Gas–liquid chromatographic analyses of 1,2-ethanediol monoethers and monoesters

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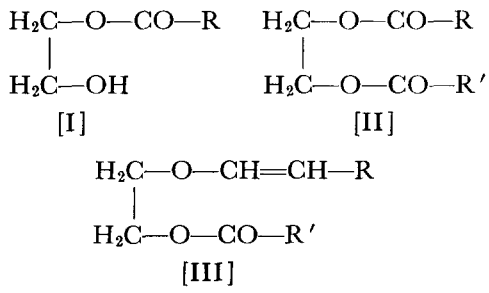
ABSTRACT Synthetic mixtures of saturated and unsaturated monoethers and monoesters of 1,2-ethanediol, ranging in chain length from 12 to 20, were analyzed as acetates, trifluoroacetates (TFA), and trimethylsilyl (TMS) ethers by gas chromatography on polar and nonpolar liquid phases. Acetates, TFA derivatives, and TMS derivatives of the glycol ethers were eluted ahead of the corresponding glycol ester derivatives on both liquid phases. The elution order of derivatives of the same compound was found to be TMS derivative before TFA derivative before acetate on the polar liquid phase, and TFA derivative before TMS derivative before acetate on the nonpolar liquid phase. Elution orders relative to methyl stearate were also determined. With one exception, all of the derivatives, and both liquid phases, were found suitable for the quantitative analysis of diol monoethers and monoesters.

SUPPLEMENTARY KEY WORDS diol lipids · acetates · trifluoroacetates · trimethylsilyl ethers

THE OCCURRENCE of natural lipids in which the alcoholic moiety consists of short-chain diols, such as 1,2-ethanediol, propanediols, or butanediols, instead of glycerol, has been recognized only recently (1). The chemical and physical properties of these compounds (2, 3) are similar to those of the glycerol-derived lipids and diol lipids had therefore, escaped detection for a long time. The separation of diol lipids from the bulk of lipid material is difficult and their presence has been demonstrated by indirect means only. Intact individual classes or molecular species of diol lipids have not been isolated from natural sources.

Diols have been detected after hydrolysis of lipid extracts from animal (4–7) and plant (5, 6, 8) tissues, as well as from microorganisms (6, 9, 10). Analyses of the hydrolysis products from neutral or polar lipid fractions revealed that diol lipids occur in nature as monoesters [I] (9), diesters [II] (5–7), alk-1-enyl monoether monoesters [III, "neutral diol plasmalogens"] (6), or as phospholipids of unknown structure (7).



Alkyl ethers of short-chain diols have not been detected as yet in biological materials.

Methods previously used for the detection of diols in lipid hydrolysates involved thin-layer (4), paper (4, 9, 10), and gas–liquid chromatography of the free diols (11, 12) or of diol derivatives (6, 13, 14).

The present communication describes the quantitative GLC analysis of saturated and unsaturated monoethers and monoesters of 1,2-ethanediol. Three derivatives of these model compounds were examined on polar and nonpolar liquid phases.

EXPERIMENTAL METHODS

Syntheses of the long-chain monoethers and monoesters of 1,2-ethanediol used in this study have been described previously (2). Trifluoroacetic anhydride was purchased

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Abbreviations: GLC, gas–liquid chromatography; TFA, trifluoroacetyl; TMS, trimethylsilyl; SE-30, methyl silicone polymer; EGSS-X, ethylene glycol succinate–methyl silicone polymer.

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from Eastman Organic Chemicals, Rochester, N.Y.; hexamethyldisilazane was purchased from Peninsular Chemical Research, Gainesville, Fla.; trimethylchlorosilane was obtained from K & K Laboratories, Inc., Plainview, N.Y. Other reagents and materials were reagent grade or better and were used without further purification.

Acetates (15), trifluoroacetates (TFA derivatives) (16), and trimethylsilyl ethers (TMS derivatives) (17) were prepared according to methods described previously.

GLC analyses were carried out on an Aerograph model 204 gas chromatograph. A 152 cm × 3 mm O.D. (1.75 mm I.D.) Pyrex column packed with 15% ethylene glycol succinate–methyl silicone polymer (EGSS-X) coated on 100–120 mesh Gas-Chrom P, and a 5 ft × 1/8 inch stainless steel column packed with 5% methyl silicone polymer (SE-30) coated on 60–80 mesh Chromosorb W were used in these analyses. Column operating temperatures are given in the figure legends. Flash heater and detector temperatures were maintained at 275 and 250°C, respectively. Helium served as carrier gas at a flow rate of 40–60 ml/min. Oxygen and hydrogen flame gases were regulated to give maximum detector sensitivity. Peak areas were measured with a Datex model DIR-1 digital integrator.

RESULTS AND DISCUSSION

Trifluoroacetates

Mixtures of TFA derivatives of glycol monoethers and of glycol monoesters, ranging in chain length from 12 to 20, are resolved completely by gas chromatography on both polar and nonpolar liquid phases (Fig. 1). The monounsaturated compounds of each class are well resolved from the corresponding saturated ones on the EGSS-X column, and are partially resolved on the SE-30

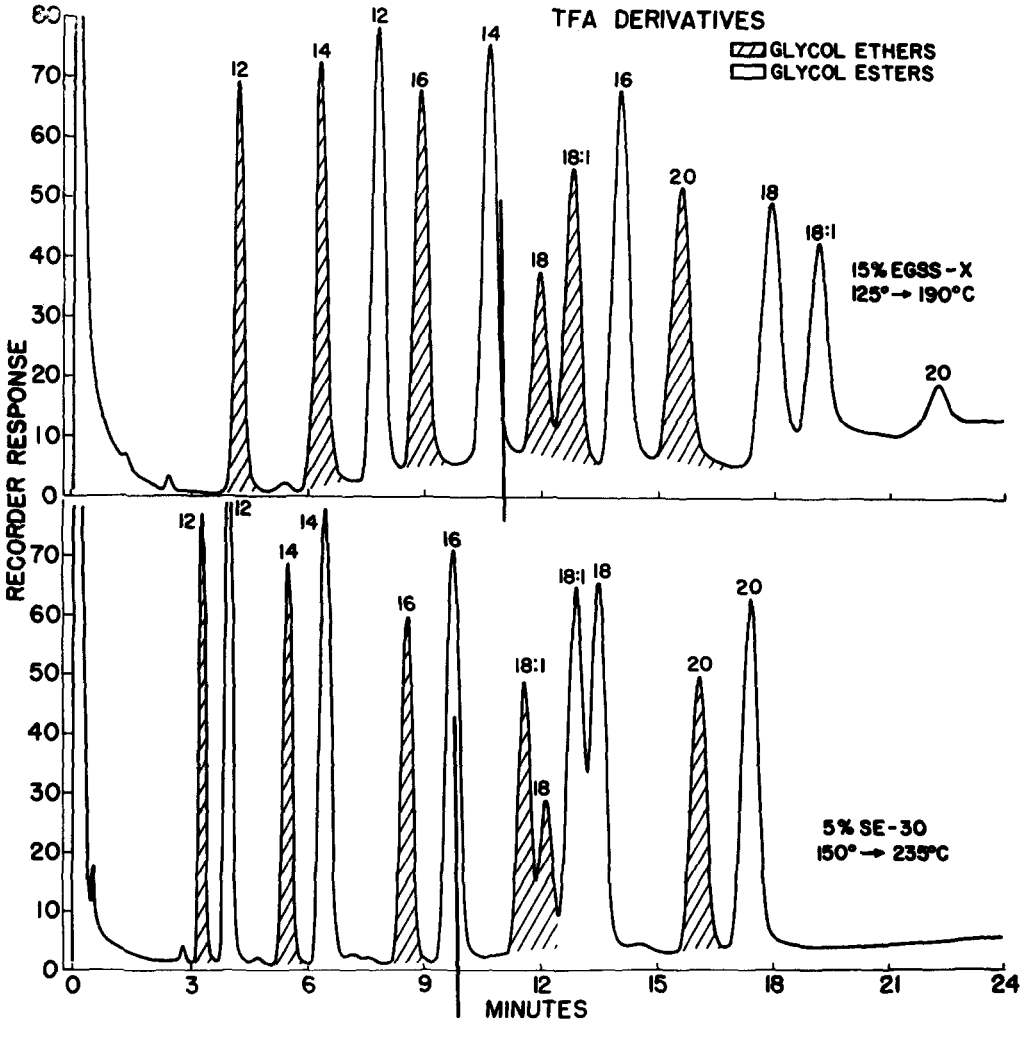

FIG. 1. Typical chromatograms of TFA derivatives of glycol monoethers and glycol monoesters obtained on polar and nonpolar columns. The approximate retention times of methyl stearate are indicated by vertical lines.

column. On either liquid phase the glycol ether trifluoroacetates are eluted ahead of the TFA derivatives of glycol esters of corresponding chain lengths, but the difference in elution time between ethers and esters is more pronounced on the polar phase.

Trimethylsilyl Ethers

The separations of the TMS ethers of glycol ethers and glycol esters obtained on the EGSS-X and the SE-30 columns are shown in Figs. 2 and 3, respectively. The elution order of the TMS derivatives is similar to that of the TFA derivatives. On the SE-30 column, TMS ethers of glycol esters are eluted midway between the glycol ether derivatives of the same chain lengths and the next higher homologues having two methylene groups more, while the TFA derivatives of the glycol esters are eluted immediately after the glycol ether of the same chain length (Figs. 1 and 3). The TMS derivatives of saturated and the corresponding unsaturated glycol esters were resolved on the polar liquid phase, but the glycol ethers were only partially resolved. Similarly, TMS derivatives of saturated and unsaturated glyceryl monoethers and long-chain alcohols were previously shown to be difficult to separate on a polar liquid phase (16, 18). Sahasrabudhe and Legari (19) have also shown that saturated and unsaturated TMS derivatives as well as the isomers of propane-1,2-diol monoesters are not resolved on a nonpolar liquid phase.

Acetates

Typical chromatograms of the acetates of a glycol ether and glycol ester mixture obtained on the polar and on the nonpolar liquid phases are shown in Figs. 2 and 3, respectively. Acetates required the EGSS-X column to be operated at a higher temperature than for the TFA or TMS derivatives of the glycol esters if elution was to be achieved in a reasonable length of time. Saturated and unsaturated glycol ethers and esters were completely resolved on the polar phase, but were only partially resolved on the nonpolar liquid phase. The acetates of both the glycol ethers and esters gave unsymmetrical peaks on the SE-30 column, the glycol esters tailing most

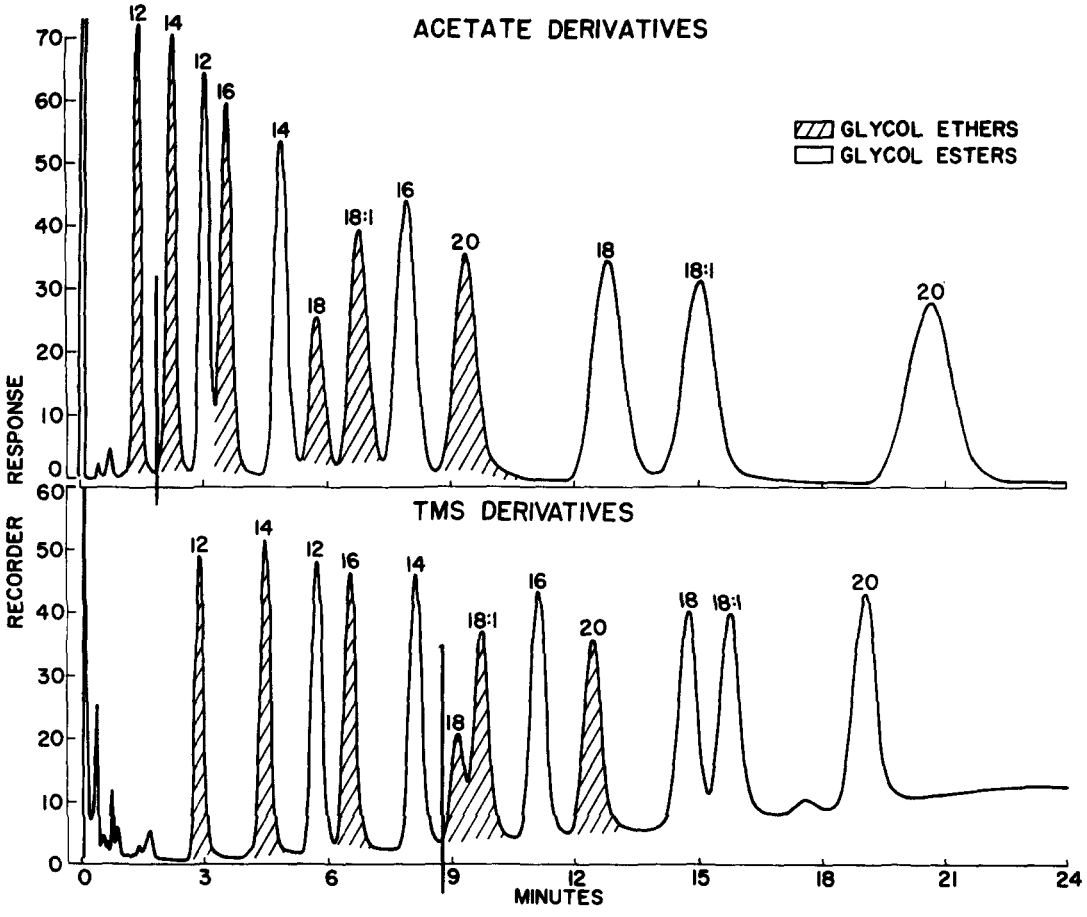

FIG. 2. Representative chromatograms of the acetate and TMS derivatives of glycol monoethers and glycol monoesters obtained on the 15% EGSS-X column. Column temperature was programmed from 125 to 185°C at approximately 3°C/min for the analysis of the TMS derivatives and maintained isothermally at 195°C for the analysis of the acetates. The approximate retention times of methyl stearate are indicated by vertical lines.

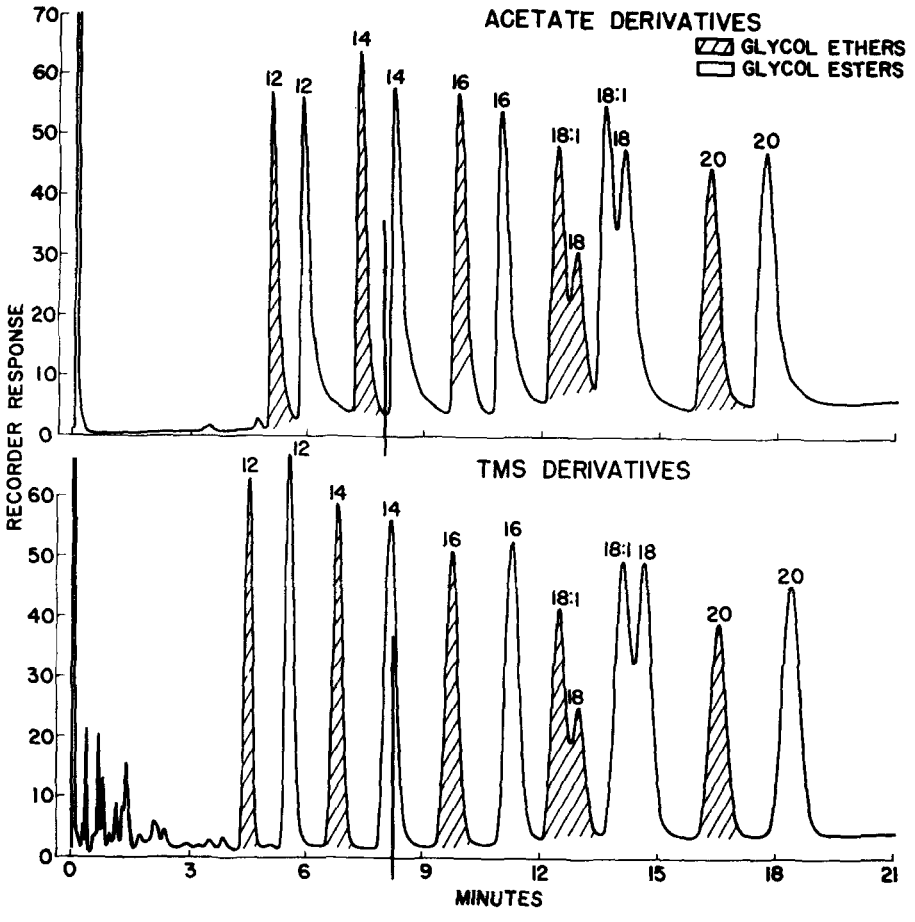

FIG. 3. Gas–liquid chromatograms of the acetate and TMS derivatives of glycol monoethers and glycol monoesters obtained on the 5% SE-30 column. Column temperature was programmed from 150 to 235°C at approximately 4°C/min. The approximate retention times of methyl stearate are indicated by vertical lines.

severely. Changes in flow rate and column temperature failed to improve peak symmetry.

Relative Elution Order

Expectedly, the trifluoroacetates, acetates, and TMS ethers of glycol ethers are eluted ahead of the corresponding glycol esters on both liquid phases (Figs. 1–3). The elution order of the three derivatives relative to each other on EGSS-X is: TMS derivative before TFA derivative before acetate. The sequence of elution on SE-30 is: TFA derivative before TMS derivative before acetate. The TMS derivatives are eluted immediately *before* and the acetates are eluted *with* the TFA derivatives having two methylene groups more.

Mixed derivatives of the same compound were eluted in the expected order on the polar phase, but the retention times on the nonpolar liquid phase were inversely proportional to the weight of the derivative. Discussions of the factors that contribute to retention times of TFA and TMS derivatives obtained on nonpolar liquid phases that are not proportional to molecular weights have appeared and are applicable to the present observations (20, 21).

Retention times observed under temperature-programmed conditions are usually not reproducible; thus elution times must be related to a common internal standard such as methyl stearate. Methyl stearate had approximately the same, but slightly shorter, retention times than the TMS derivative of the octadecyl glycol ether on the polar column, but eluted midway between the TMS derivatives of the tetradecyl and hexadecyl glycol ethers on the nonpolar column. Methyl stearate was eluted immediately after the TMS derivative of the glycol tetradecanoate on the polar column, but both were eluted as a single peak on the SE-30 column. The approximate elution times of methyl stearate relative to each of the derivatives for both the polar and nonpolar liquid phases are indicated in each of the figures.

Quantification

The gas-chromatographic analyses of the TMS ethers and acetates of glycol ethers and glycol esters give quan-

TABLE 1 KNOWN AND EXPERIMENTALLY DETERMINED VALUES FOR TMS, ACETATE, AND TFA DERIVATIVES OF GLYCOL ETHER AND ESTER STANDARD MIXTURES OBTAINED BY GAS CHROMATOGRAPHY ON POLAR AND NONPOLAR LIQUID PHASES

Glycol Lipid	Carbon Chain	Standard Mixture		Found† for Standard Mixture on:				
				5% SE-30‡			15% EGSS-X§	
		Weight	Moles*	TMS	Acetate	TFA	TMS	Acetate
		%				*area* %		
Monoether	12:0	13.4	16.8	15.7	14.9	15.5	14.6	14.6
	14:0	18.0	20.1	19.9	19.9	20.5	19.4	19.5
	16:0	19.5	19.7	20.2	20.5	21.1	20.1	19.8
	18:0	9.0	8.2	8.2	8.7	7.7	8.2	9.8
	18:1	19.2	17.8	17.7	17.4	16.7	18.1	17.0
	20:0	20.8	17.5	18.3	18.6	18.5	19.6	19.2
Monoester	12:0	13.2	16.5	14.8	14.3	16.4	14.1	14.2
	14:0	14.8	16.6	16.0	15.0	17.2	15.3	15.6
	16:0	16.3	16.6	16.8	16.3	17.2	16.4	17.0
	18:0	17.9	16.6	17.6	18.2	17.0	17.0	17.8
	18:1	17.4	16.3	16.7	16.7	16.6	17.5	16.0
	20:0	20.4	17.4	18.1	19.5	15.6	19.7	19.5

* Weight and mole percentages of the parent compounds.
† Area percentages were determined with a digital integrator and represent the mean of two determinations.
‡ Column temperature was programmed from 150 to 235°C at approximately 4°C/min.
§ Column temperature was programmed from 125 to 185°C at approximately 3°C/min when TMS derivatives were analyzed. Acetates were analyzed isothermally at 195°C.

titative and reproducible results. Values determined by GLC on polar and nonpolar liquid phases for both derivatives agree quite well with the known percentages of the standard mixtures, as shown in Table 1.

Analyses of the trifluoroacetates of glycol ethers and glycol esters on polar liquid phases constitute an exception. The results obtained for the TFA derivatives of both ethers and esters on the EGSS-X column did not give quantitative values and therefore are not shown in Table 1. As is evident from a comparison of the chromatograms in Fig. 1, loss, predominantly of the higher molecular weight glycol esters, was encountered when analyses were performed on EGSS-X. Reproducible and quantitative chromatographic analyses of TFA derivatives of glycol ethers were achieved after the EGSS-X column had been conditioned with trifluoroacetic anhydride (16). Recovery of the higher molecular weight glycol esters was also greatly improved after the column had been conditioned, but some loss of the higher esters still occurred. Quantitative results on nonpolar liquid phases and the apparent sample decomposition on polar liquid phases, similar to that observed here for the TFA derivatives of the glycol esters, has previously been reported for TFA derivatives of certain amino acids (22).

Free glycol monoethers and monoesters had long retention times and did not give quantitative results on either liquid phase.

Applicability of the Method

The described GLC procedure for the quantitative analysis of various derivatives of 1,2-ethanediol monoethers and monoesters is also applicable for the analysis of monoethers and monoesters of other short-chain alcohols such as propane-, butane-, and pentanediols. Gas chromatography in combination with other analytical and chemical methods will permit the separation, identification, and quantitation of most naturally-occurring diol lipids. Diol alk-1-enyl monoether-monoesters (neutral diol plasmalogens) yield diol monoesters on mild acid treatment, or diol monoethers after lithium aluminum hydride reduction and subsequent hydrogenation. Similarly, diol monoether-monoesters yield diol monoethers upon acid hydrolysis or hydrogenolysis. Diol monoesters are obtained by the action of lipase on diol diesters. Diol phospholipids could be converted to monoethers or monoesters in a similar manner.

Despite the structural simplicity of diol lipids, and the fact that these compounds occur widely in nature, the biological significance and metabolism of diol lipids are completely unknown. At present, difficulties in the study of these compounds are largely analytical.

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REFERENCES

1. Bergelson, L. D. 1968. *In* Progress in the Chemistry of Fats and Other Lipids. R. T. Holman, editor. Pergamon Press, Oxford. In press.
2. Baumann, W. J., H. H. O. Schmid, H. W. Ulshöfer, and H. K. Mangold. 1967. *Biochim. Biophys. Acta.* **144:** 355.

3. Baumann, W. J., and H. W. Ulshöfer. 1968. *Chem. Phys. Lipids*. **2:** 114.
4. Carter, H. E., P. Johnson, D. W. Teets, and R. K. Yu. 1963. *Biochem. Biophys. Res. Commun*. **13:** 156.
5. Bergelson, L. D., V. A. Vaver, and N. V. Prokazova. 1964. *Dokl. Akad. Nauk SSSR*. **157:** 122.
6. Bergelson, L. D., V. A. Vaver, N. V. Prokazova, A. N. Ushakov, and G. A. Popkova. 1966. *Biochim. Biophys. Acta*. **116:** 511.
7. Vaver, V. A., S. I. Shtshennikov, and L. D. Bergelson. 1967. *Biokhimya*. **32:** N5.
8. Ukita, T., and A. Tanimura. 1961. *Chem. Pharm. Bull. Tokyo*. **9:** 43.
9. Asselineau, J. 1961. *Biochim. Biophys. Acta*. **54:** 359.
10. Demarteau-Ginsburg, H., and A. M. Miquel. 1962. *Bull. Soc. Chim. Biol*. **44:** 679.
11. Vaver, V. A., A. N. Ushakov, and L. D. Bergelson. 1968. *Izv. Akad. Nauk SSSR, Ser. Khim*. In press.
12. Assman, K., O. Serfas, and G. Geppert. 1967. *J. Chromatog*. **26:** 495.
13. Vaver, V. A., A. N. Ushakov, and L. D. Bergelson. 1967. *Izv. Akad. Nauk SSSR, Ser. Khim*. 1187.
14. Vaver, V. A., A. N. Ushakov, and L. D. Bergelson. 1967. *Izv. Akad. Nauk SSSR, Ser. Khim*. 1645.
15. Fritz, J. S., and G. H. Schenk. 1959. *Anal. Chem*. **31:** 1808.
16. Wood, R., and F. Snyder. 1966. *Lipids*. **1:** 62.
17. Wood, R. D., P. K. Raju, and R. Reiser. 1965. *J. Am. Oil Chemists' Soc*. **42:** 161.
18. Wood, R. 1968. *J. Gas Chromatog*. **6:** 94.
19. Sahasrabudhe, M. R., and J. J. Legari. 1968. *J. Am. Oil Chemists' Soc*. **45:** 148.
20. Wood, R. D., P. K. Raju, and R. Reiser. 1965. *J. Am. Oil Chemists' Soc*. **42:** 81.
21. Wood, R., E. L. Bever, and F. Snyder. 1966. *Lipids*. **1:** 399.
22. Blau, K., and A. Darbre. 1965. *J. Chromatog*. **17:** 445.