Sperm Whale Oil.
Part 3: Alkanes and Alcohols

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ABSTRACT

Pristane has been isolated from sperm whale oil. The monounsaturated alcohols have been isolated and the position of the double bond determined by a modified von Rudloff oxidation technique. Several isomers have been found for each chain length suggesting that many of the alcohols have a dietary origin.

INTRODUCTION

The number of lipid biochemical studies of marine organisms is increasing from year to year, partly because of commercial considerations since the value of fish oils places them 10th in the world production and partly because the complexity of the problems stimulates research workers. Investigations into sperm whale, *Physeter macrocephalus*, have been reported since 1920 and have been reviewed by Hilditch and Williams (1) and by Lovern (2). Direct gas liquid chromatography (GLC) of spermaceti has been reported by Holloway (3), while a study of the saturated, mono- and diunsaturated esters of a commercial sperm whale oil indicated that they are produced in a random fashion (4). Hansen and Cheah (5) examined samples of head and blubber oils and suggested that dietary sources of lipid were more important than de novo synthesis. An interesting feature of their work was the finding that the C_{14}-monounsaturated acid in blubber comprised two isomers with double bonds at the 5 and the 9 positions. Sano (6) has found that, amongst the minor acids in sperm oil, 3, 8, 12 trimethyltridecanoic acid represents 0.005% of the total acids. More recently lipolysis results (7) suggest that there is a common pool of fatty acids which can be incorporated into both glycerices and wax esters. In an attempt to extend our studies of sperm whale oil we have analyzed the hydrocarbon fraction and the alcohols from the ester fraction.

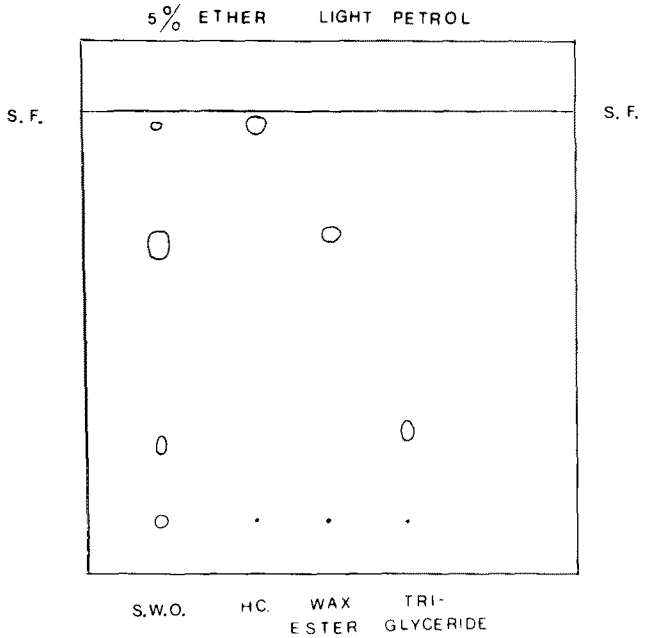

FIG. 1. Thin layer chromatography of sperm whale oil.

MATERIALS AND METHODS

The samples of refined sperm whale oil were supplied by Highgate and Job, Liverpool, England. All the organic solvents except *t*-butanol were of Reagent Grade and the inorganic reagents of laboratory grade.

Saponification of Sperm Whale Oil

Commercial sperm whale oil (60 g) was heated with 0.5% methanolic potassium hydroxide (750 cc) over a

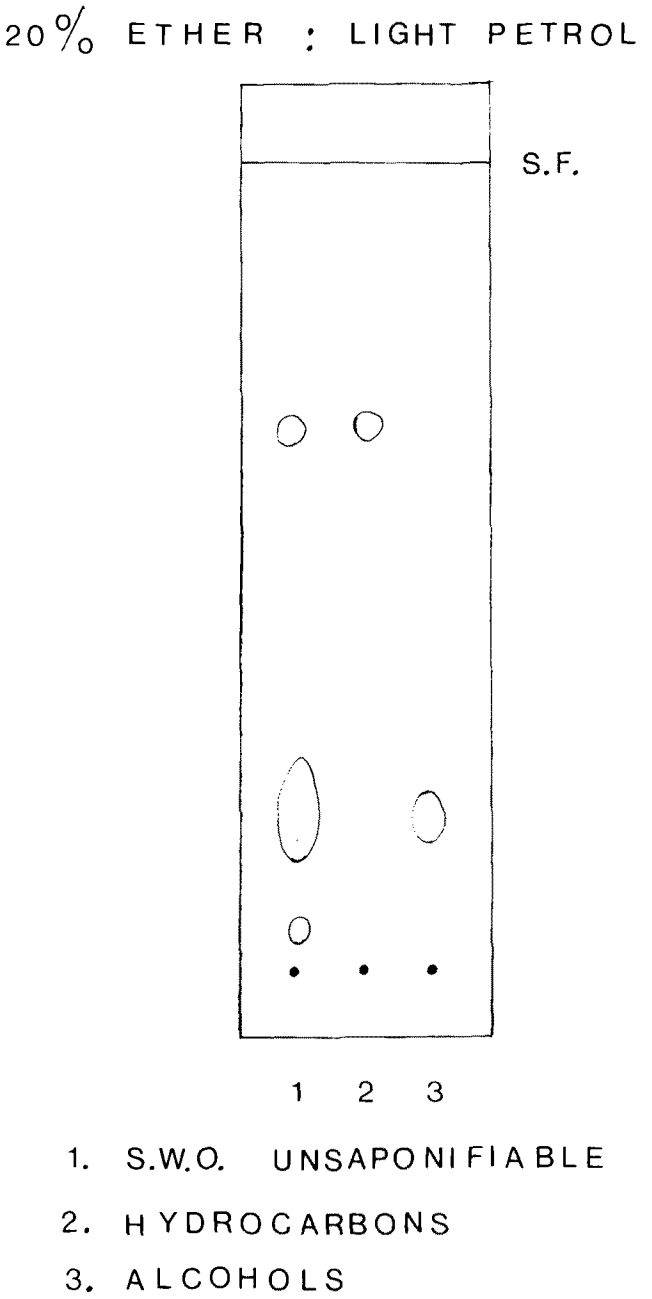

FIG. 2. Thin layer chromatography of sperm whale oil unsaponifiables.

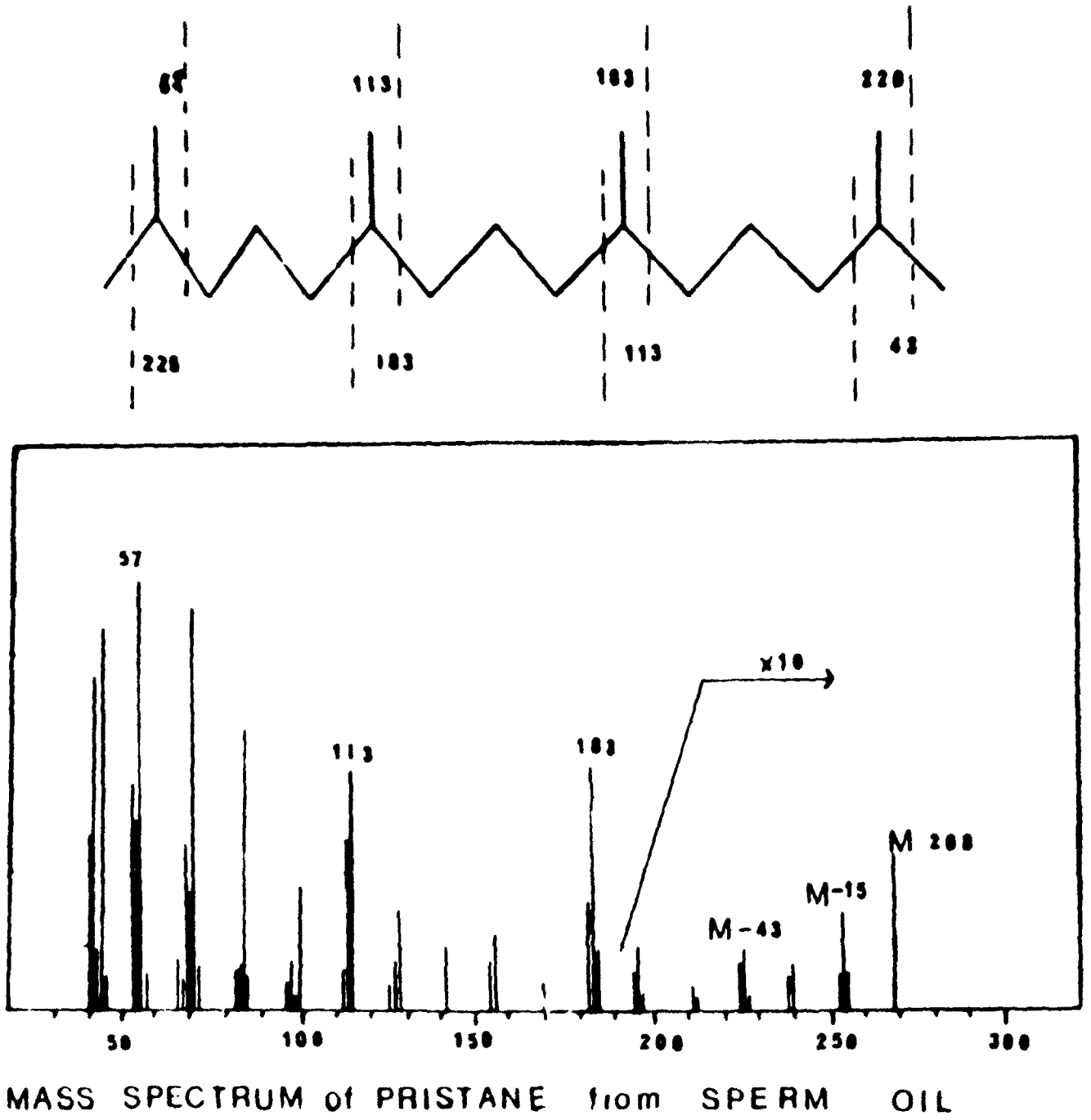

FIG. 3. Mass spectrum cleavage pattern of pristane isolated from sperm whale oil.

steam bath for 2.5 hr, after which the methanol (500 cc) was distilled off and water (750 cc) added. This solution was extracted with ether (4 x 300 cc), and the ether extracts were combined and back-washed with water and finally dried over sodium sulphate. The unsaponifiable matter (18.4 g) was obtained by removal of the solvent.

Acetylation of Sperm Whale Oil Alcohols

The unsaponifiable matter (10 g) was dissolved in pyridine (300 cc) and acetic anhydride (100 cc) and stirred overnight. To the reaction mixture was added water (700 cc), and the mixture was extracted with ether (3 x 200 cc). The ether extracts were washed with water, dried over sodium sulphate, and the solvent was removed. The resultant mixture of acetates, hydrocarbons, unreacted alcohols and more polar material (10.1 g) could then be separated by column chromatography.

Column Chromatography

The acetylated unsaponifiable matter (5 g) was chromatographed on silica gel (60 g) (0.2-0.5 mm particle size) to yield the hydrocarbons (30 mg) and the acetates (4.8 g).

Gas Liquid Chromatography

Preparative GLC of the acetates was accomplished on a 20% Apiezon L on Phase Sep N (44-60 mesh) column (5 ft x 3/8 in.) in a Pye 105 model instrument. Collection of fractions up to chain length C_{18} was achieved isothermally at 250 C, and the remaining fractions were collected on temperature programming up to 280 C. Analytical GLC was performed on a 1% Apiezon L column (on Gas Chrom Z) (80-100 mesh) in a Pye 104 dual column instrument.

Thin Layer Chromatography (TLC)

Individual fractions from the preparative scale GLC separation (20 mg) were chromatographed on silver nitrate (16.7%) silica gel (0.25 mm) with petroleum ether-diethyl ether 90:10 as the eluting solvent.

Von Rudloff Oxidant

Stock oxidant solution was prepared from sodium

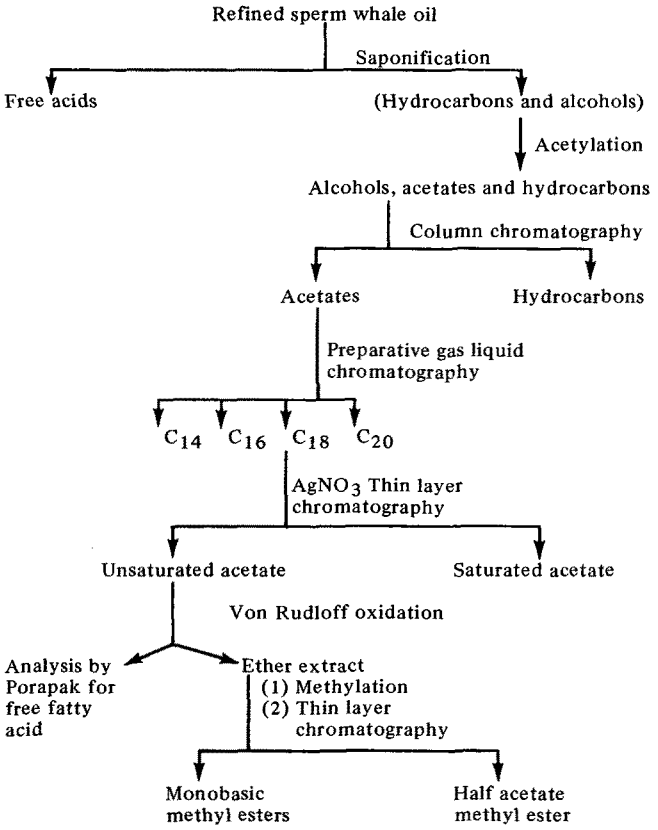

FIG. 4. Analytical procedure for separation and identification of individual alcohols from sperm whale oil.

periodate (0.2 M) and potassium permanganate (0.005 M).

Purification of tert-butanol: *t*-butanol (650 cc) was refluxed with potassium hydroxide (2 g) and potassium permanganate (2 g) for 2 hr and then distilled through a 6 in. column packed with glass helices.

Modified von Rudloff oxidation: the C_{18} acetates (4 mg) were dissolved in purified *t*-butanol (1 cc) and stock oxidant solution (2 cc), and the reaction mixture was shaken for 1 hr.

Direct analysis of oxidation products: an aliquot (20 μliters) was withdrawn from the von Rudloff oxidation mixture (3000 μliters) and injected directly on to a Porapak Q (80-100 mesh) column (18 x 3/16 in.) at 100 C. The temperature was held at 100 C until the solvent had been eluted and then increased to 230 C at a rate of 6 C/min.

Analysis of monobasic and half acetylated monobasic acids: the von Rudloff oxidation products were extracted with diethyl ether (4 x 10 cc) and methylated with diazomethane. The methylated products were separated by TLC into methyl esters and half acetylated methyl esters, each of which was then examined by GLC on Apiezon L columns.

Mass Spectrometry

Mass spectrometry was achieved on an A.E.I. MS 9 instrument.

RESULTS

Thin layer chromatography showed that sperm whale oil consisted of four fractions (Fig. 1): hydrocarbons (0.18 wt%), wax esters (76 wt%), triglycerides (22.6 wt%), and more polar material (1.2 wt%). In order to obtain sufficient hydrocarbons for further analysis, a preliminary enrichment was made by saponification of the oil to yield the "unsaponifiable matter." This unsaponifiable matter was made up of three fractions as shown by TLC (Fig. 2): the hydrocarbons, the free alcohols, and a more polar fraction.

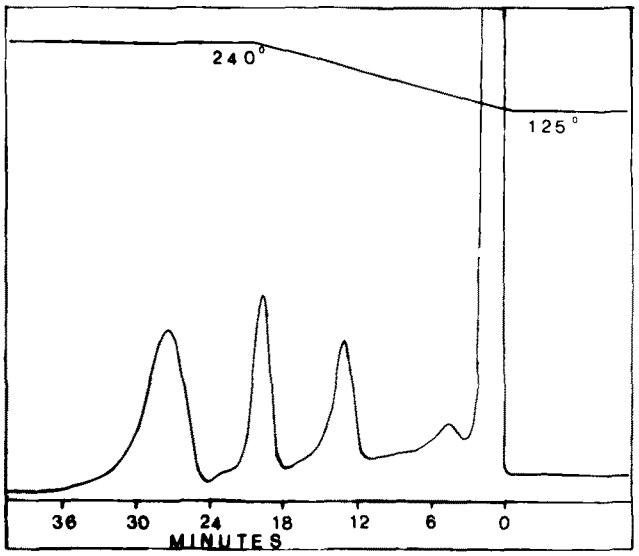

FIG. 5. Gas liquid chromatography separation of propionic, pentanoic, heptanoic and nonanoic acids on Porapak Q.

TABLE I

Sperm Whale Oil Alcohols

Compound	Per cent
12:0	0.5
14:0	15.0
14:1	5.0
15:0	1.5
16:0	29.1
16:1	15.2
17:0	1.0
18:0	3.5
18:1	21.3
19:0	1.0
20:1	6.8

By column chromatography on silica gel, it was possible to isolate 15 mg of hydrocarbons from 3.15 g of "unsaponifiable matter." No significant quantities of unsaturated hydrocarbons could be detected on $AgNO_3$ TLC (8) and the IR spectrum indicated that this fraction was free from carbonyl containing contaminants.

Analysis of the hydrocarbons by GLC at three different temperatures, 150 C, 200 C and 260 C, showed that there was only one major component with a retention index (9) of 1700, i.e., it cochromatographs with *n*-heptadecane. Several other minor components with retention indices of 1780, 1880, 1990, 2610, 2700, 2780, 2860, 3050 and 3340 were also present. The major component was subjected to gas chromatography and mass spectrometry, and the spectrum indicated that the molecular weight was 268 which corresponds to $C_{19}H_{40}$. The retention index considered with this molecular weight suggests that the major alkane is branched. The whole mass spectrum (Fig. 3) is identical with that of the branched chain alkane, pristane. In order to ensure that the saponification step had *not* introduced any artifacts, a small quantity of the hydrocarbon fraction was isolated by TLC from sperm whale oil itself and proved to consist of pristane and no other significant component as shown by GLC.

The alcohols were acetylated in the presence of acetic anhydride and pyridine, and analyzed by GLC on an Apiezon L column at 185 C. Under normal conditions, GLC indicates (Table I) that only the even chain homologues are unsaturated with C_{16} and C_{18} as the major monoene components. However when preparative scale GLC was used to isolate fractions of differing molecular

TABLE II

Sperm Whale Monounsaturated Alcohols

Chain length	Double bond position (% of each isomer)							Published isomer distribution (reference)
	ω.11	ω.10	ω.9	ω.8	ω.7	ω.6	ω.5	
14	---		48		40		12	ω.9(21)
16	---		71		24		5	ω.7(21)
18	4	Trace	84	Trace	9	Trace	3	ω.9(22)
20	3		73		24			

weights, further examination (Fig. 4) by analytical GLC indicated that there were two peaks in the C_{17} region, one of which is presumably an unsaturated alcohol. Very small quantities of branched chain saturated and unsaturated alcohols have been noted, but their structure has not yet been fully elucidated. The individual fractions which had been separated according to chain length were then chromatographed on $AgNO_3$ TLC to remove the saturated acetates. Individual monounsaturated alkyl acetates were obtained which were at least 97% homogenous with respect to chain length.

In order to determine the position of the double bond in each of the alkyl acetates it was decided to use the von Rudloff oxidation technique (10). The major disadvantage of this procedure is claimed to be the isolation of the short chain fatty acids which are formed. Exhaustive ether extraction for up to 24 hr (11) has been suggested. To eliminate this time loss a modified procedure was devised which permitted the analysis by GLC from aqueous solution of the liberated free fatty acids. Analysis of the fatty acids (up to decanoic acid) in the presence of water and *t*-butanol has been achieved on a Porapak Q resin bead column (Fig. 5) with coefficients of variation no greater than ±3.5% (12).

An aliquot from the oxidation mixture of one of the sperm whale alkylacetates was injected directly on to the Porapak Q column to determine the proportions of short chain fatty acids. In addition the remainder of the von Rudloff oxidation mixture was extracted with ether, and the cleavage products were methylated with diazomethane. The methyl esters were separated by TLC from the acetoxy methyl esters, both of which were analyzed by GLC on an Apiezon L column. The proportions of the acetoxy methyl esters confirmed the results of the double bond position obtained by direct fatty acid analysis. Each homolog (Table II) appears to consist of a mixture of double bond positional isomers.

DISCUSSION

Pristane has been isolated previously from the liver oils of lemon shark (13), smooth dogfish (13), nurse shark (13) and basking shark (13,14), herring oil (15) and zooplankton (16). Avigan and Blumer (17) have shown that phytol can be converted to pristane by copepods. More recently Ackman (18) has shown that pristane is present in fresh water fish oils usually at the level of 0.0001 wt%, while in whole body herring oil the proportion is as high as 0.107 wt%. The slightly higher value of 0.17 wt% in sperm whale oil may be due to the fact that the sperm whale is at the head of the food chain which would lead to a concentration of the hydrocarbon.

The alcohols appear to be quite distinct from those found in the plant kingdom where only one major unsaturated isomer for each chain length is common and from those found in gouramis eggs (19). Each homolog in sperm whale oil consists of a number of double bond isomers with the C_{18} showing the widest range of ω5 to ω11 (similar to the situation for fatty acids in skin) (20). Most desaturases are highly specific with regard to the positions from which they remove hydrogens indicating the sperm whale isomers cannot be the result of a faulty desaturation step.

The major constituent for each chain length is the ω9 isomer suggesting that these homologs constitute one biosynthetic family, but the presence of the other isomers can be explained in one of two ways. The most popular theory for the biosynthesis of fatty alcohols assumes that the acids and alcohols are in reversible equilibrium (19) and that the acids are produced by the normal de novo processes. In the only recent study of the positional isomers in the fatty acids of sperm oil, Hansen and Cheah (5) have found that the C_{14} acid is a mixture of ω9 and ω5 isomers, but the C_{18} is ω9. If Schlenk's findings of reversible equilibrium for acids and alcohols is applicable here, our findings can only be explained on the basis that much of the alcohol fraction is derived from dietary lipids.

ACKNOWLEDGMENTS

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