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Ester and Ether-Linked Lipids in the Mandibular Canal of a Porpoise (*Phocoena phocoena*). Occurrence of Isovaleric Acid in Glycerolipids*

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ABSTRACT: High proportions of isovaleric acid (40.5 mole %) and long-chain iso acids, such as isopentadecanoic acid (4.8 mole %), are present in the neutral glycerolipids of the mandibular canal of the porpoise (*Phocoena phocoena*). Although isovaleric acid, a product of leucine metabolism, is readily esterified in triglyceride biosynthesis the isopentyloxy structure was not detected in the alkyl chains of glyceryl ethers or the dialkoxypentane fraction of the diol lipids. These findings suggest that isovaleric acid, unlike longer chain structures, is not readily reduced and incorporated into alkyl moieties. The apparent absence in the mandibular canal of C_{20} and C_{22} unsaturated acids characteristic of marine organisms suggests that lipid biosynthesis is not significantly dependent on dietary polyenoic acids.

The mandible of the porpoise (*Phocoena phocoena*) contains a cavity filled with fatty tissue. This tissue plays an important yet undefined role in the echolocation system of this cetacean (Norris, 1964). The presence of such a deposit in the mandible may imply a biochemical function for lipids in sound transmission that is reminiscent of the role for these compounds in the photochemistry of sight (Adams, 1969; Erhardt *et al.*, 1966). Nevertheless, very little is known about the composition of the mandible tissue. Early workers, however, have noted large proportions of isovaleric acid in the total lipids of some cetaceans (Hilditch and Williams, 1964).

Two recent papers from this laboratory have revealed that the mandible tissue contains unique diol lipids (dialkoxyalkanes) (Varanasi and Malins, 1969) and unusual wax

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esters composed of long-chain alcohols and high proportions of isovaleric acid (Varanasi and Malins, 1970). Furthermore, both long-chain acids and alcohols of the wax esters contained significant amounts of iso structures.

In the present paper we show evidence for esterification of isovaleric acid, a product of leucine metabolism, in the formation of neutral glycerolipids. No evidence was found, however, to suggest that isovaleric acid is reduced to form alkoxy chains of either glyceryl ethers or dialkoxyalkanes. The principal component of the dialkoxyalkanes is now shown to be a 1,5-dialkoxypentane.

Materials and Methods

A porpoise (*P. phocoena*) was caught in a trawl net in the Moray Firth, Scotland, April 1967. The mandible was excised and the fatty tissue from a mandibular canal (*ca.* 30 cm^3) was extracted by the method of Hanson and Olley (1963). The wet tissue contained 85% lipid.

Thin-layer plates, precoated with silica gel G (Brinkman Instrument Co., Westbury, N. Y.), were used for all separation and purification procedures. The indicators 2,7-dichlorofluorescein and iodine (Malins *et al.*, 1965) were employed for the detection of lipid fractions.

Several milligrams of dialkoxyalkanes (0.5% of the total lipid) were isolated from *Phocoena phocoena* mandible lipid by previously described separation-reaction-separation techniques (Varanasi and Malins, 1969). The dialkoxyalkanes (I), which had a similar migration rate to pure marker dihexadecyloxyethane in hexane–diethyl ether (80:20, v/v), were further characterized by infrared spectrometry, nuclear magnetic resonance spectrometry, and mass spectrometry as reported previously (Varanasi and Malins, 1969).

The dialkoxyalkanes (I) were hydrogenated in hexane (Guyer *et al.*, 1963) over PtO_2. To determine the composition of the alkyl chains and the structure of the diol backbone of I it was necessary to analyze the hydrogenated dialkoxyalkanes (II) *via* two degradative reactions. To obtain the composition of the alkyl chains, fraction II was refluxed with 50% hydriodic acid for 24 hr (Guyer *et al.*, 1963) and the reaction product was extracted with diethyl ether. The organic phase was washed with water, a saturated solution of K_2CO_3, and 50% $Na_2S_2O_3$, respectively. The alkyl iodides obtained from the cleavage of the ether linkage of II were found to have a migration rate equal to that of marker *n*-octadecyl iodide when chromatographed in hexane on thin layers of silica gel G. The alkyl iodides were analyzed by gas–liquid chromatography at 180° with a Barber-Colman instrument (Model No. 5000, Rockford, Ill.) using conditions previously described by Malins *et al.* (1965).

To obtain the diol backbone of I, the ether linkage of II was cleaved with boron trichloride according to the method of Kates *et al.* (1965). The reaction products were partitioned between petroleum ether (bp 30–60°) and water. Diols (III) from the aqueous phase were purified by thin-layer chromatography using chloroform–methanol (85:15, v/v) as developing solvent (Bergelson *et al.*, 1966). Pure C_2–C_5 diols (Distillation Products Industries, Rochester, N. Y.) were chromatographed on the same plate as markers. The diols (III) were further characterized by low-temperature thin-layer chromatography of the dinitrato derivatives using 1,5-dinitratopentane as marker. Diacetate derivatives (IV) synthesized from III by the method of Bergelson and coworkers (1966), were analyzed by gas–liquid chromatography (Malins *et al.*, 1965) at a column temperature of 109°.

Triglycerides (84% of the mandible lipid) were separated by thin-layer chromatography in hexane–diethyl ether (90:10, v/v). Triglycerides were transesterified by heating with butanol and a catalytic amount of sulfuric acid in a sealed ampoule at 95° for 2 hr (Kuksis and Breckenridge, 1965). Butyl esters were purified by thin-layer chromatography in hexane–diethyl ether (98:2, v/v) at low temperature (2°) to prevent losses of short-chain components (Varanasi and Malins, 1970). Butyl esters were analyzed by gas–liquid chromatography at column temperatures of 90° (short-chain components) and of 148° (long-chain components). Quantitation of the butyl esters was based upon internal standards.

The mandible lipid (4.0 g) was chromatographed on five preparative plates (2 mm in thickness) using hexane–diethyl ester–acetic acid (80:20:1, v/v) as developing solvent. A section of the adsorbent that included 1.0 cm above and below marker 1-*O*-hexadecyldioleylglycerol was scraped from the plate and eluted with diethyl ether. This fraction was refluxed in ethanolic KOH solution for 1 hr. Glyceryl ethers (45 mg, 0.12 μmole) were isolated from the nonsaponifiable fraction by thin-layer chromatography (Malins *et al.*, 1965). The purity of these compounds was established by analyses of isopropylidene derivatives *via* the complementary use of chromatographic techniques and infrared spectrometry (Hanahan *et al.*, 1963). The composition of the glyceryl ethers was obtained *via* the analysis of isopropylidene derivatives by gas–liquid chromatography (Malins *et al.*, 1965).

Results and Discussion

Dialkoxyalkanes. Prior to the detection and isolation of the dialkoxyalkanes from *P. phocoena* (Varanasi and Malins, 1969), naturally occurring diol lipids containing an alkoxy chain had not been reported. Bergelson and coworkers (1966) detected the acyl and alk-1-enyl derivatives *via* the analysis of hydrolytic products of neutral lipid fractions from various natural products. The isolation of these labile diol lipids in intact form by thin-layer chromatography was hampered by the presence of large proportions of triglycerides of similar migration rate. However, diesters of diols were detected in the presence of triglycerides by high-temperature gas–liquid chromatography (Bergelson *et al.*, 1966). The dialkoxypentanes of *P. phocoena* were readily isolated from contaminating saponifiable lipids (*e.g.*, wax esters) by thin-layer chromatography after hydrolysis.

The composition of alkyl chains of the hydrogenated dialkoxyalkanes (II) comprised 86.4% 18:0, 7.2% 16:0, and 2.7% 14:0 structures. The high percentage of octadecyl iodide demonstrates that the dialkoxyalkanes (I) contain predominantly C_{18} chains, reminiscent of the composition of the glyceryl ethers of previously studied marine and terrestrial species (Hallgren and Larsson, 1963; Malins and Wekell, 1969). Usually, selachyl alcohol (18:1) is the major component of these glyceryl ethers. However, attempts to analyze unsaturated alkoxy structures, such as 18:1, were hampered by extensive decomposition of olefinic linkages on treatment of I with hydrogen iodide (Hanahan, 1965) and boron trichloride.

The relative retention times of several synthetic diol diacetates, together with diacetates (IV) derived from the dialk-

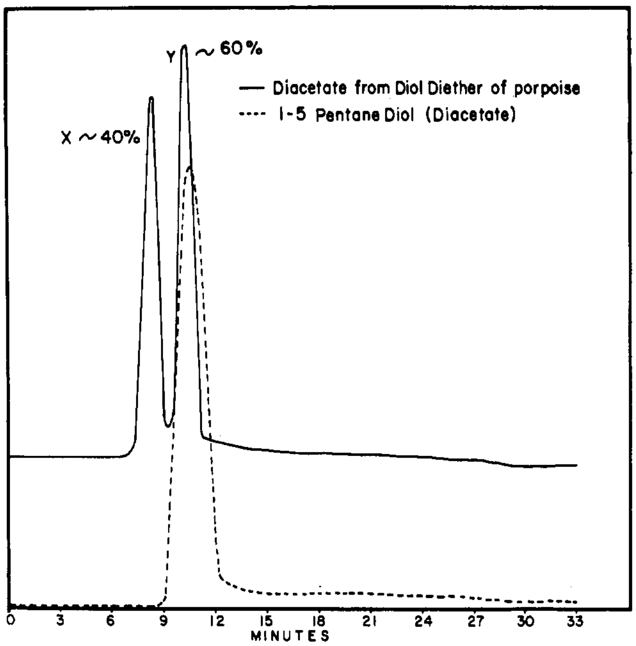

FIGURE 1: Gas–liquid chromatogram (diethylene glycol succinate 5%, 109°) of diacetates (IV) compared to that of 1,5-pentane diacetate.

TABLE I: Gas Chromatographic Analysis of Synthetic Polyol Acetates Together with Diacetates Obtained from the Dialkoxyalkanes from Mandible Lipids of *P. phocoena*.

	Relative Retention Times	
Acetates of Polyols	Synthetic Specimen	*P. phocoena* Mandible Lipids
1,2-Ethanediol	0.15	
1,2-Propanediol	0.13	
1,3-Propanediol	0.21	
1,2-Butanediol	0.12	
1,3-Butanediol	0.17	
1,4-Butanediol	0.30	
2,3-Butanediol	0.08	
1,5-Pentanediol	0.44	0.44 (60%)
2,3-Pentanediol	0.14	
2,4-Pentanediol	0.15	
1,6-Hexanediol	0.66	
Glycerol	1.00	
Unknown		0.35 (40%)

TABLE II: Triglycerides and Glyceryl Ethers from the Mandible Lipid of *P. Phocoena.*

Chain	Triglycerides (mole %)	Glyceryl Ethers (mole %)
5:0 br	40.5[a]	
8:0	1.2[a]	
9:0		0.4
10:0 br	0.3[a]	0.7[b]
10:0	0.5	0.4
11:0 br	0.7[a]	
11:0	0.3	
12:0 br	1.6[a]	4.3[b]
12:0		0.1
12:1		0.5
Unknown	1.7	
13:0 br	1.3[a]	
13:0		3.3
14:0 br	1.5[a]	
14:0	8.0	2.4
14:1[c]	1.0	
15:0 br[c]	4.8[a]	
15:0	2.2	1.7
16:0 br	2.8[a]	4.3[b]
16:0	8.4	43.0
16:1	13.1	
17:0		2.8
17:?	1.7	
18:0	1.0	7.5
18:1	7.4	27.0

[a] Iso structure. [b] Specific branched structure not determined. [c] Resolved only after hydrogenation.

oxyalkanes (II), are given in Table I. A typical gas chromatogram of the diacetates is shown in Figure 1. Chromatography at two different column temperatures revealed the presence of only two diol diacetates. Peaks X and Y represented 40 and 60% of the chromatographed mixture, respectively. The relative retention time of Y corresponds to that of the diacetate of pure 1,5-pentane diol. The retention time of X does not correspond to the retention time of any diol diacetate given in Table I. Peak X might therefore contain 1,2-, 1,3-, or 1,4-pentane diacetates, or a longer chain homolog. The lack of standard diols, other than those shown in Table I, has not allowed us at present to further confirm the identity of X. Also, the small amount of dialkoxyalkanes in the mandible lipid has so far hampered our efforts to isolate and identify the structure. The evidence indicates that the major component of the dialkoxyalkane fraction (I) is a 1,5-dialkoxypentane containing two C_{18} chains.

Experiments with rat liver indicated that 1,2-propanediol may be derived from acetol phosphate (Groth *et al.*, 1952; Rudney, 1954). However, studies relating to the biosynthesis of higher and lower diol homologs have not been reported in the literature. It is conceivable that the biosynthesis of the diol backbone of the 1,5-dialkoxypentanes may involve deamination of glutamic acid.

Metabolic interrelations between ester and ether structures of glycerolipids have been proposed (Snyder, 1969). In all probability, analogous relations exist between the alkyl, alk-1-enyl, and acyl structures of the diol lipids. Clearly the mandible lipid of the porpoise (*P. phocoena*) provides an interesting opportunity for further research on the metabolism of the recently discovered diol lipids.

Triglyceride Fraction. We attempted to separate triglycerides and diacylglyceryl ethers by thin-layer chromatography as previously described (Malins *et al.*, 1965). However, no distinct separation was obtained. A high proportion of isovaleric acid in these fractions, together with long-chain acids, undoubtedly hampered chromatographic resolution. Although diacylglyceryl ethers could not be separated from triglycerides, it was of particular importance to ascertain the presence of isovaleric acid in the triglyceride fraction (97% triglycerides and 3% diacylglyceryl ethers).

The data in Table II reveal an impressive amount (40.5 mole %) of isovaleric acid in the triglyceride fraction. It is apparent that a significant proportion of triglycerides contain isovaleric acid on at least two positions of glycerol. Also present are considerable amounts of long-chain iso acids, such as isopentadecanoic acid (4.8 mole %). As proposed in a previous publication, these iso acids may be synthesized by chain elongation of isovaleric acid (Varanasi and Malins, 1970).

In contrast to most marine species, detectable amounts of polyunsaturated acids were not present in the triglycerides of the mandibular canal. Furthermore, no evidence was found for more than trace amounts of fatty acids having chain lengths greater than 18 carbon atoms. A lack of characteristic C_{20} and C_{22} polyenoic acids in the triglycerides may indicate that the fat body of the mandibular canal is not significantly dependent on lipids of the food chain.

The high proportion of isovaleric acid in the triglycerides, together with long-chain acids, implies that certain glycerides possess a measurable optical activity. The mandible lipid is therefore a desirable material for studies on the optical rotation of natural glycerides (Schlenk, 1965).

In the absence of appropriate metabolic studies it is reasonable to assume that the triglycerides are biosynthesized *via* acylation of α-glycerophosphate with isovaleryl-CoA according to the well-known pathway described by Kennedy and Weiss (1956). The latter precursor is a catabolite of leucine (Christophe, 1963). The question of the site of biosynthesis of the glycerolipids containing isovaleric acid is of considerable interest. It is unknown whether this acid is esterified in the liver and transported to the mandibular canal or whether it is esterified in the acoustic organ itself. Early evidence presented by Lovern (1934) on the fatty acids of *Phocoena communis* indicates that isovaleric acid is not present in the hepatic lipids. Accordingly, further work with these cetaceans may well reveal that the unusual glycerides containing iso structures are biosynthesized at the site of occurrence.

Glyceryl Ethers. Although diacylglyceryl ethers could not be isolated, glyceryl ethers were obtained by saponification of a fraction migrating on thin-layer plates 1 cm above and below pure marker 1-*O*-hexadecyldioleylglycerol. However, by analogy with the triglycerides, esterified moieties of diacylglyceryl ethers might be expected to contain high proportions of isovaleric acid.

The analysis of the glyceryl ethers is presented in Table II. Chimyl alcohol (16:0) is the major component; however, a significant amount of selachyl alcohol (18:1) is also present. This finding may be contrasted with analyses of a number of other marine species in which selachyl alcohol is the major constituent (Hanahan *et al.*, 1963; Malins *et al.*, 1965; Malins and Wekell, 1969). It is particularly noteworthy that the isopentyl chain, which predominates in the acyl groups of the triglycerides, was not detected in the alkoxy chains of either the glyceryl ethers or the dialkoxyalkanes. This finding is consistent with results obtained from the analysis of wax esters from porpoise mandible lipids (Varanasi and Malins, 1970), which indicate that the isopentyl chain is primarily associated with fatty acids. It appears therefore that isovaleric acid is not extensively reduced and incorporated into alkyl moieties. The selective incorporation of the isopentyl chain into glycerolipids suggests a unique relationship between the metabolism of leucine and lipid in this mammal.

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