Quantitative analysis and comparison of the physical properties of *O*-alkyl and *S*-alkyl monoethers of glycerol

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ABSTRACT A homologous series (C_{10}, C_{12}, C_{14}, C_{16}, C_{18}) of synthetic *O*-alkyl and *S*-alkyl ethers of glycerol was analyzed by gas–liquid chromatography (GLC) and thin-layer chromatography (TLC), and examined by IR and n.m.r. spectroscopy; the physical properties of the *O*-alkyl and *S*-alkyl ethers were compared. Isopropylidene derivatives of the glycerol ethers and thioethers were quantitatively analyzed by GLC on polar and nonpolar liquid phases. On a medium polar liquid phase (ethylene glycol succinate), mixtures of the *O*-alkyl and *S*-alkyl ethers were completely resolved. Isopropylidene derivatives of glycerol ethers and of thioethers could be separated as classes (though not into individual homologues) by TLC. *O*-hexadecyl and *S*-hexadecyl ethers of glycerol are easily distinguished by IR and n.m.r. spectroscopy.

SUPPLEMENTARY KEY WORDS glycerol 1-ethers · 1-thioethers · isopropylidene derivatives · gas–liquid chromatography with sulfur filter · thin-layer chromatography · IR spectroscopy · n.m.r. spectroscopy

THE OCCURRENCE, biological effects, and metabolism of naturally occurring alkyl and alk-1-enyl monoethers of glycerol have been reviewed by Snyder (1). Hemopoietic, radioprotective, wound healing, and bacteriostatic properties, as well as antitumor activity, have been attributed to these compounds (for review see reference 1), but their therapeutic effectiveness often appears minimal and even questionable. The thioethers of glycerol have been reported to exhibit certain desirable pharmacological properties (2) and might prove more effective than their *O*-alkyl analogues.

The *S*-alkyl glycerol ethers were once considered to be valuable models for studying the metabolism of *O*-alkyl ethers because the molecule could be labeled with three different radioactive atoms at three different sites: the ether linkage, the hydrocarbon chain, and the glycerol moiety. However, studies with the hydrocarbon chain labeled indicate that the *S*-ethers are metabolized differently from the *O*-ethers: a high percentage of the label was found in the urine of rats after ingestion of the *S*-alkyl ethers (3). The *S*-alkyl glycerol ethers have not been found in nature; however, this may be due to the lack of methods and sufficient knowledge of their physical and chemical properties to distinguish them from *O*-alkyl glycerol ethers. This communication describes the physical properties of the *S*-alkyl ethers, their quantitative analysis, and a method for the separation of a mixture of *O*-alkyl and *S*-alkyl ethers of glycerol.

EXPERIMENTAL PROCEDURE

Materials

The *S*-alkyl glycerol ethers were synthesized from 1-thioglycerol and the desired alkyl bromide by a modification of the Williamson reaction (4) similar to that described by Lawson, Getz, and Miller (5). The final purification was achieved by TLC on Silica Gel G in hexane–diethyl ether 80:20. Isopropylidene derivatives of the glycerol ethers were prepared by a method described previously (6). Purity was greater than 98% as determined by the gas–liquid chromatographic procedures described later in this section. The method of

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; EGSS-X, ethylene glycol succinate–methyl silicone polymer; EGS, ethylene glycol succinate; SE-30, methyl siilcone polymer.

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synthesis, purity, and the chemical and physical properties of the *O*-alkyl ethers used in this study have previously been reported (7). Both the *O*-alkyl and *S*-alkyl glycerol ethers are racemic mixtures of the 1- and 3-isomers (*sn* nomenclature).

Reagent grade diethyl ether was purchased from Fisher Scientific Co., Inc. Solvents used were glass-distilled and were purchased from Burdick and Jackson, Inc., Muskegon, Mich. Other reagents were reagent grade or better and were used without further purification.

Gas–Liquid Chromatography

A dual column Aerograph Model 204 gas chromatograph (Varian Aerograph, Walnut Creek, California) with hydrogen flame ionization detectors was used to analyze the isopropylidene derivatives of the glycerol ethers. Three different columns (5 ft × $^1/_8$ inch) were used, containing different liquid phases, as follows: (A) 5% methyl silicone polymer (SE-30) coated on 60–80 mesh Chromosorb W (Varian Aerograph); (B) 15% ethylene glycol succinate–methyl silicone polymer (EGSS-X) coated on 100–120 mesh Gas-Chrom P (Applied Science Laboratories, State College, Penna.); and (C) 15% ethylene glycol succinate (EGS) coated on 80–100 mesh D-dusted Gas-Pack WAB (Chemical Research Services, Inc., Addison, Ill.). The EGSS-X and EGS columns were Pyrex and the SE-30 column was stainless steel. The EGS and EGSS-X columns were operated isothermally at 175 and 185°C, respectively, unless otherwise indicated. The SE-30 column was temperature programmed manually from 150 to 225°C at approximately 4°C/min. The flow rate of helium carrier gas was 40–60 ml/min. Oxygen and hydrogen flow rates were regulated to give maximum detector sensitivity. Injector and detector temperatures were maintained at 290 and 250°C for all analyses. Peak areas were determined with a Datex Model DIR-1 digital integrator (Conrac Corp., Duarte, Calif.) and by the triangulation method. Percentages represent the mean of three determinations.

Thin-Layer Chromatography

Silica Gel G layers, some impregnated with sodium arsenite and boric acid, were prepared as described previously (7). The glycerol ethers were chromatographed in chloroform–methanol 98:2 and their isopropylidene derivatives in hexane–diethyl ether 90:10.

IR Spectroscopy

The IR spectra were recorded with a Perkin-Elmer Model 337 spectrophotometer (Norwalk, Conn.). 500-mg KBr discs (13 mm) containing 1.0–1.2 mg of sample were used to obtain the spectra.

Nuclear Magnetic Resonance Spectroscopy

The n.m.r. spectra were obtained with a Varian A-60 high resolution spectrometer (Varian Associates, Palo Alto, Calif.). Spectra of the *S*-alkyl ethers were recorded from a 15% solution of each compound in carbon tetrachloride at 40°C. The *O*-alkyl ether spectra were recorded earlier (7) on the same instrument under similar conditions. The resonance peak of tetramethylsilane was assigned the value of zero parts per million (ppm).

RESULTS AND DISCUSSION

Gas–Liquid Chromatography

Hanahan, Ekholm, and Jackson (8) were the first to employ the isopropylidene derivatives of glycerol ethers for GLC analyses. However, the quantitative aspects were not investigated. Isopropylidene derivatives of both *O*-alkyl and *S*-alkyl ethers of glycerol give quantitative results on polar and nonpolar liquid phases (Table 1). Experimental values agreed more closely with known weight percentages than with mole percentages.

Mixtures of isopropylidene derivatives of *O*-alkyl and *S*-alkyl ethers can be analyzed simultaneously by GLC. Representative chromatograms obtained for such mixtures on all three liquid phases are shown in Fig. 1. The *O*-alkyl and *S*-alkyl glycerol ethers were completely resolved on the EGS column (Fig. 1C). A glycerol thioether, which differs from its *O*-alkyl ether analogue by a molecular weight of 16, was expected to be eluted between the *O*-alkyl ether with the same carbon chain length and the next higher homologue, but in fact it was

TABLE 1 QUANTIFICATION OF STANDARD MIXTURES OF ISOPROPYLIDENE DERIVATIVES OF *O*-ALKYL AND *S*-ALKYL GLYCEROL ETHERS BY GLC

No. of Carbons in Side-Chain	Known Percentages		Percentages Found with Each Phase		
	Mole	Weight	EGS*	EGSS-X†	SE-30‡
			O-alkyl ethers		
10	15.5	12.2	11.2	14.7	12.2
12	18.5	16.4	16.5	17.5	16.7
14	20.3	19.9	20.6	20.3	20.0
16	21.8	23.4	23.9	22.5	23.6
18	24.0	28.1	27.7	25.0	27.5
			S-alkyl ethers		
10	14.1	11.2	12.6	13.1	12.2
12	15.9	14.0	14.8	14.3	14.9
14	18.6	18.1	18.2	17.7	18.5
16	25.2	26.6	26.0	26.0	26.3
18	26.2	30.1	28.2	28.9	27.9

* At 175°C. Percentages determined with a digital integrator.
† At 185°C. Percentages determined by triangulation.
‡ Manually programmed from 150 to 225°C at approximately 4°C/min. Percentages determined with a digital integrator.

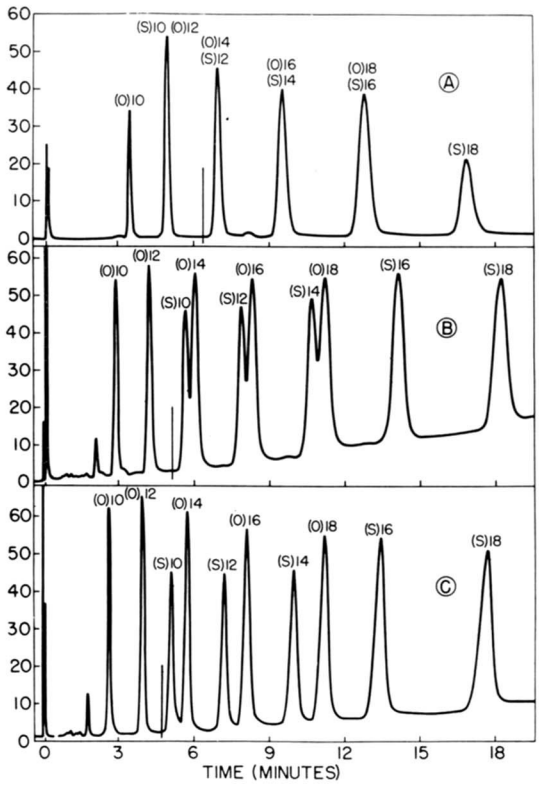

FIG. 1. Gas–liquid chromatograms of isopropylidene derivatives of mixtures of *O*-alkyl and *S*-alkyl glycerol ethers on SE-30 (*A*), EGSS-X (*B*), and EGS (*C*). Peaks are identified by a letter and a number; (*O*) and (*S*) represent *O*-alkyl and *S*-alkyl ethers respectively and the number represents the number of carbon atoms in the aliphatic chain. SE-30, temperature programmed from 150 to 225°C; EGSS-X and EGS, temperature manually programmed from 125 to 185°C. The approximate retention times of methyl stearate are indicated by the vertical lines.

eluted later. The retarded elution due to the replacement of an oxygen atom with a sulfur atom is probably attributable to changes in solubility, vapor pressure, and polarity of the molecule. On polar columns, the thioethers were eluted immediately before *O*-alkyl ethers that contained four more carbon atoms in their chain.

Isopropylidene derivatives of *S*-alkyl glycerol ethers were also analyzed by GLC using a Melpar flame photometric detector equipped with a sulfur filter, which allowed nanogram quantities of sulfur-containing compounds to be detected in the presence of other organic compounds, including *O*-alkyl glycerol ethers. This equipment is ideally suited for examining naturally occurring lipids for thioethers. We are indebted to Dr. J. F. O'Donnell, Tracor Inc., Austin, Tex. for these analyses.

Thin-Layer Chromatography

In general, it would be necessary to separate mixtures of the *O*-alkyl and *S*-alkyl ethers by TLC before GLC analysis, because unsaturation in the carbon chain causes overlapping of peaks, and simultaneous analysis of the two lipid classes is difficult. Several solvent systems and adsorbents at various stages of activation were tried for the separation of free *O*-alkyl and *S*-alkyl ethers. Boric acid-impregnated plates, used for the separation of isomeric glycerol ethers (7), gave marginal separation (the thioethers appeared between 1- and 2-isomers), but the system was unsatisfactory for mixtures of various chain lengths because of spot elongation and incomplete resolution. Isopropylidene derivatives could, however, be separated by TLC as shown in Fig. 2. Unexpectedly, the isopropylidene derivatives of the thioethers move faster than the *O*-alkyl ethers, although they would be expected to be more polar.

IR Spectroscopy

Infrared spectra of the *O*-hexadecyl and *S*-hexadecyl ethers of glycerol are compared in Fig. 3. The spectrum of the *O*-alkyl ether is practically identical with that reported previously (7). Major differences in the absorption patterns of the *O*-alkyl and *S*-alkyl glycerol ethers appear between 7.0 and 15.0 μ. The *O*-alkyl ether exhibits strong ("ether") absorption at 8.85 μ, whereas the thioether absorbs (less strongly) at 9.0μ. Primary hydroxyl absorption at 9.4 μ, prominent in the *O*-alkyl ether, is weaker in the thioether. The thioether also shows strong absorption at 6.98 and 11.4 μ, which is ab-

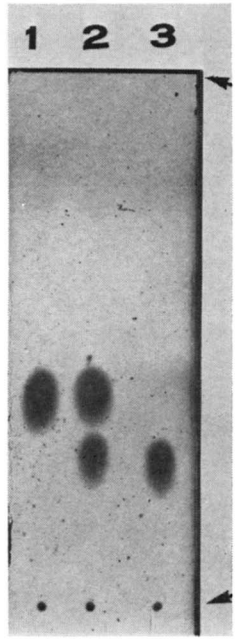

FIG. 2. Thin-layer chromatogram of isopropylidene derivatives of *O*-alkyl and *S*-alkyl glycerol ethers on Silica Gel G in hexane–diethyl ether 9:1. (*1*) *S*-alkyl ethers (C_{10}, C_{12}, C_{14}, C_{16}, and C_{18}); (*3*) *O*-alkyl ethers (C_{10}, C_{12}, C_{14}, C_{16}, and C_{18}); and (*2*) mixture of (*1*) and (*3*). The origin (bottom) and solvent front (top) are indicated by arrows.

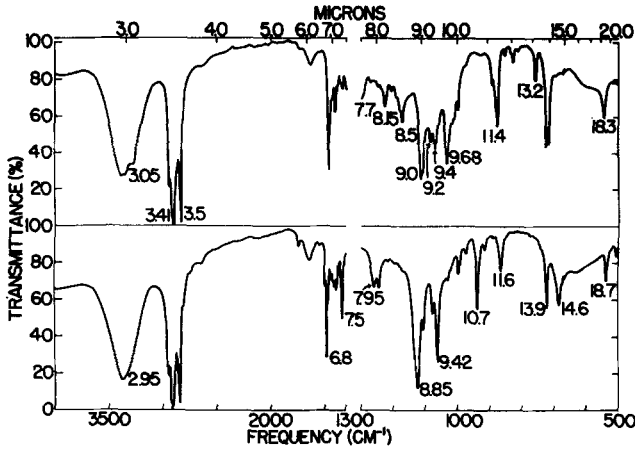

FIG. 3. IR spectra of 1-hexadecyl glycerol thioether (top) and 1-hexadecyl glycerol ether (bottom).

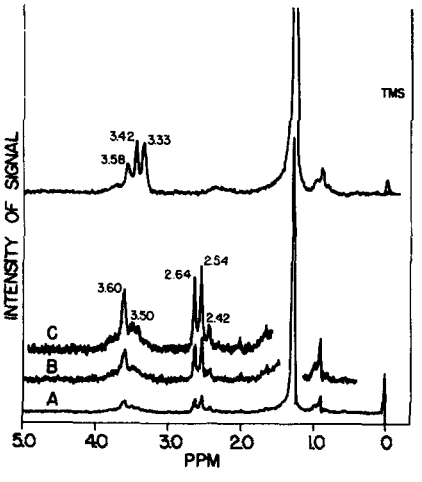

FIG. 4. Proton resonance spectra of 1-hexadecyl glycerol ether (top) and 1-hexadecyl glycerol thioether (bottom) obtained at 60 mHz. Amplification: (*A*) 3.2, (*B*) 8.0, and (*C*) 16.0.

sent from the *O*-alkyl ether spectrum; other characteristic peaks of the thioether are at 7.7, 8.15, 8.5, 13.2, and 18.3 μ.

Nuclear Magnetic Resonance Spectra

The n.m.r. spectra of *O*-hexadecyl and *S*-hexadecyl glycerol ethers are shown in Fig. 4. The thioether spectrum is readily distinguishable from the ether spectrum by the shift upfield of the resonance of the methylene protons adjacent to the sulfur atom (2.64, 2.54, and 2.42 ppm). Hydroxyl proton resonance appears at 3.6 ppm in the presence of the proton resonance of the glycerol methylene vicinal to the sulfur linkage. Spectra of the *O*-alkyl ethers of glycerol have been discussed previously (6). The glycerol thioethers appear to be useful for studying the complex splitting patterns exhibited by glycerol in lipid molecules.

Significance of the Methods

N.m.r. and IR spectroscopy, GLC, and TLC allow one to isolate, characterize, identify, quantify, and distinguish the *O*-alkyl and S-alkyl ethers of glycerol. The methods can be used to determine the occurrence of thioethers in nature and to purify these ethers and their derivatives for other biological investigations.

This investigation was supported in part by the U.S. Atomic Energy Commission and by National Institutes of Health Grant GM 12562-04.

Manuscript received 19 December 1968; accepted 7 March 1969.

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