2. Balch, R. T., Sugar Research Foundation (N.Y.), Technol. Rept. Ser. No. 3 (1947).
3. Baltes, J., Fette u. Seifen, *55*, 517–528 (1953).
4. Brockmann, H., and Schodder, H., Ber. *74B*, 73–78 (1941).
5. Chibnall, A. C., Piper, S. H., Pollard, A., Williams, E. F., and Sahai, P. N., Biochem. J., *28*, 2189–2207 (1934).
6. Cole, L. J. N., "Chromatographic Procedures for the Isolation of the Original Constituents of Natural Waxes with Special Reference to the Study of Ouricuri Wax," thesis, The Ohio State University, Columbus (1956).
7. Farmer, E. H., Koch, H. P., and Sutton, S. A., J. Chem. Soc., 541–547 (1943).
8. Horn, D. H. S., and Matic, M., J. Sci. Food Agr., *8*, 571–576 (1957).
9. Kreger, D. R., Rec. Trav. Bot. Néerland., *41*, 606–736 (1948), (pub. 1949).
10. Kreger, D. R., and Schamhart, C., Biochim. et Biophys. Acta *19*, 22–44 (1956).
11. Lely, J. A., and Van Rijssel, T. W., Philips Techn. Rev., *13*, 47–54 (1951).
12. Malkin, T., Progr. in Chem. Fats Lipids, *1*, 1–17 (1952).
13. Piper, S. H., Chibnall, A. C., Hopkins, S. J., Pollard, A., Smith, J. A. B., and Williams, E. F., Biochem. J., *25*, 2072–2094 (1931).
14. Piper, S. H., Chibnall, A. C., and Williams, E. F., Biochem. J., *28*, 2175–2188 (1934).
15. Schuette, H. A., and Baldinus, J. G., J. Am. Oil Chemists' Soc., *26*, 530–532, 651–652 (1949).
16. Spickett, R. G., Mfg. Chemist, *28*, 165–169 (1957).
17. Trillat, J. J., Oléagineux, *2*, 68–75 (1947).
18. Warth, A. H., "The Chemistry and Technology of Waxes" (2nd ed.), Reinhold Publishing Corporation, New York, 1956.
19. Wijnberg, A., "Over Rietwas en de Mogelijkheid Zijner Technische Gewinning," thesis, Technical University, Delft, the Netherlands (1909).

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Gas-Liquid Chromatography of Fatty Derivatives. II. Analysis of Fatty Alcohol Mixtures by Gas-Liquid Chromatography[1]

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IN A RECENT REPORT from this laboratory (3) gas-liquid chromatography was applied to the analysis of saturated fatty alcohols. The separations were made by using either silicone grease or Carbowax 4000 monostearate as the partitioning agent and C-22 firebrick as the inert support. The analysis of the mixed saturated and unsaturated components in alcohols derived from soybean and linseed oil was only partially successful in that the separation of the unsaturated alcohols was incomplete.

The analysis of mixtures of saturated and unsaturated fatty acids in the form of methyl esters has recently been made practical by Lipsky (4) and by Orr and Callen (6) through the use of certain polyesters as partitioning liquids. Preliminary work showed that these polyesters appeared to be singularly selective toward unsaturated components in mixtures with saturates. It was logical therefore to expect that they would perform similarly toward the unsaturated alcohols if they, in turn, were converted to esters.

In this study it was demonstrated that these polyesters are effective in the separation of both saturated and unsaturated fatty alcohols and that vapor-phase chromatography can be the basis for an analysis which would otherwise be impractical, if not impossible.

Experimental

Preparation of Alcohol Standards. In order to prove the feasibility of separating quantitatively mixtures of unsaturated alcohol acetates on polyester columns, it was necessary to prepare the individual alcohols in as nearly pure a state as possible. Reduction of the corresponding methyl esters by lithium aluminum hydride was chosen as the approach for two reasons: a) the methyl esters are more easily purified than the alcohols; and b) the reduction is essentially quantitative. The methyl esters were supplied by the Hormel Institute. Methyl linoleate and linolenate as supplied were made by debromination of tetrabromostearic and hexabromostearic acids, respectively.

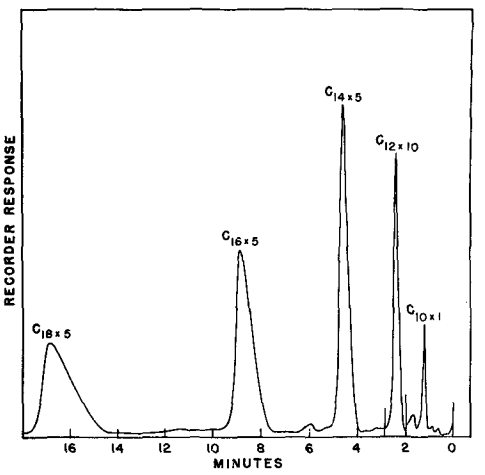

FIG. 1. Chromatogram of a mixture of normal C_{10} through C_{18} alcohol acetates, attenuation as noted.

In order to check the purity of the methyl esters they were chromatographed according to the method described by Orr and Callen (6). The methyl stearate, methyl oleate, and methyl linolenate showed only one component by gas chromatography; the methyl linoleate contained 1.5% of methyl oleate. A correction was applied for this oleate contamination in subsequent studies of blends.

The procedure used for the lithium aluminum hydride reduction was essentially that of Nystrom and Brown (5). Ethyl ether was refluxed over lithium aluminum hydride and distilled into a second flask containing twice the theoretical amount of lithium aluminum hydride required for the reduction. After solution was effected, the methyl ester was added

[1] ADM Technical Talk No. 166.

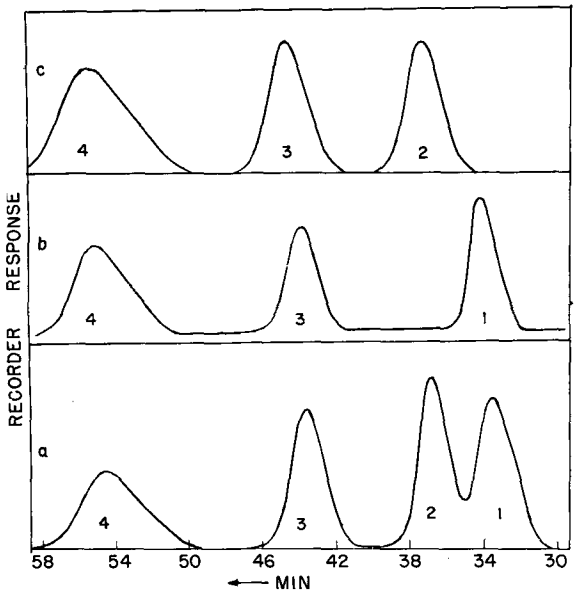

FIG. 2. Separation of mixtures of stearyl, oleyl, linoleyl, and linolenyl alcohol acetates. Peaks: 1, stearyl; 2, oleyl; 3, linoleyl; 4, linolenyl.

slowly. The reduction was probably completed almost instantly, but a gentle reflux was maintained for 30 min. to insure the maximum conversion to alcohols and consequently to reduce the amount of contaminants. Atmospheric moisture was excluded at all times. The complex was decomposed with 10% aqueous sulfuric acid, and the resulting alcohol was isolated by extraction with ethyl ether.

The alcohols were purified by chromatographing each over a 2.5 by 12-cm. column of activated alumina. The crude reduction mixture was placed on the column in a carbon tetrachloride solution, and the unreacted ester was eluted with the same solvent. The alcohols were stripped from the column with methanol and recovered. The linoleyl alcohol contained less than 0.1% of conjugated diene as measured by the absorbance at 232 mμ. The linolenyl alcohol contained slightly less than 1% of conjugated diene, which probably resulted from autoxidation. The initial gas-liquid chromatograms of the acetates of the alcohols showed that the linoleyl alcohol contained less than 1% of oleyl alcohol, and this correction was subsequently made in the known mixtures. The stearyl, oleyl, and linolenyl alcohols were chromatographically pure.

The alcohols were converted to the acetates by gently refluxing with an equal weight of acetic anhydride, after which the excess of anhydride was hydrolyzed. The product was washed with warm water. The acetates were then dried in a vacuum oven at 60°C. and stored under nitrogen at 0°C. when not in use. Weighed mixtures of these alcohol acetates were used in the following work.

Gas-Liquid Chromatography. A Beckman GC-2 was used without modification. Four filaments constituted the reference and sample detectors (two of each), and the bridge was operated at 200 ma. Helium was the carrier gas, and both detector and column were maintained at 228°C. With an inlet pressure of 30 p.s.i.g. and the outlet at atmospheric pressure the carrier flow rate through a nine-foot column was 42 ml./min., as measured by a wet test meter at 25°C. The sample size was two microliters. Peak areas were measured with a compensating planimeter and were defined by drawing tangents to the points of inflection.

Early chromatograms made by using Reoplex 400 revealed a tendency of the polyester to drip slowly from the column, giving so-called "railroad tracks" in the chromatogram. Resoflex 446 was found to perform satisfactorily, being stable at temperatures up to 260°C. for short periods of time and at 230°C. for weeks of operating time.

A nine-foot column on ¼-in. O.D. stainless steel was packed with 15.1 g. of absorbent, which consisted of 15% Resoflex 446 on a support of 60–80 mesh Johns-

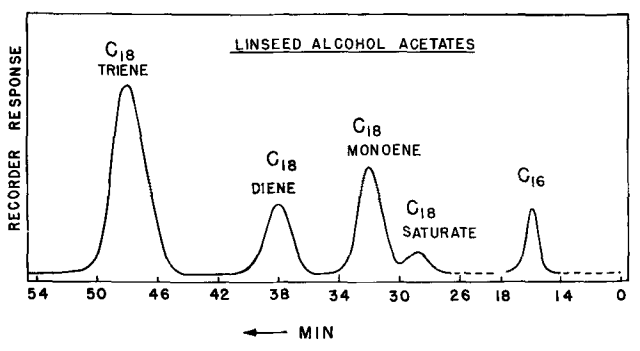

FIG. 3. Chromatogram of linseed alcohol acetates.

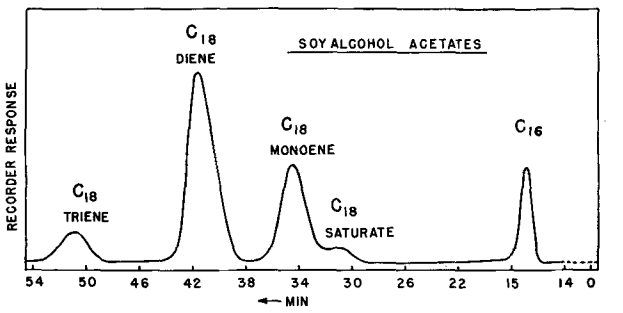

FIG. 4. Chromatogram of soy alcohol acetates.

Manville Chromosorb W. The Chromosorb was initially deactivated by washing with concentrated hydrochloric acid, 10% sodium hydroxide, and water, in order, followed by heating at 200°C. for 2 hrs. The screened inert material was added to the polyester dissolved in methylene chloride, and the excess solvent was allowed to evaporate. After packing, the coiled column was equilibrated in the instrument at 220°C. for 8 hrs.

Before chromatographing the alcohol acetates, the behavior of the column was first checked by evaluating the separation obtained with known mixtures of the methyl esters of stearic, oleic, linoleic, and linolenic acids. The separation obtained on the column was comparable to that obtained by Lipsky (4) and Orr and Callen (6). However, over a temperature range of 208–230°C., it was found that the analyzed area percentages for each peak were in good agreement with the actual weight percentages. This was contrary to previous findings, which indicated a decrease in the recovery of slow movers with an increase in column temperature.

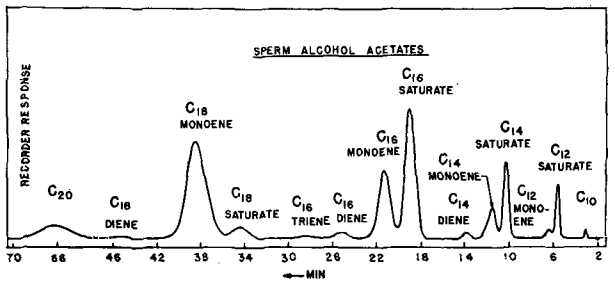

FIG. 5. Chromatogram of sperm alcohol acetates.

Results and Discussion

After the behavior of the column toward the unsaturated fatty methyl esters was observed, a known mixture of saturated alcohol acetates was run to determine the optimum operating conditions.

Figure 1 is a reproduction of the chromatogram that resulted from a mixture of the normal alcohol acetates with chain lengths of 10 through 18. Through the use of weighed mixtures of alcohol acetates it was easily shown there was no significant difference in the actual percentages by weight and the peak area percentages obtained by gas chromatography. With all operating parameters constant, the retention time of a given saturated alcohol acetate is approximately that of the next higher methyl ester; for example, the C_{16} alcohol acetate emerges at the time to be expected for the C_{17} methyl ester. In Figure 2a the separation of the four C_{18} alcohol acetates is shown. The number of theoretical plates, for linoleyl acetate, calculated by the method of Keulemans (2) is 2,200.

Figure 2b is the curve obtained for stearyl, linoleyl, and linolenyl acetates, and Figure 2c is that of the three unsaturated acetates alone. Mixtures of stearyl and oleyl alcohols offer the most difficult problem in separation. These chromatograms were run so that the relationship of peak areas to percentage composition could be studied.

The results of the study are summarized in Table I.

TABLE I

Analysis of Known Mixtures of Alcohol Acetates

Mixture	Component	Present Wt. %	Found area (%)			
			Run 1	2	3	Avg.
1	Stearyl	25.3	28.0	28.4	28.7	28.4
	Oleyl	25.0	24.9	24.7	24.5	24.7
	Linoleyl	24.9	23.9	23.7	23.2	23.6
	Linolenyl	24.8	23.2	23.2	23.6	23.3
2	Oleyl	26.2	27.9	27.6		27.8
	Linoleyl	31.2	29.9	30.3		30.1
	Linolenyl	42.6	42.2	42.1		42.1
3	Stearyl	28.5	31.2	30.2		30.7
	Linoleyl	29.4	27.4	27.9		27.6
	Linolenyl	42.1	41.4	41.9		41.6

Operating Conditions

Column: 9 ft. of Resoflex 446 on Chromosorb W. (15% by wt.)
Temperature: 226°C.
Carrier Gas: 42 ml./min., inlet—30 p.s.i.g.
Sample: 0.002 ml.

The first mixture shows the effect of a relatively high level of saturated alcohol on the relationship between peak area and weight percentage. Mixtures 2 and 3 were run to determine if the differences were caused by poor peak sharpness of stearyl and oleyl alcohol. When stearyl acetate is absent and resolution is not a problem, as in mixture 2, agreement is good. With stearyl acetate present and oleyl acetate absent, peaks are still sharp, but the correlation between weight percentages and peak areas is similar to that encountered in the first mixture.

Attempts to improve the accuracy by varying the column temperature were equally unsuccessful (Table II). Since it might have been possible that the slow-moving components were lost on the column, because of preferential interaction, an increase in the operating temperature should have increased this loss. However, as shown in Table II, recoveries at higher temperatures are essentially the same.

The high values obtained for stearyl alcohol are as yet unexplainable, but the phenomena are associated with the amount of the saturated component present. As the level of saturated component decreases, the difference between the weight percentage and area percentage also decreases.

TABLE II

Analysis of Known Mixtures of Alcohol Acetates
Effect of Variation of Temperature

Mixture	Component	Present Wt. %	Found area (%)		
			231°C.		242°C.
1	Oleyl	35.3	36.8	36.5	36.3
	Linoleyl	17.7	16.6	16.4	16.7
	Linolenyl	47.0	46.6	47.1	47.0
			226°C.		238°C.
2	Stearyl	42.8	44.7	45.4	45.3
	Linoleyl	16.9	16.0	15.6	15.5
	Linolenyl	40.3	39.3	39.0	39.1

Operating Conditions

Column: 9 ft. of Resoflex 446 on Chromosorb W. (15% by wt.)
Temperature: 226°C.–242°C.
Carrier Gas: 42 ml./min., inlet—30 p.s.i.g.
Sample: 0.002 ml.

Despite this problem the method constitutes a workable analysis, at least as reliable as the ultraviolet spectrophotometric technique. The method was applied to the analysis of alcohols derived from linseed, soy, and sperm oils. These alcohols were prepared by reduction of the oils with lithium aluminum hydride. The peaks in these chromatograms (Figure 3, 4, 5) were identified by reference to the pure components. Small amounts of C_{14} and C_{20} were found in the chromatograms of the alcohols derived from soy and linseed. These are not shown in the figures, having been removed to facilitate reproduction. They show up well in the sperm oil alcohol acetate.

In Table III is a comparison of the composition of the alcohols of linseed and soy with the corresponding fatty acids. There is apparently little change in the pattern. It should be noted that the acids are calculated as methyl esters and the alcohols as their

TABLE III

Comparison of Composition of Acids of Linseed and Soy with Corresponding Alcohols

Major component [c]	Linseed			Soy		
	Acid [a]	Alcohol [b]		Acid [a]	Alcohol [b]	
		1	2		1	2
C_{16}	6.7	6.8	6.7	10.9	11.4	11.3
C_{18}	4.3	4.5	4.5	3.6	4.2	4.0
C_{18} Monoene	21.9	21.8	22.2	25.3	24.8	25.1
C_{18} Diene	15.5	15.6	15.7	51.0	50.3	50.5
C_{18} Triene	51.5	51.3	50.9	9.3	9.3	9.1

[a] Analyzed and calculated as methyl esters.
[b] Analyzed and calculated as acetates.
[c] Trace amounts of C_{14}, C_{20} were not used in the calculations.

acetates. A slight change in the composition will be effected by converting these values to the original acids and alcohols.

Conclusions

Gas-liquid chromatography has been shown to be applicable to the analysis of fatty alcohols. Through the use of polyester columns these alcohols have been separated according to chain length and degree of unsaturation. A study has been made of the relationship between peak areas of the chromatograms and the actual weight percentages of the four C_{18} alcohols found in the fatty alcohols derived from linseed oil. Fatty alcohols, prepared from soybean, linseed, and sperm oil have been prepared and analyzed by the proposed procedure.

Craig and Murty (1) have recently reported that polyesters based on succinic acid are preferable for the liquid phase of the chromatographic column to those made from adipic in that they afford a better separation of methyl stearate from methyl oleate. Conversely adipic columns gave a more effective separation of the esters of linolenic and arachidic acids.

The application of these polyesters to the analysis of fatty alcohol acetates is expected to improve their separation in a similar fashion, but further work is indicated in the search for a liquid phase that will permit both separations in the minimum time.

REFERENCES

1. Craig, B. M., and Murty, N. L., Can. J. Chem., *36*, 1297 (1958).
2. Keulemans, A. I. M., Gas Chromatography, Reinhold Publishing Corporation, New York, 1957.
3. Link, W. E., Hickman, H. M., and Morrissette, R. A., J. Am. Oil Chemists' Soc., *36*, 20 (1959).
4. Lipsky, S. R., Chem. Eng. News, *36*, (No. 5), 48 (1958).
5. Nystrom, R. F., J. Am. Chem. Soc., *69*, 1197, 2548 (1947).
6. Orr, C. H., and Callen, J. E., J. Am. Chem. Soc., *80*, 249 (1958).

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The Occurrence of Higher Fatty Acids in Corn Pollen[1, 2]

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IN RECENT YEARS the lipide constituents of pollen have been the subject of several investigations. From the ether extracts of corn pollen Anderson (1) isolated a mixture of two phytosterol palmitates, a saturated hydrocarbon, a C_{30}-saturated alcohol, and a phosphatide. Previously Miyake (2) reported the presence of phytosterol. A summary by Lunden (3) reports the occurrence of other acids, hydrocarbons, and sterols in the pollen of other plants. Since little information is available on the fatty acid content of corn pollen, a study was made to identify these lipides in the saponifiable fraction of corn pollen.

Two kg. of freshly collected corn pollen, *Zea mays* (variety Ohio M-15 hybrid field corn), were placed in a 5-liter flask. The pollen was covered with ethyl ether and permitted to stand for a day at room temperature. Periodically during extraction the ether was kneaded into the dough-like mass of pollen. The extract was then decanted and replaced with fresh ether, and this procedure of extraction and decantation was subsequently repeated at daily intervals until 10 fresh portions of ether had been used for each flask of pollen. The combined ether extracts were concentrated by distilling off the ether. The last traces of solvent were removed by evacuating the flask containing the extract. From 7.89 kg. of pollen were obtained 304 g. of ether-soluble oil.

The saponifiable fraction was obtained by refluxing a solution of the lipides (214.6 g.) in 400 ml. of 1.5 *N* ethanolic potassium hydroxide for 8 hrs. The hydrolyzate was diluted with 1,600 ml. of water, and the unsaponifiable fraction was removed by extraction with Skellysolve "B." Acidification of the aqueous-

[1] This investigation was supported by a grant from the National Science Foundation.
[2] Journal Article No. 2139 of the Michigan Agricultural Experiment Station.

TABLE I
Fractionation of the Methyl Esters of the Fatty Acids in Corn Pollen

Fraction number	Boiling point in degrees C. at 2–3 mm. of pressure[a, b]	Weight of fraction in grams	Iodine number	Refractive index at 25°C.	Saponification equivalents of methyl esters		Melting point of derivative, degrees C.		Acid identified
					Found	Calcd.	Observed[b]	Reported	
Original methyl ester	.....	100.2	142	1.468	293	.....	.....	.....	.....
1	155–162 (155)	10.1	20	1.444	262	270.5	86[c]	86	Palmitic
2	162–168 (165)	17.1	80	1.450	280	270.5 294.5	86[c] 114[e]	86 114.7–115.2	Palmitic Linoleic
3	168–176 (174)	16.9	128	1.455	281	270.5 296.5 294.5	86[c] 132[d] 114[e]	86 132 114.7–115.2	Palmitic Oleic Linoleic
4	176–180 (177)	23.5	217	1.468	291	298.5 296.5 292.5	90[c] 132[d] 181[f]	90 132 181.5–181.6	Stearic Oleic Linolenic
5	180–184 (184)	9.1	210	1.469	293	298.5 294.5 292.5	90[c] 114[e] 181[f]	90 114.7–115.2 181.5–181.6	Stearic Linoleic Linolenic
Residue	.....	23.6	.....	.....	.....	.....	.....	.....	.....

[a] Figures in parentheses show temperature in which major portion of the fraction was distilled.
[b] Uncorrected.
[c] *p*-Bromophenacyl ester (5)
[d] Hydroxy acid (6).
[e] Tetrabromo-addition compound (7).
[f] Hexabromo-addition compound (7).