Fatty Acid Compositions of Indian Shark Liver Fats

S. P. PATHAK and L. M. DEY, Department of Industrial Chemistry, Banaras Hindu University, Banaras, India

TSUJIMOTO (7, 8), AFTER INTENSIVE STUDY of a number of marine fish fats, came to the conclusion that Elasmobranch fish liver fats can be classified roughly into four groups on the basis of unsaponifiables and saturated fatty acids contents. In the first three groups there are different proportions of unsaponifiable matter and different degrees of unsaturation in the fatty acids. But, in all the three groups, only about 20% of the fatty acids are saturated.

Although Tsujimoto predicted the occurrence of the fourth group, very little data were available until the recent work by Pathak *et al.* in this laboratory definitely proved the occurrence of such fats in Asiatic varieties of shark liver. The data have now been extended by the analysis of liver fats from two varieties of Elasmobranch fish caught on the West Coast of India, namely, *Carcharias limbatus* and *Pristis cuspidatus*. These liver fats belong to the fourth group because they were found to contain about 40% of saturated fatty acids.

Experimental

The following experimental procedure for the investigation of the component fatty acids of both the fats was adopted.

Carcharias limbatus Liver Fat. A sample weighing 250 g. of the fat was dissolved in 10 volumes of acetone and was kept for about a month for the separation of the phosphatides. Then 6 g. of phosphatides were precipitated and were removed by filtration. The glyceride portion (230 g.) was saponified by refluxing it with 50 g. of potassium hydroxide dissolved in 1,150 ml. of 95% alcohol for about 45 min. At the end of this time about half of the alcohol was removed by distillation, and the soap solution was diluted with water. The unsaponifiable matter was removed from the soap solution by extracting it with ether, and the fatty acids were then recovered by acidifying the soap solution with dilute mineral acid. The fatty acids thus obtained were resolved into different fractions, first, by the lithium salt-acetone method (9) and then by Hilditch's modified lead salt-alcohol method (2). The results are recorded in Table I.

TABLE I

Separation of the Acids of *Carcharias limbatus* Liver Fat

Group	Description	Weight		Iodine value
		g.	%	
A	Lead salt-alcohol insoluble.....	86.0	42.5	16.6
B	Lead salt-alcohol soluble but lithium salt-acetone insoluble.	55.7	27.6	126.1
C	Lithium salt-acetone soluble....	60.3	29.9	227.3

Each group of acids was separately converted into methyl esters on taking the usual precautions for handling unsaturated acids (1). The esters were then fractionally distilled under reduced pressure through an electrically heated and packed column. The iodine values and the saponification equivalents of each of the sub-fractions were then determined. Since prolonged storage might cause structural and other changes, the highly unsaturated fraction C was analyzed first, followed by B, and then the most saturated fraction A. (The ester fractionation data are not given in order to save space.)

The compositions of each ester sub-fraction were determined from their saponification equivalents and iodine values, according to the method recommended by Hilditch (2). The mean unsaturation of each of the unsaturated esters was determined by the usual interpolation and extrapolation methods, from which the mean equivalent of each of the homologous groups follows. The final composition of the original fat was computed from these figures in relation to the crystallization chart, whiich is recorded in Table I.

Pristis cuspidatus Liver Fat. The experimental procedure was essentially the same as for *Carcharias limbatus* liver fat and is summarized in Tables III and IV.

TABLE II

Component Acids in Groups A, B, and C and in the Whole Fat (*Carcharias limbatus* Liver Fat)

Acids	A (42.5%)	B (27.6%)	C (29.9%)	Total	Fatty acids ex-N.S.[a]	
					(% wt.)	(% mol.)
Myristic	1.78	0.81	—	2.59	2.65	3.29
Palmitic	17.75	0.89	—	18.64	19.06	21.09
Stearic	14.81	0.04	—	14.85	15.19	15.14
Arachidic	0.68	—	—	0.68	0.69	0.63
Unsaturated						
C_{14}	0.07	0.43	—	0.50	0.51	0.64
C_{16}	1.59	4.99	1.64	8.22	8.40	9.37
C_{18}	4.83	13.69	3.71	22.23	22.73	22.87
C_{20}	0.96	5.96	6.19	13.11	13.40	12.39
C_{22}	—	0.78	12.82	13.60	13.90	11.85
C_{24}	—	—	3.39	3.39	3.47	2.73
Non-saponifiables	0.03	0.01	2.15	2.19	—	—
Average Number of Double Bonds in Unsaturated Acids						
C_{14}	1.0	1.0		1.0		
C_{16}	1.0	1.0	1.0	1.0		
C_{18}	1.0	1.1	2.1	1.3		
C_{20}	1.0	2.7	3.3	2.9		
C_{22}	—	3.0	3.9	3.9		
C_{24}	—	—	4.5	4.5		

[a] Excluding unsaponifiables.

Discussion

In Tsujimoto's classification of fish liver fats the fourth group was distinguished by a high content (about 50%) of saturated fatty acids. However until recently very few data about such fats were available. Recent work definitely proves the existence of such a group. Fats from *Galeocerdo rayneri* (4), *Galeocerdo tigrinus* (5), and *Carcharias melanopterus* (6) contain about 41%, 43%, and 34% of saturated acids with about 59%, 57%, and 66% of unsaturated acids, respectively.

The comparison (Tables II and IV) of *C. limbatus* and *P. cuspidatus* liver fats shows great similarities in their compositions although they belong to different families. The total saturated acid contents are 38% and 42%, respectively. Palmitic acid is the predomi-

TABLE III

Separation of the Acids of *Pristis cuspidatus* Liver Fat

Group	Description	Weight		Iodine value
		g.	%	
A	Lead salt-alcohol insoluble.....	60.7	40.4	3.8
B	Lead salt-alcohol soluble but lithium salt-acetone insoluble..	68.3	45.5	108.1
C	Lithium salt-acetone soluble....	21.1	14.1	170.4

TABLE IV

Component Acids in Groups A, B, and C and in the Whole Fat (*Pristis cuspidatus* Liver Fat)

Acids	A (40.4%)	B (45.5%)	C (14.1%)	Total	Fatty acids ex-N.S.[a]	
					(% wt.)	(% mol.)
Myristic	1.39	0.36	—	1.75	1.78	2.19
Palmitic	22.56	1.84	—	24.39	24.76	27.15
Stearic	13.25	1.14	—	14.39	14.61	14.44
Arachidic	1.01	—	—	1.01	1.03	0.92
Unsaturated						
C_{14}	—	0.32	—	0.32	0.32	0.40
C_{16}	0.34	3.90	2.10	6.44	6.44	7.12
C_{18}	1.19	19.54	2.62	23.35	23.71	23.62
C_{20}	0.65	13.30	3.82	17.77	18.05	16.42
C_{22}	—	4.09	3.21	7.30	7.41	6.26
C_{24}	—	—	1.86	1.86	1.89	1.48
Unsaponifiables	0.02	1.01	0.49	1.52	—	—
Average Number of Double Bonds in Unsaturated Acids						
C_{14}		1.0		1.0		
C_{16}	1.0	1.0	1.3	1.1		
C_{18}	1.0	1.1	1.8	1.2		
C_{20}	1.0	1.5	3.1	1.8		
C_{22}		3.4	4.8	4.0		
C_{24}			5.5	5.5		

[a] Excluding unsaponifiables.

nant saturated acid with subsidiary amounts of stearic acid and minor amounts of myristic and arachidic acids. Among the unsaturated acids which form about 62% and 58%, respectively, of the total fatty acids, the acids of C_{18}, C_{20}, and C_{22} series predominate whereas C_{24} acids are present in subordinate proportions and C_{14} acids are present in only minor quantities. Thus our findings give additional proof for the existence of a fourth group among the Elasmobranch fish liver fats, as predicted by Tsujimoto.

The most striking feature in the case of *Pristis cuspidatus* liver fat is its high (12.5%) content of unsaponifiable matter. Fortunately another sample of liver fat from *P. cuspidatus* caught on the East (Malabar) Coast has been studied by Pathak and Suwal (6) and was found to contain only a negligible quantity of unsaponifiable matter. Comparison of the fatty acid compositions of the two fats (Table V) shows that the unsaponifiable matter, which is probably chiefly selachyl-glyceryl ethers, bear an important relationship to the fatty acid composition. With the increase of unsaponifiable matter the saturated acid content increases from 37% to 42% whereas the unsaturated acid content decreases from 63% to 58%. The marked fall in the content of the most highly unsaturated acid (C_{24},–11H) from 4.6% to 1.9% is particularly noteworthy. The unsaturated C_{20} and C_{22} acid contents increase from 16.4% and 5.2% to 18.1% and 7.4%, respectively, and again the unsaturated C_{16} and C_{18} acids decrease from 8.2% and 28.5% to 6.4% and 23.7%, respectively, with no marked change in the unsaturated C_{14} acid content. In the case of saturated acids every individual acid increases to some extent. It is quite clear that there is a definite tendency for the fat to be more saturated with the appearance of unsaponifiables, which support Lovern's view (3) that "disappearance of polyethenoid unsaturation in the higher fatty acids accompanied by the appearance of alcohol ethers of selachyl type are both to be regarded as evidence of an unusual tendency towards unsaturation or hydrogenation in fish liver fats." The general pattern of the liver fats is similar.

TABLE V

Fatty Acid Compositions of *Pristis cuspidatus* Liver Fats

Reference Unsaponifiables, % Acids	(6) 1–2%	(author) 12.5%
Myristic	1.2	1.8
Palmitic	22.9	24.8
Stearic	12.7	14.6
Arachidic	0.1	1.0
Unsaturated		
C_{14}	0.2	0.3
C_{16}	8.2	6.4
C_{18}	28.5	23.7
C_{20}	16.4	18.1
C_{22}	5.2	7.4
C_{24}	4.6	1.9
Average Number of Double Bonds in Unsaturated Acids		
C_{14}	1.0	1.0
C_{16}	1.0	1.1
C_{18}	1.1	1.2
C_{20}	2.7	1.8
C_{22}	3.7	4.0
C_{24}	5.6	5.5

Summary

Liver fats of *Carcharias limbatus* and *Pristis cuspidatus* from the West Coast of India have been studied.

Lithium salt-acetone and lead salt-alcohol methods were adopted for the resolution of the fatty acids. An efficient, electrically heated and packed column has been employed for fractionation of the methyl esters of fatty acids.

These fats have been found to have a high content of saturated fatty acids and thus belong to the fourth group of Tsujimoto's classification of Elasmobranch fish liver fats.

Analyses of the liver oils from the same species of fish show that there is a tendency towards saturation or hydrogenation of polyethylenic acids accompanied by an increasing concentration of unsaponifiable matter, which is mainly alcohol ethers of the selachyl type, in accordance with Lovern's views.

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REFERENCES

1. Bjarnason, O. B., and Meara, M. L., J. Soc. Chem. Ind., *63*, 61 (1944).
2. Hilditch, T. P., "The Chemical Constitution of Natural Fats," John Wiley and Sons Inc., New York, N. Y. (1947).
3. Lovern, J. A., Biochem. J., *31*, 755 (1937).
4. Pathak, S. P., Agarwal, C. V., and Mathur, S. S., J. Am. Oil Chemists' Soc., *29*, 593 (1952).
5. Pathak, S. P., and Pande, G. D., *ibid.*, *32*, 229 (1955).
6. Pathak, S. P., and Suwal, P. N., *ibid.*, *31*, 332 (1954).
7. Tsujimoto, M., Chem. Umsch. Fette, *39*, 50 (1932).
8. Tsujimoto, M., J. Chem. Soc. Ind., *51*, 317T (1932).
9. Tsujimoto, M., J. Chem. Soc. Ind., Japan, *23*, 272 (1920).

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