# notes on methodology

## Gas–liquid chromatography of dialkyl, alkyl acyl, and diacyl derivatives of glycerol

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SUMMARY Dialkyl, alkyl acyl, and diacyl glycerols were resolved as trimethylsilyl ethers and as acetates by gas–liquid chromatography on a nonpolar stationary phase (OV-1). The two types of derivatives proved suitable for quantitative gas chromatographic analysis.

SUPPLEMENTARY KEY WORDS trimethylsilyl ethers · acetates · thin-layer chromatography

DIALKYL ETHERS of glycerol are known to be constituents of various biological materials, in which they occur as phospholipids. Sehgal, Kates, and Gibbons (1) have isolated di-*O*-phytanyl glycerol ether from the lipids of *Halobacterium cutirubrum*, where it occurs in the glycerophosphatides (2, 3). Marinetti, Erbland, and Stotz (4) detected 1,2-dialkyl glycerol ethers in hydrolyzates of hydrogenated bovine heart lipids, and Popović (5) found the same compounds in human heart muscle. More recently, evidence for the occurrence of dialkyl glycerophosphatides in rat brain was reported (6). Neutral derivatives of dialkyl glycerol ethers, such as dialkyl acyl glycerols, have not been conclusively identified in biological materials. Recently, a comprehensive review on glycerol ethers was published (7).

Dialkyl glycerol ethers were first synthesized by Kates, Chan, and Stanacev (8). We have synthesized a series of 1,2-dialkyl glycerol ethers differing in chain length by only two carbon atoms (9, 10). Such a homologous series is ideally suited for studying the gas chromatographic behavior of these compounds and for developing methods for their analysis.

The present communication describes the gas–liquid chromatographic analysis of 1,2-dialkyl glycerol ethers as trimethylsilyl derivatives and as acetates. The usefulness of these derivatives for the separation, isolation, and quantification of dialkyl, alkyl acyl, and diacyl glycerols is evaluated. The methods described will greatly aid in identifying these compounds in animal tissues and in other biological materials.

*Materials.* 1,2-Dialkyl glycerol ethers (9, 10), glycol monoethers, and glycol monoesters (11) were synthesized as described previously. Alkyl acyl glycerols were a gift from Doctors E. O. Oswald and C. Piantadosi, University of North Carolina, Chapel Hill, N.C. Di- and triglycerides were purchased from The Hormel Institute Lipids Preparation Laboratory, Austin, Minn. Hexamethyldisilazane was obtained from Peninsular Chemical Research, Gainesville, Fla.; trimethylchlorosilane was purchased from K & K Laboratories, Inc., Plainview, N.Y. Other chemicals and reagents were reagent grade or better and were used without further purification.

*Preparation of Derivatives.* Trimethylsilyl (TMS) ethers were prepared as described previously (12). Acetates were prepared according to the procedure described by Fritz and Schenk (13).

*Thin-Layer Chromatography.* Layers of Silica Gel G (0.25 mm thick) were spread on glass plates (20 cm × 20 cm) with a modified Colab applicator (14). The chromatoplates were air-dried and then activated for 15 min at 110°C. Development was carried out with hexane–

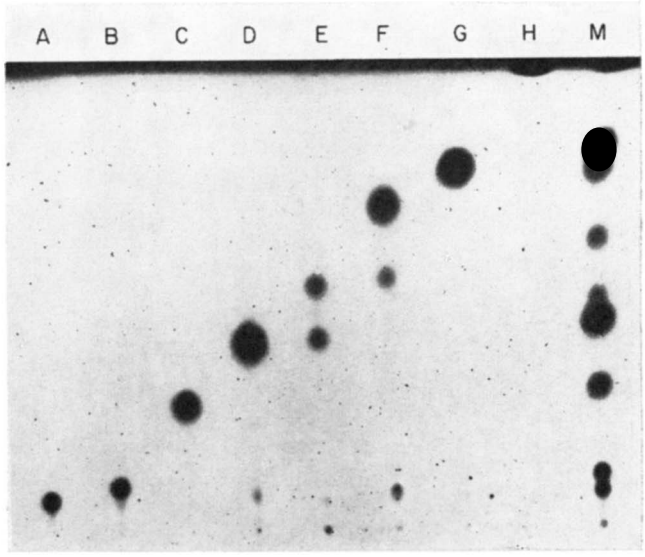


FIG. 1. Thin-layer chromatogram of dialkyl, alkyl acyl, and diacyl glycerols and related lipids. *A*, 1-monopalmitin; *B*, glycerol 1-hexadecyl ether; *C*, glycol monomyristin; *D*, tetradecyl glycol ether; *E*, 1,2- and 1,3-dipalmitin (1,2-isomer is the lower spot); *F*, 1-octadecyl-2- and 3-hexadecanoyl glycerols (1,2-isomer is the lower spot); *G*, 1-octadecyl-2-hexadecyl glycerol ether; *H*, tripalmitin; and *M*, mixture of *A*–*H*. Silica Gel G, hexane–diethyl ether–methanol 80:20:5.

Abbreviations: DE, dialkyl glycerol ether; DG, diglyceride; TG, triglyceride; TMS, trimethylsilyl; AC, acetate; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; OV-1, methyl silicone polymer.

* Under contract with the U. S. Atomic Energy Commission.

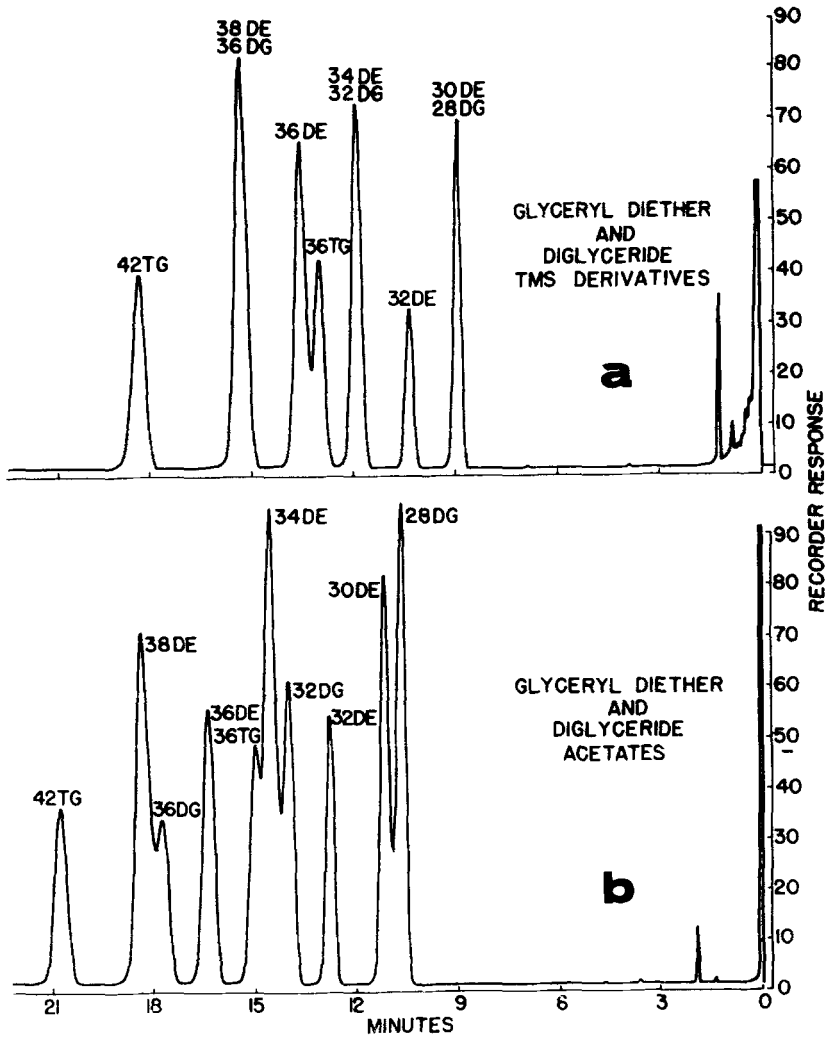


FIG. 2. Chromatograms (GLC) of trimethylsilyl ethers (*a*) and acetates (*b*) of 1,2-dialkyl glycerol ethers (DE) and 1,2-diglycerides (DG). Carbon numbers given represent the number of carbon atoms in the long-chain moieties of the parent glycerol compound. The retention times of trilaurin (36TG) and trimyristin (42TG) are given as standards. 1% OV-1 on Gas-Chrom Q, 150–275°C at 5°C/min.

diethyl ether–methanol 80:20:5 in an unlined tank. Fractions were detected by spraying with chromic–sulfuric acid and charring.

*Gas–Liquid Chromatography.* Analyses were carried out with an Aerograph model 204 instrument (Varian Aerograph, Walnut Creek, Calif.) modified for the analysis of triglycerides and other high molecular weight compounds (R. Wood, R. D. Harlow, and F. Snyder, data to be published). A Pyrex column (70 cm × 4 mm O.D., 2.5 mm I.D.) packed with 1% OV-1 coated on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories Inc., State College, Pa.) was used. Column oven temperature was manually programmed from 150 to 275°C at a rate of approximately 5°C/min. Flash heater and detector temperatures were maintained at 275 and 300°C, respectively. Helium served as carrier gas at a flow rate of 100 ml/min. Flow rates of hydrogen and oxygen were 50 and 300 ml/min, respectively. Peak areas were measured with a Datex model DIR-1 digital integrator (Conrac Corporation, Elmsfort, N.Y.).

*Results and Discussion.* Dialkyl glycerol ethers, alkyl glycerol ethers, and glycol monoethers, which can be obtained from naturally occurring lipids by hydrolysis or by reduction with lithium aluminum hydride (15), are well resolved from each other by adsorption chromatography. The thin-layer chromatogram (Fig. 1) depicting the separation of disubstituted glycerols and related lipids demonstrates, however, that 1-alkyl-2-acyl glycerols overlap with 1,3-diglycerides. The lipid classes isolated by thin-layer chromatography can be further resolved by GLC.

The TMS ethers and acetates of dialkyl, alkyl acyl, and diacyl glycerols were analyzed by GLC under conditions similar to those employed for the analysis of tri-

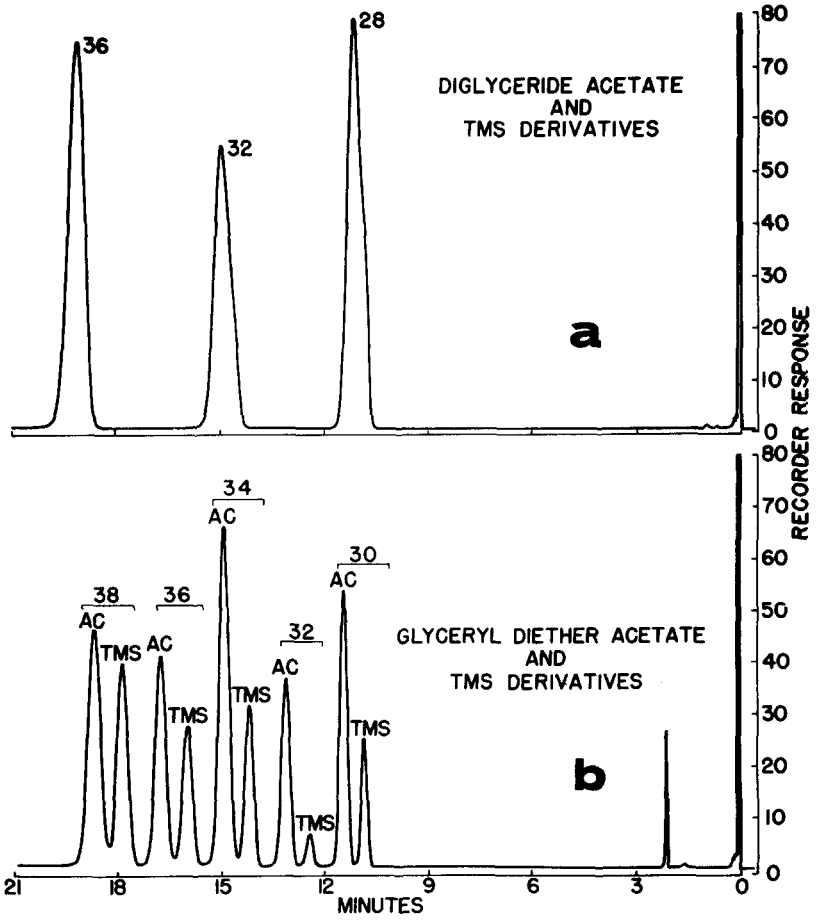


FIG. 3. Chromatograms (GLC) of TMS ethers and acetates of diglycerides (*a*) and of dialkyl glycerol ethers (*b*) analyzed as mixtures. Experimental conditions as for Fig. 2.

glycerides (16). When mixtures of derivatives of isomeric diacyl glycerols, namely 1,2- and 1,3-diglycerides, were chromatographed, broadened or shouldered peaks were obtained but complete resolution could not be achieved. Mixtures of derivatives of 1,2- and 1,3-alkyl acyl glycerols behaved in an analogous manner. Resolution of the isomeric dimyristin acetates has been reported previously (16), but a much longer column containing a higher percentage of liquid phase than that employed in the present study was used.

The TMS derivatives of dialkyl glycerol ethers exhibit retention times identical with those of the diglyceride derivatives that have two fewer methylene groups (Fig. 2a). However, the *acetates* of dialkyl glycerol ethers and diglycerides can, as a rule, be separated within each compound class and from each other (Fig. 2b). A comparison of the two chromatograms in Fig. 2 demonstrates that both types of derivatives are eluted later than triglycerides having the same carbon numbers. Interestingly, the retention times of acetates are longer than those of the TMS derivatives, although the acetates have the lower molecular weights.

The TMS derivatives and acetates of alkyl acyl glycerols are eluted between those of the dialkyl glycerol ether of the same carbon number and the next higher homologue, and between the diglyceride of the same carbon number and the preceding homologue. This is in agreement with the retention times reported for the acetates of disubstituted glycerols derived from ox brain glycerophosphatides (17). The ability to resolve the derivatives of alkyl acyl glycerols from those of diglycerides allows one to determine the percentages of each released from phosphatides by enzymatic hydrolysis.

On the nonpolar stationary phase used in this investigation, the TMS derivatives and acetates of diglycerides have very similar retention times and cannot be separated from each other (Fig. 3a). However, in the case of 1,2-dialkyl glycerol ethers the TMS derivative is eluted ahead of the corresponding acetate (Fig. 3b) despite the higher molecular weight of the TMS derivative. This is in contrast to the elution order expected for a separation on a nonpolar liquid phase, which usually separates according to molecular weights. The same elution order was also observed for the derivatives of alkyl acyl glycerols, but

TABLE 1 QUANTITATIVE DETERMINATION OF 1,2-DIALKYL GLYCEROL ETHERS AS TRIMETHYLSILYL DERIVATIVES AND ACETATES BY GLC

| Chain Length | | Carbon | Trimethylsilyl Ethers | | | Acetates | | |
|---|---|---|---|---|---|---|---|---|
| 1-Position | 2-Position | Number | Weight | Mole | Found | Weight | Mole | Found |
| | | | | % | | | % | |
| 18 | 12 | 30 | 16.8 | 18.7 | 17.0* | 18.1 | 20.0 | 18.1* |
| 16 | 16 | 32 | 8.6 | 9.1 | 8.6 | 14.5 | 15.2 | 15.3 |
| 18 | 16 | 34 | 23.3 | 23.5 | 22.8 | 24.9 | 24.9 | 24.9 |
| 18 | 18 | 36 | 19.8 | 19.1 | 21.4 | 17.7 | 16.9 | 18.8 |
| 18 | 20 | 38 | 31.5 | 29.6 | 30.6 | 24.8 | 23.1 | 22.8 |

Analyses were carried out on a Pyrex column packed with 1% OV-1. The temperature was programmed from 150 to 285°C at approximately 5°/min.
* Mean values.

the TMS derivatives and acetates were only partially resolved.

TMS ethers of dialkyl, alkyl acyl, and diacyl glycerols are eluted earlier than expected on the basis of molecular weight, relative to a triglyceride.

Recently it has been observed (18) that extremely unsymmetrical triglycerides, e.g., butyrates of long-chain diglycerides, show retarded elution times. We have observed a similar effect for diglyceride acetates. Further retardation of elution is observed in compounds of equivalent molecular weights when an ether function at the glycerol moiety is substituted for an ester group.

Shorter retention times of the TMS derivatives and the longer retention times of the acetates, relative to triglycerides, are probably the result of changes in vapor pressure, partition coefficient, and the shapes of the molecules.

Gas chromatographic analyses of both TMS derivatives and acetates of 1,2-dialkyl glycerol ethers give accurate and reproducible results. As shown in Table 1, the "area percentages" found resemble more closely the weight percentages than mole percentages. The two derivatives were equally suitable for the quantification of diacyl glycerols (19, 20), as reported previously by other investigators.

The technical assistance of R. D. Harlow and Nelson Stephens is appreciated.

*Manuscript received 5 July 1968; accepted 18 September 1968.*

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