Synthesis of ^{35}S- and ^{14}C-Labeled Glyceryl Thioethers

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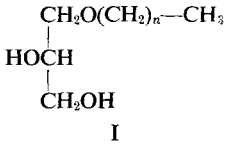
Abstract □ The racemic ^{35}S- and ^{14}C-labeled alpha derivatives of octadecyl glyceryl thioether and hexadecyl glyceryl thioether of high specific activity and purity were prepared. Synthesis was carried out by treating the appropriate alkyl halide with an excess of 1-thioglycerol in the presence of KOH or NaOH. The labeled glyceryl thiomonoethers (*S*-ethers) were isolated and purified from the reaction mixtures by silicic acid column chromatography. The radiopurity was determined by zonal TLC.

Keyphrases □ Glyceryl thioether, ^{35}S-, ^{14}C-labeled—Synthesis □ Column chromatography—Separation □ TLC—identity □ Scintillometry, liquid—radioactivity, purity

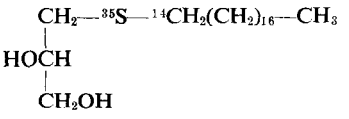
A significant part of glyceryl derivatives encountered in natural lipids belong to the *O*-ether series. Tsujimoto and Toyama (1) were the first to report the occurrence of glyceryl ether in the nonsaponifiable fractions of liver lipids of elasmobranch fishes. The three most common glyceryl ethers (I) are 16:0-1, 18:0-1, and 18:1-(9c)-1, and commonly referred to as chimyl alcohol, batyl alcohol, and selachyl alcohol, respectively.



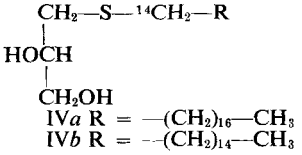
I

n = 15 (16:0-1)
n = 17 (18:0-1)
n = 17 (18:1-1)

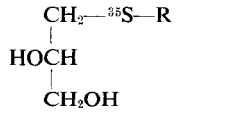
In connection with the authors' interests (2) on the biosynthesis and mechanism of biocleavage of the ether linkage in glyceryl ethers, they have prepared glyceryl thiomonoethers (II–IV) labeled with ^{35}S and ^{14}C in the long aliphatic hydrocarbon chain in order to compare their metabolic fate with that of oxygen analogs (I).



II



IV*a* R = —$(CH_2)_{16}$—CH_3
IV*b* R = —$(CH_2)_{14}$—CH_3



III*a* R = —CH_2—$(CH_2)_{16}CH_3$
III*b* R = —CH_2—$(CH_2)_{14}$—CH_3

The synthetic scheme used in the preparation of the labeled glyceryl thioethers was based on the Williamson reaction for the synthesis of ethers in general. The authors have used a modification of this reaction as have others (3) for the preparation of unlabeled glyceryl thiomonoethers. The chemical and physical properties (4) and the *in vivo* metabolism (5) of glyceryl thioethers have just recently been reported by the present authors.

EXPERIMENTAL

Material—The 1-bromohexadecane and 1-bromooctadecane were white label chemicals (Eastman). Unlabeled 1-thioglycerol (95% pure)[1] was used as were labeled halides and ^{35}S-1-thioglycerol (85% pure).[2]

Chromatography—*Silicic Acid Columns*—The silicic acid was washed with distilled water several times to remove fine particles. It was filtered and dried at 110° for 48 hr.; 10 g. of the silicic acid was used to prepare columns (20 × 200) having good separation properties and a fast flow rate (2–3 ml./min.).

Thin-Layer (TLC)—TLC plates were prepared with Silica Gel G as described by Mangold (6). All of the labeled compounds in Table I had R_f values indentical to the corresponding unlabeled compounds.

Radiopurity and Specific Activity Determinations—Zonal profile scans (7) (Fig. 1. A = DL-1-^{35}S-thiohexadecyloxy-2,3-propanediol; B = DL-1-^{35}S-thio-1′-^{14}C-octadecyloxy-2,3-propanediol) were prepared after resolution of the ^{35}S- and ^{14}C-labeled compounds on Silica Gel G in a solvent system of ethanol–ammonium hydroxide–water (80:4:16) and diethyl ether–water (100:0.5). All radioassay measurements were made in liquid scintillation spectrometers having efficiencies of 62% for ^{35}S and 75% for ^{14}C in a scintillation solution described earlier (8). Efficiency determinations were based on standard compounds, 0.01 *N* $H_2{}^{35}SO_4$ in 0.1 *N* HCl (Amersham/Searle Corp.) and toluene-^{14}C.[2]

Synthesis—Optimum synthesis conditions were determined by making several unlabeled glyceryl thioethers to standardize the yield and purity before the actual labeled materials were used. The melting points were taken (Mel-Temp apparatus) and agree with the literature (3). Carbon and hydrogen analyses were performed.[3] Physical constants are listed in Table I.

DL-1-^{35}S-Thio-1′-^{14}C-octadecyloxy-2,3-propanediol (II)—Using a 30-ml. reaction filter flask equipped with magnetic stirrer, 264 mg. (2.4 mmoles) of ^{35}S-thioglycerol (8.2 mc.) in 5 ml. of methanol and 114.8 mg. (0.34 mmole) of octadecyl-1-^{14}C-bromide (2.00 mc.) and 218.6 mg. (0.66 mmole) of unlabeled octadecyl bromide in 12 ml. of hexane were stirred under nitrogen. Next 2.6 ml. of 1 *N* alcoholic KOH were added and the reaction mixture stirred at room temperature for a period of 24 hr. Additional (23 ml.) ethanol was added from time to time in order to keep the reaction mixture homogeneous. After completing the reaction, the mixture was cooled and diluted with 20 ml. of cold distilled water; the resulting precipitate was filtered under nitrogen, then washed with 60 ml. of cold distilled water and finally dried *in vacuo* over P_2O_5. The white precipitate (340 mg.) was dissolved in 10 ml. of warm benzene and applied to a 10-g. silicic acid column. Three fractions were eluted and collected with the following solvents: Fraction I—150 ml. benzene; Fraction II—150 ml. chloroform–benzene (75:25 v/v); Fraction III–130 ml. chloroform–methanol (2:1 v/v). Fraction III contained 262 mg. of labeled glyceryl thioether derivative (72% yield).

DL-1-^{35}S-Thiooctadecyloxy-2,3-propanediol (IIIa)—An analogous procedure was used for the preparation of IIIa with the following exceptions: 132 mg. (1.19 mmoles) of -^{35}S-thioglycerol (3.5 mc.) in 2.25 ml. of methanol, 248 mg. (0.74 mmole) of octadecyl bromide and 1.8 ml. of 1 *N* alcoholic KOH. After purification by silicic acid column chromatography, Fraction III contained 111.8 mg. (37%) of labeled glycerol thioether.

[1] Evans Chemetics, Inc., New York, N. Y.
[2] New England Nuclear Corp., Boston, Mass.
[3] Spang Microanalytical Laboratory, Ann Arbor, Mich.

Table I—Thioglyceryl Ethers

Compound	M.p., °C.	Yield, %	Radio Purity, %	Specific Activity, mc./m*M*	Anal., % Calcd.	Found
II	75–75.5[a]	72	>98	^{35}S-3.50 ^{14}C-1.35	C, 69.93 H, 12.29 8.89	C, 69.88 H, 12.34 S, 8.90
III*a*	75–75.5	37	>98	^{35}S-2.84	C, 69.93 H, 12.29 S, 8.89	C, 69.87 H, 12.40 S, 8.88
III*b*	75–76.5[b]	25	>98	^{35}S-2.07	C, 68.61 H, 12.12 S, 9.64	C, 68.56 H, 12.22 S, 9.62
IV*a*	75–75.5	66	>99	^{14}C-1.21	C, 69.93 H, 12.29 S, 8.89	C, 69.90 H, 12.41 S, 8.87
IV*b*	75–76.5	79	>99	^{14}C-1.23	C, 68.61 H, 12.12 S, 9.64	C, 68.54 H, 12.23 S, 9.61

[a] *Reference* 3, m.p. 74–75°. [b] *Reference* 3, m.p. 76–77°.

DL-1-^{35}S-Thiohexadecyloxy-2,3-propanediol (IIIb)—The same procedure as described for the preparation of II was used with the following exceptions: 134 mg. (1.19 mmoles) of -^{35}S-thioglycerol (3.5 mc.) in 2.5 ml. of methanol, 218 mg. (0.6 mmoles) of hexadecyl bromide and 1.8 ml. of 1 *N* alcoholic KOH. After purification by silicic acid column chromatography, Fraction III contained 83 mg. (25%) of labeled glycerol thioether.

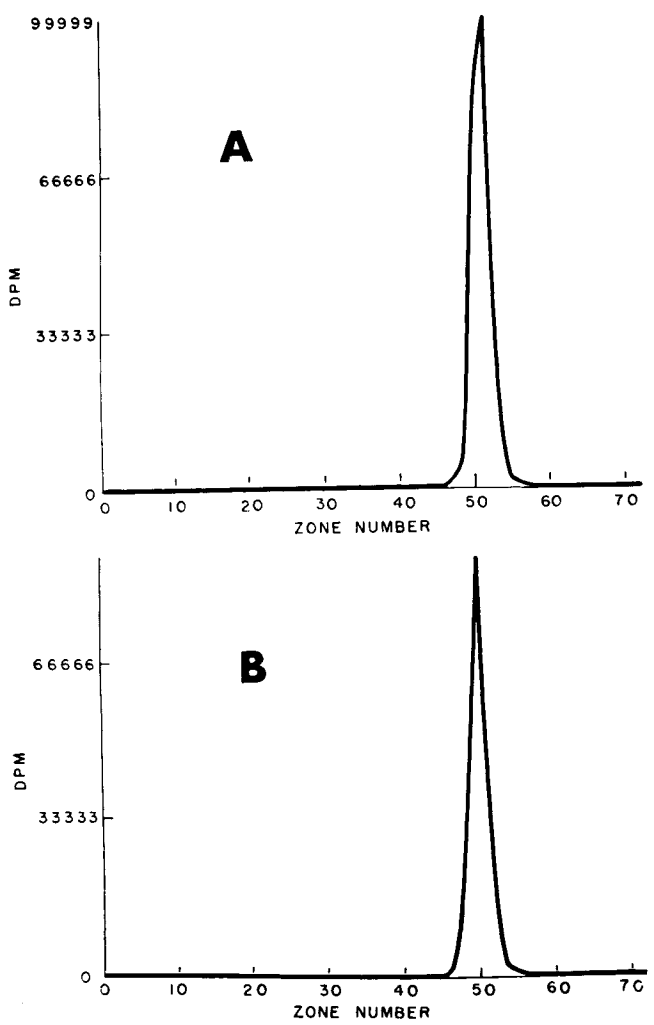


Figure 1—*Zonal profile scans (2-mm.) of thin-layer chromatograms used to resolve (A)* DL-**1**-^{35} *S-thiohexadecyloxy-2,3-propanediol and (B)* DL-*1*-^{35}*S-thio-1'*-^{14}*C-octadecyloxy-2,3-propanediol. Chromatography was carried out on Silica Gel G in a solvent system of ethanol–ammonium hydroxide–water, 80–4–16 (v/v).*

DL-1-Thio-1'-^{14}C-octadecyloxy-2,3-propanediol (IVa)—The same procedure as described for the preparation of II was used with the following exceptions: 174 mg. (1.55 mmoles) of 1-thioglycerol in 5 ml. of methanol, 85 mg. (0.26 mmole) of octadecyl-1-^{14}C-bromide (2.00 mc.), 180 mg. (0.54 mmole) of unlabeled octadecyl bromide, and 2.0 ml. of 1 *N* NaOH. After purification by silicic acid column chromatography, Fraction III contained 208 mg. (66%) of labeled glyceryl thioether.

DL-1-Thio-1'-^{14}C-hexadecyloxy-2,3-propanediol (IVb)—The same procedure as described for the preparation of II was used with the following exceptions: 172 mg. (1.53 mmoles) of 1-thioglycerol, 69.4 mg. (0.23 mmole) of hexadecyl-1-^{14}C-bromide, 174.7 mg. (0.57 mmole) of unlabeled hexadecyl bromide in 5 ml. of methanol, and 2.0 ml. of 1 *N* NaOH. After purification by silicic acid column chromatography, 266 mg. (79%) of labeled glyceryl thioether was obtained.

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