vacuum. The column was developed with two successive 500 ml. portions of Skellysolve B, followed by two 500 ml. portions of 2 per cent acetone in Skellysolve B. These eluates were collected separately and were evaporated to dryness. After weighing, the residues were dissolved in hot acetone, and the precipitates which formed on cooling were filtered. The yield and melting points of the fractions thus obtained are shown in Table 1.

Based on chromatographic behavior, as presented earlier in Plates I and II, it was assumed that most of the paraffin and ester were eluted by the first 500 ml. of eluant (fraction A), and that most of the alcohol was eluted by the first 500 ml. of 2 per cent acetone in Skellysolve B (fraction C). Fraction A thereafter was used as a source of paraffin and ester. Fraction B probably contained a mixture of the materials found in fractions A and C, and therefore was not studied further. Fraction C was used as a source of alcohol. Fraction D had a slight yellow color, indicating it was probably contaminated with other lipid material. It was not used in subsequent work.

Table 1. Resolution of crude wax by adsorption on tricalcium phosphate.

Fraction :	Eluting Agent :	Yield :	Melting Point
A	500 ml. Skellysolve B	0.1610 g	67-76° C
B	500 ml. Skellysolve B	0.0116 g	72-78° C
C	500 ml. 2% acetone in Skellysolve B	0.1358 g	82-83° C
D	500 ml. 2% acetone in Skellysolve B	0.0158 g	83-84° C

Paraffin. As was noted above, tricalcium phosphate did not appear to be capable of separating completely the paraffin and ester, while either magnesia or silicic acid seemed suitable. Therefore, 0.2 gram of fraction A was dissolved in 100 ml. of Skellysolve B and was chromatographed on a silicic acid:Supercel column in the manner previously described. Table 2 shows that the paraffin (fraction A-1) was separated easily from the ester by the use of this adsorbent.

Table 2. Resolution of fraction A by adsorption on silicic acid.

Fraction :	Eluting Agent :	Yield :	Melting Point
A-1	100 ml. Skellysolve B	0.0120 g	59-60° C
A-2	500 ml. Skellysolve B	0.0000 g	---
A-3	500 ml. Skellysolve B	0.0260 g	77-84° C
A-4	500 ml. Skellysolve B	0.0900 g	72-76° C
A-5	500 ml. Skellysolve B	0.0680 g	67-68° C
A-6	500 ml. 2% acetone in Skellysolve B	0.0000 g	---

Further attempts to purify fraction A-1 on silicic acid columns were unsuccessful. However, after chromatographing it on a magnesia column it melted at 62.5-63.0° C. This material was warmed with concentrated sulfuric acid at 100° C. for four days. This treatment caused some charring, indicating the presence of compounds other than paraffins. However, the melting point was not changed. The infrared absorption spectrum of the acid-treated material (Plate III) contained only peaks which are characteristic of hydrocarbons. Carbon and hydrogen analyses of the acid-treated paraffin produced the following results: C, 84.74 per cent; H, 14.70 per cent. The theoretical values for n-nonacosane (C<sub>29</sub>) are C, 85.29 per cent, and H, 14.71 per cent. 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 3 with those of n-nonacosane and with those of a mixture of synthetic paraffins prepared by Piper, et al. (8). The constants of the acid-treated paraffin are quite similar to those of the mixture. Therefore, it was concluded that the paraffin of sorghum grain wax probably is a mixture of n-heptacosane, n-nonacosane, and n-hentriacontane.

Table 3. Physical data on paraffins.

Sample	Melting Point °C	Transition Point °C	Long Spacing °A
Isolated paraffin treated with H <sub>2</sub> SO <sub>4</sub>	62.5-63.0	52.7-53.5	37.76
n-Nonacosane	63.4-63.6	57.3	38.68
Equimolar mixture of C <sub>27</sub> , C <sub>29</sub> , C <sub>31</sub> paraffins	63.5-63.6	52.3	37.75

EXPLANATION OF PLATE III

Infrared spectra of sorghum grain wax components

Fig. 1. Paraffin

Fig. 2. Ester

Fig. 3. Alcohol

## PLATE III

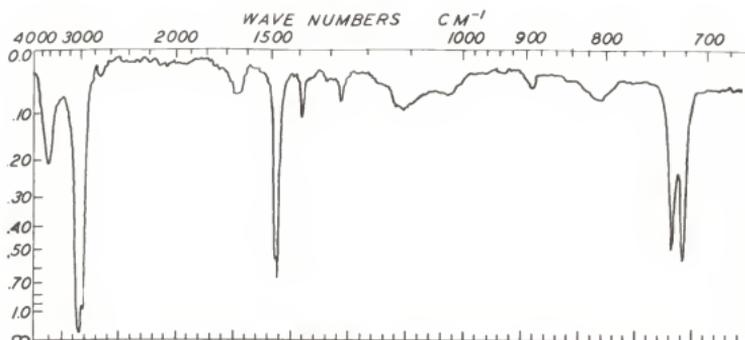


Fig. 1

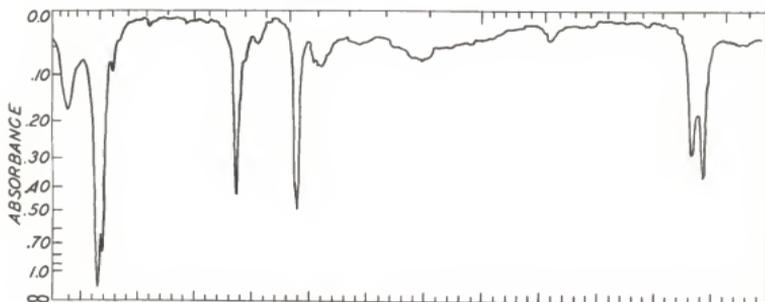


Fig. 2

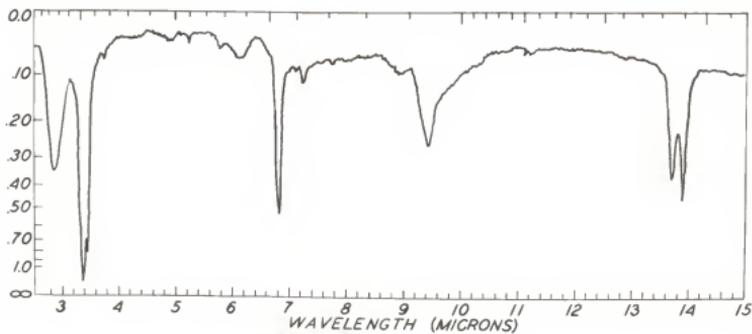


Fig. 3

Alcohol. Fraction C (Table 1) was subjected to additional chromatography in an effort to increase its purity. Two tenths gram of the fraction was chromatographed on a 1 x 15 inch column of silicic acid. Table 4 shows that only a trace of low melting impurity was removed by this treatment. The melting point of the principle fraction was not changed appreciably by this adsorption.

Table 4. Purification of fraction C by adsorption on silicic acid.

Fraction :	Eluting Agent :	Yield :	Melting Point
C-1	100 ml. Skellysolve B	0.0000 g	---
C-2	500 ml. Skellysolve B	0.0000 g	---
C-3	500 ml. Skellysolve B	trace	72-74° C
C-4	500 ml. Skellysolve B	0.0000 g	---
C-5	500 ml. Skellysolve B	0.0000 g	---
C-6	500 ml. 2% acetone in Skellysolve B	0.0230 g	81-82° C
C-7	500 ml. 2% acetone in Skellysolve B	0.1540 g	82.5-83.2° C
C-8	500 ml. 2% acetone in Skellysolve B	0.0000 g	---

The infrared absorption spectrum of fraction C-7 contained peaks at 2.9 $\mu$  and 9.4 $\mu$ , which are characteristic of alcohols (Plate III). Carbon and hydrogen values obtained on this fraction were: C, 81.70 per cent; H, 14.21 per cent. Theoretical values for octacosanol are: C, 81.95 per cent; H, 14.15 per cent.

An acetate of fraction C-7 was prepared by the method of Koonce and Brown (6). A mixture of the alcohol, acetic anhydride, and anhydrous sodium acetate was refluxed for eight hours. Water was added, and the precipitate which formed was filtered and recrystallized from acetone. It was purified further by adsorption on tricalcium phosphate and elution with Skellysolve B. The melting point of the acetate was 66.7-67.5°C.

The alcohol was converted to a fatty acid by the method of Pollard, et al. (10). Fraction C-7 was dissolved in glacial acetic acid and chromic acid was added. After heating at 100° C for one hour, the mixture was poured into water and was extracted with warm benzene. The benzene extract was evaporated to dryness and the residue was extracted with hot ether. After filtering, the ether filtrate was shaken with ethanolic sodium hydroxide and allowed to stand for three hours. The mixture was centrifuged and the precipitate washed twice with ether. The precipitate was acidified with HCl and the liberated fatty acid was extracted with ether. The ether was evaporated and the residue was crystallized three times from acetone. The acid thus obtained had a melting point of 85.5-86.5° C.

The long spacing of fraction C-7 was obtained from its X-ray diffraction pattern.

The data obtained on the alcohol and on its derivatives are compared in Table 5 with the data of Piper, et al. (9) for n-octacosanol and for a mixture of normal primary alcohols.

Table 5. Physical properties of various alcohols and their derivatives.

Sample	: Melting : Point °C	: Acetate : m.p. °C	: Acid : m.p. °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 <sub>26</sub> , 40% C <sub>28</sub> , 40% C <sub>30</sub> )	82.3	65.5	84.8	78.9

It was concluded from these data that the free alcohol of sorghum grain wax is probably a mixture containing n-hexacosanol, n-octacosanol, and n-triacontanol.

Ester. Although Blair, et al. (1) used magnesia as the adsorbent for purifying alfalfa ester, repeated efforts to purify sorghum grain ester in this manner failed. Silicic acid, however, proved to be an excellent adsorbent for this purpose. Table 2 shows the results of chromatographing fraction A (Table 1) on silicic acid. In addition to removing paraffin impurities from the ester fraction, it appears that there was some resolution of the ester fraction itself. In an attempt to resolve further the ester fraction, 0.2 g of fraction A-4 (Table 2) was chromatographed again on a 1 x 15 inch column of silicic acid. The results are presented in Table 6.

It appears that there was indeed slight resolution of the ester fraction by this column, suggesting that it is 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 purposes. Subsequent work, therefore, was carried out on the total ester by combining fractions A-3, A-4, and A-5 (Table 2). This combination seemed justified since the infrared spectra of these fractions were essentially identical. Each contained a peak at 5.75  $\mu$ , indicating the presence of a carboxyl group, but showed no other functional group peaks. Plate III shows the absorption spectrum of fraction A-3.

Table 6. Resolution of Fraction A-4 on silicic acid.

Fraction :	Eluting Agent :	Yield :	Melting Point
A-4-a	100 ml. Skellysolve B	0.0000 g	----
A-4-b	500 ml. Skellysolve B	0.0000 g	----
A-4-c	100 ml. Skellysolve B	0.0000 g	----
A-4-d	100 ml. Skellysolve B	0.0000 g	----
A-4-e	100 ml. Skellysolve B	0.0000 g	----
A-4-f	100 ml. Skellysolve B	0.0000 g	----
A-4-g	100 ml. Skellysolve B	0.0000 g	----
A-4-h	100 ml. Skellysolve B	0.0015 g	73-81°C
A-4-i	100 ml. Skellysolve B	0.0180 g	70-76°C
A-4-j	100 ml. Skellysolve B	0.0142 g	70-72°C
A-4-k	100 ml. Skellysolve B	0.0171 g	70-74°C
A-4-l	100 ml. Skellysolve B	0.0170 g	67-73°C
A-4-m	100 ml. Skellysolve B	0.0180 g	70-74°C
A-4-n	100 ml. Skellysolve B	0.0180 g	67-68°C
A-4-o	100 ml. Skellysolve B	0.0140 g	68-72°C

Table 6. (Continued)

Fraction :	Eluting Agent :	Yield :	Melting Point
A-4-p	100 ml. Skellysolve B	0.0107 g	67-70°C
A-4-q	100 ml. Skellysolve B	0.0081 g	67-68°C
A-4-r	100 ml. Skellysolve B	0.0060 g	66-67°C
A-4-s	100 ml. Skellysolve B	trace	63-66°C
A-4-t	100 ml. Skellysolve B	0.0000 g	----

Attempts to obtain a good saponification number for the combined fraction failed, due to the extreme difficulty of accomplishing complete saponification of wax esters (7). However, a modification of the method of Chibnall, et al. (4) gave sufficient saponification for isolation and identification of the products. This method consists essentially of alcohol interchange to produce a long chain alcohol and the ethyl ester of the fatty acid. The ethyl ester then is saponified to form a soap of the fatty acid.

Five-tenths gram of the combined ester (m.p. 68-70°C) was placed in 100 ml. of ethanol to which had been added 3.25 g of sodium. The mixture was refluxed for eight hours and then was extracted with three portions of Skellysolve B. These extracts were combined and concentrated to 50 ml., and the resulting solution was poured into 50 ml. of 10 per cent ethanolic KOH solution. After refluxing for one hour, three grams of  $\text{CaCl}_2$  were added and refluxing was continued for two hours. Water was added and the mixture was extracted with hot benzene. The

benzene extract was evaporated to dryness and the residue was recrystallized from acetone.

The alcohols thus isolated had a melting point of 81.5-82.5°C. The acetate was prepared and had a melting point of 65.5-66°C. The alcohol was converted to an acid by chromic acid oxidation. The melting point of this acid was 85-87°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 4).

The saponification mixture, from which the alcohols had been removed by benzene extraction, contained a precipitate of the calcium salts of the fatty acids. This precipitate was removed by filtration and was washed twice with boiling benzene. The precipitate was acidified with 10 per cent HCl, the fatty acids were extracted with ether, and the ether solution was evaporated to dryness. After recrystallization from acetone, the fatty acids melted at 79-84°C. Further purification was accomplished by agitating with ethanolic NaOH to form the insoluble sodium soaps. The precipitate was washed with ether and acidified. The fatty acids were extracted with ether and the solution was evaporated to dryness. The residue had a melting point of 84-86°C. (Compare with the data of Table 5). Repetition of this technique did not sharpen the melting point. The neutralization equivalent of this material was 430. The neutralization equivalent of octacosanoic acid is 424, and that of triacontanoic acid is 452.

From the melting point and neutralization equivalent, it was concluded that the fatty acid component of the wax ester probably is a mixture of n-hexacosanoic, n-octacosanoic, and n-triacontanoic acids.

## DISCUSSION

Warth (13) has reported the following composition for carnauba wax: esters, 80 per cent, free alcohols, 12 per cent, paraffins, 1 per cent, lactone, 3 per cent, and resins, 4 per cent. From the present study, the composition of sorghum grain wax appears to be: esters, 49 per cent, free alcohols, 46 per cent, and paraffins, 5 per cent. It is recognized that sorghum grain wax may contain other components, since minor constituents might have been lost during its purification.

The components of the two waxes which perhaps cause the principle differences in their physical properties would seem to be esters and free alcohols. If the percentage of free alcohols could be reduced in sorghum grain wax, the properties of the modified wax might be more similar to those of carnauba wax.

This study was not concerned with the practical aspects of modifying sorghum wax. The data indicate, however, that the free alcohols could be removed with little difficulty. Removal of either of the other major components from the wax would be more difficult. It would seem to the writer that additional research is warranted, and should be directed toward removal of the alcohol fraction on a sufficiently large scale that the physical properties which are desirable in commercial waxes (hardness, luster, abrasion resistance, etc.) could be determined.

## SUMMARY

Sorghum grain wax (m.p. 80-84°C) was obtained by extraction of the grain with Skellysolve B and precipitation with acetone. By correlating the chromatographic behavior of this wax with the chromatographic behavior of pure alfalfa wax components, it was concluded that the sorghum grain wax contained paraffin, ester, and alcohol.

The paraffin was isolated and purified by chromatography on silicic acid and magnesia columns. After treatment with concentrated sulphuric acid, the paraffin had a melting point of 62.5-63.0°C. The melting point, transition point, and X-ray diffraction pattern of this paraffin indicate that it is probably a mixture of n-heptacosane, n-nonacosane, and n-hentriacontane.

The alcohol (m.p. 82.5-83.5°C) was isolated by chromatography on tricalcium phosphate and purified with silicic acid columns. The melting point and X-ray diffraction pattern of the alcohol, and the melting points of the acetate and the acid derived from the alcohol, identified it as probably a mixture of n-hexacosanol, n-octacosanol, and n-triacontanol.

The ester was isolated and purified by means of silicic acid columns. Saponification yielded a mixture of aliphatic alcohols and a mixture of fatty acids which appear to contain primarily 26, 28, and 30 carbons.

## ACKNOWLEDGEMENTS

The author gratefully acknowledges the counsel and helpful assistance given by his major instructor, Dr. H. I. Mitchell. Appreciation is also expressed to Dr. R. D. Dragsdorf for the X-ray photographs and their interpretation, and to Steven Plovan for his assistance in obtaining the infrared data.

## LITERATURE CITED

- (1) Blair, E. H., Mitchell, H. L., Silker, R. E.,  
Ind. Eng. Chem., 45, 1104 (1953).
- (2) Bunger, W. B., Kummerow, F. A.,  
Oil Chem. Soc. J. 28, 121 (1951).
- (3) Channon, H. J., Chibnall, A. C.,  
Biochem. J., 23, 168 (1925).
- (4) Chibnall, A. C., Piper, S. H., Pollard, A., Smith, J. A. B.,  
Biochem. J., 25, 2095 (1931).
- (5) Chibnall, A. C., Latner, S. L., Williams, E. F., Ayre, C. A.,  
Biochem. J., 28, 313 (1934).
- (6) Koonce, S. D., Brown, J. B.,  
Oil and Soap, 21, 231 (1944).
- (7) Markley, K. S.,  
"Fatty Acids," p. 578, Interscience Publishers,  
New York, 1947.
- (8) Piper, S. H., Chibnall, A. C., Hopkins, S. J., Pollard, A.,  
Smith, J. A. B., Biochem. J., 25, 2072 (1931).
- (9) Piper, S. H., Chibnall, A. C., Williams, E. F.,  
Biochem. J., 28, 2175 (1934).
- (10) Pollard, A., Chibnall, A. C., Piper, S. H.,  
Biochem. J., 25, 2111 (1931).
- (11) Pollard, A., Chibnall, A. C., Piper, S. H.,  
Biochem. J., 27, 1889 (1933).
- (12) Schuette, H. A., Baldinus, J. G.,  
Oil Chem. Soc. J., 26, 651 (1949).
- (13) Warth, A. H.,  
"The Chemistry and Technology of Waxes," p. 104,  
Reinhold, New York, 1947.
- (14) Zweig, S., Taub, A.,  
Ind. Eng. Chem., Anal. Ed., 12, 9 (1940).

FRACTIONATION OF SORGHUM  
GRAIN WAX

by

JACK LEE DALTON

B. S., Nebraska State Teachers College,  
Chadron, Nebraska, 1953

---

ABSTRACT OF A THESIS

submitted in partial fulfillment of the  
requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE  
OF AGRICULTURE AND APPLIED SCIENCE

1958

Because of the high cost of carnauba wax, there has been an increasing interest in developing a substitute for it. It has been reported that sorghum grain wax is similar in some respects to carnauba wax, but there are some differences in their physical properties. If the compounds in sorghum grain wax could be determined, their proportions perhaps could be altered to produce a wax with more of the characteristics of carnauba wax. The present investigation is concerned with isolation and identification of the components of sorghum grain wax.

Sorghum grain wax (m.p. 80-84°C.) was obtained by extraction of the grain with Skellysolve B and precipitation with acetone. The chromatographic behavior of this wax on columns of tricalcium phosphate, magnesia, and silicic acid was correlated with the chromatographic behavior of pure paraffin, ester, and alcohol from alfalfa wax. A fraction of the sorghum wax was lightly adsorbed on the columns, and was eluted with small quantities of Skellysolve B. This behavior identified the fraction as paraffin. A second fraction was more tightly adsorbed, and was eluted with larger quantities of Skellysolve B. It was concluded that this fraction was ester. A third fraction was adsorbed strongly, and 2 per cent acetone in Skellysolve B was required for its elution. It was concluded that this fraction was alcohol.

The paraffin was isolated and purified by chromatography on silicic acid and magnesia columns. After treatment with

concentrated sulfuric acid, the paraffin had a melting point of 62.5-63.0°C. The melting point, transition point, and X-ray diffraction patterns of this paraffin indicate that it is probably a mixture of n-heptacosane, n-nonacosane, and n-hentriacontane.

The alcohol (m.p. 82.5-83.5°C.) was isolated by chromatography on tricalcium phosphate and purified with silicic acid columns. The melting point and long spacing of the alcohol, and the melting points of the acetate and the acid derived from the alcohol identified it as probably a mixture of n-hexacosanol, n-octacosanol, and n-triacontanol.

The ester was isolated and purified by means of silicic acid columns. Saponification of the ester yielded a mixture of alcohol and fatty acid. Physical constants of the alcohol and its derivatives indicated that it is a mixture of alcohols similar to the mixture found as free alcohol. The fatty acids were difficult to characterize because of the small quantity available. However, it was decided that they probably are a mixture of n-hexacosanoic, n-octacosanoic, and n-triacontanoic acid.

The wax contains 5 per cent paraffin, 49 per cent ester, and 46 per cent free alcohol. This is quite different from the composition of carnauba wax which contains 1 per cent paraffin, 80 per cent ester, and 12 per cent free alcohol. It is possible that removal of the free alcohol from sorghum grain wax to increase the percentage of ester might produce a

wax with more of the properties of carnauba wax. From this study, it is concluded that the free alcohol could be removed easily by the use of an adsorbent.