Hydrocarbons and Alcohols of Basking Shark and Pig Liver Lipids

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Abstract

Four samples of the unsaponifiables of basking shark liver oil were adsorbed on alumina and eluted to yield Fractions 1–5, inclusive. Analyses by temperature programmed GC and by silica gel chromatography showed hydrocarbons in the first four fractions with squalene increasing to Fraction 3 and the pristane level being highest in Fraction 1. Aside from pristane and squalene, other hydrocarbons occurred at levels of 420–750 mg% in the oils on a weight basis, of which about 60% constituted a series of n-paraffins (relative carbon number range: 15.0–38.0) together with smaller amounts of at least one branched chain saturated group. Unsaturated hydrocarbons eluted mainly after squalene. The oils contained up to 460 mg% sterol and 78–270 mg% alcohols of C_{10} to C_{30}, the ratio of saturated to unsaturated members being about 1.6. The composition of the unsaponifiable lipids of pig liver was quite different from that of the marine oils. It contained 10.6% sterol in addition to 400 mg% alcohols, the latter consisting of 81.8% saturated components (C_{12} to C_{31}; ratio of saturated: unsaturated members, 4.4). The hydrocarbons comprised 450–700 mg% of the unsaponifiable mixture and squalene, paraffins and additional unsaturated components occurred at levels of 20.6, 24.4 and 11.9 mg%, respectively. The saturated hydrocarbons were high in normal homologs of relative carbon number range, 15 to 36; pristane could not be detected.

Introduction

Saturated hydrocarbons are quite widespread in many animal sources and have been shown to be definite components of scalp or hair lipids (1). Recently, the paraffinic types in wool fat have been characterized by Simmonds et al. (2).

As the sebum saturated hydrocarbons occur together with large amounts of squalene, it was thought that other natural mixtures of lipids high in this component might likewise present a variety of paraffinic hydrocarbons for correlative studies. For this purpose, the hydrocarbon make-up of the basking shark liver oil appeared very promising. In addition to squalene, this lipid contains pristane (2,6,10,14-tetramethylpentadecane), a component also occurring in lanolin (3). In regard to basking shark liver oil, the studies of Blumer and co-workers are pertinent (4–8). In contrast to the unsaturated fatty acids which are changed, there was little structural alteration in the hydrocarbons of zooplankton on which the shark fed and the hydrocarbon distribution served as an index of the food source and the feeding grounds of the animal. The squalene level was quite high in the liver lipids, although it was present in negligible amounts in the zooplankton.

In the present study, basking shark liver oil processed from four different fishing expeditions was saponified and the unsaponifiable portion chromatographed over alumina, thereby yielding the hydrocarbons, alcohols and sterols. Emphasis was directed to the saturated hydrocarbons. Parallel experiments were carried out with a mammalian liver lipid mixture as represented by the pig. The composition of alcohols was also ascertained for the two lipid types.

Experimental Procedures

Starting Lipids

Basking shark liver oil comprised four batches, Sample M originating from J. C. Martens & Co., Bergen, Norway, which was also the source of purified pristane, and the other three from Albert Quast, Hamburg, Germany. Liver pooled from 12 pigs was obtained fresh from the abattoir, care being taken to prevent any external contamination. The tissue was blended with absolute methanol, an equivalent volume of chloroform then being added. The contents were stirred frequently at room temperature for a period of three days, filtered and the residue exhaustively extracted with chloroform-methanol (1:1). The extract was rid of solvent in an all glass rotary vacuum evaporator and the residue taken up in petroleum ether (b 30–60 C), washed with portions of water and then dried over anhydrous sodium sulfate. Removal of the solvent yielded the lipids with a recovery of about 5% based on the liver. Each of the solvents was distilled prior to use and all glassware and equipment were repeatedly rinsed with the solvents.

For the saponification of the liver lipids with alcoholic sodium hydroxide, chromatography of the unsaponifiable components (UNS) over alumina and separation of the hydrocarbons by silica gel column chromatography and for the analysis of sterol and squalene, the procedures described previously were employed with little modification (1,9–11). Several of the methods are exemplified below. The alcohols were converted to the esters by treatment with acetic anhydride in pyridine and analyzed by GC over polar and nonpolar packings; the unsaturated esters were hydrogenated employing Adams' platinum oxide catalyst (10). The presence of hydrocarbons in the various fractions was verified by the infrared spectra and the substantiation of several of the normal paraffins was accomplished by mass spectrometry.

Gas Chromatography

Samples were submitted to temperature programming in a Barber Colman gas chromatograph model 5000 with hydrogen flame detector. The rate of heating was 2 C/min. The U-shaped glass column of 72 × 0.6 in. OD, was packed with 3% SE-30 on 60–80 mesh Gas Chrom P. The heater and detector temperatures were 320 and 350 C, respectively, and the column was varied over 175–310 C; the carrier gas was He at 15 lb pressure. With the system containing 15% DEGS on 60–80 mesh Gas Chrom P, the column, injector and detector temperatures were 210, 210 and 250 C, in the order stated; He was introduced at 70 ml/min. The samples were dissolved

in ethyl ether and volumes of 3 μl injected. With several hydrocarbon cuts, the SE-30 column temperature was maintained at 240 C. Normal hydrocarbons of C_{29} and greater (1) were employed as standards and tentative assignment for each peak obtained.

Basking Shark Liver Oil

Sample M, Saponification. Oil in the amount of 200 g was refluxed with a solution of 300 ml 20% sodium hydroxide in 95% ethanol for 16 hr, after which the mixture was taken up in 3.5 l water and extracted repeatedly with ethyl ether. The ethereal extract (volume: 4 l) was washed with portions of water and dried over anhydrous sodium sulfate. Removal of solvent from the filtrate gave rise to 54.1 g UNS or a recovery of 27.1%. The fatty acids obtained from the saponifiable portion by treatment with mineral acid, ether extraction and washing the latter solution with water weighed 122.6 g (61.3%).

Chromatography of UNS Over Alumina. A solution of 47.8 g UNS in 4.8 l petroleum ether was passed over 1.92 kg Alcoa F-20 and the column was eluted with 1.4 l each of the following, the percentages being on a volume basis: petroleum ether, 5% chloroform + 95% petroleum ether, 10% chloroform + 90% petroleum ether, chloroform, and absolute methanol. Removal of the solvents led to Fractions 1–5, inclusive, in the recoveries shown in Table I. Based on GC over SE-30, Fraction 1 contained pristane and squalene in amounts of 18.9% and 81.1%, eluting at $C_{17.5}$ and C_{28}, respectively. Except for traces of n-C_{19}, it was difficult to measure any of the other peaks. Fractions 2 and 3 each contained 95–97% squalene and 8–9 other minor peaks with relative C numbers ranging from 16.5 to 30.4. Fraction 4 was lower in squalene content but displayed several peaks up to $C_{29.3}$ and Fraction 5 was composed of sterol and alcohols.

Chromatography of Fractions 1 and 2 Over Silica Gel. A total of 35.0 g Fraction 1 in 3.5 l petroleum ether was passed over a water-cooled column containing 1.05 kg Davison's silica gel (100–200 mesh). The column was then eluted with 2.1 l each of petroleum ether, benzene, ethyl ether, acetone and absolute methanol. Thirty-three 350 ml fractions were collected, the distribution of lipid removed by the first two media being shown in Table I. The composition of cuts 1–5 employing petroleum ether and of cuts 16 and 19, eluted with benzene and ethyl ether, respectively, are presented in Table II.

In a similar manner, 3.01 g of Fraction 2 dissolved in 300 ml petroleum ether was adsorbed on 90 g silica gel and eluted with the same solvents, 50 ml cuts being collected. The recoveries of lipid were 4.8%, 78.4%, 0.6%, 1.3% and 1.1% employing the above five media in the order given. Saturated hydrocarbons, notably pristane, occurred at a level of 0.10% of Fraction 2. The cuts contained C_{28} (squalene) and to a lesser extent, $C_{29.3}$, $C_{29.5}$ and $C_{29.8}$.

Samples 1, 2 and 3. Three additional batches of liver oil were also saponified and fractionated (Table I). Silica gel chromatography of the respective fractions from treatment with alumina gave analytical data similar to those for Sample M.

Silica Gel Chromatography of Fractions 2, 3 and 4 of Sample 1. Petroleum ether-eluted cuts from silica gel chromatography of Fraction 2 were high in pristane except for the first two, which also contained other paraffins, all of which were normal (Table III). The main portion eluted by benzene comprised squalene with smaller amounts of other olefins, especially, the $C_{29.3}$. Cuts from Fraction 3 contained 99–99.9% squalene with minor quantities of $C_{20.8}$, $C_{21.8}$ and $C_{29.5}$. Fraction 4 (12 mg) adsorbed on silica gel yielded no residues with petroleum ether, acetone or methanol. Benzene removed lipid which progressively fell in squalene content and increased in a $C_{29.3}$ component. Based on mass spectrometry, the latter appeared to be cyclic with a mass number of 426.

Shark Liver Alcoholic Components. The sterol content of Fraction 5 from each of the shark UNS was quite high, that derived from sample M being 64.5%. GC analyses of alcohols as based on total sterol and without sterol in Fraction 5 are presented in Table IV.

Pig Liver Lipids

Saponification. A 69.3 g sample of pig liver lipids was saponified by refluxing with 280 ml of 20% alcoholic sodium hydroxide for 24 hr. Processing of the mixture yielded 9.0 g UNS or a recovery of 13.0% (small losses).

Chromatography of UNS Over Alumina. The chromatography of UNS over alumina gave rise to Fraction 5 almost exclusively except for hydrocarbons in amount of 450–700 mg/100 g. In one run employing 4 g UNS, the yields were 7.6, 6.5, 1.9 and 1.5 mg for Fractions 1–4, respectively, with a recovery of 85% in Fraction 5 which analyzed 96% sterol. GC analysis of Fractions 1–4, inclusive, was carried out with the DEGS column. Fraction 1 displayed 35 peaks of which the C_{31}-peak material was composed of 2.5% squalene and 3.0% of alkane and 70% of

TABLE I

UNS and Alumina-Chromatographed Fractions From Four Basking Shark Liver Oil Samples

Sample	M	1	2	3
Liver Oil Saponified, g	200.0	75.5	47.6	40.3
UNS, g (%)	54.1(27.1)	12.9(17.1)	13.4(28.2)	10.2(25.3)
Fatty Acids, g (%)	122.6(61.3)	57.6(76.3)	31.2(65.5)	24.8(61.5)
Alumina-Chromatographed Fractions				
UNS Sample, g	47.8	11.9	10.0	8.0
Fraction 1[a], g (%)	41.1(86.0)	7.2(60.5)	8.1(81.0)	4.2(53.0)
Fraction 2[b], g (%)	3.7(7.7)	2.2(18.4)	0.40(4.0)	1.6(20.0)
Fraction 3[c], mg (%)	54.0(0.11)	1210.0(10.2)	238.0(2.4)	987.0(12.3)
Fraction 4[d], mg (%)	66.4(0.14)	17.4(0.15)	5.7(0.06)	5.0(0.06)
Fraction 5, mg (%)	996.0(2.1)	180.4(1.5)	218.9(2.2)	229.0(2.9)

[a] For samples 1, 2 and 3 over SE-30, the fractions contained $C_{17.5}$: 37.0%, 24.5% and 33.3%; C_{19}: 2.0%, 0.7% and 1.4%; and C_{28} + squalene: 60.0%, 73.4% and 65.2%, in the order stated.

[b] GC analysis of the fractions of samples 1, 2 and 3 over SE-30 showed the presence of 97.5%, 97.7% and 97.2% of C_{28} + squalene and 0.9%, 1.2% and 0.7% pristane, respectively. Relative percentages of the remaining peaks were: $C_{16.5}$, 0.1–0.2%; $C_{20.5}$, 0.1–0.4%; C_{21}, T–0.1%; C_{23}, 0.2–0.3%; $C_{25.5}$, 0.1–0.2%; $C_{26.5}$, T–0.3%; and $C_{29.8}$, 0.1–0.3%.

[c] For samples 1, 2 and 3, the C_{28} + squalene contents were 96.0%, 93.6% and 95.1% and the $C_{17.5}$: 0.1%, 0.7% and 1.0%, respectively, as ascertained over SE-30. The additional peaks were as follows: $C_{20.2}$, 0.3–0.6%; $C_{25.5}$, 0.5–0.9%; $C_{26.5}$, 0.2–0.8%; $C_{27.5}$, 0.2–0.4%; $C_{29.5}$, 0.2–0.3%; and $C_{30.4}$, 0.2–0.4%.

[d] The C_{28} + squalene contents were 33.0%, 49.7% and 58.0%; $C_{29.3}$: 61.7%, 23.7% and 26.2%; and C_{19}: 0.6%, 10.4% and 12.1% for the fractions from samples 1, 2 and 3, inclusive. The other peaks, employing the SE-30 column constituted: $C_{19.5}$, 0.1–2.0%; $C_{20.8}$, 0.3–1.6%; $C_{23.8}$, 0.5–1.3%; $C_{25.5}$, 0.2–1.3%; $C_{26.5}$, 0.2–0.5%; $C_{27.5}$, T-0.1%; $C_{29.5}$, 0.1–0.8%; and $C_{30.1}$, 0.1–1.8%. The $C_{26.8}$-component (1.8%) occurred in sample 1.

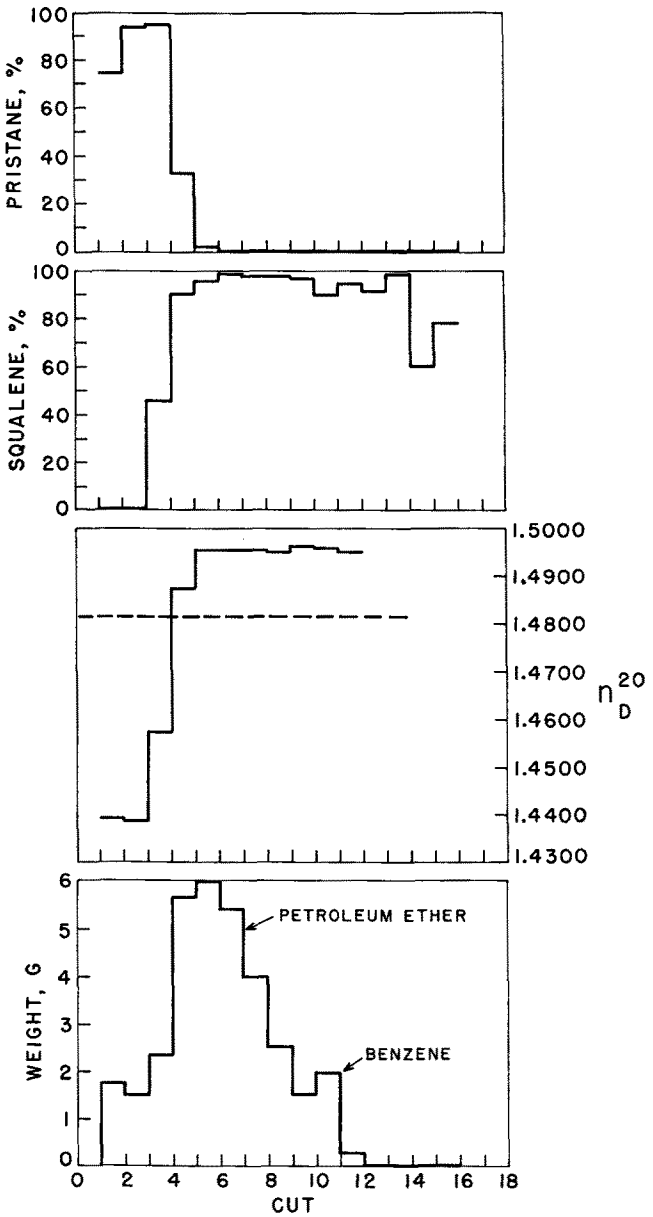

FIG. 1. Distribution of weights, indices of refraction and pristane and squalene contents of cuts from chromatography of 35.0 g Fraction 1 (alumina) of basking shark liver oil M-UNS over silica gel. Cuts 1–10 and 11–16, inclusive were eluted with petroleum ether and benzene, respectively.

the entire fraction comprised n-paraffins and branched chain homologs. Fraction 2 contained 77.8% C_{31} + squalene in addition to 1.6% n-paraffins (C_{20}–C_{27}), 0.2% $C_{28.4}$, 13.9% $C_{31.6}$ and 4.0% C_{34}. Fraction 3 showed the presence of 57.5% squalene, 27.3% $C_{31.6}$ and 15.1% $C_{33.6}$, while Fraction 4 was free of squalene but displayed 14 peaks of C_{20} to $C_{32.8}$.

Silica Gel Chromatography of Alumina Fraction I. Fraction 1 (7 mg) was submitted to chromatography over silica gel and the column eluted with petroleum ether (5 cuts) and benzene (8 cuts). Paraffinic hydrocarbons were high in the fraction and accounted for most of the petroleum ether-eluted cuts as noted in Table V, temperature programming being conducted over SE-30.

Alcohol Composition. GC analysis of the alcohol acetates was carried out on the residual portion of Fraction 5 following the removal of the sterol by fractional crystallization from methanol. The percentage composition of the sample containing 70% sterol appears in Table VI and is given in terms of the alcohols as such.

TABLE II

Silica Gel Chromatographed Cuts from Fraction 1 (Alumina) of Basking Shark Liver Oil M UNS (SE-30)[a]

Rel. C No.	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Cut 16	Cut 19
15.0	1.3						
15.4	0.5						
15.6	0.3						
15.8	0.2						
16.0	0.2						
16.2	0.4						
16.5	0.2						
17.0	0.2						
17.5[b]	75.0	93.7	94.1	33.1	1.4		
18.0	0.4						
18.5	3.0			0.2			
19.0	11.7	0.8	1.0	0.3			
19.5	0.2						
20.0	0.2	0.3	0.1				
20.5	0.2						
21.0	0.6	0.1	0.1				
21.3	0.2						
21.5	T						
22.0	0.4	0.1	0.1				
22.5	0.3						
23.0	0.3	0.3	0.4	0.2			
24.0	0.3	0.1	T				
24.8	T						
25.0	0.3	0.1	T				
26.0	0.4	0.1	0.2				
26.5	T						
27.0	0.2	0.3	0.4				
27.5	T					0.7	
28.0	0.5	0.7	0.5	66.2[c]	90.4[c]	78.5[c]	94.8[c]
28.5	T						
29.0	0.2	0.4	0.5		2.9	1.4	0.3
29.5	T					19.4	1.7
30.0	0.3	0.6	0.4		4.4		
30.5	T						
31.0	0.2	0.8	0.8		0.9		
31.5	T						
32.0	0.2	0.4	0.2				
33.0	0.2	0.4	0.5				
34.0	0.2	0.3	0.2				
35.0	0.2	T	0.1				
36.0	0.2		0.1				
37.0	0.2						
38.0	0.2						

[a] Petroleum ether-eluted cuts except for samples 16 and 19 which were removed with benzene and ethyl ether, respectively. The overall percentage recoveries of lipid eluted from Fraction 1 by petroleum ether, benzene, ethyl ether, acetone and methanol were 92.7, 6.6, 0.07, 0.01 and 0.04, in the order stated. In all tables, the GC values are area %.
[b] Pristane.
[c] Squalene.

Discussion

The present methods allowed for the concentration and enrichment of the hydrocarbons of the basking shark liver oil. The main portion consisted of squalene and pristane; other saturated hydrocarbons occurred, including normal homologs as well as branched chain members. Thus, as noted from Table II, petroleum ether-elution of the silica gel column treated with Fraction 1 (alumina) of oil M yielded 5 cuts with pristane making up 37% of the fraction or 3.5% as based on the oil. Cuts 1–3, inclusive, contained other saturated hydrocarbons at 0.30%, 0.07% and 0.06% in terms of oil M of which the main portion was n-paraffinic, C_{19} being quite prominent in cut 1

TABLE III

Normal Paraffinic Hydrocarbons of Fraction 2 of Basking Shark Liver Oil 1-UNS as Separated by Silica Gel Chromatography[a]

Relative C No.	Cut 1	Cut 2
17.5 (Pristane)	53.9	69.7
19.0	3.0	
20.0	3.0	
21.0	4.6	
22.0	6.1	
23.0	6.1	
24.0	6.1	
25.0	4.6	
26.0	4.6	3.0
27.0	3.0	6.0
28.0	3.0	9.0
29.0	1.5	9.0
30.0	T	3.0

[a] Composition of cuts 1 (11.9 mg) and 2 (4.1 mg) of a total of 16 eluted by petroleum ether; the remaining cuts (total lipid: 70.0 mg) contained pristane almost exclusively.

TABLE IV

Basking Shark Liver Oil Alcohols Based on Fraction 5 Analysis (DEGS)[a,b]

Relative C No.	Sample 1		Sample 2		Sample 3	
	Sterol-free	With sterol	Sterol-free	With sterol	Sterol-free	With sterol
10.0	0.6	0.2	0.6	0.2	0.8	0.3
11.0	1.0	0.3	0.6	0.2	0.8	0.3
12.0	2.0	0.6	1.1	0.4	1.3	0.5
13.0	1.0	0.3	0.6	0.2	0.8	0.3
14.0	18.1	5.4	12.6	4.2	14.4	5.3
15.0	0.6	0.2	1.8	0.6	1.3	0.5
16.0	2.0	0.6	1.8	0.6	1.3	0.5
16.4[c]	0.6	0.2	0.6	0.2	0.3	0.1
17.2	0.3	0.1	0.6	0.2	0.5	0.2
18.0	0.3	0.1				
18.4	1.0	0.3	0.6	0.2	0.5	0.2
18.8[c]	1.7	0.5	1.1	0.4	1.3	0.5
19.8			1.1	0.4	0.8	0.3
20.8[c]	2.3	0.7	0.6	0.2	0.5	0.2
22.3[c]	6.7	2.0	1.5	0.5	2.2	0.8
23.5	1.7	0.5	1.1	0.4	1.3	0.5
24.5	1.7	0.5	1.8	0.6	1.3	0.5
24.6	6.7	2.0	3.5	1.2	4.5	1.7
25.6			3.8	1.3	2.2	0.8
26.0	12.4	3.7	10.7	3.6	10.8	4.0
26.4			0.3	0.1	0.3	0.1
27.0			0.8	0.2		
27.5[c]	0.6	0.2	0.8	0.3	1.9	0.7
28.6[c]	22.9	6.8	34.7	11.6	33.2	12.3
29.2	15.2	4.5	17.3	5.8	17.2	6.4
Sterol		70.0		66.4		62.8

[a] Percentages are given in terms of Fraction 5.
[b] Oil M resembled sample 3 in alcohol and sterol composition except for the following at levels of 0.1–0.2% based on Fraction 5 and including the sterol: $C_{20.2}$, $C_{21.0}$, $C_{21.8}$, $C_{22.8}$, $C_{24.0}$ and $C_{27.0}$.
[c] Unsaturated component.

(11.7%); the range in carbon number was 15 to 38 with a total of 43 peaks in cut 1. For this latter cut, excluding pristane, the ratio of normal to branched saturated components was 4:1 and subsequent cuts as well as the initial ones from silica gel chromatography of Fraction 2 were high in straight chain hydrocarbons, as shown in Table III. The overall content of pristane-free paraffinic hydrocarbons in the oil as exemplified by sample M, was 440 mg/100 g. The unsaturated components, aside from squalene, amounted to 300–400 mg/100 g and in the case of oil M, of the 312 mg/100 g, the $C_{29.3}$ and $C_{29.5}$ components contributed 38 and 14 mg/100 g, respectively. Small amounts of zamene or C_{19}-monoolefins and C_{20}-diolefins as described by Blumer et al. (6–8)

TABLE V

Composition of Hydrocarbons of Pig Liver Fraction 1 Separated by Silica Gel Chromatography (SE-30)[a]

Relative C No.	Cuts 1 + 2	Cut 3
15.0	1.1	
16.0	1.7	1.4
17.0	3.3	1.4
18.0	4.0	5.7
18.6	1.1	
19.0	5.0	7.1
19.6	1.1	
20.0	4.5	7.1
20.5	1.1	2.9
21.0	4.0	5.7
21.6	1.1	
22.0	8.5	5.7
22.5	2.2	
23.0	4.5	7.1
23.5	1.1	1.4
24.0	3.3	5.7
24.5	1.1	1.4
25.0	4.0	4.3
25.5	1.1	
26.0	4.0	5.7
26.5	0.5	
27.0	6.2	2.9
28.0 + Squalene	5.6[b]	15.7[c]
29.0	5.0	5.7
30.0	4.5	4.3
31.0	5.6	1.4
32.0	4.0	4.3
33.0	3.3	1.4
34.0	2.9	1.4
35.0	2.2	
36.0	1.9	

[a] Fractionation of 7.0 mg product of which cuts 1, 2 and 3 obtained with petroleum ether comprised the main portion of the product. Cuts 4 and 5 (petroleum ether) yielded little lipid. Of the benzene-eluted hydrocarbons, cuts 6–8, inclusive, contained 33.3% $C_{27.3}$ and 66.6% C_{28} + squalene, while combined cuts 9–12 analyzed for 29.4% $C_{20.5}$, 29.4% C_{28} + squalene and 41.1% $C_{29.3}$.
[b] Squalene is absent.
[c] About 50% of this peak is due to squalene.

TABLE VI

Pig Liver Alcohols as Determined by GC of Residual Fraction 5 Acetates (DEGS)[a]

Relative C No.	%
12.0	1.1
13.0	0.9
14.0	13.7
15.0	2.7
16.0	12.4
17.0	1.7
18.0	4.5
18.3[b]	9.2
19.4	3.1
20.0	2.5
21.0	0.9
21.4	0.9
22.0	1.3
22.6	1.8
23.4[b]	0.9
24.0	1.3
25.0	9.9
25.6	2.8
26.0	1.1
27.0	8.6
27.4[b]	7.3
28.0	6.7
28.3[b]	T
30.0	2.2
30.3	2.9

[a] Residue analyzing for 70% sterol and remaining after crystallization of most of the sterol. Percentages are in terms of the alcohols in Fraction 5 alone.
[b] Unsaturated.

also appeared to be present but no attempt was made to isolate them for further confirmatory study. The data for the 4 oils are summarized in Table VII.

Fraction 5 from chromatography of UNS over alumina served as a convenient source for the analysis of sterol and alcohols. The shark liver oils displayed sterol and alcohol ranges of 0.37–0.46% and 0.20–0.27%, respectively, the contents being correspondingly lower for oil 1 (Table VII). The percentage compositions in terms of saturated straight and branched chain and unsaturated members and the ratio of saturated to olefinic alcohols were 38.0, 26.6, 34.8 and 1.85 for sample 1; 30.8, 30.1, 39.3 and 1.55 for sample 2; and 31.5, 28.6, 39.4 and 1.53 for sample 3, in the order stated. Oil M simulated the last one in alcohol composition except for minor amounts of additional components, all saturated (Table IV). The main straight chain saturated alcohols were C_{14} and C_{26}, and $C_{24.6}$ and $C_{29.2}$, were the predominant branched types; of the unsaturated members, C_{16}, C_{18}, C_{20}, C_{22}, C_{27} and C_{28}, the last one ranged highest in amount.

The UNS of the pig liver lipids contained principally sterol and alcohols (Fractions 1–4, inclusive, from alumina chromatography; 437 mg/100 g UNS) representing a total of 56.9 mg/100 g as based on the initial lipids and accordingly, far under the levels observed with the shark liver oils. Of the hydrocarbons, 20.6, 24.4 and 11.9 mg/100 g constituted squalene, paraffins and additional olefins including

TABLE VII

Summary of Analytical Findings for Basking Shark Liver Oils (Percentages Based on the Initial Samples)[a]

Component, %	Sample M	Sample 1	Sample 2	Sample 3
Squalene	20.8 (20.5)	10.9	18.4	16.5
Pristane	4.4 (3.5)	3.9	5.6	4.5
Other Hydrocarbons, mg[b]	(752)	720	550	420
Paraffins, mg	(440)			
Unsaturated, mg	(312)[c]			
Sterol, mg	370	182	410	460
Alcohols, mg	200	78	210	270
Fatty acids[d]	61.3	76.3	65.5	61.5

[a] The values in parentheses refer to those determined by further fractionation over silica gel.
[b] Paraffinic hydrocarbons constituted 55–60% of the mixture free of pristane and squalene.
[c] Of this, the $C_{29.3}$ and $C_{29.5}$ components comprised 38 and 14 mg% respectively.
[d] Recovered from the saponifiable portion.

the $C_{29.3}$-component (Table V). Fraction 1 contained 2.5% squalene and the remaining hydrocarbons, mainly n-paraffins (69.6%) and branched saturated homologs (27.7%); the relative carbon number ranged from 15 to 36 and the presence of pristane could not be substantiated. The preponderance of straight chain homologs can also be noted from the petroleum ether-eluted cuts from silica gel chromatography of Fraction 1. Thus, cuts 1 + 2 (31 peaks; SE-30 column; Table V) contained 89.1% straight chain as compared to 10.4% branched saturated hydrocarbons and a similar ratio obtained with cut 3 except for the presence of a small amount of squalene. It might be pointed out that the overall hydrocarbon content in the pig liver lipids ranged from 60 to 90 mg/100 g, corresponding to 30–45 mg/kg fresh tissue. The precautions taken to prevent contamination notwithstanding, the hydrocarbons may be regarded as bona fide components considering the presence of squalene as such and at a level approaching that of the paraffins.

The sterol and alcohol contents of the pig liver lipids, 10.6% and 0.40%, respectively, ranged higher than those of the basking shark liver oil, and the alcohols from the mammalian source consisted of 81.8% saturated and 18.4% unsaturated members or a ratio of 4.4 as compared to a mean value of 1.6 for the shark product. The saturated alcohols of the hog lipid were mainly straight chain and especially prominent in C_{14}, C_{16}, C_{25}, C_{27} and C_{28}. Of the unsaturated alcohols, C_{18} and C_{27} were relatively high and C_{23} and C_{27}, the lowest.

Marine lipid hydrocarbons have been isolated by several workers and the literature with emphasis on pristane has been reviewed by Blumer et al. (5). The presence of pristane, phytadienes and C_{19}-monoolefins with the pristane skeleton in basking shark liver oil is on a nutritional basis, owing their origin to zooplankton (4–8). The n-paraffins (C_{15} and C_{17}) occurred in comparable amounts in both zooplankton and the liver oil of a young shark (11–18/10,000 parts pristane) as was also the case of pristane (8). Minute amounts of octadecane and nonadecane were reported in a commercial pristane sample of shark origin (12), and low levels of several straight chain paraffins and polyenes, in shark and herring oils (13). In this regard, herring oil was shown to contain hydrocarbons (0.05%) of C_{14} to C_{33} including pristane and squalane, the mixture without prior GC analysis, having been hydrogenated and the normal and branched chain paraffins separated by urea complexation; higher homologs as C_{27}, C_{29} and C_{31} were thought to represent unsaturated hydrocarbons (14). In relation to the alcohols, the presence of phytol as such could not be demonstrated in the present study but on the assumption that the C_{20} unsaturated peak corresponds to this component, it would occur at a level of 13 ppm in the marine oils. In an earlier report by Sörensen and Mehlum (15), a basking shark liver oil containing 84% squalene and 14% pristane, analyzed for 0.4% cholesterol in agreement with the current data. However, hexadecanol, octadecanol and octadecenol, obtained by distillation of the oil, occurred in far greater amount than in the four oils investigated, the levels being 0.6%, 0.4% and 0.3%, respectively. The separation of basking shark liver alcohol groups has also been attempted by TLC (13,16). Although not as clear-cut as the fatty acids, the composition of alcohols would also reflect dietary status of the animal in addition to species differences. In this respect, a comprehensive parallel study of the alcohols of the shark liver and zooplankton, especially, as represented by the copepod genus, *Calanus*, would be very timely.

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