SYNTHESIS AND PROPERTIES OF ^{35}S, ^{14}C AND ^{3}H LABELED S-ALKYL GLYCEROL ETHERS AND DERIVATIVES

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Radioactive S-alkyl glycerol ethers have been synthesized with ^{35}S, ^{14}C and ^{3}H labels as well as ^{3}H/^{35}S double labels.

The synthesized compounds were converted to various derivatives which can serve to characterize the S-alkyl glycerol ethers. These included the isopropylidene derivative, oxidation with periodate to the aldehyde followed by reduction with LiAlH$_4$ to the alcohol, and reaction of the alcohol with acetic anhydride to form the acetate derivative.

Chemical analysis, IR, NMR, zonal TLC profile scans and GLC showed all the products to be >99% pure.

The GLC behavior of the aldehyde and acetate derivatives of both S-alkyl glycerol ethers and O-alkyl glycerol ethers on EGSS-X was compared.

1. Introduction

S-Alkyl glycerol ethers have recently been identified as naturally occurring lipids in heart tissue [1,2]. The synthesis of alkyl glycerol thioethers [3] and their metabolism in the rat [4] and *Tetrahymena pyriformis* [5] has been described. Some preliminary work on the biosynthesis of S-alkyl glycerol ethers has been carried out [6–8].

During the course of isolating and investigating the biosynthesis of these ethers several sulfur containing lipids have been synthesized and characterized. Earlier work by Piantadosi et al. [3] describe the synthesis of radiolabeled S-alkyl glycerol ethers containing a double label of ^{35}S and ^{14}C. However, since the energy spectra of these isotopes are almost identical one cannot differentiate between them using standard liquid scintillation techniques [9]. We therefore synthesized S-alkyl glycerol ethers with a double label of ^{35}S and ^{3}H.

Various derivatives, useful for the characterization of S-alkyl glycerol ethers have also been synthesized and some of their spectral and chromatographic properties examined.

$CH_3(CH_2)_nCH_2OH \longrightarrow CH_3(CH_2)_nCH_2Br$
II

$CH_3(CH_2)_nCH_2SH$
III

CH_2SH
$CH_2OH \xrightarrow{\text{II}} CH_2-S-CH_2(CH_2)_nCH_3$
CH_2OH CH_2OH IV
                        CH_2OH

$CH_2-S-CH_2(CH_2)_nCH_3$ $CH_2-S-CH_2(CH_2)_nCH_3$
$\overset{C}{\underset{H}{\diagdown}}O$ VI $HC-O\diagdown\diagup CH_3$
                                         $H_2C-O\diagup C\diagdown CH_3$
                                                    V

$CH_2-S-CH_2(CH_2)_nCH_3$ $CH_2-S-CH_2(CH_2)_nCH_3$
$H_2COH \longrightarrow$ $H_2C-O-C CH_3$
                                            O
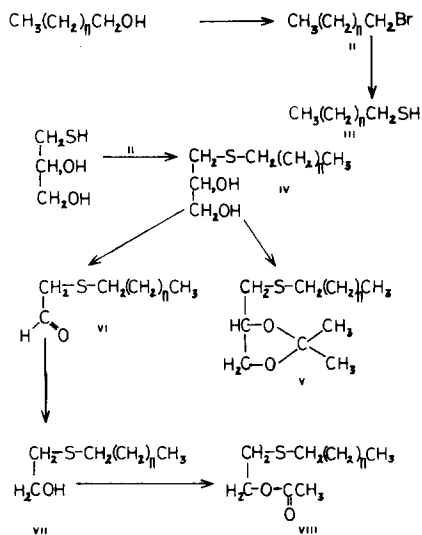VII   VIII

Fig. 1. General reactions and structures for compounds described in the text.

The different reactions and products described in this paper are summarized in fig. 1.

II. Experimental

A. Materials

All solvents and chemicals used in this work were of the highest purity. Solvents and alkyl bromides were purchased from Eastman. Aldrich 1-thioglycerol was used and silica gel G, gas liquid column packing and hexadecanol were from Applied Science. Radio-labeled compounds were obtained from New England Nuclear.

IR spectra were made neat, between KBr plates, on a Perkin-Elmer 457 spectrophotometer. NMR spectra were made in $CDCl_3$ or CCl_4 on a Varian A60A instrument with tetramethylsilane as an internal standard. Elemental analyses were performed by MHW Laboratories, Garden City, Michigan. GLC was carried out on a 6 ft column of 15% EGSS-X on Gas Chrom P as previously described [1] using a Barber-Colman 5360 instrument. Unless indicated the column temperature was 195°C. When radioactive samples were chromatographed standards were added prior to injection so that enough material was present for detection. A 10 : 1 stream splitter was used and a Packard Model 850 fraction collector was adapted to the instrument. The effluent gas was trapped in glass cartridges containing p-terphenyl crystals coated with DC-550 (Packard Instrument Co.). Each cartridge was placed

directly in a counting vial and 15 ml of scintillation cocktail added, and the vials shaken vigorously for 1 min. Radioactivity recoveries were from 75% to 80%. Radioactivity measurements were made using a Searle Analytic Isocap 300 instrument with a cocktail consisting of 0.4% diphenyloxazole and 0.1% p-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene in toluene. Efficiencies determined by an automatic external standard were 89% for ^{14}C and ^{35}S alone and 38% for ^{3}H, 67% for ^{35}S in the double labeled experiments. TLC radioactivity zonal profile scans were made using a Varian Aerograph LB 2723 instrument. Autoradiograms were made using Kodak No Screen X-ray film against a TLC plate using another glass plate to hold the film in close contact. The plates and film were placed in an autoradiogram holder (Supelco, Inc.) and placed in the refrigerator for 1 to 4 days. The film was developed using Kodak X-ray developer and fixer.

1. [9, 10-³H] or [1-¹⁴C] Hexadecanol (I)

[9, 10-^{3}H] palmitic acid (250 mCi/m mole) or [1-^{14}C] palmitic acid (53 mCi/m mole) was reduced to the alcohol and the product isolated and purified as previously described [10].

2. [9, 10-³H] or [1-¹⁴C] 1-Bromohexadecane (II)

Bromoalkanes are most conveniently prepared by saturating the corresponding alcohol with dry hydrogen bromide at 100–120°C [11]. For larger amounts of unlabeled material, hexadecanol was kept at 110°C, in a 10 ml round bottom flask, and flushed with HBr produced from tetrahydronaphthalene and bromine [12]. After 3 h excess HBr is displaced with N_2 and the bromide dissolved in hexane. Unreacted alcohol is removed using a 1 × 5 cm column of silica gel G and eluting with 40–50 ml of hexane. The bromide comes off the column in the hexane while the alcohol remains on the column. For the microsynthesis of labeled bromide, labeled hexadecanol was dissolved in 1.5 ml of 48% HBr and the generated HBr bubbled though this solution. The purity of the labeled 1-bromohexadecane was >99% as checked by a zonal profile scan of a TLC plate developed in hexane. All of the radioactivity cochromatographed on GLC with standard 1-bromohexadecane. Unlabeled 1-bromohexadecane was synthesized with a yield of 79%.

3. [9, 10-³H] 1-Hexadecanothiol (III)

This compound was prepared from the above bromide essentially as described by Urquhart et al. [13], typically 6 μmoles of [9, 10-^{3}H] 1-bromohexadecane and 16 μmoles of thiourea were dissolved in 2 ml of ethanol and refluxed in a nitrogen atmosphere for 3 h. Then 0.05 ml of 10% NaOH was added and the refluxing continued for another 2 h, after which 5 ml of water were added and the mixture acidified with 5N H_2SO_4. The 1-hexadecanethiol was extracted with hexane and purified

by TLC with hexane as the developing solvent. In the event that any disulfide was formed it was converted to the thiol by reduction with $LiAlH_4$ or $NaAlH_2(OCH_2$ $CH_2OCH_3)_2$ (vitride) [14]. It should be noted that double labeled [^{35}S-9, 10-^{3}H] 1-hexadecanethiol was synthesized using [^{35}S] thiourea.

The yield for this synthesis was 89% based on results using unlabeled materials. The radiopurity was found to be >99% using a zonal profile scan of a TLC plate developed in hexane. All of the radioactivity co-chromatographed on GLC with standard 1-hexadecanethiol.

4. DL-1-[^{35}S-9, 10-^{3}H]-Thiohexadecyloxy-2, 3-propanediol (IV)

(S-Alkyl glycerol ether). Using [^{35}S] thioglycerol (10 mCi) and the previously synthesized [9, 10-^{3}H] 1-bromohexadecane (5 mCi), double labeled S-alkyl glycerol ether was synthesized in 83% yield using essentially the method described by Piantadosi et al. [3]. ^{14}C labeled alkyl glycerol thioether can be synthesized using ^{14}C labeled 1-bromohexadecane and thioglycerol. Following purification on a silicic acid column [1] the radiopurity of the product was found to be >99% by a zonal profile scan of a TLC plate developed in hexane : diethyl ether : acetic acid (30 : 70 : 1, v/ v/v). In addition, all the radioactivity was found to be associated with the various derivatives now to be described. *Anal.* Calcd: C, 68.62; H, 12.03; S, 9.64. Found: C, 68.59; H, 12.17; S, 9.59. These values are from synthetic non-radioactive products.

5. DL-1-[^{35}S-9, 10-^{3}H]-Thiohexadecyloxy-2, 3 isopropylidene propanediol (V)

(S-Alkyl glycerol ether isopropylidene derivative). This derivative has been synthesized from the corresponding S-alkyl glycerol ether in 97% yield. The thioether (up to 20 mg) was dissolved in 2.5 ml of acetone and 1.5 μl of 60% perchloric acid added and the mixture kept at room temperature for 30 min with intermittent stirring. The mixture was neutralized with 1N NH_4OH, 2 ml of water was added and the product extracted with $CHCl_3$. The small amount of unreacted thioether was removed by TLC of the product in hexane: diethyl ether : acetic acid (30 : 70 : 1 v/v/v). This procedure was a modification of that reported by others [15]. Following purification a zonal profile scan showed the radiopurity to be >99%. On GLC of the product all the radioactivity was associated with standard material. Isopropylidene derivatives of S-alkyl glycerol ethers with alkyl chainlengths of 14, 16, 17 and 18 carbons have all been synthesized in about the same yields. It was found that isopropylidene derivatives could be converted back to the ethers in essentially 100% yield by exposure to HCl gas [16].

6. [^{35}S-9, 10-^{3}H] Hexadecyl thioacetaldehyde (VI)

Oxidation of the corresponding S-alkyl glycerol ether with $NaIO_4$ gave the S-alkylthioacetaldehyde derivative an 83% yield. Typically, up to 10 mg of S-alkyl

glycerol ether was dissolved in 1 ml of alcoholic periodate solution (95% ethanol saturated with $NaIO_4$) and kept at room temperature for 30 min with intermittent mixing. Then 2 ml of water was added and 1 ml of each of 1 N $NaAsO_2$ and 5 N H_2SO_4. The aldehyde derivative was extracted with hexane: diethyl ether (1 : 1 v/v) or $CHCl_3$ and purified by TLC in hexane : chloroform : methanol (75 : 25 : 2 v/v/v) [17]. A zonal profile scan and GLC of purified radiolabeled product showed it to be >99%.

7. [^{35}S-9, 10-^{3}H] Hexadecyl ethylene thioglycol (VII)

The acetaldehyde derivative just described was reduced to the corresponding alcohol using vitride [14]. The yield for this reaction was 86%. Products were purified by TLC in diethyl ether : 30% ammonium hydroxide (100 : 0.25 v/v). A zonal profile scan of the radiolabeled material showed purities of >99%. *Anal.* Calcd: C, 71.52; H, 12.57; S, 10.61. Found: C, 71.36; H, 12.64; S, 10,69. Values from synthetic non-radioactive product.

8. [^{35}S-9, 10-^{3}H] Hexadecyl ethylene thioglycol acetate (VIII)

The acetate derivative of the corresponding S-ethanol-alkylthiol was made in quantitative yields. The alcohol was placed in a 5 ml microflex tube and 2 ml of acetic anhydride added and 0.5 ml of pyridine. The tube was sealed and kept in a 100°C oven for 1 h. After cooling, 2 ml of water was added and the product extracted with diethyl ether and purified by chromatography in a 2 × 15 cm silicic acid column. Elution was with 60 ml of hexane: ether (2 : 1 v/v); the first 15 ml were discarded. A zonal profile scan of the purified material and GLC showed it to be >99% pure.

II. Results and discussion

The TLC zonal profile scans of synthesized compounds I, II and III are shown in fig. 2. The R_f values of the compounds using hexane as the developing solvent were: hexadecanol (I), 0; 1-hexadecanethiol (III), 0.44 and 1-bromohexadecane (II), 0.65.

The IR spectra for compounds I, II and III gave the expected results. III showed a weak absorption band at 2570 cm^{-1}, characteristic of S-H stretching.

The IR spectrum and NMR spectrum of DL-1-S-thiohexadecyloxy-2, 3-propanediol (IV) were identical to that obtained by Wood et al. [15], who compared the spectra of S-alkyl and O-alkyl glycerol ethers.

We determined the NMR spectrum of IV in $CDCl_3$ and it can be described as follows: $T_{TMS}^{CDCl_3}$ 3.65 (br.m., 3, -CH_2-CHOH-CH_2OH); 2.55 (br.m., 6, -CH_2-S-CH_2-CHOH-CH_2OH); 1.8-0.7 (sh.m., 31, -(CH_2)$_{14}$-CH_3.

We do not completely agree with the spectrum interpretation of Wood et al. [16].
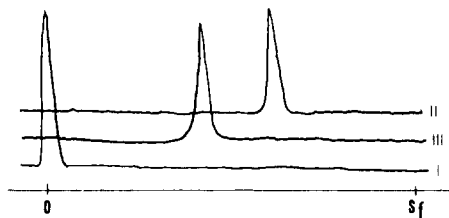
Fig. 2. TLC distribution of radioactivity found in purified I, II and III following synthesis from palmitate-1-[14]C. The TLC plate was developed in hexane, O, origin; Sf, solvent front.

They assigned the chain methylene protons α to S to resonance at 2.5 ppm and the hydroxyl proton resonance at 3.6 ppm, in the presence of the glycerol methylene protons α to S. No interpretation was given for the protons on the hydroxyl carbons (-CHOH-CH$_2$OH). These protons, which are α to a hydroxyl, exhibit resonance at 4.0–3.7 ppm when attached to a secondary carbon, and at 3.7–3.4 ppm when on a primary carbon. Their position changes very little with concentration or solvent [18]. On the other hand, both methylenes α to O in the O-alkyl ethers [15] exhibit resonance in the same region (3.4 ppm), which is the normal value. The methylene of the glycerol α to S is only in β position to the OH and these protons generally absorb in the 1.4 ppm region. This means that there is little effect from the hydroxyl. Methylene protons α to S generally absorb at 2.5 ppm [18]. Therefore the two methylenes α to S should be assigned to 2.5 ppm since they too will have little effect from the OH, as is indicated in the spectrum of the oxygen analogue.

It was pointed out by Wood et al. [16] that attempts to separate S-alkyl and O-alkyl ethers by TLC were unsuccessful, but that the corresponding isopropylidene derivatives were separated. We reported [6] a TLC system consisting of hexane : diethyl ether : acetic acid (30 : 70 : 1 v/v/v) which would separate the O and S ethers. Another system, which gives an even better separation of the ethers, is composed of diethyl ether: 30% aq. NH$_4$OH (100 : 0.25 v/v). This same system can be used to separate several of the derivatives described in this paper. The respective R_f values are: 1-hexadecylthioglycerol (IV), 0.29; 1-hexadecylglycerol, 0.16; hexadecyl ethylene thioglycol (VII), 0.61; hexadecyl ethylene thioglycerol acetate (VIII), 0.76; thiohexadecyloxy-2, 3 isopropylidene propanediol (V), 0.76. However, as can be seen from the R_f values, one cannot separate the acetate derivative (VIII) from that of the isopropylidene (V). We found it to be also possible to separate the alkyl thioacetaldehyde (VI) from the alkoxy acetaldehyde by TLC using a solvent system of hexane : diethyl ether : chloroform (70 : 25 : 5, v/v/v). The R_f values of the S and O aldehydes are 0.60 and 0.34 respectively.

The GLC behavior and mass spectra of thioalkyl isopropylidene propanediol (V) has already been reported [1,15].

The infrared spectrum of VI showed the most typical absorption peaks to be the carbon-hydrogen absorption of the aldehyde function at 2710 cm^{-1}, the carbonyl
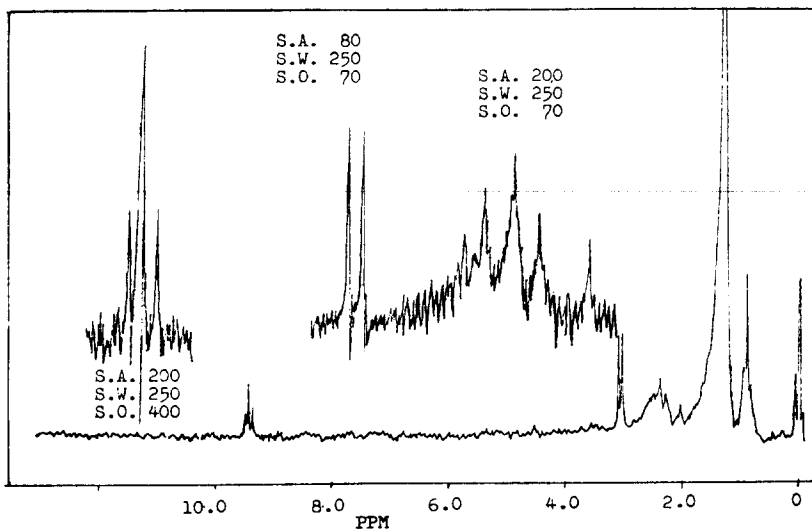
Fig. 3. NMR spectrum of hexadecyl-thioacetaldehyde (VI) made in CCl₄ on a Varian A60A instrument.

absorption at 1725 cm^{-1} and absorption at 670 cm^{-1}, which is most probably the C-S.

In the oxidation reaction, there was a possibility that the sulfide moiety had been oxidized to a sulfoxide. However, this was completely eliminated by the NMR spectrum which is illustrated in fig. 3. The following resonance values can be observed: $T_{TMS}^{CCl_4}$ 9.50 (s.t., J = 4 Hz, 1, -CHO); 3.08 (d, J = 4 Hz, 2, -S-CH₂-CHO); 2.4 (br.t., J = 7 Hz, 2, -CH₂-CH₂-S-); 2.15-1.15 (sh.m., 28, -(CH₂)₁₄); 0.90 (br.t., J = 4 Hz, 3, -CH₃).

From this spectrum it can be concluded that an aldehyde was formed and that no sulfur oxidation had occurred. The NMR spectrum shows a very distinctive triplet

Table 1

Retention times of alkyl thioacetaldehydes (ASA) and alkoxy acetaldehydes (AOA) [a].

Alkyl (Chain length)	ASA (Min)	AOA (Min)
14:0	2.60	0.85
16:0	4.15	1.67
17:0	5.24	—
18:0	6.57	2.68
18:1	—	3.10

[a] Using a 6 ft column of 15% EGSS-X on Gas Chrom-P at 195°C and a nitrogen carrier gas flow rate of 60 ml/min.

Table 2
Retention times of alkyl ethylene thioglycerol acetates (AETA) and alkyl ethylene glycol acetates (AEGA) [a]

Alkyl (Chain length)	AETA (Min)	AEGA (Min)
14:0	5.44	2.55
16:0	8.76	4.58
17:0	11.02	—
18:0	13.68	7.57
18:1	—	8.66

[a] Using a 6 ft column of 15% EGSS-X on Gas Chrom-P at 195° C and a nitrogen carrier gas flow rate of 60 ml/min.

at 9.50 ppm with a coupling constant of 4 Hz. This is strong evidence for the presence of an aldehyde proton coupled to a methylene. The spectrum also shows a sharp doublet at 3.08 ppm ($\alpha'CH_2$) with the same coupling constant of 4 Hz. If the S had been oxidized to S = 0, the αCH_2 would probably have been a multiplet and the $\alpha'CH_2$ shifted down field with a more complicated splitting pattern.

The GLC behavior of several alkyl thioacetaldehydes and alkoxy acetaldehydes were compared. The retention times are given in table 1. The aldehyde derivative is also useful for quantitations as the p-nitrophenylhydrazone [19] using the maximum absorbance wavelength of 380 nm.

The IR spectrum of VII was essentially identical to that of VI, except that the carbonyl absorption had disappeared and hydroxyl absorption appeared at 3440 cm^{-1}.

VII is most conveniently analyzed by GLC following conversion to the acetate (VIII). Table 2 gives the retention times of S-alkyl ethylene thioglycerol acetates and, for comparison, the retention times of the acetate derivative of O-alkyl ethylene glycols. As observed with the isopropylidene derivatives [15] the retention times of the sulfur compounds are about double that of the oxygen analog of the same chain length.

Methods for the synthesis of O-alkyl ethers and their derivatives have been well discussed [20]. We have applied some of these methods and developed some modified procedures for the synthesis of S-alkyl glycerol ethers and derivatives. Using radioactive precursors, radiopurities of >99% have been obtained. It is hoped that the methods described in this paper will lead to an increased interest in the biosynthesis and metabolism of the newly discovered S-alkyl glycerol ethers.

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