Unusual Wax Esters from the Mandibular Canal of the Porpoise (*Tursiops gilli*)*

Usha Varanasi and Donald C. Malins

ABSTRACT: Wax esters from the mandibular canal of the porpoise (*Tursiops gilli*) contain large amounts of isovaleric acid (43.6%) and a small amount of isopentyl alcohol (2.6%). Accordingly, the wax esters are primarily isovaleryl derivatives of fatty alcohols. High percentages of long-chain acids and alcohols containing iso structures were also present. The active involvement of isovaleric acid in wax ester biosynthesis suggests that this acid might be elongated to form branched fatty acids which are readily incorporated into wax esters directly and after reduction to alcohols.

The composition (Malins and Wekell, 1969; Lee *et al.*, 1970) and metabolism (Malins, 1966; Friedberg and Greene, 1967; Sand *et al.*, 1969) of wax esters commonly found in a variety of aquatic species has generated keen interest in recent years. Studies so far indicate that the principal components of these wax esters are long-chain fatty acids and alcohols (Nevenzel *et al.*, 1965; Iyengar and Schlenk, 1967; Sand and Schlenk, 1969) that are interconverted *in vivo* (Sand *et al.*, 1969). Because porpoises are rich in lipids containing isovaleric acid (Hilditch and Williams, 1964), it appeared likely that these cetaceans synthesize unusual short-chain wax esters containing the isopentyl structure. Accordingly, wax esters from the mandibular canal of the porpoise (*Tursiops gilli*) were analyzed. The results indicate that the isopentyl structure is incorporated into the wax esters, thereby suggesting a significant role for isovaleric acid in the metabolism of this lipid class.

Materials and Methods

A porpoise (*T. gilli*) was caught near Penguin Banks, Molakai, Hawaii in October 1969. The mandible was excised, fatty tissue (220 g) from the mandibular canal was removed, and the lipids (154 g) were extracted by the method of Hanson and Olley (1963).

The mandible lipids were fractionated in unlined tanks on thin-layer plates precoated with silica gel G (Brinkman Instrument Co., Westbury, N. Y.). Lipid fractions, detected with iodine or 2,7-dichlorofluorescein, were scraped off the plates and eluted with glass-distilled diethyl ether.

Gas–liquid chromatography was conducted with a Barber-Colman instrument, Model No. 5000 (Nuclear-Chicago, Des Plaines, Ill.), using a 6 ft × 0.25 in. o.d. glass column packed with 2.5% diethylene glycol succinate on 80–100 mesh Chromosorb G. Long-chain acetates and butyl esters were analyzed by gas–liquid chromatography at column temperatures of 130 and 148°, respectively. Short-chain components, however, were resolved at column temperatures of 98 and 90°, respectively. The gas–liquid chromatography peaks were identified by the use of pure standards. Quantitative analyses of total fractions were accomplished by basing calculations upon common peaks and internal standards. Unsaturated components were quantitated by the difference between total gas–liquid chromatography patterns before and after hydrogenation. Results of the analyses by gas–liquid chromatography of standard mixtures of butyl esters and acetates agreed with the known composition within 3%.

Isolation of Wax Esters. The mandible lipids (3.0 g) were chromatographed on six preparative plates (2 mm in thickness) in hexane–diethyl ether (95:5, v/v). Wax esters were eluted from the adsorbent with diethyl ether and further purified by thin-layer chromatography in hexane–benzene–acetic acid (60:40:1, v/v) to remove sterol esters and other contaminants (Hansen and Mead, 1965). The wax esters (I, 264.4 mg) were analyzed by infrared spectrometry using a Baird-Atomic spectrophotometer, Model NK1 (Baird-Atomic Inc., Cambridge, Mass.). The infrared spectrum of I, which exhibited a strong ester carbonyl (C=O) absorption at 1735 cm^{-1} and a C—O absorption band at 1175 cm^{-1}, was consistent with the spectrum of octadecyl palmitate with the exception of a doublet at 1360–1378 cm^{-1}. This doublet was attributed to *gem*-dimethyl groups of the hydrocarbon chains. The infrared spectrum of isopentyl decanoate exhibited a similar doublet at the same wavelengths.

Acids and Alcohols from Wax Esters. When methyl isovalerate was chromatographed on thin-layer plates at 2°, substantial losses (50%) were sustained. Butyl isovalerate was recovered in 90% yield under the same conditions. Accordingly, butanolysis was employed for the analysis of acids derived from wax esters. However, it was preferable to obtain the alcohols from the wax esters *via* methanolysis because butanol was not readily removed without losses of short-chain alcohols. Furthermore, isopentyl alcohol was recovered quantitatively from thin-layer plates after low-temperature chromatography.

Wax esters were heated at 95° for 1 hr in a sealed ampoule with 1 ml of butanol and a catalytic amount of sulfuric acid (Kuksis and Breckenridge, 1965). Butyl esters (II) were obtained by low-temperature thin-layer chromatography of

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the reaction mixture in hexane–diethyl ether (95:5, v/v) at 2° using butyl oleate as a marker.

The wax esters were heated at 95° for 2 hr in a sealed ampoule with freshly prepared boron trifluoride–methanol solution (Sand *et al.*, 1969). The reaction product was chromatographed at 2° on thin-layer plates (0.2 mm in thickness) in hexane–diethyl ether (95:5, v/v). The alcohol fraction (III) was eluted from the adsorbent with diethyl ether and the solvent was removed by fractional distillation at atmospheric pressure.

Fraction III was heated at 95° for 1 hr with acetic anhydride in a sealed ampoule (Sand *et al.*, 1969). The acetates (IV) were analyzed directly by gas–liquid chromatography. Also, IV was purified by low-temperature thin-layer chromatography using hexane–diethyl ether (95:5, v/v) and then analyzed by gas–liquid chromatography. The results revealed no appreciable change in composition of the acetates. When isopentyl acetate was subjected to similar conditions, almost quantitative recoveries were obtained.

Results and Discussion

The studies with *T. gilli* revealed that wax esters from the mandibular canal have an unusual composition (Table I). It is generally assumed from a number of studies (Malins and Wekell, 1969; Nevenzel, 1970) that naturally occurring wax esters are composed primarily of acids and alcohols having long hydrocarbon chains. Nevertheless, early work on the fractional distillation of lipids from the pilot whale (Tsujimoto and Koyanagi, 1937) suggested the presence of hexadecyl isovalerate. A notable feature of *T. gilli* is the synthesis of relatively short-chain wax esters containing large amounts of isovaleric acid (43.6%). Also of interest is the incorporation of smaller amounts of isopentyl alcohol (2.6%) into the wax esters. It is apparent, therefore, that the wax esters are primarily isovaleryl derivatives of fatty alcohols. The analyses shown in Table I reveal the presence of high percentages of both even- and odd-chain fatty acids and alcohols containing iso structures (*gem*-dimethyl groups). Furthermore, in common with the coelacanth, *Latimera chalumnae* (Nevenzel *et al.*, 1966), the wax esters from the mandibular canal of *T. gilli* do not contain detectable amounts of polyunsaturated structures. Unlike the lipids of previously studied marine organisms (Malins and Wekell, 1969), the wax esters of the porpoise are composed predominantly of saturated acids and alcohols.

Fatty acids and alcohols are believed to be freely interconverted in the biosynthesis of wax esters, although the extent of reduction and oxidation appears to depend upon the structure of the hydrocarbon chains (Sand *et al.*, 1969). The high percentages of iso structures in the long-chain acids and alcohols (Table I) suggest that isovaleric acid might be elongated to form branched fatty acids that are incorporated into wax esters directly and after reduction to alcohols. In the absence of suitable metabolic studies, however, other percursors of the iso structures cannot be ruled out (Kaneda, 1966; Asselineau and Bennet, 1964).

In marine animals, the importance of dietary fatty acids on lipid structure and composition has been extensively documented (Malins and Wekell, 1969). The large proportion of isovaleric acid, presumably derived from leucine metabolism (Christophe, 1963), demonstrates a tendency in *T. gilli* to minimize the incorporation of dietary fatty acids into wax esters. The extensive distribution of iso structures in the acids and alcohols lends support to this argument because branched fatty acids do not occur in significant amounts in the marine food chain.

TABLE I: Acids and Alcohols from Wax Esters of the Mandibular Canal of *T. gilli*.

	Acids		Alcohols	
Structure	Rel Retention Time	Mole %	Rel Retention Time	Mole %
5:0 (iso)	0.12[a]	43.6	0.13[b]	2.6
6:0 (iso)	0.16[a]	1.5		
11:0 (iso)	0.08	1.6		
12:0 (iso)	0.11	1.0		
12:0	0.13	1.6	0.11	0.3
Unknown	0.16 [c]	0.4		
13:0 (iso)	0.16 [c]	0.6		
14:0 (iso)	0.22	0.2	0.18	Trace
14:0	0.25	5.5	0.23	0.9
14:1	0.27	1.2		
15:0 (iso)	0.32	6.6	0.27	21.0
15.0	0.37	1.9	0.32	2.0
15:1	0.40	2.3		
16:0 (iso)	0.45	4.2	0.38	20.9
16:0	0.52	9.7	0.47	33.5
Unknown	0.56	3.5		
16:1			0.57 [c]	2.3
17:0 (iso)	0.66	1.8	0.57 [c]	3.0
17:0	0.72	0.7	0.68	1.0
18:0	1.00	4.3	1.00	3.2
18:1	1.10	5.3	1.13	9.3
20:0	2.00	2.6		

[a] Values based on retention time of added butyl nonanoate. All other values are relative to butyl stearate. [b] Value based on retention time of added decyl acetate. All other values are relative to octadecyl acetate. [c] Bracketed pairs were resolved only after hydrogenation.

The biosynthesis of long-chain wax esters in the dogfish (*Squalus acanthias*) is thought to take place, without activation, in an environment from which water is essentially excluded (Friedberg and Greene, 1967). The reaction is believed to occur *via* the interaction of two nonpolar compounds and an enzyme in a micellar state. However, wax ester biosynthesis involving polar short-chain acids, such as isovaleric acid, may occur *via* formation of the acyl coenzyme A in relatively hydrophilic media.

It was postulated that the lipid of the mandibular canal of the porpoise is instrumental in the transmission of sound waves to the inner ear (Norris, 1964). The unique structure of the wax esters of *T. gilli* may be related to the function of the mandible lipid as a wave guide. Comparisons of wax esters from acoustic and nonacoustic organs might provide further clues to the role of wax esters in the life process of porpoises and other cetaceans (U. Varanasi and D. C. Malins,

unpublished results). Furthermore, it would appear that the mandibular canal of *T. gilli* affords a favorable raw material for future studies on the elusive role of isovaleric acid in lipid metabolism.

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Arrangement of Fatty Acyl Groups in Phosphatidylethanolamine from a Fatty Acid Auxotroph of *Escherichia coli**

David F. Silbert

ABSTRACT: The positional distribution of fatty acyl groups in phosphatidylethanolamine from an unsaturated fatty acid auxotroph of *Escherichia coli* has been investigated following growth of the mutant on unsaturated fatty acids of widely differing structure. *cis*-Octadecenoic acids with an ethylenic bond in the center of the hydrocarbon chain were incorporated into the 1 and 2 position of the phosphatide to an extent approaching that found in the same phospholipid from the prototrophic strain. Cis unsaturated fatty acids of shorter chain lengths or with a double bond close to the carboxyl terminus were assimilated almost exclusively into the 2 position with the result that the normal proportion of molecules containing two unsaturated residues was reduced markedly. When trans unsaturated fatty acids were provided as supplement for the auxotroph, they were found as major components of both the 1 and 2 positions. *trans*-Monoenoic and saturated fatty acids share similar physical properties. The behavior of most of these molecules containing trans unsaturated fatty acyl residues might be expected to correspond to that of a molecular species possessing only long-chain saturated fatty acids. Hence, virtually all of the heterogeneity conferred on the phospholipid by virtue of the combined presence of cis unsaturated and saturated fatty acids is lost. Starvation of the auxotroph for all types of unsaturated fatty acids led to increased synthesis and incorporation of *n*-tetradecanoic and *n*-hexadecanoic acids into the phospholipids, and to a reduction of preexisting molecules containing unsaturated residues by dilution and possibly turnover. These results are discussed in relation to their bearing on membrane function and on the specificity of fatty acyl transferases involved in phospholipid biosynthesis.

The isolation and characterization of mutants in fatty acid metabolism from *Escherichia coli* strain K-12 offer a biological system that is potentially very useful for examining controlled alterations in the physiological properties of membrane lipids in relation to their effects on membrane function (Silbert and Vagelos, 1967; Silbert *et al.*, 1968;

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