• TECHNICAL

# Gas-Liquid Chromatographic Analysis of Cyclopropenoid Fatty Acids

E. L. SCHNEIDER, SOOK P. LOKE, and D. T. HOPKINS,
Ralston Purina Company, St. Louis, Missouri 63199

## Abstract

A method is described for the analysis of cyclopropenoid fatty acids in oils. The method consists of reacting the methyl esters of the cyclopropenoid fatty acids with silver nitrate in methanol to form ether and ketone derivatives. The derivatives formed from the cyclopropenoid fatty acids are separated from the methyl esters of the normal fatty acids by gas-liquid chromatography on a 15% diethylene glycol succinate column. The method is applicable to oils containing from 0.01% to 100% of cyclopropenoid fatty acids. The derivatives of oils containing low levels of cyclopropenoids are separated from the normal methyl esters by alumina chromatography prior to gas-liquid chromatography. Studies on the quantitative aspects of the derivative formation, alumina chromatography, and gas-liquid chromatography are reported. Analyses for total cyclopropenoid fatty acid content of cottonseed oil and *Sterculia foetida* oil by the gas-liquid chromatographic and hydrobromic acid titration procedures showed good agreement. Replicate analyses of a sample of *Sterculia foetida* oil for malvalic and sterculic acid gave coefficients of variation of 6.04% and 1.17%, respectively.

## Introduction

At present two methods for the analysis of cyclopropenoid fatty acids are in general use: hydrogen bromide titration (1) and the spectrophotometric Halphen test (2). While both of these methods give reproducible results on the total cyclopropene content, neither yields data on the chain length of the individual fatty acids.

The application of gas-liquid chromatography to the analysis of cyclopropenoid fatty acids, while offering great potential, has enjoyed only limited success owing to the thermal instability of the cyclopropene ring. To overcome this, several investigators proposed hydrogenation followed by gas-liquid chromatography (3–5). This technique may give several peaks for each of the cyclopropenoid fatty acids unless conducted under very closely controlled conditions. In addition, it does not allow the analysis of the other unsaturated fatty acids.

In a recent paper gas-liquid chromatography of methyl mercaptyl derivatives of cyclopropenoid fatty acids was proposed as a method of analysis (6). However, the authors stated that the procedure can not be applied to the analysis of cottonseed oils containing low levels of cyclopropenoid fatty acids. We experienced difficulty in achieving quantitative conversion of the cyclopropenoid fatty acids to the mercaptyl derivatives without also obtaining side reactions with other unsaturated fatty acids, linoleic acid in particular. An analysis utilizing the reaction of the cyclopropenoid fatty acids with silver nitrate–silica gel, followed by hydrogenation and gas-liquid chromatography also has been proposed (7).

Kircher (8) investigated the reaction of sterculene with silver nitrate. When the reaction was carried out in methanol, the principal product was an ether, identified as 9-methoxymethyl-9-octadecene. A small amount of a ketone, identified as 9-methylene-10-octadecanone also was produced. Identification of these reaction products was confirmed by various techniques. Kircher showed the utility of this reaction for the qualitative analysis of the cyclopropenoid fatty acids in *Sterculia foetida* oil and proposed that this reaction be utilized for the quantitative analysis of cyclopropenoid fatty acids. It was the purpose of this investigation to explore this proposal.

## Procedures

### Preparation of the Methyl Esters

Methyl esters were prepared from oils by refluxing approximately 100 mg of the oils with 5 ml of a solution of 1% sodium methoxide in methanol (9). After 20 min, the solution was cooled, flooded with 15 ml of distilled water, and the methyl esters extracted twice with 10 ml of petroleum ether (BP 30–60). The esters were dried over anhydrous sodium sulfate, and evaporated just to dryness in a gentle stream of nitrogen.

### Derivative Formation

The methyl esters were reacted with 15 ml of anhydrous methanol saturated with silver nitrate. The reaction was carried out for 2 hr at 30C, or 20 hr at room temperature. The normal methyl esters and the reaction products from cyclopropenes were recovered from the reaction mixture by adding 30 ml of distilled water and by extracting twice with 10 ml of petroleum ether. The combined ether fractions were dried over anhydrous sodium sulfate and evaporated to a small volume in a stream of nitrogen. For those oils that contained a large amount of cyclopropenoid fatty acids, the petroleum ether solution of methyl esters and reaction products were suitable for direct injection into the gas-liquid chromatograph.

### Alumina Chromatography

When analyzing oils that contain low levels of cyclopropenes (<5.0%), the normal methyl esters and the reaction products of cyclopropenes must be separated to prevent overloading of the gas-liquid chromatographic column. This was accomplished by transferring the petroleum ether solution to a 12 mm by 450 mm glass chromatographic column packed with 8 g of freshly prepared Woelm Neutral alumina,[1] activity grade II. The normal methyl esters were eluted with 200 ml of 1% ethyl ether in petroleum ether. The reaction products were then eluted with 150 ml of chloroform. After evaporation to a small volume and the incorporation of suitable internal standards, each of these fractions was ready for injection into the gas-liquid chromatograph.

TABLE I

Effect of Time and Temperature on Completion of Reaction Between Methyl Ester of *Sterculia foetida* Oil and Saturated Methanolic Silver Nitrate

| Equivalent chain length | Fatty acid | Area per cent Reaction temperature | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Ambient (24C) | | | | | 30C | | | |
| | | Reaction time | | | | | | | | |
| | | 0 | 1 hr | 2 hr | 4 hr | 20 hr | 1 hr | 2 hr | 4 hr | 20 hr |
| 16.0 | Palmitic | 22.9 | 22.8 | 22.4 | 21.9 | 22.0 | 21.6 | 21.7 | 21.7 | 20.9 |
| 18.0 | Stearic | 2.0 | 2.3 | 1.8 | 1.6 | 1.8 | 1.6 | 1.7 | 1.9 | 1.8 |
| 18.5 | Oleic | 11.0 | 9.8 | 9.3 | 9.1 | 9.8 | 8.9 | 8.9 | 9.3 | 9.3 |
| 19.5 | Linoleic | 12.7 | 8.9 | 8.9 | 8.7 | 8.9 | 8.9 | 8.7 | 8.8 | 8.7 |
| 20.1 | | 9.2 | Trace | ..... | ..... | ..... | ..... | Trace | ..... | ..... |
| 20.5 | | 37.1 | 3.8 | 3.4 | 2.7 | 1.5 | 3.0 | 2.4 | 2.4 | 1.8 |
| 21.7 | [a] | ..... | 5.8 | 6.0 | 5.9 | 6.3 | 5.9 | 5.9 | 5.7 | 6.1 |
| 21.9 | | 5.1 | 0.8 | 0.5 | 0.4 | ..... | 0.4 | 0.4 | 0.2 | Trace |
| 22.7 | [b] | ..... | 41.2 | 43.1 | 45.2 | 45.5 | 44.9 | 45.4 | 45.1 | 46.4 |
| 23.6 | | ..... | 0.1 | 0.1 | Trace | Trace | 0.1 | 0.1 | Trace | 0.1 |
| 24.5 | [c] | ..... | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.6 | 0.5 | 0.4 |
| 25.5 | [d] | ..... | 4.1 | 4.1 | 4.1 | 3.8 | 4.3 | 4.2 | 4.3 | 4.5 |
| Total | Cyclopropenoid derivatives [e] | ..... | 51.5 | 53.6 | 55.6 | 56.0 | 55.5 | 56.1 | 55.6 | 57.4 |

[a] Ether derivative of methyl malvalate.
[b] Ether derivative of methyl sterculate.
[c] Ketone derivative of methyl malvalate.
[d] Ketone derivative of methyl sterculate.
[e] Total amount of compounds with equivalent chain lengths of 21.7, 22.7, 24.5, 25.5.

### Gas-Liquid Chromatography

A Varian Aerograph Model 1200 flame ionization unit[2] was employed in this work. The column, 10 ft by 0.125 in. od stainless steel tubing packed with 15% stabilized DEGS on Anakrom A,[3] was operated isothermally at 190C. Nitrogen at a flow rate of 40 ml/min was the carrier gas. A 1 mv recorder was used for graphic presentation of the data and an Infotronics CRS 11 AB/H digital integrator[4] was used to obtain peak areas.

### Calculations

For samples not subjected to alumina chromatography, the weight per cent of any given component was determined by dividing the area of the component by the sum of the areas of all detected components and multiplying by 100.

When it was found necessary to isolate the reaction products of the cyclopropenes from the normal methyl esters, an internal standard consisting of a known amount of methyl heneicosanoate or methyl tricosanoate was added to each of the eluates from the alumina column prior to gas-liquid chromatography. The peak area of each component in the chromatogram was compared to the internal standard and, from this data, the composition of the original mixture was calculated.

Methyl heneicosanoate and methyl tricosanoate were chosen for internal standards because of their close structural relationship to the other components encountered in the analysis, ready availability, and lack of occurence in natural products. Other internal standards can be used provided there is little or no overlap of the standard and the components in the sample mixture.

Through the use of the digital integrator, it was possible to accurately integrate the areas of the wide range of peaks encountered in the analysis. No attenuator adjustments were required during an analysis, since the integrator was capable of handling signals over the range of 0.005 to 125 mv. The recorder attenuator was adjusted to give adequate presentation on the recorder.

## Results and Discussion

To utilize the reaction of silver nitrate with the cyclopropenoid fatty acids as a method of estimation of these acids, further work was needed on the quantitative aspects of the reaction. The time required to achieve reaction completion was studied with a sample of *Sterculia foetida* oil, which was chosen because of its high cyclopropenoid fatty acid content and ready availability. Methyl esters of the fatty acids were allowed to react with silver nitrate for varying lengths of time at each of two temperatures. Reaction products were extracted from the methanol–silver nitrate solution and injected into the gas-liquid chromatograph. Equivalent chain lengths of the resolved methyl esters were calculated with a method similar to Miwa's (10). Peak area per cents were calculated from data obtained from the digital integrator. The data are summarized in Table I.

A reduction of peak area per cent with time was observed for those peaks having equivalent chain lengths of 19.5, 20.1, 20.5, 21.9, and 23.6. Four new peaks appeared and increased with reaction time. These peaks had equivalent chain lengths of 21.7, 22.7, 24.5, and 25.5. The peaks at equivalent chain lengths of 21.7 and 22.7 represent the ethers that are reaction products of malvalate and sterculate, respectively. The peaks with equivalent chain lengths 24.5 and 25.5 represent ketones that are reaction products of malvalate and sterculate. These peaks correspond to those obtained by Kircher. Reaction times of 1 hr or greater at 30C, or 4 hr or greater at room temperature were sufficient to convert the methyl esters of the cyclopropenoid fatty acids to their corresponding derivatives. Twenty hours at room temperature was chosen as a convenient reaction time for subsequent work.

A sample of methyl sterculate of very high purity was reacted with silver nitrate–methanol as previously described. Reaction products were extracted and injected into the gas-liquid chromatograph. The chromatogram of these reaction products is shown in Fig. 1. The peaks obtained from the ether and ketone reaction products differed in equivalent chain length from those previously obtained. This difference was traced to a change in the material used to pack the column. (All equivalent chain lengths are reproducible within one batch of packing material, but differ slightly when different batches of column packing material are used.) The ether had an equivalent chain length of 22.3 and comprised 81.99% of the total area. The ketone had an equiv-

[1] Alupharm Chemicals, P.O. Box 30628, New Orleans, Louisiana.
[2] Varian Aerograph, 2700 Mitchell Drive, Walnut Creek, California.
[3] Analabs, P.O. Drawer 5397, Hamden, Connecticut.
[4] Infotronics Corporation, 7800 Westglen, Houston, Texas.

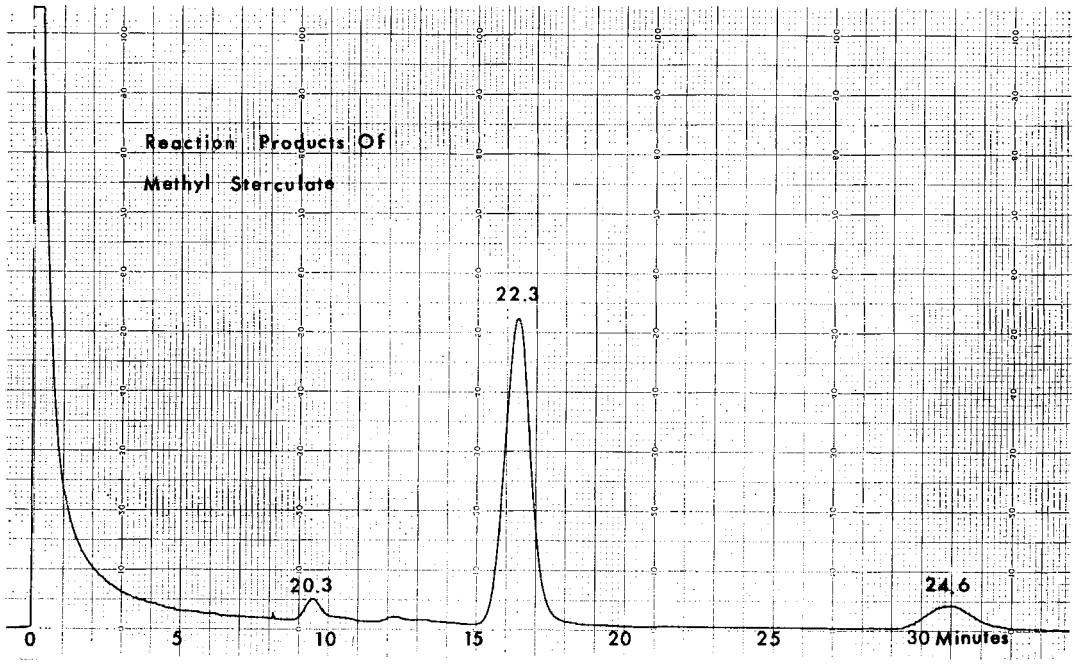


FIG. 1. Gas-liquid chromatogram of the reaction products of methyl sterculate. Peaks are listed by equivalent chain length as follows: 20.3, degradation peak of unreacted methyl sterculate; 22.3, ether reaction product; 24.6, ketone reaction product.

alent chain length of 24.6 and comprised 13.92% of the total area. The total area per cent for the two peaks was 95.91%. The only other major peak observed had an equivalent chain length of 20.3 and comprised 3.46% of the total area. This peak corresponded to a peak observed when the unreacted methyl sterculate was injected directly into the chromatograph. The reaction conditions selected gave essentially quantitative conversion to the derivatives and are suitable for an analytical procedure.

To establish the relationship of the peak area response for the ether or ketone to that of the methyl esters of a normal fatty acid, quantities of the ether and the ketone were prepared and purified by alumina chromatography. A mixture consisting of equal weight quantities of methyl palmitate and the ether derivative was subjected to gas-liquid chromatography. The peak area response of the ether derivative relative to that of methyl palmitate was 1.03 and 1.05 on successive trials. Identical results were obtained when an equal weight mixture of the ketone derivative and methyl palmitate was subjected to gas-liquid chromatography.

The silver nitrate–methanol procedure was used to analyze a sample of *Sterculia foetida* oil in replicate on different days to study the precision of the method. Cyclopropenes were also measured by hydrogen bromide titration. The results are listed in Table II. The values obtained for total cyclopropene level were consistent with values obtained by hydrogen bromide titration; the precision of the analysis for malvalate and sterculate by the silver nitrate–methanol method was good. A typical chromatogram of the analysis of *Sterculia foetida* oil is shown in Fig. 2.

To apply the silver nitrate–methanol procedure to the analysis of oils containing low levels of cyclopropenoid fatty acids, some provision must be made to handle the wide range of concentrations encountered in the analysis. Cyclopropenoid fatty acids may comprise 0.05% to 25% or more of the total fatty acid. If the methyl esters of the normal fatty acids are removed from the cyclopropene reaction products, the gas-liquid chromatography analysis can be achieved under optimal conditions, and smaller amounts of the reaction products can be detected without overloading the chromatographic column with normal fatty acid esters.

TABLE II
Fatty Acid Analysis of *Sterculia foetida* Oil

| Equivalent chain length | Fatty acid | Per cent | | | | | | | Mean | SD | CV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 16.0 | Palmitic | 22.0 | 22.0 | 21.6 | 21.7 | 21.7 | 20.6 | 22.3 | | | |
| 18.0 | Stearic | 1.57 | 1.78 | 1.57 | 1.87 | 1.71 | 1.81 | 2.01 | | | |
| 18.5 | Oleic | 9.04 | 9.85 | 8.86 | 9.27 | 8.94 | 9.26 | 8.95 | | | |
| 19.3 | Linoleic | 8.69 | 8.86 | 8.88 | 8.87 | 8.66 | 8.73 | 9.23 | | | |
| 20.3 | | 2.70 | 1.49 | 3.02 | 2.40 | 2.41 | 1.84 | 2.06 | | | |
| 21.3 | [a] | 5.86 | 6.33 | 5.85 | 5.71 | 5.89 | 6.11 | 4.00 | | | |
| 21.6 | | 0.35 | ..... | 0.42 | 0.20 | 0.39 | 0.30 | ..... | | | |
| 22.3 | [b] | 45.2 | 45.5 | 44.9 | 45.1 | 45.4 | 46.4 | 42.1 | | | |
| 23.2 | | 0.03 | Trace | 0.05 | Trace | 0.08 | 0.13 | ..... | | | |
| 23.6 | [a] | 0.45 | 0.41 | 0.45 | 0.51 | 0.51 | 0.36 | 1.52 | | | |
| 24.6 | [b] | 4.06 | 3.78 | 4.30 | 4.30 | 4.21 | 4.45 | 7.76 | | | |
| Total malvalate | [c] | 6.31 | 6.74 | 6.30 | 6.22 | 6.46 | 6.47 | 5.52 | 6.29 | 0.38 | 6.04% |
| Total sterculate | [d] | 49.26 | 49.28 | 49.20 | 49.40 | 49.61 | 50.85 | 49.86 | 49.64 | 0.58 | 1.17% |
| Total cyclopropenoid fatty acids | | 55.57 | 56.02 | 55.50 | 55.62 | 56.07 | 57.32 | 55.38 | 55.93 | 0.67 | 1.20% |
| HBr | [e] | 52.0 | | | | | | | | | |

[a] Derivative of malvalic acid.
[b] Derivative of sterculic acid.
[c] Total of ECL 21.3 and 23.6.
[d] Total of ECL 22.3 and 24.6.
[e] Calculated as sterculate.

Separation of the reaction products from the normal fatty acid methyl esters was attempted by column chromatography with Alcoa F-20 Alumina, Merck No. 71707 Alumina, and Woelm Neutral Alumina. The methyl esters of the normal saturated and unsaturated fatty acids were eluted with 1% to 5% ethyl ether in petroleum ether, depending on the alumina used. The reaction products of the cyclopropenoid fatty acids were eluted with chloroform. The Woelm Neutral Alumina gave the best separation and most consistent results. Data showing the methyl ester composition of a sample of a reacted *Sterculia foetida* oil before and after alumina chromatography are in Table III. No significant changes in the ester composition occurred during chromatography. Alumina column fractionation provided a convenient and efficient means of separating the reaction products of the cyclopropenoid fatty acid esters from the methyl esters of the normal fatty acids.

TABLE III

Composition of Reaction Products of Methyl Esters of *Sterculia foetida* Oil and Methanolic Silver Nitrate Before and After Alumina Chromatography

| Equivalent chain length | Fatty acid | Before alumina chromatography Area per cent | After alumina chromatography Area per cent 1% Ethyl ether fraction | After alumina chromatography Area per cent Chloroform fraction |
|---|---|---|---|---|
| 14.0 | Myristic | 0.1 | 0.1 | |
| 15.0 | ..... | Trace | Trace | |
| 15.4 | ..... | Trace | Trace | |
| 16.0 | Palmitic | 21.2 | 20.6 | |
| 16.6 | Palmitoleic | 0.3 | 0.3 | |
| 17.0 | ..... | Trace | 0.1 | |
| 17.6 | ..... | 0.1 | 0.1 | |
| 18.0 | Stearic | 2.0 | 2.0 | |
| 18.5 | Oleic | 8.4 | 8.8 | |
| 19.3 | Linoleic | 8.9 | 9.0 | |
| 20.3 | ..... | 1.7 | 2.0 | |
| 21.3 | [a] | 5.1 | | 4.9 |
| 22.3 | [b] | 45.4 | | 45.2 |
| 23.2 | ..... | .1 | 0.1 | |
| 23.6 | [a] | .4 | | 0.6 |
| 24.6 | [b] | 6.3 | | 6.2 |

[a] Derivative of malvalic acid.
[b] Derivative of sterculic acid.

TABLE IV

Analysis for Cyclopropenoid Fatty Acids in *Sterculia foetida* Oil Diluted with Various Amounts of Corn Oil

| Equivalent chain length | Fatty acid | Per cent *Sterculia foetida* oil 100 | 10 | 1 |
|---|---|---|---|---|
| | | Per cent corn oil 0 | 90 | 99 |
| 16.0 | Palmitic | 22.1 | 12.8 | 12.5 |
| 16.6 | Palmitoleic | 0.2 | 0.1 | ..... |
| 18.0 | Stearic | 2.0 | 2.4 | 2.5 |
| 18.5 | Oleic | 8.9 | 29.0 | 31.0 |
| 19.3 | Linoleic | 9.2 | 48.3 | 51.2 |
| 20.0 | | 0.1 | 0.6 | 0.7 |
| 20.4 | | 2.1 | 1.6 | 0.9 |
| 21.4 | [a] | 4.0 | 0.3 | 0.04 |
| 22.0 | | ..... | 0.7 | 0.8 |
| 22.4 | [b] | 42.1 | 3.2 | 0.32 |
| 23.6 | [a] | 1.5 | 0.1 | 0.003 |
| 24.6 | [b] | 7.8 | 0.9 | 0.07 |
| Total | Malvalate | 5.5 | 0.4 | 0.043 |
| Total | Sterculate | 49.9 | 4.1 | 0.38 |
| Total | Cyclopropenoid fatty acids | 55.4 | 4.5 | 0.423 |

[a] Derivative of malvalic acid.
[b] Derivative of sterculic acid.

To study the recovery of the cyclopropenoid fatty acids when this silver nitrate–methanol method was applied to oils with varying levels of cyclopropenes, a series of synthetic samples was prepared using corn oil with different amounts of *Sterculia foetida* oil added. A sample of each of the synthetic oils was analyzed. A known amount of an internal standard was added to the two fractions from the Woelm Alumina chromatography prior to gas chromatography so that the composition of the original mixture could be calculated from data on the two fractions. Results of this study are shown in Table IV.

The recoveries of the cyclopropene reaction products from the synthetic samples were reasonable and the procedure can be used to measure the cyclopropenoid fatty acids of cottonseed oils containing approximately 0.5% total cyclopropenoid fatty acids. A sample of cottonseed oil was analyzed by silver nitrate–methanol as well as by by the hydrogen bromide titration method. The data are reported in Table V.

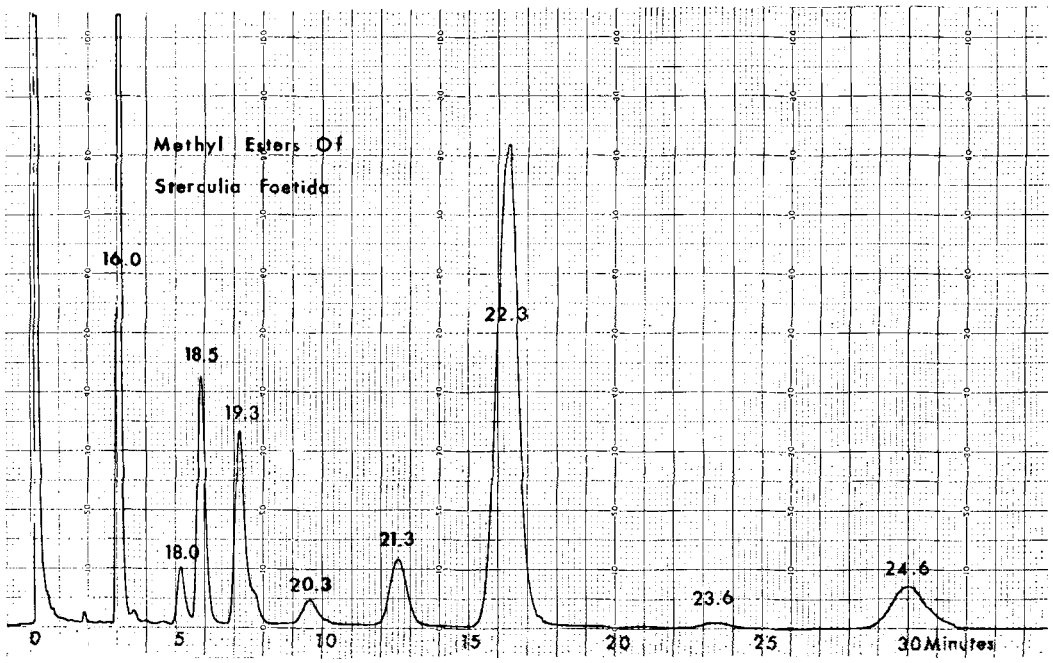


FIG. 2. Gas-liquid chromatogram of the reacted methyl esters of *Sterculia foetida*. Peaks are listed by equivalent chain length as follows: 16.0, methyl palmitate; 18.0, methyl stearate; 18.5, methyl oleate; 19.3, methyl linoleate; 20.3, methyl linolenate; 21.3, ether derivative of methyl malvalate; 22.3, ether derivative of methyl sterculate; 23.6, ketone derivative of methyl malvalate; 24.6, ketone derivative of methyl sterculate.

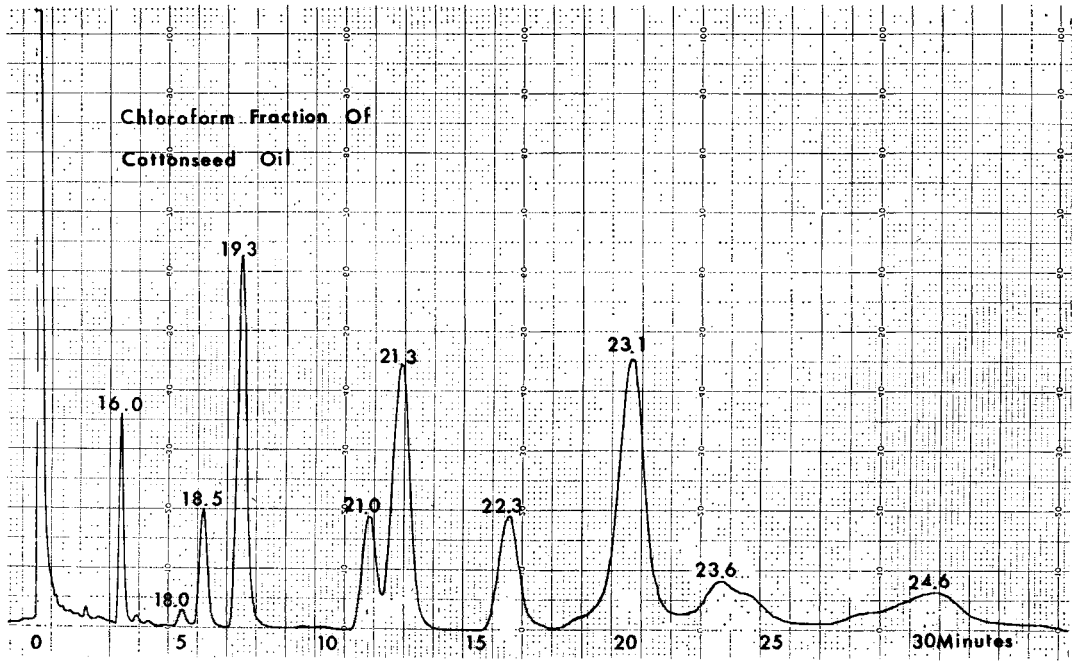


FIG. 3. Gas-liquid chromatogram of the chloroform fraction of the methyl esters of reacted cottonseed oil. Peaks are listed by equivalent chain length as follows: 16.0, methyl palmitate; 18.0, methyl stearate; 18.5, methyl oleate; 19.3, methyl linoleate; 21.0, methyl heneicosanoate, internal standard; 21.3, ether derivative of methyl malvalate; 22.3, ether derivative of methyl sterculate; 23.1, unidentified; 23.6, ketone derivative of methyl malvalate; 24.6, ketone derivative of methyl sterculate.

Similar values for the cyclopropenoid fatty acids were obtained by both procedures. A chromatogram of the chloroform fraction of this sample is shown in Fig. 3.

In an effort to establish a lower limit of detection of the method, a sample of corn oil containing 0.10% *Sterculia foetida* oil was analyzed. No changes in the procedures were employed other than to increase the sample size taken for analysis to 0.5 g. The chromatogram of the chloroform fraction of this sample is shown in Fig. 4. While precise quantitation was not done on this sample, integrator values indicated that the sterculate was present at about 0.04%, or approximately the expected level. The peak corresponding to the ether reaction product from malvalic acid (ECL 21.3) was clearly discernible and represents a theoretical amount of 0.006% malvalic acid. As a result of this limited study, we must conclude that the lower limit of detection of cyclopropenoid fatty acids by the silver nitrate–methanol method is below 0.01%. Further work remains to be done to determine the quantitative aspects of the analysis at this low level and to establish the lowest limit of detection.

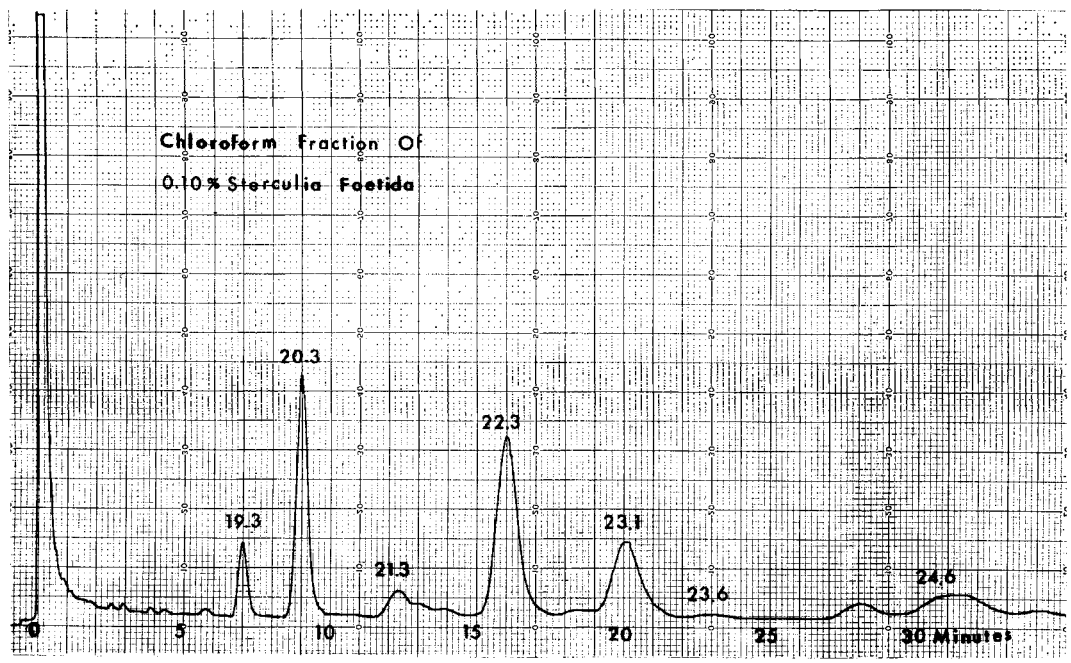


FIG. 4. Gas-liquid chromatogram of the chloroform fraction of methyl esters of 0.10% *Sterculia foetida* oil in corn oil. Peaks are listed by equivalent chain length as follows: 19.3, methyl linoleate; 20.3, methyl linolenate; 21.3, ether derivative of methyl malvalate; 22.3, ether derivative of methyl sterculate; 23.1, unidentified; 23.6, ketone derivative of methyl malvalate; 24.6, ketone derivative of methyl sterculate.

TABLE V
Fatty Acid Composition of Cottonseed Oil

| Equivalent chain length | Fatty acid | Per cent |
|---|---|---|
| 14.0 | Myristic | 0.77 |
| 16.0 | Palmitic | 21.0 |
| 16.6 | Palmitoleic | 0.69 |
| 17.0 | | 0.10 |
| 17.6 | | 0.07 |
| 18.0 | Stearic | 2.46 |
| 18.5 | Oleic | 19.3 |
| 19.3 | Linoleic | 54.1 |
| 20.0 | | 0.21 |
| 20.2 | | 0.19 |
| 21.3 | [a] | 0.27 |
| 22.0 | | 0.09 |
| 22.3 | [b] | 0.13 |
| 23.1 | | 0.44 |
| 23.6 | [a] | 0.08 |
| 24.6 | [b] | 0.07 |
| Total | Malvalic | 0.35 |
| Total | Sterculic | 0.20 |
| Per cent cyclopropenoid as measured by HBr | | 0.53 |

[a] Derivative of malvalic acid.
[b] Derivative of sterculic acid.
[c] Calculated as malvalic acid.

The conversion of the cyclopropenoid fatty acids to stable derivatives suitable for gas-liquid chromatography can be accomplished selectively and quantitatively. The individual cyclopropenoid fatty acids may be determined by chromatography of their corresponding derivatives using techniques similar to those employed for the analysis of methyl esters of normal fatty acids. The analysis described has been in use in our laboratory for several months for the analysis of seed oils containing cyclopropenoid fatty acids at levels ranging from 50% down to fractions of 1%. The procedure is simple, and yields reproducible results on all of the samples we have analyzed.

ACKNOWLEDGMENT

The authors thank Mr. R. O. Feuge, Southern Utilization Research and Development Division, for providing purified methyl sterculate.

This work was supported by the So. Reg. Utiliz. & Dev. Div., USDA under Contract No. 12-14-100-8882.

REFERENCES

1. Magne, F. C., J. A. Harris, R. A. Pittman and E. L. Skau, JAOCS *43*, 519–524 (1966).
2. Deutschman, A. J., Jr., and I. S. Klaus, Anal. Chem. *32*, 1809–1810 (1960).
3. Wilson, T. L., C. R. Smith, Jr., and K. L. Mikolajczak, JAOCS *38*, 696–699 (1961).
4. Cornelius, J. A., and G. Shone, Chem. Ind. 1246–1247 (1963).
5. Cornelius, J. A., T. W. Hammonds and G. G. Shone, J. Sci. Food Agr. *16*, 170–172 (1965).
6. Raju, P. K., and R. Reiser, Lipids *1*, 1, 10–15 (1966).
7. Johnson, A. R., J. A. Pearson, F. S. Shenstone, A. C. Fogerty, K. E. Murray and B. H. Kennett, Ibid. *2*, 316–322 (1967).
8. Kircher, H. W., JAOCS *42*, 899–903 (1965).
9. Luddy, F. E., R. A. Barford and R. W. Riemenschneider, Ibid. *37*, 447–451 (1960).
10. Miwa, T. K., Ibid. *40*, 309–313 (1963).

[Received February 16, 1968]