Component Fatty Acids of Marine Fish Liver Oils

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LIVER OILS of the Asiatic varieties of Elasmobranch fish have been studied mostly in Japan (Tsujimoto and co-workers, for example). From the semi-quantitative data obtained from these studies (10) a fourth class of the Elasmobranch fish liver oil has come into being. This particular group of the liver oils is characterized by the high content of the saturated acids [nearly 50% of the total fatty acids in the white shark *Carcharias gangeticus* (9) and 64% in the liver oil of the Chinese fan fish (12); the former consist mostly of palmitic and the latter stearic acid]. Detailed analysis of two samples of Indian shark liver oils, one from the Bay of Bengal and the other from the Arabian Sea, but of the same species *(Galeocerdo rayneri)*, showed the total saturated acid contents to be Ca. 40% in both the cases, consisting mainly of palmitic acid (7). The present study has been undertaken to supply more data to the above body of information. The two liver oils studied (one from the shark *Carcharias melanopterus* and the other from the saw-fish, *Pristis cuspidatus*, Bay of Bengal) proved to belong to Tsujimoto's fourth group of Elasmobranch fish liver oil.

Experimental

The *Carcharias melanopterus* liver oil and the *Pristis cuspidatus* liver oil, herein referred to as oil No. 1 and oil No. 2, respectively, were supplied to us by U. S. Kini, manager, Government Oil Factory, Kozhikode, Calicut, Madras. The experimental procedure noted below has been followed for the investigation of the two oils.

The oil was dissolved in acetone (10 times) and kept at 0°C. for two weeks, and the precipitated phosphatides were removed by filtration through a Buchner. To the glyceride portion was added a minimum quantity of potassium hydroxide (25 g. per 100 g. of the oil) dissolved in 95% alcohol (500 c.c. per 100 g. of the oil) and heated for three-quarters of an hour on a water bath, after which nearly 50% of alcohol was distilled off and the resulting soap solution was cooled and diluted with water. It is preferable to risk the chance of slightly incomplete saponification rather than to incur the rearrangement of some of the highly unsaturated components. The unsaponifiable matters were removed from the soap solution with ether, and the fatty acids were recovered after decomposing the soap solution with dilute mineral acid. The mixed acids obtained were resolved into different fractions according to varying degrees of unsaturation, first by the lithium salt acetone method (11) and then by the Hilditch's modified lead salt alcohol method (2). The results are summarized in Table I.

Each group of the acids was separately converted into methyl esters, taking the precautions suggested by Bjarnason and Meara (1) and fractionated through Longenecker's E.H.P. column (3). The mean unsaturations and mean molecular weights of each of the sub-fractions were determined. The most unsaturated fraction, C, was taken up first without any loss of time, then the less unsaturated fraction B, and lastly the least unsaturated fraction A. However the ester-fractionation data, along with saponification equivalents and iodine values, are given in the alphabetical order in Table II.

Carcharias Melanopterus

TABLE II

Fractionation Data of the Methyl Esters A, B, and C Acids

No.	Liver oil No. 1			Liver oil No. 2		
	g.	S.E.	I.V.	g.	S.E.	I.V.
	Methyl esters of A acids					
A1	3.19	254.9	1.1	3.14	259.6	0.0
A2	4.62	264.8	1.1	3.88	271.0	0.0
A3	4.85	271.1	1.6	3.83	271.9	0.0
A4	4.88	273.0	2.0	3.64	273.8	0.1
A5	5.50	275.2	2.0	3.86	274.5	0.1
A6	4.90	279.2	2.1	4.81	275.1	0.1
A7	4.33	280.7	3.2	4.07	275.7	0.4
A8	3.72	289.2	5.1	3.05	277.3	2.0
A9	4.06	296.3	7.6	2.77	287.8	3.2
A10	3.65	302.2[a]	12.0	4.11	295.4	5.1
A11	....	....	....	5.65	297.8[b]	7.0
	Methyl esters of B acids					
B1	3.32	245.8	21.2	2.90	262.3	45.3
B2	3.99	264.1	67.4	3.82	269.7	62.2
B3	4.12	272.9	79.2	3.96	281.0	70.3
B4	3.87	274.5	85.1	4.88	288.3	76.9
B5	3.91	280.3	93.5	5.03	293.8	81.1
B6	3.29	286.3	98.4	5.39	399.4	85.4
B7	4.57	293.6	106.9	5.17	299.6	92.6
B8	4.17	293.7	119.8	4.89	300.1	101.3
B9	3.72	302.2	166.7	4.41	300.4	111.4
B10	4.76	321.8	254.8	4.65	318.0	165.8
B11	4.24	334.9[a]	165.0	3.55	325.8[b]	159.4
	Methyl esters of C acids					
C1	3.74	280.2	136.9	3.45	278.7	125.5
C2	3.93	288.9	173.1	3.54	306.6	163.3
C3	4.45	307.2	238.6	3.87	315.2	217.7
C4	5.08	321.3	268.2	4.46	329.5	251.0
C5	4.67	335.3	277.6	3.94	354.5	261.7
C6	4.27	340.2	291.6	2.29	360.0	220.9
C7	4.25	348.0	325.7	2.37	365.4	136.8
C8	3.31	354.0	252.0	2.76	372.3[b]	137.4
C9	3.23	356.6	146.7	....	....	....
C10	4.92	362.4[a]	124.7	....	....	....

[a] S. E. of esters, after extracting the unsaponifiable matter with ether, A10, 299.0; B11, 327.7; C10, 358.0.
[b] S. E. of esters, after extracting the unsaponifiable matter with ether, A11, 296.2; B11, 319.6; C8, 365.9.

The compositions of each of the ester-fractions were calculated from the saponification equivalent and the iodine value according to the method of Hilditch (2). As usual, the mean unsaturation, expressed as the fractional number of hydrogen atoms short of saturation, for example, —2.0H (monoethenoid), was determined by interpolation or extrapolation from the

TABLE I

Crystallization of the Mixed Fatty Acids

Fraction	Description	Liver oil No. 1			Liver oil No. 2		
		g.	%	I.V.	g.	%	I.V.
A	Lithium soaps insol. in acetone, but lead salts insoluble in ethanol.....	57.0	27.4	8.2	54.0	32.6	1.2
B	Lithium soaps insol. in acetone, lead soaps soluble in ethanol.....	71.0	34.1	130.2	81.0	47.4	110.0
C	Lithium soaps soluble in acetone.....	80.0	38.5	244.0	35.0	20.0	195.6

Carcharias Melanopterus Liver Oil No. 1

TABLE III

Component Acids of Fractions (Increments % wt.)

Acids	A (27.4%)	B (34.1%)	C (38.5%)	Total	Acids ex. N. S.[a]	
					% wt.	% mol.
Saturated						
C_{14}	1.49	1.61	....	3.10	3.11	3.87
C_{16}	15.78	2.64	....	18.42	18.45	20.50
C_{18}	8.91	0.57	....	9.48	9.49	9.50
C_{20}	0.08	....	....	0.08	0.08	0.07
Unsaturated						
C_{14}	0.02(—2.0)	0.78(—2.0)	....	0.80	0.80	1.01
C_{16}	0.33(—2.0)	8.22(—2.0)	2.24(—2.3)	10.79(—2.1H)	10.80	12.10
C_{18}	0.73(—2.0)	12.35(—2.9)	6.54(—4.7)	19.62(—3.6)	19.65	19.92
C_{20}	0.04(—2.0)	6.40(—4.9)	8.73(—7.0)	15.17(—6.1)	15.20	14.12
C_{22}	....	1.46(—7.1)	15.62(—9.0)	17.08(—9.0)	17.10	14.68
C_{24}	....	....	5.31(—11.0?)	5.31(—11.0?)	5.32	4.23
Unsaponifiables	0.02	0.07	0.06	0.15	....	....

[a] Excluding non-saponifiable matter.

ester fractions in each of the groups B and C from which the mean equivalent of each of the homologues ester groups (C_{16}, C_{18}, C_{20}, etc.) follows. The final fatty acid composition of the original oil was built up from these figures, giving the data in Tables I and II, and is recorded in Table III for oil No. 1 and Table IV for oil No. 2.

Discussion

The two liver oils, oil No. 1 and oil No. 2, from the livers of *Carcharias melanopterus* and *Pristis cuspidatus,* respectively, belonging to the Carchariidae and Pristidae families, had iodine values 140.5 and 92.9, respectively. This difference in their unsaturations is clearly manifest when we compare the component fatty acids of the two oils (Table V). The *C. melanopterus* (shark) and *Pristis cuspidatus* (saw-fish) are of the same order *Selachii* and were caught off the Madras coast.

Great similarities in the component acids of the two oils, though of different families, become apparent on perusal of Table V. The total saturated acid contents are 31.1 and 36.9% for the liver oils No. 1 and 2, respectively. The predominant saturated fatty acid is palmitic acid (18.4% in oil No. 1 and 22.9% in oil No. 2) with subsidiary amounts of stearic (9.5% and 12.7%) and minor amounts of myristic acids (3.1% and 1.2%, respectively). Arachidic acid is present in traces only (0.1% in both cases). Unsaturated acid contents are 68.9 and 63.1%, respectively. The unsaturated acids are mainly C_{16} (10.8 and 8.2%), C_{18} (19.7 and 28.5%), C_{20} (15.2 and 16.4%), with traces of C_{14} monoethenoids (0.8 and 0.2%, respectively). Besides these, oil No. 1 contains C_{22} 17.1% and C_{24} 5.3%, and oil No. 2 contains C_{22} 5.2% and C_{24} acids 4.6%. The degree of unsaturation of the different groups of unsaturated acids is varying. The acids in oil No. 1 (I.V. 140.5 are more unsaturated than the corresponding acids in oil No. 2 (I.V. 92.5).

The Elasmobranch fish liver oils have been classified into four broad groups by Tsujimoto (10). The first three groups differ in the unsaturated acids contents, their actual proportions and degrees of unsaturations. But all these three groups of liver oils are typically "marine" type in their fatty acid compositions, where the saturated acid content is always around 20% of the total fatty acids. The fourth group of liver oils is characterized by remarkably high proportions of saturated acids content. The liver oils of the shark, *Carcharius gangeticus,* is claimed by Tsujimoto (9) to contain 50% of saturated acids, mainly palmitic, with 50% of unsaturated acids, almost wholly hexedecenoic and oleic. The liver oil from the Chinese fan-fish, *Dasyatis akejei* (12), was similarly found to contain 65% of saturated acids in its total fatty acids, with stearic (46%) predominating, and 19% of palmitic acid. The unsaturated C_{18} acids were found to be 30% with the mean unsaturation —3.6H. As previously mentioned, the above data have been obtained from partially quantitative analysis of the oil. The first detailed analysis of type of oil is from the Indian shark, *Galeocerdo rayneri* (8), family Carchariidae. The fatty acid compositions of two samples of *G. rayneri* liver oils (i. Bombay sample and ii. Madras sample), two samples of *C. melanopterus* livers (the first one being Bombay sample and the second one Madras sample No. 1) and *P. cuspidatus* liver oil (oil No. 2) are given in Table V in the order mentioned above. The total saturated acids are 41, 40, 34, 31, and 37%, respectively, with about 25, 24, 19, 18, and 23% of palmitic acid; and about 11, 14.5, 9, 9.5, and 13% stearic acids. Myristic acid is present in minor amounts only (3.3, 1.5, 4.4,

Pristis Cuspidatus Liver Oil No. 2

TABLE IV

Component Acids of Fractions (Increments % wt.)

Acids	A (32.6%)	B (47.4%)	C (20.0%	Total	Acids ex. N. S.[a]	
					% wt.	% mol.
Saturated						
C_{14}	0.82	0.39	....	1.21	1.21	1.51
C_{16}	20.03	2.87	....	22.90	22.93	25.14
C_{18}	11.06	1.64	....	12.70	12.72	12.57
C_{20}	....	0.07	....	0	0.07	0.07
Unsaturated						
C_{14}	....	0.25(—2.0)	....	0.25(—2.0)	0.25	0.31
C_{16}	0.05(—2.0)	6.66(—2.0)	1.44(—2.0)	8.15(—2.0)	8.16	9.02
C_{18}	0.62(—2.0)	24.95(—2.0)	2.92(—4.3)	28.49(—2.2)	28.53	28.43
C_{20}	....	10.50(—4.9)	5.85(—5.9)	16.35(—5.3)	16.36	14.98
C_{22}	....	....	5.16(—7.4)	5.16(—7.4)	5.17	4.36
C_{24}	....	....	4.60(—11.0?)	4.60(—11.0?)	4.60	3.61
Unsaponifiables	0.02	0.07	0.03	0.12	....	....

[a] Excluding non-saponifiable matter.

TABLE V

Component Acids (% Wt.) of Shark (and Related) Fish Liver Oils

Acids	*Galeocerdo rayneri*			*Carcharias melanopterus*		*Pristis cuspidatus*
	i	(7)	ii	(8)	(author) Oil No. 1	(author) Oil No. 2
Lauric	0.4		....	Trace	....	....
Myristic	3.3		1.5	4.4	3.1	1.2
Palmitic	24.9		23.6	18.5	18.4	22.9
Stearic	11.1		14.5	9.0	9.5	12.7
Arachidic	1.2		0.3	1.8	0.1	0.1
Unsaturated						
C_{12}	0.1		....	....	....	....
C_{14}	1.1		0.2	2.9	0.8	0.2
C_{16}	11.2		10.9	12.7	10.8	8.2
C_{18}	19.6		23.3	20.0	19.7	28.5
C_{20}	22.3		11.6	19.0	15.2	16.4
C_{22}	4.8		12.2	7.3	17.1	5.2
C_{24}	....		1.9	4.4	5.3	4.6
Mean unsaturation of						
C_{12}	—2.0H		....	....	....	....
C_{14}	—2.0H		—2.0H	—2.0H	—2.0H	—2.0H
C_{16}	—2.6H		—2.0H	—2.1H	—2.1H	—2.0H
C_{18}	—3.9H		—2.6H	—4.0H	—3.6H	—2.2H
C_{20}	—7.0H		—5.8H	—6.8H	—6.1H	—5.3H
C_{22}	—10.6H?		—8.4H	—9.9H	—8.8H	—7.4H
C_{24}	....		—11.0H?	—11.0H?	—11.0H	—11.0H?

3.1, and 1.2%) along with traces of arachidic acid in all the five oils.

The total unsaturated acids are 59, 60, 66, 69, and 63%, respectively, consisting of 11, 11, 13, 11, and 8% of unsaturated C_{16} acids, and 20, 23, 20, 20, and 28.5% of unsaturated C_{18} acids; along with 27, 26, 31, 37.6, and 27% of C_{20} and above polyethenoids. Traces of C_{14} monoethenoids occur in all the five cases. C_{24} polyethenoids (1.9, 4.4, 5.3, and 4.6%) occur except in *G. rayneri* 1. It is clear from the above that, though there are naturally subordinate differences in the actual proportions of the various acids, the over-all pattern of distribution of the saturated acids as well as the different groups of unsaturated acids and their degree of unsaturations is, broadly speaking, very similar. This is as to be expected in that fats, in their fatty acid compositions, have been found to align themselves in groups according to their biological origin. Such specific effects connected with phytogenetical relationships are now very well recognized in the vegetable fats and also animal (land and aquatic) fats.

The saturated acid content (Ca. 40%) in the above cases is abnormally high in comparison to that of a typical marine fat (Ca. 20%). This shows evidence of bio-hydrogenation which takes the form of simultaneous hydrogenation of polyethylenic and monoethylenic members in the normal preformed marine fat. Such selective modification of the ingested fat [the main source of depot fats in aquatic animals seems to be a characteristic of this particular group (IV group) of Elasmobranch fish (4)].

The relatively less amount of saturated acids (31%) content in oil No. 1 is noteworthy, as compared to the usual saturated acid content (Ca. 40%). This deficit has been made up in the C_{20} and above polyethenoids. This brings to mind the New Zealand shark, *Galeorhinus australis,* liver oil (6) which was found to contain 23% of saturated acids and 42% of C_{20} and above polyethenoids, the latter closely resembling the figure 38% for oil No. 1. Lovern (5) has remarked, "What is certain is that Elasmobranch oils may be of diversified compositions, but it is possible that fats exist with compositions intermediate between the types (the four groups mentioned above) and that a gradual transition could be shown from one extreme to another." Such a possibility is pointed out by the present study. At present the data available are still too sparse to allow any definite conclusion.

Summary

1. Two liver oils (Elasmobranch) from *Carcharias melanopterus* and *Pristis cuspidatus,* caught off the Madras coast are studied, and their component fatty acids are reported.

2. The mixed acids were separated into three groups (varying unsaturation) of acids, and their methyl-esters were fractionated.

3. The liver oils are found to belong to the fourth group of Tsujimoto's classification of Elasmobranch fish liver oils. *Carcharias melanopterus* liver oil contains 31.1% unsaturated acids (myristic 3.1, palmitic 18.4, stearic 9.5, and 0.1% arachidic) and 68.9% unsaturated acids (C_{16} 10.8, C_{18} 19.7, C_{20} 15.2, C_{22} 17.1, C_{24} 5.3%, and traces of C_{14} monoethenoid). *Pristis cuspidatus* liver oil contains 36.9% saturated acids (myristic 1.2, palmitic 22.9, stearic 12.7, and arachidic 0.1%) and 67.1% unsaturated acids (C_{16} 8.2, C_{18} 28.5, C_{20} 16.4, C_{22} 5.2, C_{24} 4.6%, and traces of C_{14} monoethenoid). The unsaturations of the different groups of acids are almost of the same order.

4. The abnormal content of saturated acids can be explained by the process of bio-hydrogenation. The relatively less amount of saturated acids in *Carcharias melanopterus* liver oil along with its higher content of polyethylenic acids (C_{20} and above) points strongly to the possible presence of intermediate types of fats among the four groups of Elasmobranch oils.

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