Discussion

In feeding studies with dihydroxystearic acid triglycerides, Harris et al. found that rats fed this material grew better than their controls (2). This is in contrast to the present results with dihydroxystearic acid, but the fact that feeding of an acid gives different effects from feeding of its triglycerides has been observed repeatedly.

The weight increase after the initial weight depression, and the more normal weights of livers and adrenals in the animals killed at age 305 days, shows adaptation to the diet. We have observed this adaptation in animals fed diets containing oxidized fats (10). In the metabolism of dihydroxystearic acid, little of the ingested material was excreted but, on the other hand, none was deposited in the depot fat.

From these studies, it appears that the intake of dihydroxystearic acid does not have severely toxic effects. This would be in line with a previous observation by Larson et al., who found that the long term feeding of epoxidized soybean oil was well tolerated by rats (11). On the other hand, Nightingale et al. observed that the feeding of 25% of triglycerides containing ⅓ dihydroxystearic acid suppressed the vitamin K content of the rats' intestinal flora to such an extent that a severe disturbance of the blood clotting mechanism resulted (3).

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REFERENCES

1. Swern, Daniel, Eastern Regional Research Laboratory, U.S.D.A., personal communication.
2. Harris, R. S., H. Sherman, and E. E. Lockhart, Arch. Biochem., *5*, 63 (1944).
3. Nightingale, G. E. E. Lockhart, and R. S. Harris, *Ibid.*, *12*, 381 (1947).
4. Greenspan, F., Ind. Eng. Chem., *39*, 847 (1947).
5. Kaunitz, H., C. A. Slanetz, R. E. Johnson, and V. K. Babayan, J. Nutr., *73*, 386 (1961).
6. Kishimoto, Y., and N. S. Radin, J. Lip. Res., *1*, 72 (1959).
7. Zucker, L. M., and T. F. Zucker, J. Gen. Physiol., *25*, 445 (1942).
8. Kaunitz, H., C. A. Slanetz, and R. E. Johnson, J. Nutr., *64*, 513 (1958).
9. Kaunitz, H., C. A. Slanetz, R. E. Johnson, and V. K. Babayan, *Ibid.*, *70*, 521 (1960).
10. Kaunitz, H., C. A. Slanetz, R. E. Johnson, J. Guilmain, H. B. Knight, D. H. Saunders, and D. Swern, *Ibid.*, *60*, 237 (1956).
11. Larson, P. S., J. K. Finnegan, H. B. Haag, R. B. Smith, Jr., and G. R. Hennigar, Toxicol. & Appl. Pharmacol., *2*, 649 (1960).

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Gas Chromatography of *Cis-Trans* Fatty Acid Isomers on Nitrile Silicone Capillary Columns[1]

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Abstract

Three nitrile silicone polymers have been evaluated as liquid phases for gas chromatographic separation of the geometric isomers of methyl oleate, methyl linoleate, and methyl linolenate on capillary columns. A polymer of β-cyanoethylmethylsiloxane proved the most effective. This liquid phase separated oleate from elaidate, resolved the four geometric isomers of linoleate into three peaks, and divided the eight geometric isomers of linolenate into six peaks. Two other copolymers of dimethylsiloxane and β-cyanoethylmethylsiloxane gave poorer resolution of *cis-trans* isomers, but showed different elution patterns for the geometric isomers of linoleate and linolenate.

Introduction

OVER THE past few years, many workers have reported the ability of high-resolution gas liquid chromatography (GLC) columns to separate the *cis* and *trans* isomers of unsaturated fatty methyl esters. Apiezon or polyester liquid phases have usually been employed to make these separations. Previous accomplishments in this field were summarized in a recent review (1).

During recent investigations of the properties of several polar GLC liquid phases, it was noted that capillary columns coated with nitrile silicone polymers exhibited a remarkable ability to separate *cis* and *trans* isomers. We decided to investigate this effect further. This report describes the evaluation of three nitrile silicone polymers as capillary column liquid phases for separating the geometric isomers of methyl oleate, methyl linoleate, and methyl linolenate.

Procedures

Materials. The preparation and characterization of the pure fatty acid isomers and their mixtures used in this study have been previously described (1,2).

Gas Chromatography. A Barber-Colman Model 20 gas chromatograph equipped with a capillary column and an argon ionization detector was used for all gas chromatography analyses. Samples were injected into the flash vaporizer at 275–300C. By means of sample dilution with petroleum ether and a stream splitting arrangement, approximately 0.001 to 0.010 μl of methyl esters was placed on the capillary column. The detector cell was equipped with a radium ionization source and maintained at 220–240C. The ionization voltage applied to the cell electrodes was 1100 v. A scavenging flow of argon (55–65 ml/min) through the detector maintained an effective cell volume of a few μl.

Three nitrile silicones were evaluated as GLC liquid phases:

A. General Electric nitrile silicone XE-60, a copolymer of 50 mole % dimethylsiloxane and 50 mole % β-cyanoethylmethylsiloxane (3). This

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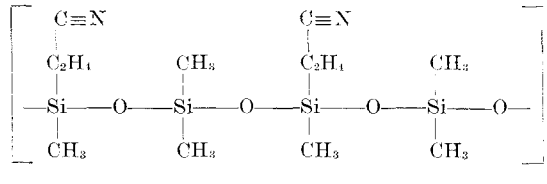
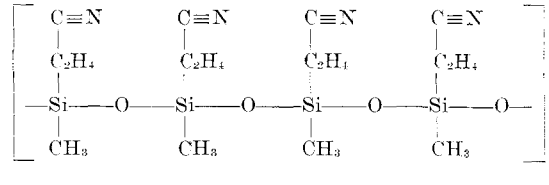
material can be approximately represented by the formula:



B. General Electric experimental nitrile silicone 238-149-99, a polymer made from 100% β-cyanoethylmethylsiloxane (3). This material can be approximately represented by the formula:



C. A 1:1 by weight measure of the two General Electric nitrile silicones XE-60 and 238-149-99.

These three materials will subsequently be referred to as 50%, 100%, and 75% nitrile silicones respectively, according to the number of silicon atoms which have a cyanoethyl group attached.

Each nitrile silicone was coated on a 200 ft stainless steel capillary column having an internal diameter of 0.010 in. The coating procedure of Litchfield, Reiser, and Isbell (1) was employed using a 9–14% (w/v) solution of the nitrile silicone in acetone. Columns were conditioned for 6 hr at 80C, 6 hr at 120C, 6 hr at 150C, 6 hr at 175C, and overnight at 200C before being used. Inert gas was kept flowing through the columns whenever they were above room temperature.

All analyses were run at 200C with an argon flow of 0.45–0.51 ml/min through the column (achieved with 20 psig head pressure on the column). Under these conditions, methyl stearate had an approximate elution time (measured from point of injection) of 41 min on the 50% nitrile column, 27 min on the 75% nitrile column, and 17 min on the 100% nitrile column. However, these elution times diminished as the columns aged.

Nitrile silicone capillary columns operated at 200C had a column life roughly equivalent to diethylene glycol succinate polyester (DEGS) capillary columns operated at 175C. Maximum column life was 1–2 months, and the best separations of geometric isomers were obtained during the first 5–10 days of use. Nitrile silicone capillary column life could probably be lengthened considerably by operating at 175C or below.

GLC peaks were identified by comparison with the retention times of pure materials or by the "mixed chromatogram" techniques previously described for the geometric isomers of methyl linoleate and methyl linolenate (1,2). Equivalent chain length (ECL) values for each peak were computed essentially according to the method of Miwa, Mikolajczak, Earle, and Wolff (4). Retention times for this calculation were measured from the solvent front. (With 200 ft capillary GLC columns, as much as 8 min may elapse between sample injection and appearance of the solvent front on the chromatogram. If the retention times of the saturated, straight-chain esters are plotted against chain length on semi-logarithmic graph paper, a straight line is obtained only if retention times are measured from the solvent front.)

Results

The equivalent chain length values for all fatty methyl esters tested on the three columns are listed in Table I.

Separation of Stearate, Oleate, Linoleate, and Linolenate. Methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate could be resolved on all three columns, but the best separation was achieved on the 100% nitrile column. A typical chromatogram with the 100% nitrile silicone is shown in Figure 1. The separation and elution order on this column were approximately equivalent to results obtained with a DEGS polyester capillary.

Separation of Oleate and Elaidate. Methyl oleate and methyl elaidate could be separated on all three columns with the elaidate eluting first each time. On the 50% nitrile column, methyl elaidate had the same elution time as methyl stearate. On the 100% nitrile column, however, a complete separation of methyl stearate, methyl oleate, and methyl elaidate was obtained (Fig. 2). Results on the 75% nitrile column were similar to those on the 100% nitrile column but had slightly poorer resolution.

Separation of Linoleate Geometric Isomers. Methyl 9,12-linoleate has four possible geometric isomers: *cis-cis, cis-trans, trans-cis,* and *trans-trans.* These isomers could be partially resolved on all three nitrile silicone columns. Unexpectedly, the elution pattern of the various isomers was different on each column. On the 50% nitrile column, three peaks were eluted (Fig. 3A): a) the 9-*trans*, 12-*trans* isomer; b) the 9-*cis*, 12-*trans* isomer; c) the 9-*trans*, 12-*cis* and 9-*cis*, 12-*cis* isomers combined. On the 75% nitrile column, four peaks were eluted (Fig. 3B): a) the 9-*trans*, 12-*trans* isomer; b) the 9-*cis*, 12-*trans* isomer; c) the 9-*trans*, 12-*cis* isomer; d) the 9-*cis*, 12-*cis* isomer. Unfortunately, the 75% nitrile column did not resolve the last three peaks sufficiently for accurate quantitation of their areas. On the 100% nitrile column, three main peaks were eluted (Fig. 3C): a) the 9-*trans*, 12-*trans* isomer; b) the 9-*cis*, 12-*trans* and 9-*trans*, 12-*cis* isomers together; c) the 9-*cis*, 12-*cis* isomer.

Separation of Linolenate Geometric Isomers. Methyl linolenate has eight possible geometric isomers: one all-*cis* isomer, three mono-*trans* isomers, three di-*trans* isomers, and one all-*trans* isomer. Only methods for preparing the all-*cis* and all-*trans* isomers were avail-

TABLE I

Equivalent Chain Length Values (4) of Various Fatty Methyl Esters on Nitrile Silicone Capillary Columns

Compound	Liquid phase		
	50% Nitrile silicone	75% Nitrile silicone	100% Nitrile silicone
Methyl stearate	18.00	18.00	18.00
Methyl oleate	18.06	18.33	18.48
Methyl elaidate	18.00	18.13	18.26
Methyl 9-*cis*,12-*cis*-octadecadienoate	18.33	18.77	19.21
Methyl 9-*cis*,12-*trans*-octadecadienoate	18.24	18.62	18.99
Methyl 9-*trans*,12-*cis*-octadecadienoate	18.30	18.68	19.02
Methyl 9-*trans*,12-*trans* octadecadienoate	18.07	18.39	18.64
Methyl 9-*cis*,12-*cis*,15-*cis*-octadecatrienoate	18.70	19.20	20.04
Methyl 9,12,15-octadecatrienoate	18.54	18.97	19.66
(three mono-*trans* isomers)	18.67	19.12	19.85
	18.70	19.13	19.85
Methyl 9,12,15-octadecatrienoate	18.43	18.81	19.43
(three di-*trans* isomers)	18.54	18.92	19.53
	18.54	18.92	19.53
Methyl 9-*trans*,12-*trans*,15-*trans*-octadecatrienoate	18.26	18.57	19.13

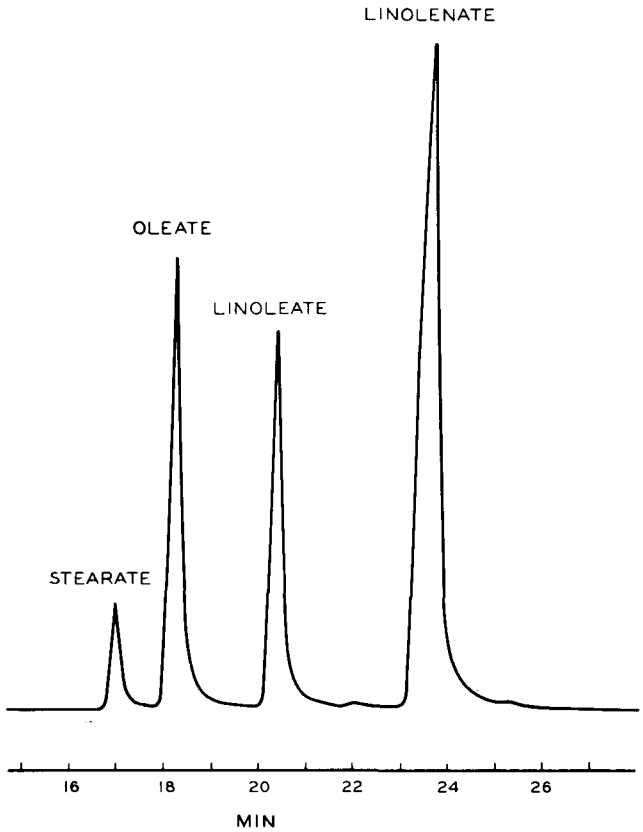


FIG. 1. Separation of methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate on a 200-ft capillary column coated with 100% nitrile silicone.

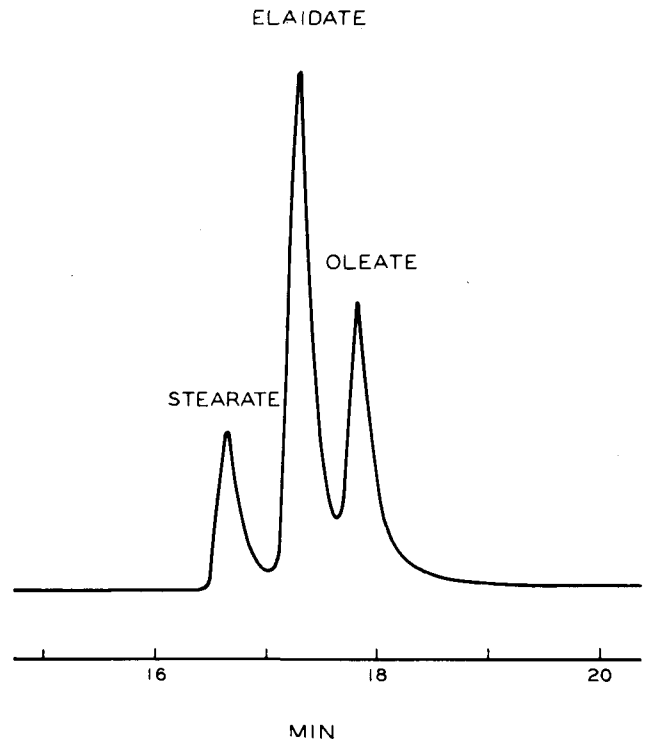


FIG. 2. Separation of methyl stearate, methyl elaidate, and methyl oleate on a 200-ft capillary column coated with 100% nitrile silicone.

able in the literature. Therefore, peaks were identified by the previously described indirect method (1) which only distinguishes the eight isomers as all-*cis*, mono-*trans*, di-*trans*, or all-*trans*.

The elution pattern of the linolenate geometric isomers also changed as the amount of nitrile in the liquid phase was varied. On the 50% nitrile column, four main peaks were eluted (Fig. 4A): a) the all-*trans* isomer; b) one di-*trans* isomer; c) two di-*trans* and one mono-*trans* isomers together; d) two mono-*trans* and the all-*cis* isomers together. The resolution of these four peaks was quite adequate to allow their quantitation. On the 100% nitrile column, however, six peaks were eluted (Fig. 4B): a) the all-*trans* isomer; b) one di-*trans* isomer; c) two di-*trans* isomers together; d) one mono-*trans* isomer; e) two mono-*trans* isomers together; f) the all-*cis* isomer. The resolution of these six peaks was adequate to allow their quantitation. Results on the 75% nitrile column were similar to those on the 100% nitrile column but had slightly poorer resolution.

Discussion

Nitrile silicones are effective GLC liquid phases for resolving many geometric isomers of fatty methyl esters on capillary columns. Furthermore, they separate the geometric isomers of oleate, linoleate, and linolenate as well as or better than DEGS polyester or Apiezon L capillary columns (1,2).

Of the three nitrile silicones evaluated, the most polar (the 100% β-cyanoethylmethylsiloxane polymer) is the most versatile. Not only will it resolve the most individual peaks, but it does so more rapidly than the other two silicones. None of the three liquid phases completely resolves all isomers (see Table I). Despite the overlapping of linoleate and linolenate isomers on the 100% nitrile column, complete quantitative analysis is still possible. For example, *cis-trans* isomerized linseed oil (a mixture of palmitate, stearate, and the geometric isomers of oleate, linoleate, and linolenate) could still be quantitatively analyzed using this technique. First, chromatography on a packed, DEGS polyester column would indicate the total amounts of 16:0, 18:0, 18:1, 18:2, and 18:3 that are present. A chromatogram run on the 100% nitrile capillary column would determine the content of stearate, elaidate, and oleate. Next, pure 18:2 and 18:3 fractions could be isolated by preparative gas chromatography (a normal packed DEGS polyester GLC column will give good separation of 18:0, 18:1, 18:2, and 18:3, but is unable to distinguish between geometric isomers), countercurrent distribution (5), or chromatography of mercuric acetate adducts (6). The content of each of the four isomers of linoleate could then be computed from chromatograms of the pure 18:2 fraction on both the 50% and 100% nitrile capillaries. Finally, analysis of the pure 18:3 fraction on the 100% nitrile column would indicate the amounts of all-*trans*, di-*trans*, mono-*trans*, and all-*cis* linolenate isomers present. There is some tendency of peaks to tail on these nitrile silicone capillary columns (see Fig. 1,2,3, and 4), and this should be taken into account when quantitating peak areas.

Increasing the nitrile content of the silicone polymer decreases the elution time for any given methyl ester. For example, methyl stearate has an elution time (measured from point of injection) of ca. 41 min on the 50% nitrile column, 27 min on the 75% nitrile column, and 17 min on the 100% nitrile column. Similar trends for the geometric isomers of linoleic and linolenic acids are shown in Figures 3 and 4.

Nitrile silicone columns apparently elute related geometric isomers in the order of the number of *trans* bonds they contain. This is best illustrated with linolenate where the all-*trans* isomer elutes first, next

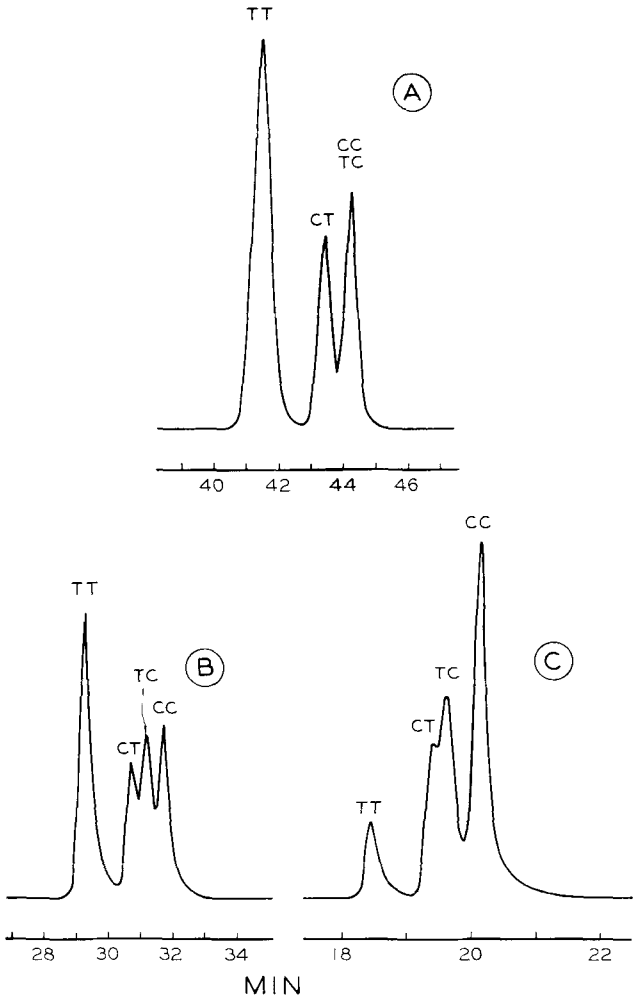


FIG. 3. Separation of the four geometric isomers of methyl 9,12-linoleate on 200-ft capillary columns coated with: (A) 50% nitrile silicone, (B) 75% nitrile silicone, and (C) 100% nitrile silicone. TT=9-*trans*, 12-*trans* isomer. CT=9-*cis*, 12-*trans* isomer. TC=9-*trans*, 12-*cis* isomer. CC=9-*cis*, 12-*cis* isomer.

the di-*trans* isomers, then the mono-*trans* isomers, and finally the all-*cis* isomer. This same pattern is repeated with linoleate: the 9-*trans*, 12-*trans* isomer elutes in the first peak, and the 9-*cis*, 12-*cis* isomer elutes in the last peak. The different elution patterns of linoleate and linolenate geometric isomers with the different nitrile silicones are also consistent with this elution scheme.

The elution order of geometric isomers might possibly be attributed to the formation of a π-complex between the nitrile groups in the silicone and the double bonds in the methyl esters. It has been demonstrated (7,8) that a nitrile group will attract the π-electrons in an olefin to form a stable π-complex. This nitrile/olefin π-complex is quite similar to that formed between silver ion and an olefin (9). The Ag^+/olefin π-complex is known to be stronger with *cis* than with *trans* double bonds, and this property has been used to separate the geometric isomers of oleic, linoleic, and linolenic acids by various forms of chromatography (10,11,12). The elution order of geometric isomers using silver ion chromatography is identical with their elution order on our nitrile silicone GLC columns. One can speculate that the nitrile/olefin π-complex may also show selectivity for *cis* and *trans* isomers, and thus account for their order of elution from a nitrile silicone GLC column.

Further evidence for the ability of the nitrile/olefin π-complex to perform GLC separations has been reported by Bayer, Hupe, and Mack (13). These workers found that nitrile GLC liquid phases were extremely effective in resolving aliphatic, olefinic, and aromatic compounds of almost identical boiling points. These desirable GLC separation characteristics were apparently due to the formation of a π-complex between the nitrile groups in the liquid phase and the π-electrons of the olefinic and aromatic compounds.

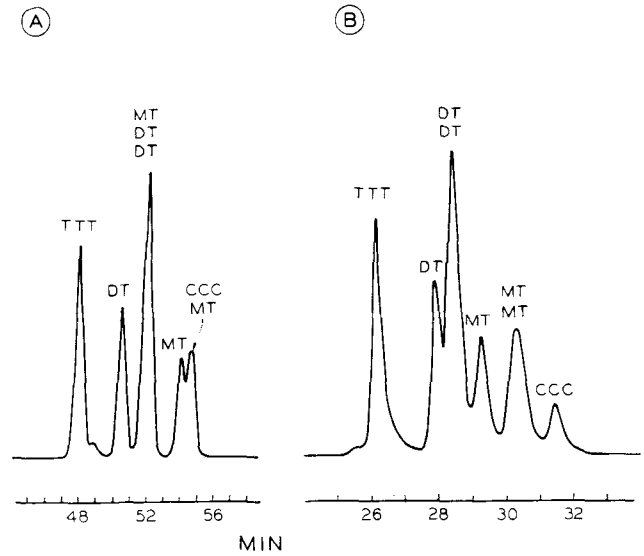


FIG. 4. Separation of the eight geometric isomers of methyl 9,12,15-linolenate on 200-ft capillary columns coated with: (A) 50% nitrile silicone, and (B) 100% nitrile silicone. TTT=all-*trans* isomer. DT=di-*trans* isomers. MT=mono-*trans* isomers. CCC=all-*cis* isomer.

It would be naive to think that GLC elution order is determined by only one factor. Many other considerations such as relative vapor pressures of the esters, polarity of the esters, polarity of the silicones, etc. undoubtedly also play a role in determining elution order. But a π-complex effect may well be the major factor which enables nitrile silicone GLC liquid phases to separate geometric isomers so effectively.

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REFERENCES

1. Litchfield, Carter, R. Reiser, and A. F. Isbell, JAOCS, *40*, 302 (1963).
2. Litchfield, Carter, A. F. Isbell, and R. Reiser, JAOCS, *39*, 330 (1962).
3. Obtained from A. C. Martellock, Silicone Products Dept., General Electric Co., Waterford, N. Y.
4. Miwa, T. K., K. L. Mikolajczak, F. R. Earle, and I. A. Wolff, Anal. Chem. *32*, 1739 (1960).
5. Scholfield, C. R., E. P. Jones, J. Nowakowska, E. Selke, B. Sreenivasan, and H. J. Dutton, JAOCS *37*, 579 (1960).
6. Kuemmel, D. F., Anal. Chem. *34*, 1003 (1962). Jantzen, E., H. Andreas, K. Morgenstern, and W. Roth, Fette, Seifen, Anstrichmittel *63*, 685 (1961).
7. Merrifield, R. E., and W. D. Phillips, J. Am. Chem. Soc. *80*, 2778 (1958).
8. Bauer, R. H., Anal. Chem. *35*, 107 (1963).
9. Winstein, S. and H. J. Lucas, J. Am. Chem. Soc. *60*, 836 (1938).
10. Dutton, H. J., C. R. Scholfield, and E. P. Jones, Chem. Ind. No. 46, 1874 (1961).
11. de Vries, B., *Ibid.* No. 24, 1049 (1962).
12. de Vries, B., JAOCS, *40*, 184 (1963).
13. Bayer, E., K. P. Hupe, and H. Mack, presented at International Symposium on Advances in Gas Chromatography, Houston, Texas, 1963.

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