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NATURAL OCCURRENCE OF S-ALKYL GLYCEROL ETHERS IN EHRLICH ASCITES CELLS

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Abstract—1. *S*-alkyl glycerol ethers were isolated by thin-layer chromatography from the total lipids of Ehrlich ascites cells following reduction with vitride.

2. Gas–liquid chromatography of the isopropylidene derivatives showed the principal component to be 16:0 (83%) with lesser amounts of 18:1 (14%) and 18:0 (2%).

3. Quantitative analysis yielded values of about 2 μmoles/100 mg lipid for the isolated thioethers.

INTRODUCTION

Bovine and human heart have been shown to contain *S*-alkyl glycerol ethers (glycerol thioethers) as components of the non-polar lipids, resembling the *O*-alkyl glycerol ethers in composition (Ferrell & Radloff, 1970; Ferrell, 1973). The biosynthesis of *S*-alkyl glycerol ethers by liver microsomes has been found to occur by a pathway analogous to the biosynthesis of *O*-alkyl glycerol ethers (Ferrell & Desmyter, 1974). The occurrence and biosynthesis of *O*-alkyl glycerol ethers in Ehrlich ascites cells is well established (Wood, 1973; Hajra, 1973). This paper describes the isolation of *S*-alkyl glycerol ethers from Ehrlich ascites cells.

EXPERIMENTAL PROCEDURES

Materials

All solvents and chemicals used were of the highest possible purity. Silica gel G and GLC column packing were purchased from Applied Science Laboratories. Vitride was purchased from Eastman Kodak Co.

S-alkyl glycerol ethers of varying chain lengths were synthesized as we described earlier (Ferrell *et al.*, 1976).

Methods

The Ehrlich ascites tumor cells (a hypotetraploid strain) were grown in the abdominal cavities of 54 Swiss mice and were harvested 7 days after inoculation. A total of 250 ml of fluid was collected and the cells obtained by centrifugation at 2000 *g* for 2 min.

The lipids were extracted from the cells using the Bligh and Dyer method (Bligh & Dyer, 1959). A total of 1.63 g of lipid was obtained. This total lipid was reduced (in several aliquots) with vitride essentially as described by others (Wood & Snyder, 1968). The main difference in the procedure was substituting chloroform–methanol (4:1 v/v) for diethyl ether as the extracting solvent. We reported earlier that diethyl ether does not adequately extract the *S*-alkyl glycerol ethers (Ferrell & Desmyter, 1974). The various extractions were combined and evaporated to dryness using a rotary evaporator. The residue was dissolved in chloroform and subjected to TLC with hexane:diethyl ether:acetic acid (30:70:1 v/v/v) as the developing solvent. The lipids were visualized under u.v. light after spraying with 0.005% rhodamine 6-G in 78% aq. methanol. This solvent separates the *O*-alkyl and *S*-alkyl glycerol ethers (Ferrell, 1973), however the *O*-alk-1-enyl (plasmalogens) and *S*-alkyl glycerol ethers chromatograph together. Therefore, the *S*-alkyl glycerol ether band was scraped from the plate, eluted with chloroform and the solvent evaporate under N_2. The residue was then exposed to HCl gas for 35 min, a procedure we have shown to destroy any *O*-alk-1-enyl glycerol ethers (Ferrell *et al.*, 1970). The products were then separated by TLC using diethyl ether:30% aq. NH_4OH (100:0.25 v/v). We have shown this developing solvent to also work well for the separation of *S*- and *O*-alkyl glycerol ethers yielding respective R_F values of 0.29 and 0.16 (Ferrell *et al.*, 1976). The *S*-alkyl glycerol ether band was scraped, eluted and evaporated to dryness under N_2. The isopropylidene derivatives were synthesized and purified by TLC as described earlier (Ferrell *et al.*, 1976). These isopropylidene derivatives were qualitatively analyzed by GLC as described previously (Ferrell, 1973). Peak areas were determined as the product of peak height and width at half peak height. Percentages were calculated in terms of peak areas. Peak identification was made by co-chromatography with synthetic standards.

Quantitative analyses were made as hydrazones (Ferrell *et al.*, 1969) following periodate oxidation (Gelman & Gilbertson, 1969) of the *S*-alkyl glycerol ethers.

RESULTS AND DISCUSSION

Following the exposure to HCl gas a hydrazone analysis for *O*-alk-1-enyl glycerol ethers was negative. NMR and IR spectra of the isolated *S*-alkyl glycerol ether band was identical to that obtained with purified synthetic standards (Wood *et al.*, 1970; Ferrell *et al.*, 1976).

The results of three separate isolations using 500 mg samples of total lipids from the Ehrlich ascites cell are given in Table 1. The principal component is the 16:0 compound with significant amounts of 18:1 also being present as well as small amounts of 18:0.

The quantitative analysis of the *S*-alkyl glycerol ethers from the three 500 mg aliquots give values ranging from 2.16 μmole/100 mg lipid to 2.7 μmole/100 mg lipid. This represents approx 0.8–1%

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Table 1. Distribution of *S*-alkyl glycerol ethers in Ehrlich ascites cells

Shorthand designation	Experiment A	B	C
16:0	83.6	82.9	83.8
18:0	2.4	2.8	2.1
18:1	14.0	14.3	14.1

of the total lipid. In contrast, the sum of the *O*-alkyl and *O*-alk-1-enyl glycerol ethers in Ehrlich ascites cells is of the magnitude of 2.3% of the total lipids (Howard *et al.*, 1973). In human heart, the approximate value for *S*-alkyl glycerol ethers was 0.09 μmole/100 mg lipid (Ferrell, 1973).

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