ISOLATION OF ETHYLENE GLYCOL FROM THE LIPIDS OF BEEF LUNG

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Extensive studies of the isolation of glycerides from mammalian tissue have demonstrated that triglycerides are the most widely occurring with lesser amounts of di- and monoglycerides respectively. It appears, however, that glycerol is the only polyhydroxy alcohol normally found in glyceride fractions from mammalian sources. Recently, Damareau-Ginsburg and Miguel (1962) reported the isolation from Mycobacterium avium of a wax fraction which on saponification yielded glycerol and ethylene glycol identified by means of the benzoate derivatives.

During the isolation of complex lipids from a commercial beef lung extract (Teets et al., 1963) a fraction was encountered which showed a strong ester band in the infrared and which was not readily hydrolyzed under the conditions of Schmidt et al. (1946). Hydrolysis of this fraction with methanolic potassium hydroxide yielded mainly palmitic and stearic acids as the ether soluble constituents. Sterol tests were negative. Paper chromatography of the water-soluble fraction showed the presence of glycerol plus a second substance which had the same R_f as that of ethylene glycol in three solvent systems. A more detailed study of this

problem was undertaken and for this purpose extracts of fresh beef lung were prepared.

Fresh beef lung (30 lb.), transported in dry ice, was finely ground in an electric mincing machine and extracted four times with reagent-grade acetone by stirring at room temperature for 24 hours. The remaining tissue was then re-extracted four times with anhydrous ether. The combined ether extract after evaporation, representing a total of 14.5 g. of lipid (213 mg. P), was loaded in benzene solution onto a column of Mallinkrodt silicic acid (430 g.) prepared after removal of the fines by repeated suspension in methanol and activation by heating in vacuo (80 mm.) at 140° for 12 hours. The column was eluted with benzene and 50 ml. fractions were collected.

The first 1550 ml. of eluent were combined into three fractions: A (1-12), B (13-17), C (18-31). Fraction A appeared to consist mainly of hydrocarbons and was not investigated further.

Fractions B (0.87 g.) and C (3.01 g.) were characterized by infrared and by thin layer chromatography on Silica Gel G developed with 5% methanol in benzene (iodine detection of spots). Fraction B gave typical triglyceride properties and Fraction C showed in addition di- and monoglycerides. A series of spots in the tri-glyceride region were apparent in B and to a lesser extent in C.

Fractions B and C were then treated with 1 N methanolic KOH (90% MeOH) at 37° for 24 hours. The hydrolysates were acidified to pH 1 with 5 N HCl and extracted four times with diethyl ether. The aqueous phase was stirred with an excess of Amberlite MB-3 and allowed

to stand before filtering from the resin. The clear filtrate was reduced on a rotary evaporator at 40° (14 mm.) and the residue dissolved in ethanol for thin layer chromatography on Silica Gel G. The plates were chromatographed in chloroform: methanol:formic acid (65:25:10), and developed by spraying with periodate and benzidine.

Table I shows R_f values of compounds obtained from fractions B and C compared with standards.

TABLE I

Thin Layer Chromatography on Silica Gel G in $CHCL_3$:MeOH:HCO_2H (65:25:10)

Compounds	R_f	Fraction	R_f
Glycerol	0.43	B	0.59
Ethylene Glycol	0.59	C	0.43, 0.59
1,2-Propanediol	0.65		
D-Glyceraldehyde	0.50		

The data indicate that fraction B yielded only ethylene glycol while fraction C gave both glycerol and ethylene glycol, with glycerol the predominant component (based on visual inspection). These results were confirmed by vapor phase chromatography of the acetates (prepared according to Shriner et al. (1957) compared with the behavior of standard samples of ethylene glycol diacetate and glycerol triacetate. Retention times were 2.2 min. and 14.3 min. respectively when chromatographed on an 8 ft. 10% QF-1 column at 140° and 75 ml./min. The solid support was 60/80

Chromosorb W which had been pretreated with hexamethyl disilazane. Fraction B showed only ethylene glycol diacetate while Fraction C gave glycerol and ethylene glycol acetates in the approximate ratio of 4:1.

In blank runs in which glycerol solutions were carried through the entire experimental procedure, no spot other than glycerol was detected by thin layer chromatography.

DISCUSSION

The above results demonstrate the existence in beef lung of a combined form of ethylene glycol which is extracted with glyceride fractions. The relative amounts of ethylene glycol-containing lipid in total lung lipid cannot be estimated from these data since partial fractionation may well have occurred in extracting the fresh lung tissue. Furthermore, differential losses of glycerol and ethylene glycol may have occurred in concentrating the aqueous solutions.

In view of the isolation of ethylene glycol from bacterial lipids by Damareau-Ginsburg and Miguel, it may be that ruminants constitute a special case due to the presence of bacteria in the rumen. On the other hand, Lovern (1956) found that the saponification of fish glyceride fractions yielded water-soluble products of hydrolysis other than glycerol which were assumed to be non-lipid contaminants. It is entirely possible that this class of compound has escaped detection due to its similarity to the glycerides in chromatographic behavior. Ethylene glycol dipalmitate synthesized in our laboratory by the method of Paquot (1947) had an R_f on thin layers of silica gel comparable to various triglyceride standards. It is also probable that appreciable

losses of ethylene glycol would occur during concentration procedures and that small amounts of the compound remaining may have been overlooked or assigned to non-lipid contaminants.

Further work is in progress to determine quantitatively the extent of the occurrence of esterified ethylene glycol in beef lung and the nature of the fatty acids involved in the ester linkage. Other tissues from beef and other animals are being examined to determine how general this occurrence may be. (In single preliminary experiments lard and beef suet were found to contain very small amounts of ethylene glycol.) The possible metabolic significance of such compounds is also under investigation.

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