Chromatographic Analysis of Polyglycerols and Their Fatty Acid Esters

M. R. SAHASRABUDHE, Research Laboratories, Food and Drug Directorate, Ottawa, Canada

Abstract

Polyglycerols and their fatty acid esters have been analyzed by gas-liquid chromatography (GLC) as trimethylsilyl ether derivatives. Linear diglycerols and triglycerols were isolated from commercial polyglycerols by vacuum distillation. Mono- and di-fatty acid esters were synthesized in the laboratory. Two isomers of diglycerol have been separated and identified. GLC analysis was carried out on columns packed with 3% JXR on Gas Chrom Q. Response factors for diglycerol and triglycerol relative to glycerol have been established. Commercial polyglycerol esters are shown to be mixtures of glycerol, free fatty acids, mono- and diglycerides, and mono- fatty acid esters of diglycerol and triglycerol. Separation of free polyglycerols and their esters is also demonstrated by thin-layer chromatography on Silica Gel-G containing 4.0% boric acid.

Introduction

POLYGLYCEROLS ARE PREPARED by acid or base-catalyzed condensation of glycerol. Linear and cyclic polyglycerols containing two and more glycerol units are described by several workers (1–13). It has been demonstrated that polyglycerols readily form esters with fatty acids (2). These esters, because of their lipophilic and hydrophilic properties, are used as emulsifiers in foods. However little information is available on the methods of analysis for these compounds. Commercial polyglycerols are mixtures of glycerol, diglycerol, triglycerol, and higher polyglycerols. Zajic (13) described a paper chromatographic procedure for the separation of polyglycerols. Siegel, Bullock, and Carter (6) described a procedure for the separation, identification, and quantitative estimation of α,α'-and α,β-diglycerol by paper chromatography. Troy and Alsop (9) developed a periodic acid oxidation method for the quantitative estimation of glycerol and diglycerol in commercial "diglycerol." A considerable portion of this product consisted of higher polyglycerols which were not identified.

Application of thin-layer (TLC) and gas chromatographic (GLC) techniques to the separation, identification, and quantitative estimation of polyglycerols and their fatty acid esters is described in this paper. Polyglycerols and their esters are analyzed by GLC as trimethylsilyl ethers (TMS).

TMS derivatives have been successfully used for the separation of phenols (14,15), carbohydrates (16), and sterols (17–19). The ease of preparation and the volatility of these derivatives provide the most suitable criteria for gas chromatographic applications. Recently Wood and associates (20) demonstrated the resolution of α- and β-isomers of monoglycerides as TMS ethers. Studies carried out in these laboratories have shown that TMS derivatives can also be used for the quantitative estimation of diglycerides as well as mixtures of mono- and diglycerides (21).

Experimental Procedure

Materials

Samples of polyglycerol were obtained commercially and fractionated by vacuum distillation on an Arthur Smith (ASCO) Rota-film Molecular Still model 50-2. The scheme of distillation of a 100-g sample of commercial triglycerol at 0.1 mm Hg is shown in Figure 1. The fractions of diglycerol and triglycerol obtained were purified further by preparative TLC. The identity of the fractions was checked by molecular weights (22), hydroxyl values (23), and elemental analysis. Purified fractions of diglycerol and triglycerol were esterified with palmitic, stearic, and oleic acids to yield mono- and di-fatty acid esters. The procedure described by Hartman (24) for the synthesis of mono- and diglycerides was used with slight modifications.

Preparation of TMS Derivatives

A 30- to 50-mg sample was dissolved in 0.5 ml of pyridine in a 5-ml conical centrifuge tube and treated with 0.2 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane. The reaction mixture was shaken for 15 to 30 sec and allowed to settle for 5 min. About 0.2 μl was injected directly into the chromatograph.

Gas-Liquid Chromatography

Gas chromatographic analysis was carried out on a Perkin-Elmer Model 800 gas chromatograph, equipped with a dual flame ionization detector and with dual 3-ft, ⅛-in. stainless steel columns packed with 3% JXR on Gas Chrom Q. Column temperature was programmed from 120 to 325C at 10C per min. Helium flow was regulated at 33 ml/min at ambient temperature.

Thin-Layer Chromatography

TLC was carried out on 250μ layers of Silica Gel G containing 4.0% boric acid, coated on 8 × 8-in. glass plates and developed with benzene-methanol 8:3. Spray reagent was dibromo(R)fluorescein. The plates were viewed under UV light. Preparative TLC was carried out on 500μ layers, and the desired areas were scraped off and eluted with ethyl alcohol and ethyl ether (1:1).

Results and Discussion

Twenty years ago Isitin (3) fractionated polyglycerols by fractional vacuum distillation. Other researchers converted polyglycerols to more volatile derivatives, such as acetates (4,12), allyl ethers, and ketals (10). In the present study, direct distillation was attempted in order to obtain pure fractions of diglycerol, triglycerol, and hexaglycerol. By stepwise distillation of a commercial sample of mixed

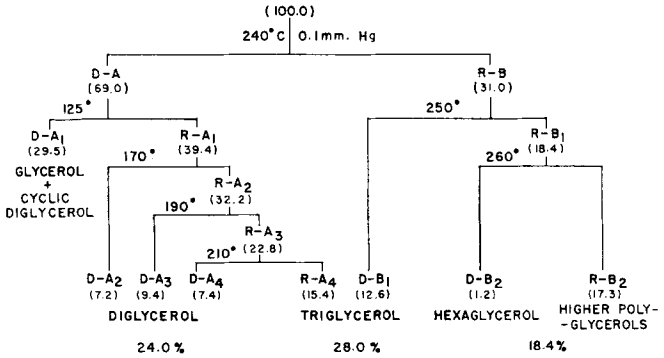

FIG. 1. Schematic diagram of distillation of 100 g of commercial polyglycerol (triglycerol) on ASCO Rota-film Molecular Still Model 50–2. D = distillate; R = residue. Figures in parentheses are weights of fractions in g.

polyglycerols, samples of diglycerol and triglycerol of 94–98% purity were obtained (Fig. 1). Diglycerol distilled from 170 to 210C while triglycerol distilled from 210 to 250C. Residual fraction R-B1 was viscous and difficult to distill under the conditions of the study, and only small quantities of hexaglycerol were obtained when distilled at 260C.

Figure 2 shows the gas chromatographic pattern of TMS derivatives of a control mixture of glycerol, diglycerol, triglycerol, and higher polyglycerols (Fraction R-B_1, Fig. 1). All polyglycerols give split-peaks. Figure 3 shows some possible isomers of diglycerol. Summerbell and his associates (7,8) assigned definite structures to some of the polyglycerol derivatives. They demonstrated that diglycerol obtained by the acid-catalyzed condensation is *trans*-2,5-bis-(hydroxymethyl)-p-dioxane. Base-catalyzed condensation yields predominantly linear polyglycerols, such as α,α'-diglycerol (3,3′ oxipropanediol) and α,β-diglycerol (2,3′ oxipropanediol). Other metameric configurations are also possible such as *cis*-2,5-, *cis*-2,6-, and *trans*-2,6-*bis*(hydroxymethyl)-p-dioxane. Samples of $\alpha'\alpha$- and α,β'-diglycerol separated by paper chromatography were obtained from Siegel (6). GLC of the TMS ethers of these compounds individually and in mixtures showed that α,β-diglycerol is eluted before the α,α'-isomer and appears as a shoulder on peak No. 3, Figure 2. Similar confirmation of the isomers of triglycerol has not been possible because of the lack of pure standards.

Figure 4 shows the resolution of polyglycerols by TLC. Samples of polyglycerols, separated by distillation (Fig. 1), were spotted on TLC plates. Spot 2

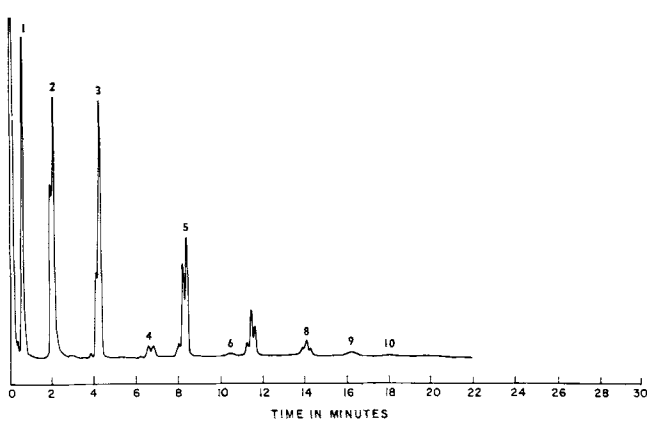

FIG. 2. TMS derivatives, polyglycerols: 1, glycerol; 2, cyclic diglycerol; 3, diglycerol; 4, unidentified; 5, triglycerol; 6–10 higher polyglycerols.

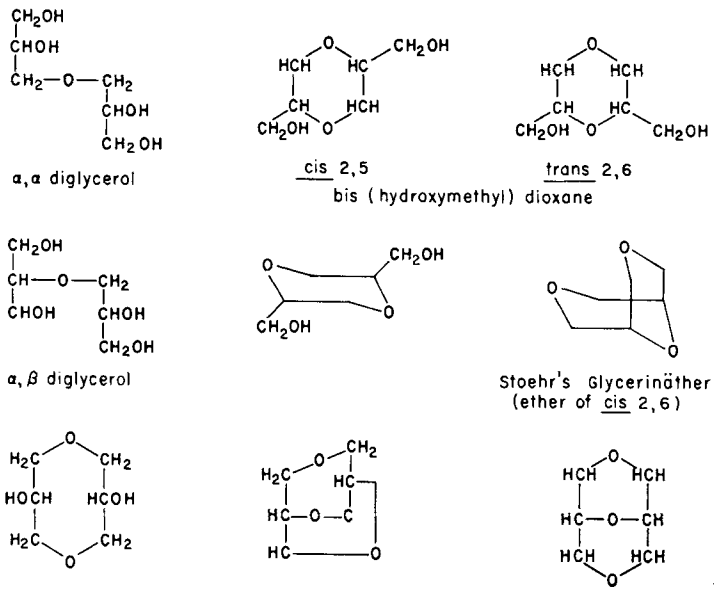

FIG. 3. Structural configurations of diglycerol.

is cyclic diglycerol, a fraction that distilled with glycerol at 125C. This compound is less polar than glycerol (Spot 1) and is assumed to be a cyclic diglycerol on the basis of its molecular weight and elemental analysis. Spots 5, 6, and 7 are commercial polyglycerol compositions which show the presence of glycerol, diglycerol, triglycerol, and hexaglycerol.

Figure 5 shows the separation of polyglycerols and their fatty acid esters by TLC and identification by GLC as TMS ethers. The sample was analyzed by preparative TLC. Fractions 1, 2, and 3 are essentially linear polyglycerols; 4 and 5 are assumed to be cyclic polyglycerols; and 6, 7, and 8 were identified as mono-fatty acid esters of triglycerol, diglycerol, and glycerol respectively.

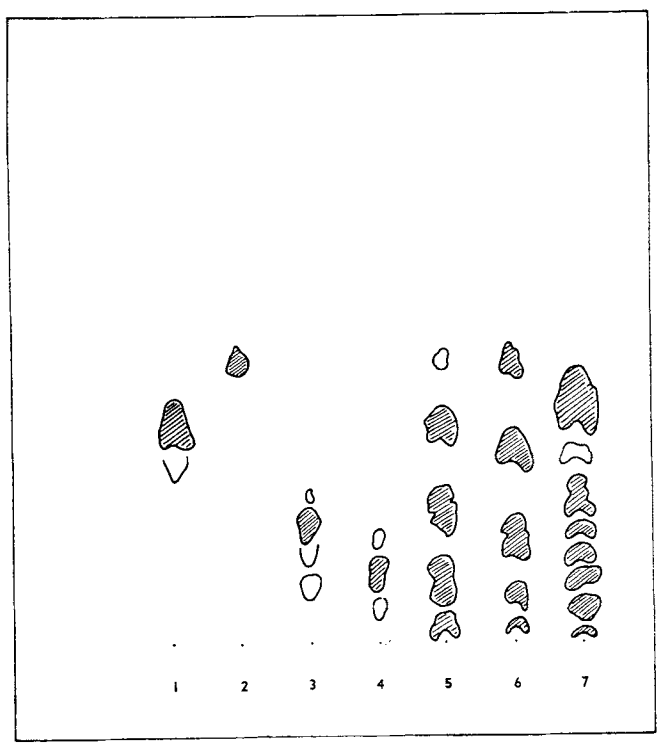

FIG. 4. TLC polyglycerols: silica gel G – 0.4% boric acid; benzene + methanol (8:3); 1, glycerol; 2, cyclic diglycerol; 3, diglycerol; 4, triglycerol; 5, 6, and 7, commercial polyglycerols.

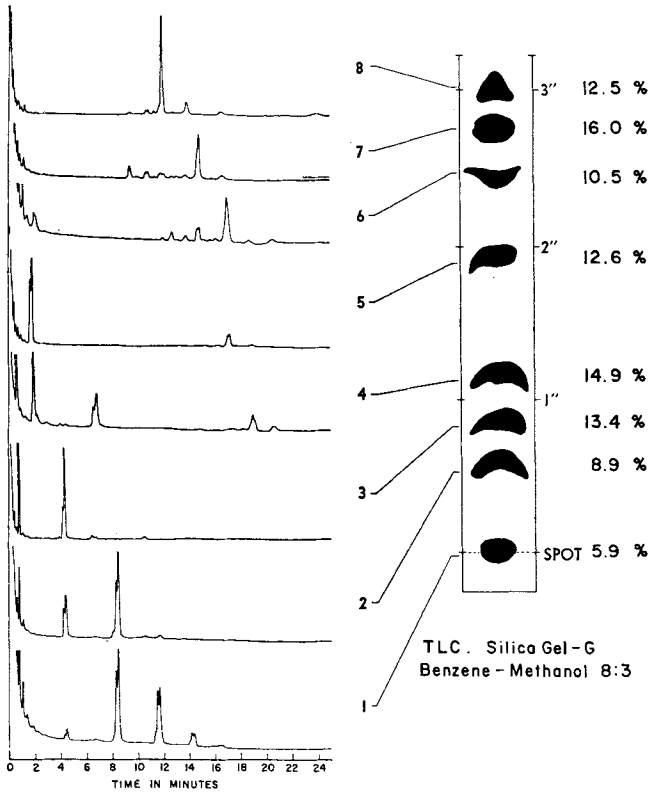

FIG. 5. Diagrammatic representation of separation of polyglycerols and polyglycerol esters by TLC and their identification by GLC: TLC fractions 1, 2, and 3, polyglycerols; 4 and 5, cyclic polyglycerols; 6, triglycerol monostearate; 7, diglycerol monostearate; 8, glycerol monostearate.

Figure 6 shows the GLC separation of polyglycerol esters as TMS ethers. Known mono- and di-fatty acid esters of glycerol, diglycerol, and triglycerol were used in this mixture.

Figure 7 shows the GLC pattern which was obtained with a commercial sample of polyglycerol esters as TMS derivatives. The identified peaks include free fatty acids, free glycerol, mono- and diglycerides, di-, tri-, and hexaglycerols, also monofatty acid esters of di- and triglycerol. Because of differences in the number of hydroxyl groups and consequently the number of TMS groups, the responses of the various polyglycerols to the hydrogen

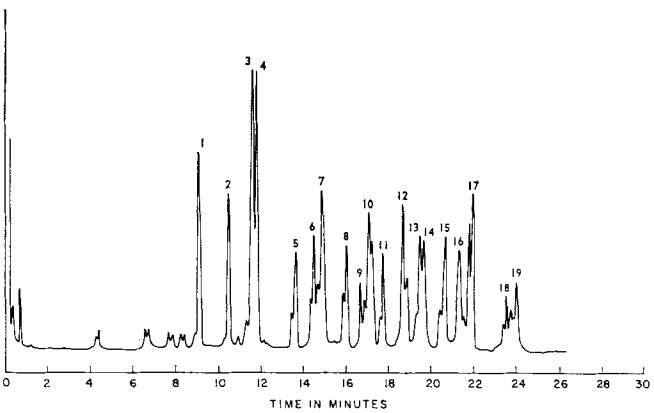

FIG. 6. GLC of TMS derivatives: α-monoglycerides 1, myristate-(M); 2, palmitate (P); 3, oleate (O); 4, stearate (S) diglycerol mono-fatty acid esters—5, P; 6, O; 7, S triglycerol mono-fatty acid esters—8, P; 9, O; 10, S α,α′ diglycerides—11, PP; 12, PS; 13, OO; 14, SS diglycerol di-fatty acid esters—15, PP; 16, OO; 17, SS triglycerol di-fatty acid esters—18, OO; 19, SS.

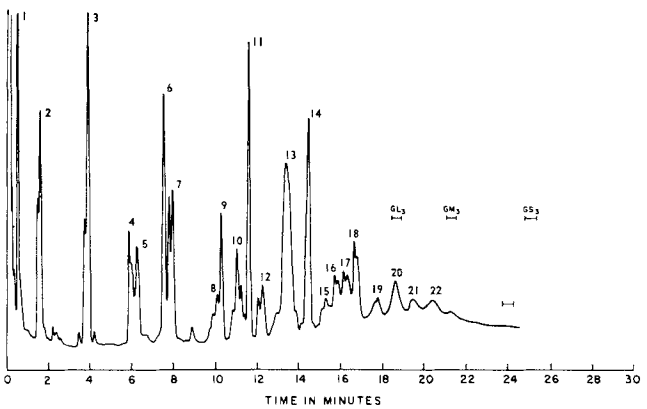

FIG. 7. TMS derivatives of a commercial sample of a polyglycerol composition: 1, glycerol; 2, cyclic diglycerol; 3, linear diglycerols; 4, palmitic acid; 5, unidentified; 6, stearic acid; 7, triglycerol; 8 and 9, glycerol monopalmitate; 10, hexaglycerol; 11, glycerol monostearate; 12, unidentified; 13 and 14, diglycerol mono-fatty acid esters; 15–18, triglycerol mono-fatty acid esters; 19–21, diglycerides; 22, diglycerol mono-fatty acid ester.

flame are different. Response factors relative to glycerol calculated as weight-to-peak-area ratios for diglycerol and triglycerol were 1.48 and 2.88 respectively.

Regulatory agencies, such as the Food and Drug Administration in the United States and the Food and Drug Directorate in Canada, require precise micro quantitative methods for the analysis of several food additives when used in mixtures. To analyze such a complex mixture, a scheme (25) has been devised employing column, thin-layer, and gas chromatographic techniques. The sample is fractionated into such lipid classes as triglycerides, diglycerides, monoglycerdes, and polyglycerols, also polyglycerol esters (26). Analysis of individual classes is achieved by GLC (21). Work on other fatty derivatives which are used as food additives is in progress and will be reported later.

ACKNOWLEDGMENTS

Technical assistance, J. J. Legari; commercial samples, Drew Chemical Corporation; suggestions and critical evaluation, W. P. McKinley.

REFERENCES

1. Howard, W. L., J. Org. Chem. *24*, 267 (1959).
2. Harris, M., U.S. Patent Nos. 2,022,766; 2,023,388 (1935).
3. Isitin, M., Ann. faculte sci. Marseille *13*, 5 (1946); C. A. *41*, 2392 (1947).
4. Rangier, M., Compt. Rend. 187, 345 (1928); C. A. *22*, 4468 (1928).
5. Roach, J. R., and H. Wittcoff, J. Am. Chem. Soc. *71*, 3944 (1949).
6. Siegel, H., A. B. Bullock and G. B. Carter, Anal. Chem. *36*, 502 (1964).
7. Summerbell, R. F., and J. R. Stephens, J. Am. Chem. Soc. *76*, 731, 6401 (1954).
8. Summerbell, R. K., and E. S. Pocklaki, JAOCS *39*, 306 (1962).
9. Troy, A., and W. G. Alsop, Ibid. *35*, 394 (1958).
10. Würzinger, J., and Gebauer, W., Brot und Geback *11*, 209 (1962).
11. Wittcoff, H., R. J. Roach and S. E. Muller, J. Am. Chem. Soc. *69*, 2655 (1947); *71*, 2666 (1949).
12. Wright H. J., and R. N. DuPuis, Ibid. *68*, 446 (1946).
13. Zajic, J., Potravinarska technologie *6*, 179 (1962).
14. Shaw, R. O. D., Anal. Chem., *35*, 1580 (1963).
15. Langer, S. H., P. Pangales and I. Wender, Chem. Ind. (London) 1664 (1958).
16. Sweeley, C. C., R. Bentley, M. M. Mikita and W. W. Wells, J. Am. Chem. Soc. *85*, 2497 (1963).
17. Luukkainen, T., W. J. A. Vander Heuvel, E. P. Haati and E. C. Horning, Biochem. Biophys. Acta *52*, 599 (1961).
18. Wells, W. W., and M. M. Mikita, Anal. Biochem. *4*, 204 (1962).
19. Mikita, M. M., and W. W. Wells, Ibid. *5*, 523 (1963).
20. Wood, R. D., P. K. Raju and R. Reiser, JAOCS *42*, 161 (1965).
21. Sahasrabudhe, M. R., and J. J. Legari, Ibid. *44*, 379 (1967).
22. McCrone, W. C. Jr., "Fusion Methods in Chemical Microscopy," Interscience Publishers Inc., Division of John Wiley and Sons Inc., New York, 1957, p. 62–63.
23. AOCS Official and Tentative Methods, 2nd ed., Chicago, 1946 method Cd 4–40.
24. Hartman, L., J. Chem. Soc. (London) 3572 (1957).
25. Sahasrabudhe, M. R., unpublished.
26. Sahasrabudhe, M. R., J. J. Legari and W. P. McKinley, J. Assoc. Off. Anal. Chem. *49*, 337 (1966).

[Received October 31, 1966]