FATTY ACIDS OF MARINE OILS AND PIG LIVER LIPIDS

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ABSTRACT. — The total fatty acids and mono-, di- and triglyceride acids of basking shark liver oil were analyzed by gas chromatography of the methyl esters over SE-30 and diethyleneglycol succinate packings. The findings were compared with those of the total acids from two commercial shark liver oil products, raw cod liver oil and carcass lipids of British Columbia salmon, red fish and Norway dog fish in addition to sperm oil and the total and glyceride-derived acids from pig liver lipids. The most prominent acids of the marine oils were 16:0, 16:1, 18:1, 20:1 and 22:1 and the ratio of olefinic to saturated acids was 4.0 but ranged lower for dog fish and sperm oils. The ratio of mono- to polyunsaturated acids was highest for sperm, salmon and Irish pale shark liver oils (4.0-4.7), lowest for cod (1.9) and intermediate for the remaining marine oils. Pig liver fatty acids showed a far greater level of saturated homologs, 16:0, 18:0, 18:1 and 18:2 being the most outstanding components.

Marine lipids in contrast to depot fats of animals, comprise excellent sources of polyunsaturated acids and generally contain relatively low levels of unsaponifiable components (Hilditch and Williams, 1963; Khalid *et al.*, 1968, among others). The iodine numbers of several oils have been correlated with the polyunsaturation of such mixtures by empirical formulas (Ackman, 1966). The polyunsaturated acid moieties of fish oils tend to accumulate in the β-position of glycerol in the glycerides, whereas, marine mammals (whale and seal), not unlike the pig, display primarily shorter fatty acid chains in this position with the longer ones, both saturated and unsaturated, occurring at the α-position (Brockerhoff and Hoyle, 1963; Brockerhoff *et al.*, 1963, 1968).

The present study was undertaken with the view of discerning the composition of the total fatty acids and the component acids of the mono-, di- and triglycerides of the liver oil of the basking shark. The latter species has been investigated in this laboratory with respect to the hydrocarbon, alcohol and sterol constitution, the unsaponifiable portion amounting up to 28% of the oil (Gershbein and Singh, 1968). For comparative purposes, the total acids from saponification of raw cod liver oil and Irish and pale shark liver oils of commerce and carcass lipids of Norway dog fish, British Columbia salmon and ocean red fish as well as sperm oil and pig liver lipids were also submitted to analysis by gas chromatography (GC) over polar and nonpolar packings.

Materials and Methods

All solvents were of AR or CP grade and were distilled prior to use. The basking shark liver oil (Sample M) originated from J. C. Martens & Co., Norway, natural winter sperm oil from Werner G. Smith, Cleveland, British Columbia salmon oil from Shafer-Haggart, Ltd., Vancouver, British Columbia and pale and Irish shark liver oils, North Sea production samples, through the distribution of Arthur Trask Co., Chicago. The latter also made available stock samples of raw cod liver oil and the fish carcass oils. Norway dog and red fish oils, the last one being a Northeastern U. S. product.

Pig liver was obtained fresh from the abattoir and the lipids extracted by blending with methanol, an equivalent volume of chloroform then being introduced. The contents were allowed to stand at 25° C with frequent stirring, filtered and the residue treated with excess 1:1 methanol-chloroform mixture. The filtrate was concentrated under vacuum and the residue taken up in petroleum ether (b. 30-60° C), washed with several portions of water and dried over anhydrous sodium sulfate. Removal of the solvent from the filtrate yielded the lipids in amount of 5% based on the starting tissue.

Total Fatty Acids

The marine oils were saponified by refluxing with 10% aqueous sodium hydroxide for a period of 16 hr. The cooled contents were extracted with portions of ether to remove the unsaponifiable material and the aqueous solution then acidified with sulfuric acid and the fatty acids removed by means of ether. The extract was washed with portions of water, dried over sodium sulfate and the fatty acids obtained by concentration of the filtrate. Hydrolysis of pig liver lipids was also affected by heating with 20% sodium hydroxide in 95% ethanol (Gershbein and Krotoszynski, 1965; Gershbein and O'Neill, 1966).

Separation of Glycerides and Free Fatty Acids

A 4% solution of basking shark liver oil or pig liver lipids in hexane was chromatographed over florisil by the method of Carroll (1961) for the isolation of free fatty acids and the glycerides. The column was eluted successively with the following media, concentration yielding the components as indicated: hexane (hydrocarbons), 5% ether in hexane (sterol esters), 15% ether in hexane (triglycerides), 25% ether in hexane (free sterol), 50% ether in hexane (diglycerides), 2% methanol in ether (monoglycerides), 4% glacial acetic acid in ether (free fatty acids) and methanol (phospholipids). The above procedure was also applied to the isolation of free acids from the Irish shark liver oil. In all cases, the order of distribution was: triglycerides $>>$ diglycerides $>$ monoglycerides.

Esterification of Acids

The free and total fatty acids were esterified by refluxing with methanol saturated with hydrogen chloride. The mono-, di- and triglycerides were transesterified by heating with sodium methoxide in methanol for 30 minutes by the method of Luddy *et al.* (1960). Excess methoxide was neutralized with 30% acetic acid and the methyl esters extracted with hexane, the mixture washed three times with 10% ethanol and the hydrocarbon removed under nitrogen. The methyl esters were analyzed directly as such or after catalytic hydrogenation of aliquots in a Parr low pressure apparatus at 25° C in the presence of Adams' platinum oxide catalyst.

Gas Chromatography

All GC analyses were carried out in a Barber Colman model 5000 gas chromatograph equipped with hydrogen flame detector. The U-shaped glass column measuring 8 ft. x ¼ inch o.d. was charged with 3% SE-30 on 60-80 mesh Gas Chrom P and programmed at 150-280° C, the rate of heating being 2°/min; the detector temperature was 300° C. The carrier gas was helium at 80 ml/min. With 15% diethyleneglycol succinate (DEGS) on 80-100 mesh Gas Chrom P, the column, injector and detector temperatures were 210, 220 and 250° C, respectively; the helium flow rate was 65 ml/min. The methyl esters were dissolved in ether and volumes of 5 μl injected. Tentative assignments for each peak were obtained from a semilogarithmic plot of relative retention time versus chain length and the degree of unsaturation of standard mixtures of methyl esters.

Results and Discussion

The composition of fatty acids obtained by saponification of the liver

oils, fish carcass lipids and sperm oil as based on GC over 15% DEGS is presented in Table 1 and for the total and glyceride acids from basking shark liver oil M, in Table 2. The total acid portions were quite similar in the number and distribution of peaks except for some differences in regard to a few components. Thus, octadecatrienoic acid was not detected in basking shark liver and salmon oils, nor the 20:4 acid in dog fish oil. The 22:2 acid occurred in the latter sample, Irish shark liver and red fish oils, the 22:3 component, in the last product, salmon oil and basking shark liver oil and the 24:1 acid, at 0.9-1.0% levels in basking and Irish shark liver oils but absent or in trace amounts in the other mixtures.

The most prominent acids accounting for at least 70% of the total comprised 16:0, 16:1, 18:1, 20:1 and 22:1. In this regard, hexadecenoic acid ranged lowest in the Irish and basking shark liver oils (2.7 and

TABLE 1.—GC Analyses of Esters of Total Fatty Acids from Fish Lipids and Sperm Oil (DEGS Column)[a]

C-No[b]	Norway Dog Fish	Irish Shark Liver Oil[c]	Pale Shark Liver Oil	British Columbia Salmon	Raw Cod Liver Oil	Red Fish Oil	Sperm Oil
10:0	0.4	0.4 (0.1)	0.1	T[d]	0.2	0.2	0.6
11:0	0.4	0.3 (0.1)	0.1	T	0.2	0.2	0.3
12:0	0.5	0.3 (0.2)	0.2	0.2	0.4	0.2	3.3
13:0	0.3	0.3 (0.1)	0.1	T	0.2	0.2	0.9
14:0	5.2	5.1 (4.2)	3.8	5.9	3.0	4.6	9.0
15:0	0.9	0.6 (0.5)	0.6	0.5	0.8	0.9	4.9
16:0	15.9	11.0 (11.7)	10.3	11.0	12.7	10.6	9.6
16:1	7.2	2.7 (2.4)	5.3	12.8	8.6	7.7	14.9
17:1	0.8	0.7 (0.4)	0.7	0.8	0.9	0.8	1.0
18:0	2.9	2.1 (2.2)	3.2	2.2	2.2	2.1	1.3
18:1	18.8	8.4 (7.0)	20.2	16.0	25.0	15.0	26.5
18:2	1.8	0.9 (0.9)	1.5	1.4	1.8	1.8	1.7
18:3	0.5	0.3 (0.1)	0.3	.....	0.4	0.6	0.9
20:1	10.6	21.5 (21.8)	17.0	18.4	9.3	18.5	8.2
20:2	1.0	0.8 (0.4)	0.8	1.0	1.6	1.0	1.0
20:3	0.5	0.6 (0.2)	0.4	0.6	0.4	0.4	1.0
20:4	.....	0.6 (0.4)	0.8	0.6	1.1	0.4	0.7
22:1	15.9	31.5 (37.5)	18.7	17.0	8.6	15.6	4.6
20:5	4.9	2.7 (2.9)	3.5	4.6	9.8	6.0	2.7
22:2	1.4	0.5 (T)	.....	.....	.....	0.6	.....
22:3	.....	.....	.....	1.3	.....	0.6	.....
24:1	.....	1.0 (T)	.....	.....	T	T	.....
22:4	2.3	3.2 (2.5)	3.3	1.1	2.0	3.3	1.3
22:5	1.9	1.8 (0.8)	1.6	1.1	1.8	2.1	1.0
22:6	5.8	2.7 (3.3)	7.4	3.5	8.9	6.3	4.3
Saturated	26.5	20.1 (19.1)	18.4	19.8	19.7	19.0	29.9
Unsaturated	73.4	79.9 (80.6)	81.5	80.2	80.2	80.7	69.8
Mono—	53.3	65.8 (69.1)	61.9	65.0	52.4	57.6	55.2
Poly—	20.1	14.1 (11.5)	19.6	15.2	27.8	23.1	14.6

a. The total acids were obtained by saponification of the oils.
b. Number of carbon atoms: number of double bonds.
c. Values in parentheses refer to the free fatty acids which were isolated by chromatography of the lipids over Florisil.
d. Trace.

3.3%) and highest in salmon and sperm oils, the levels being 12.8 and 14.9%, respectively. As with the 16:1 acid, octadecenoic acid was lower in basking and Irish shark liver oils, 4.8 and 8.4%, respectively, but otherwise, occurred at 15-26.5% in the other marine oils; the highest levels were displayed by cod liver (25.0%) and sperm oils (26.5%). The 20:1 acid was diminished in dog fish, cod and sperm oils (8.2-10.6%) as contrasted to 17-21.5% for the remaining mixtures. In the case of the 22:1 acid, the lowest amounts were noted in sperm oil (4.6%) and raw cod liver oil (8.6%), maximal levels in basking shark (30.3%) and Irish shark liver oils (31.5%) and intermediate ranges in the remaining products. Octadecenoic acid occurred at 3-9% and was higher in sperm oil (9.0%) and the 20:5 acid ranged from 2.7 to 9.8%, basking shark and cod liver oils containing 8.0 and 9.8%, respectively. A comparable level of the 22:6 acid occurred in the last oil.

The ratio of unsaturated to saturated acids was about 4:1 for the marine products except for greater saturation in the dog fish and sperm oils with values of 2.3 and 2.6, respectively. As would be expected in view of the above discussion, the distribution of unsaturated components also showed variations in type. Thus, the ratios of mono- to polyunsatur-

TABLE 2.—Basking Shark Liver Oil M Total and Mono-, Di- and Triglyceride Fatty Acid Components (DEGS).[a]

C-No.	Total	Mono-	Di-	Tri-
10:0	0.3			
11:0	0.3			
12:0	0.4			
13:0	0.3			
14:0	5.8	6.2	6.0	5.2
15:0	0.9	0.4	0.4	0.5
16:0	10.4	14.2	12.7	8.6
16:1	3.3	5.9	5.2	5.1
18:0	1.9	2.1	1.8	1.8
18:1	4.8	7.9	7.2	7.7
18:2	0.9	0.8	0.8	0.9
20:1	19.1	19.6	21.5	20.9
20:2	2.4	2.1	3.0	2.6
20:3	0.4	0.2	0.2	0.3
22:1	30.3	29.7	27.9	32.3
20:4	1.4	0.4	0.2	0.5
20:5	8.0	3.6	4.8	4.9
22:3	1.9			
24:1	0.9			
22:4	0.5	0.8	0.8	1.2
22:5	0.5	1.0	1.0	0.9
22:6	5.3	4.8	6.2	6.2
Saturated	20.3	22.9	20.9	16.1
Unsaturated	79.7	76.8	78.8	83.5
Mono-	58.4	63.1	61.8	66.0
Poly-	21.3	13.7	17.0	17.5

a. Fatty acids were obtained from the glycerides by transesterification.

ated acids were highest for sperm, salmon and Irish shark liver oils, 4.0, 4.3 and 4.7, respectively, and lowest for raw cod liver oil (1.9); the remaining marine oils displayed values of 2.6-3.2. It should be pointed out that the free fatty acids obtained from Irish shark liver oil by chromatography over florisil simulated the corresponding total acids except for an apparent increase in the monounsaturated homologs (mono-: polyunsaturation, 6.0). Of the two North Sea commercial oils, the Irish shark liver oil more closely resembled the basking shark product in fatty acid composition.

The acids obtained from transesterification of the basking shark liver oil glycerides paralleled those of the total acids in most respects. The octadecenoic acid contents (7.2-7.9%) ranged higher for the glycerides than that of the total acid mixture and the 20:5 acid, lower (3.6-4.9%). The ratios of mono- to polyunsaturated components were 4.6, 3.6 and 3.8 for the mono-, di- and triglycerides, respectively, as compared to 2.7 for the total acid content. However, the ratio of saturated to unsaturated acids was rather constant for the four batches. A comparable distribution of glycerides as well as of the component fatty acids also resulted by application of the thin-layer chromatographic method employing silica gel G to this marine oil (Singh *et al.*, 1966).

In marked contrast to the marine oils, the fatty acids of the pig liver lipids displayed greater saturation, the ratio of olefinic to saturated acids averaging 1.2 for the samples; the ratios of mono- to polyunsaturation were 1.0, 1.5, 1.7 and 1.3 for the total, free, monoglyceride and triglyceride acids, respectively (Table 3). The diglycerides were not analyzed due to overheating of the mixture during processing. The components, 22:0, 22:1 and 22:2 were detected solely in the total acids and the presence of the 22:3 homolog could not be substantiated among the free acids. The most prominent members were 16:0 (16.0-26.7%), 18:0 (12.2-22.5%), 18:1 (25.1-32.0%) and 18:2 (7.7-14.6%). The free and glyceride acids contained more hexadecanoic acid but less octadecenoic acid than the total acid sample and the octadecanoic acid content was lowest for the triglyceride fraction. It will be noted that the last acid made up only 1.3-3.2% of the various marine mixtures (Tables 1 and 2).

Aside from general species differences, the fatty acid composition is greatly dependent on the metabolic and nutritional status of the animal. Such considerations, notwithstanding, the present data are in good agreement with several prior publications, although many of the analyses did not employ GC criteria. In a report by Gelpi and Oro (1968), a South American basking shark liver oil sample showed the presence of about 18.5% each of 18:1, 20:1 and 22:1 acids in addition to 5.5% 12:0, 20.3% 16:0, 6.8% 16:1 and 3.1% 18:0; the total fatty acid content was 33.8%. Such findings are in contrast to the higher acid content of the present product, presumably of north Atlantic origin and containing lower 14:0 and 18:1 and higher 16:1 and 22:1 acid contents, among others. The analysis advanced for British Columbia salmon is at variance with

TABLE 3.—Pig Liver Free and Total Fatty Acids and Acids Derived from the Mono- and Triglycerides (DEGS).

C-No.	Total	Free	Monogly-ceride	Trigly-ceride
14:0	1.1	1.8	1.8	3.0
15:0	0.6	0.9	0.4	1.5
16:0	16.0	20.6	22.2	26.7
16:1	2.0	3.7	2.6	3.8
17:0	0.6	0.4	0.4	0.7
18:0	22.5	20.6	19.1	12.2
18:1	25.1	29.3	32.0	26.7
18:2	14.6	7.7	10.6	9.9
20:0	0.6	0.9	0.8	0.7
18:3	2.3	1.3	1.3	1.5
20:2	0.6	0.4	0.8	1.5
20:3	0.9	0.4	0.8	T
22:0	0.6	.....	.....	.....
22:1	1.1	.....	.....	.....
20:4	4.1	7.8	4.0	3.8
22:2	2.0	.....	.....	.....
22:3	1.4	.....	T	0.7
22:4	1.4	0.9	0.4	3.8
22:5	1.1	1.3	1.3	1.5
22:6	0.9	1.3	0.8	1.5
24:0	T	T	T	T
Saturated	42.0	45.2	44.7	44.8
Unsaturated	.....	.....	.....	.....
Mono-	28.2	33.0	34.6	30.5
Poly-	29.3	21.1	20.0	24.2

data reported for Coho salmon (Saddler *et al.*, 1966), the former showing higher 20:1 and 22:1 contents and diminished 18:2, 20:3, 20:4 and 22:6 levels. However, aside from the species differences, Stansby (1967) demonstrated wide variations in the fatty acid composition of Coho salmon from fresh and salt water and for fingerlings.

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