Hydrocarbons of Dogfish and Cod Livers and Herring Oil

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Abstract

Dogfish and cod liver oils and the oil from the whole herring were saponified and the hydrocarbons concentrated by chromatography of the unsaponifiable portion over alumina followed by silica gel treatment of the resulting fractions. Temperature programmed gas chromatography employing a 3% SE-30 packing was applied to the analysis of hydrocarbons of C_{14} to $C_{32.5}$. The paraffins comprised two or more groups. Dogfish liver oil gave rise to 7.62% unsaponifiables and pristane, other saturated types, squalene and an additional group, high in unsaturated components, were 193, 325, 308 and 200 mg% in this portion or 15.7, 24.8, 23.5 and 15.3 mg%, respectively, in the oil. Cod liver oil yielded 1.0% unsaponifiables of which the above hydrocarbons in the order stated amounted to 0.30%, 1.15%, 3.29% and 2.27% or 3.0, 11.5, 32.9 and 22.7 mg% in the liver oil. The unsaponifiable material of herring oil (1.35%) was prominent in paraffinic hydrocarbons, the levels of the above specified components being 16.34%, 3.51%, 0.99% and 1.41% as stated or 221, 47.4, 13.4 and 19.1 mg% in the oil. The sterol and alcoholic contents were ascertained for the three marine oils and the glyceryl ether levels found to be highest for dogfish liver oil.

Introduction

The composition of hydrocarbons and alcohols of the liver oil of the basking shark has been reported by Gershbein and Singh (1) and the findings compared with those of the corresponding lipids of the pig. In addition to pristane and squalene, the oil contained 420–750 mg% of other hydrocarbons with carbon numbers ranging from 15 to 38 as ascertained by temperature programmed gas chromatography (GC). The present investigation extends these studies to other fish oils to demonstrate paraffinic hydrocarbon types. Liver oils of the dogfish (*Squalus acanthias*) and the cod (*Gadus morhua*) and the oil from the whole herring (*Clupea harengus*) were saponified, the hydrocarbons of the unsaponifiable portion (UNS) concentrated by chromatography over alumina and paraffinic mixtures obtained by treatment with silica gel. Lambertsen and Holman (2) subjected herring oil to saponification and chromatographed the resulting UNS over alumina but the hydrocarbon mixture was hydrogenated and the straight and branched chain homologs separated by urea complexation prior to GC analysis. Alumina has also been employed as an adsorbent in the preliminary processing of fish oil hydrocarbons by Swain (3), among others. The earlier literature on marine oil hydrocarbons has been reviewed by Swain (4) and Bailey et al. (5).

Experimental Procedures

The acids obtained from saponification of the oils with aqueous alkali were converted to the methyl esters and analyzed by GC over polar and nonpolar packings, yielding the data of Table I. Hydrogenation was carried out in a Parr low pressure apparatus at 25 C in the presence of Adams platinum oxide catalyst. It must be stressed that all equipment was thoroughly defatted with chloroform-methanol (2:1) and ethyl ether and with no exceptions, the solvents were of AR grade and distilled before use. To minimize oxidation, concentration of extracts was carried out under vacuum or in a nitrogen atmosphere at temperatures under 35 C.

The procedures for the isolation of hydrocarbons and alcohols have been presented in detail previously (1,6,7). In a typical run, a mixture of 204 g herring oil and 327 ml 20% sodium hydroxide in ethanol was refluxed for 24 hr and the cooled contents taken up in 3 liters of water and extracted with six 400 ml portions of ethyl ether. The ethereal solution was washed with six 400 ml aliquots of water and the dried filtrate concentrated. The yield of UNS was 2.76 g or 1.35% based on the initial oil. Acidification of the saponifiable portion with dilute sulfuric acid followed by extraction with ether and washing with water, led to 195 g of acids. UNS was further resolved by passage of a 1% solution in petroleum ether (bp 30–60 C) over activated alumina (Alcoa F-20) and elution of the column with petroleum ether alone and containing 5% chloroform, then 10% chloroform, 100% chloroform and finally, absolute methanol. All fluid percentages are on a volume basis. Removal of the solvents under reduced pressure and nitrogen gave rise to fractions 1–5, inclusive. Hydrocarbons occurred in fractions 1, 2 and 3 and appeared in fraction 4 along with small amounts of oxygen-containing components. Fraction 5 was made up of alcohols and sterols. Thus, on chromatography of the above herring UNS in 276 ml petroleum ether over 105 g Alcoa F-20 and elution with 105 ml portions of the five solvents, 22.8% of UNS occurred in fractions 1–4, inclusive (Table II). Further enrichment of the paraffins was afforded by chromatography of fractions 1 and 2 over Davison's silica gel (200–236 mesh) and elution with petroleum ether. Other hydrocarbon components were then removed by successive treatments with benzene, ethyl ether, acetone and methanol. Sterol was analyzed both by way of digitonin and by GC.

GC analyses were carried out in a Barber Colman Gas chromatograph model 5000 with hydrogen flame detector, the borosilicate U-shaped column measuring 72 × 0.6 in. o.d. With the 3% SE-30 packing on 60–80 mesh Gas Chrom P, the heater and detector temperatures were 320 and 350 C, respectively and in the temperature programming, the column was varied over 175–310 C at the rate of 2 C/min. 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 and He was introduced at 70 ml/min. Volumes of 3 μl of each of the sample-ethyl ether solutions were injected and the resulting peaks identified tentatively on the basis of normal hydrocarbon standards up to C_{29} and greater. Glyceryl ethers were acetylated and analyzed by GC as proposed by Bloomstrand and Gürtler (8), the standards originating from Applied Science Laboratories, also the source of the fatty acid ester mixtures. As indicated in the earlier study of

TABLE II

Hydrocarbon Composition of the Marine Oils and UNS

Composition	Dogfish liver		Cod liver		Herring	
Oil saponified, g	204		234		204	
UNS recovery, g (%)	15.55	(7.62)	1.95	(0.83)[a]	2.76	(1.35)
Alumina chromatographed fractions						
UNS sample, g	11.19		1.95		2.76	
Fraction 1, mg (%)	87.4	(0.78)	87.0	(4.46)	563.1	(20.4)
Fraction 2, mg (%)	20.2	(0.18)	37.5	(1.92)	21.3	(0.77)
Fraction 3, mg (%)	16.5	(0.15)	11.0	(0.56)	28.3	(1.03)
Fraction 4, mg (%)	15.2	(0.15)	24.4	(1.25)	17.6	(0.64)
Hydrocarbons[b]						
Pristane, % in UNS (mg% in oil)	0.193	(15.7)	0.298	(3.0)	16.34	(221)
Other saturated members, % in UNS (mg% in oil)	0.325	(24.8)	1.15	(11.5)	3.51	(47.4)
Squalene, % in UNS (mg% in oil)	0.308	(23.5)	3.29	(32.9)	0.992	(13.4)
Additional homologs, % in UNS (mg% in oil)[c]	0.200	(15.3)	2.27	(22.7)	1.41	(19.1)

[a] In a second run employing 232 g oil, the recovery of UNS was 2.36 g or 1.14%.
[b] Based on GC analysis of alumina- and silica gel-treated mixtures.
[c] Contains both saturated and unsaturated types, the latter predominating.

Gershbein and Singh (1), squalene and pristane eluted from SE-30 at $C_{28.0}$ and $C_{17.5}$, respectively.

Results

The distribution of components in the hydrocarbon fractions obtained from the marine lipids is presented in Table II. The UNS yields from the dogfish and cod liver oils and herring lipids were 7.62%, 0.83% and 1.35%, respectively, of which the hydrocarbon fractions isolated by alumina chromatography comprised 1.26%, 8.19% and 22.84%, in the order stated, as based on UNS. The relative carbon numbers and area percentages of hydrocarbons in the alumina fractions from dogfish liver UNS and in the silica gel-treated cuts from fractions 1 and 2 of each of the three marine sources appear in Table III.

With cod liver UNS, squalene comprised 39.0%, 80.0%, 11.8% and 2.4% of fractions 1 to 4, respectively and pristane appeared to the extent of 9.4% in fraction 1 and 5.2% in fraction 4 but was absent from fractions 2 and 3. A $C_{30.5}$ component occurred to the extent of 73.9% of fraction 3, the remaining portion constituting a variety of hydrocarbons, each in small amount.

GC analysis of fraction 1 of herring UNS showed the following five peaks and the corresponding relative percentages: $C_{16.6}$, 2.8; $C_{17.5}$, 84.5; $C_{18.8}$, 7.0; C_{21}, 2.1 and C_{28}, 3.5; the mixture was essentially devoid of squalene. However, fraction 2 contained 54.5% squalene in addition to 10.0% pristane. The last hydrocarbon was absent among the 12 peaks of fraction 3, squalene, $C_{20.4}$, $C_{20.8}$, $C_{24.8}$, $C_{26.5}$ and $C_{30.7}$ constituting 23.8%, 8.5%, 6.8%, 5.1%, 31.4% and 4.3%, in the order stated. Pristane also occurred in fraction 4 at a level of 10.6% but squalene could not be detected.

By far the major portion of the UNS was composed of sterols, fatty alcohols and glyceryl ethers occurring in fraction 5 from alumina column elution. Thus, dogfish liver UNS contained 26.3% sterols and 71.4% alcohols and of the latter, chimyl, batyl and selachyl alcohols were 4.8%, 3.2% and 60.9%, respectively. The sterol and alcoholic contents of cod liver UNS amounted to 70.7% and 21.1%, in the order stated, with the glyceryl ethers, chimyl and selachyl alcohols at area percentages of 2.9% and 14.9%, respectively. Herring oil UNS contained 50.6% sterols and 26.9% alcohols, the latter analyzing for 1.8% chimyl and 5.2% selachyl alcohols.

TABLE I

Total Fatty Acid Composition of the Fish Oils[a,b]

Fatty acid[c]	Dogfish liver oil	Cod liver oil	Herring oil
14:0	4.0	4.2	5.7
15:0	0.6	0.6	0.6
16:0	17.8	10.6	13.3
16:1	5.7	9.5	6.5
17:0	0.5	0.2	0.5
18:0	1.4	1.4	1.1
18:1	21.8	21.1	14.7
18:2	1.1	1.0	1.1
18:3	0.5	0.4	0.4
20:1	14.4	19.0	17.9
18:4	0.5	0.8	0.8
20:2	Trace	Trace	Trace
22:1	17.3	7.3	22.8
20:4	Trace	0.4	0.3
20:5	4.3	9.5	6.6
24:1	Trace	Trace	Trace
22:4	0.5	0.4	
22:5	0.5	0.6	0.8
22:6	8.6	12.6	6.8
Saturated	24.3	17.0	21.2
Unsaturated	75.2	82.6	78.7
Mono-	59.2	56.9	61.9
Poly-	16.0	25.7	16.8

[a] The oils were saponified by heating with 10% aqueous NaOH for 16 hr.
[b] In all Tables, the GC data are area percentages.
[c] Number of C-atoms: number of double bonds.

Discussion

Of the three marine oils, the UNS yield from dogfish liver ranged highest (7.62%) but the hydrocarbon content was low. The composition is summarized in Table II and all percentages are on a weight basis. The hydrocarbon content of UNS was 1026 mg% as derived from analysis of alumina-chromatographed fractions and silica gel-treated cuts from fractions 1 and 2 or 79.3 mg% in the initial oil and almost equally distributed between the saturated and olefinic types. Silica gel provided an adequate tool toward the enrichment of paraffins and minimized the masking of certain components by the higher amounts of pristane and squalene in several fractions. Thus, as based on the alumina fractions alone, the respective contents of pristane, squalene and the remaining hydrocarbons in UNS were 290, 522 and 301 mg%, corresponding to 22.1, 39.8 and 22.9 or a total of 84.8 mg% in the oil. The dogfish paraffinic mixture was prominent in C_{19}, $C_{30.7}$ and $C_{32.5}$ in addition to pristane. As observed by Gershbein and Singh (1) for basking shark liver oil, two or more series of saturated hydrocarbons are present in each of the three marine animal oils in addition to C_{19} monoolefins and C_{20} phytadienes as identified by Blumer and Thomas (9–11). The latter unsaturated components were not further fractionated for structural study.

TABLE III

GC Analysis of Dogfish, Cod Liver and Herring Oil
Hydrocarbons Obtained by Chromatography Over Alumina and Silica Gel (SE-30)

Relative C number	Dogfish liver						Cod liver				Herring							
	Alumina fractions[a]		Fraction 1 gel cuts[b]			Fraction cut 1[c]	Fraction 1 gel cuts[d]		Fraction 2 gel cuts[e]		Fraction 1 gel cuts[f]				Fraction 2 gel cuts[g]			
	1	3	1	2	4		1	2	1	4	1	2	3	4	1	2	3	4
14.0	Trace		Trace				0.9	1.5	0.4	0.1								
15.0	0.3		Trace				2.2	1.1	0.6	0.2								
16.0	0.3	0.3	0.4	0.1			3.3	2.2	0.5	0.7	0.6	0.3						
16.6	0.5	Trace	0.6	0.3			3.7	1.8	0.7	0.3	4.5	4.8						
16.8	0.8	0.2	1.1	0.4			5.6	2.5										
17.0	0.3		1.2	0.5			2.2	13.7	1.3	0.3								
17.2	0.5		0.9	0.4														
17.4	0.3		0.8	0.3														
17.5 (pristane)	34.6	9.2	32.0	29.8	7.6	3.9	18.5	8.7	0.5	0.2	87.4	48.8	33.8	8.7	97.9	35.2	0.6	0.2
17.8													1.4					
18.0	0.6		2.1	1.1			0.7	0.5	0.7	0.7		0.4	0.6	2.5				
18.5							4.4	4.7	5.6	0.8	3.4				2.0			
18.8							12.9	17.4	7.8	1.2		32.5	10.8	0.7				
19.0	1.5		6.0	6.2	0.3						1.1				Trace	Trace		
19.5							0.7	0.3	2.7									
20.0	0.2	0.3	1.2	0.4					1.3	1.5								0.4
20.5	0.5		0.1	0.2					2.8	17.0								
20.8													3.0	3.8			9.2	12.9
21.0							0.9	0.2	5.0	1.1	1.5	4.8	1.7	1.5				
21.3	0.2																	
21.5	Trace	1.1	0.5	0.3							1.1	3.9					7.8	11.2
22.0							2.8	0.9	1.3	0.8								
22.5	0.2		0.4	0.1				1.5										
22.6	0.3		1.1															
23.0	0.6		0.4	0.4	3.8		0.9	1.1	1.5				8.4	12.5				
23.5	0.8				11.5	2.3			2.8	0.4			6.7	12.5		17.6	2.7	2.4
24.0	Trace	0.5	0.5	0.1		0.5	0.9	0.1	0.3								3.0	2.1
24.5																	1.8	1.2
25.0	0.3		0.6	0.4			1.5	0.2	0.2									
25.5																23.5		
26.0	0.6	7.4	0.9	0.4		2.7	1.5	0.2	1.6	0.7			1.0				5.7	4.7
26.5		0.9				1.9			1.6	0.7							4.6	3.5
27.0	0.6		2.5	1.1			2.2	0.7	1.1									
27.5																		
28.0 + squalene	35.2	59.3	1.4[h]	0.6[h]	40.0	72.7	1.7[h]	0.5[h]	38.9	71.1			23.7	50.1	Trace	Trace 23.5	64.4	61.2
29.0	0.5		2.9	1.9		0.7	2.6	2.5										
29.3									13.3									
29.5	Trace								3.4	0.4								
30.0	Trace	8.1	0.9	0.5				2.5	0.6	0.5								
30.7	13.6	4.6	38.0	42.5	5.8	3.9	27.7	24.8		0.7		3.7	3.3	1.3				
31.0		3.4																
32.0		0.5																
32.5	6.0	4.0	3.4	11.5	30.8	11.0	1.6	9.9	2.8	0.5		0.8	5.0	6.2	Trace	Trace		

[a] Fraction 2 contained 2.4% pristane and 87.7% squalene in addition to 0.9% $C_{23.5}$, 0.9% $C_{26.0}$, 0.4% $C_{29.5}$, 3.0% $C_{30.7}$ and 4.4% $C_{32.5}$. Fraction 4 (15.2 mg; 0.15% recovery based on UNS) showed the presence of 33 peaks ($C_{16.5}$ to C_{40}) and representative members in relative C number and area percentage were as follows: 18.8, 9.0; 19.5, 4.7; 21.5, 4.2; 25.0, 9.3; 25.8, 5.0; 27.5, 4.2; 29.0, 3.8; 29.7, 4.0; 30.0, 4.2; 30.7, 6.3 and 32.0, 4.5.

[b] Cuts from chromatography of 83.3 mg fraction 1 over silica gel and elution of the column with petroleum ether, benzene, ether, acetone and methanol, two portions being collected per solvent. A recovery of 88% resulted, the residues occurring in cuts 1–6, inclusive and weighing 39.8, 10.7, 5.5, 17.2, 0.2 and 0.2 mg, respectively. Cut 3 yielded 14 peaks of $C_{16.6}$–$C_{32.5}$ of which squalene, pristane, $C_{30.7}$ and $C_{32.5}$ amounted to 28.0%, 15.7%, 20.6% and 30.8%, respectively. Cut 5 (six peaks; $C_{17.5}$–$C_{32.5}$) contained the latter four components at levels of 69.5%, 4.6%, 1.3% and 4.5% in the order stated as well as 16.3% $C_{29.5}$. Only squalene was present in cut 6.

[c] Fraction 2 (20.2 mg) was submitted to gel treatment as in the above but lipid occurred only in the first three cuts (10.2, 5.6 and 0.5 mg, respectively). Cut 2 was similar to cut 1 in composition and cut 3 contained 98.6% squalene in addition to 1.3% $C_{23.5}$.

[d] From chromatography of 85.2 mg fraction 1 in 1.5 ml petroleum ether over 1.6 g silica gel and elution of lipid with the five solvents. Cuts 1–10 amounted to 15.7, 8.5, 13.4, 38.3, 2.9, 0.4, 0.3, 0.1, 0.2 and 0.0 mg, respectively and squalene was the only component of cuts 6–9, inclusive. Cuts 3 and 4 were comparable to cut 2 in composition except for squalene at 49.6% and 67.3% and pristane at 1.5% and 0.6% in the order given; cut 5 (11 peaks; $C_{16.6}$–$C_{32.5}$) displayed a squalene content of 89.6%.

[e] Chromatography of 35.8 mg fraction 2 gave rise to the following recoveries for the 10 usual eluates: 1.1, 7.2, 9.9, 15.5, 0.1, 0.8, 0.1, 0.2, 0.2 and 0.0 mg, respectively. Cuts 2 and 3 paralleled cut 1 in make-up but the squalene levels were 35.0% and 73.5%, in the order given. Cut 5 contained 89.4% squalene, 1.5% C_{26}, 7.1% $C_{26.5}$ and 1.5% $C_{32.5}$. Cuts 6 + 7 showed the presence of 2.7% $C_{26.5}$, 67.5% squalene, 8.1% C_{29}, 5.4% $C_{29.3}$ and 16.2% $C_{29.5}$ and cuts 8 + 9: 6.2% $C_{26.5}$, 31.2% squalene and 62.5% C_{29}.

[f] Fraction 1 in amount of 554.5 mg in 23 ml petroleum ether was passed over 10.1 g silica gel and the column eluted with 20 ml portions of the five solvents; two cuts were taken per medium. Cuts 1–5 contained 440.3, 71.8, 11.4, 12.1 and 0.2 mg, respectively and traces analyzing for squalene occurred in cuts 6 and 7. $C_{23.0}$ (1.9%), $C_{23.5}$ (1.3%) and squalene (96.7%) were present in cut 5.

[g] As per the latter experiment, 21.3 mg of fraction 2 gave rise to 1.9, 3.1, 5.0, 9.2, 1.2, 0.3 and 0.2 mg for cuts 1–7, respectively. Lipid was absent in the last three eluates and cuts 6 and 7 contained squalene almost exclusively. Cut 5 analyzed for 11.8% $C_{20.0}$, 3.1% $C_{20.8}$, 3.9% $C_{21.5}$, 1.5% $C_{26.0}$, 0.7% $C_{26.5}$ and 78.7% squalene.

[h] Squalene is absent.

Cod liver UNS occurred in lower yield and the hydrocarbon content of the oil was 70.1 mg%. The pristane level (298 mg%; 3.0 mg% in oil) was lower than that of the dogfish product but the squalene contents were comparable. In terms of the alumina fractions as such, pristane, squalene and the additional hydrocarbons comprised 0.484%, 3.42% and 3.18% or 4.0, 28.4 and 31.8 mg% of the initial oil in the order stated. The paraffinic hydrocarbons were investigated over a range of C_{14} to $C_{32.5}$ and aside from pristane, were prominent in $C_{18.8}$, $C_{30.7}$ and $C_{32.5}$ (Table III). The presence of the triolefin, gadusene ($C_{18}H_{32}$), purported to occur in cod liver oil (5) was not substantiated in this study.

The composition of the hydrocarbons of the herring oil is of interest. Although the recovery of UNS was 1.35%, the hydrocarbons made up one fourth of the latter with pristane and other saturated components predominating (Table II). In fact, the initial large cut eluted from silica gel treatment of fraction 2 with petroleum ether, invariably high in squalene, was quite elevated in pristane with only traces, if any, of squalene occurring. The overall level in the starting lipids was 300 mg% based on alumina and silica gel treatment and in terms of the alumina fractions alone, pristane, squalene and additional hydrocarbons amounted to 17.24%, 0.287% and 4.22% in UNS or in terms of the oil, 235, 9.0 and 57.0 mg%, in the order stated. Aside from pristane, saturated components of the following relative carbon numbers were prominent: 16.6, 18.8, 21.0, 21.5 and 23.0. In the experiments of Lambertsen and Holman (2), the hydrogenated straight chain components contained all members of C_{14} to C_{33}, the odd homologs being most prominent with n-C_{19} occurring at 10.5%; of the branched chain members, pristane and squalene comprised 70.0% and 13.0%.

Although complete structural configuration for the branched paraffinic types is not presently available, the hydrocarbons of the three marine oil products are rather complex and parallel each other, except possibly for a simpler homologous make-up for the herring hydrocarbon mixture. With the latter, as high as the saturated contents are, these did not approach the corresponding level of the basking shark liver oil as demonstrated by Gershbein and Singh (1). Herring carcass lipids and dogfish liver oil, like the basking shark liver oil samples, contained significant amounts of nonadecane. The distribution of such hydrocarbons as pristane is of great interest from the standpoint of the food source and feeding areas of the marine animals, considering the high pristane content of zooplankton, among other organisms.

The occurrence of glyceryl ethers in fish oils, notably, dogfish liver oil, has been investigated by several workers and is reviewed by Swain (4). The glyceryl ethers range lower among the alcohols of cod liver and herring oils and even less, in basking shark liver oil. The composition advanced in the current report for dogfish sterol and alcohols, the latter being ascertained by GC following acetylation of the fatty alcohols and glyceryl ethers of fraction 5, is in agreement with the data of Hallgren and Larsson (12) and Malins et al. (13). The latter noted higher sterol and glyceryl ether contents in the liver than in the flesh of that species. The total fatty acid composition of the three oils (Table I) is likewise in the range reported by others (14–16). In this regard, it must be pointed out that the present analytical findings pertain to the particular samples in question. Even though they represent large numbers of animals, variations in hydrocarbons and other lipid components are documented not only for different species but for a given species depending on the locality and time of collection.

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