SHORT COMMUNICATIONS

THE FATTY ACID COMPOSITION OF PAKISTANI ELASMOBRANCH FISH LIVER OILS

Part 1.—The Fatty Acids of (*Mustelus monazo*) Shark Liver Oil

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Introduction

Marine liver oils have assumed great industrial importance as a source of vitamins A and D and, as such, liver oils of cod, halibut, sword fish etc. are being produced in many countries. Sharks are abundant on the Karachi-Makran coast and, according to the statistics released by the Central Fisheries Department, the present landing of the sharks is around 10,000 tons/annum. The oil extracted from these species of sharks has been shown to contain from 2,000 to 72,000 U.S.P. units of vitamin A_1, but little attention has been given to words the investigation of the fatty acid composition of the oil. Fish oils are known to be rich in unsaturated acids and it would be of interest to know the extent of unsaturation of the oils because of the emphasis these days on the unsaturated fatty acids. We have, therefore, undertaken these studies and the present communication deals with the fatty acid composition of the *Mustelus monazo* (shark) liver oil. This oil has been particularly selected to start with because of the abundance of its source.

Materials and Methods

Ten lb. of shark (*Mustelus monazo*) livers were chopped and minced, mixed with double the amount of water and cooked for two hours at 60°C with constant stirring until all the tissues were homogenized. The mixture was allowed to stand for some time in a separating funnel. The lower layer of water was removed and the rest along with the liver tissue was centrifuged to separate the oil. It was then washed with hot water to remove impurities and non-fatty matter.[1]

The oil thus obtained was dried over anhydrous sodium sulphate, and filtered. It was kept at 15°C overnight for settling the stearine. The settled stearine was filtered off and the clear oil stored under nitrogen in a refrigerator.

The physical constants of the oil were determined by the standard *A.O.C.S.*[2] methods and the values are given in Table 1.

TABLE 1.—*Mustelus monazo* (SHARK) LIVER OIL.

Acid value	1.9
Specific gravity	.0909 at 33°C
Refractive index	1.48 at 33°C
Saponification value	146
Iodine value	193
Unsaponified matter	4.81%

Infrared Studies.—An infra-red spectrograph of the oil was taken on Leitz double beam I.R. instrument at room temperature. The absence of a band at 10.3μ established the absence of the trans double bonds.[3]

Chemical Studies.—One g. of the oil was saponified with 0.5N alcoholic KOH for one hour, the soap was diluted with water and the unsaponifiable matter extracted with diethyl ether. The saponified portion was further diluted with water and the fatty acids were liberated by the addition of 40% sulphuric acid. Nitrogen gas was bubbled through the soap solution during the addition of the acid and all the subsequent steps were carried out strictly under an inert atmosphere to avoid oxidation of unsaturated acids.

The liberated fatty acids were taken up in ether and washed thrice with water to remove mineral acid. The ether extract was dried over anhydrous sodium sulphate, filtered in a clean and tared flask and the solvent removed under nitrogen.

Gas-liquid Chromatography.—Methyl esters of the fatty acids were prepared with diazomethane[4] in ethereal solution at low temperature. The esters were analysed by gas-liquid phase chromatography using radium ionization detector unit on a SEG column (13% diethylene glycol succinate on chrom P W/W.) at 190°C. The carrier gas used was argon (flow rate 50 ml./min). The identity and the percentage composition of the component fatty acids in each oil were determined from the retention times and the peak area of the methyl esters respectively (Table 2).

TABLE 2.—G.L.C. ANALYSIS OF METHYL ESTERS ON 20% DEGS.

Peak No.*	Fatty acid	RRF	% composition
1	12:0	.1540	trace
2	14:0	.2805	6.65
3	15:0	.4041	trace
4	16:0	.5379	23.77
5	16:1	.6549	13.04
6	18:0	1.000	.80
7	18:1	1.174	42.93
8	18:2	1.527	trace
9	20:1	2.020	10.39
10	22:1	3.664	2.42
11	22:4	5.130	trace

For the evaluation of minor acids, 3μl. samples of esters (a slight overload) were applied to the polyester column. In this chromatogram, four more peaks were obtained in addition to the ones obtained previously. Numbered as 1,3,8, and 11, these were identified as C_{12}:O, C_{15}:O, C_{18}:2 and C_{22}:4 by plotting the semi-logarithmic graph in the usual manner, but no quantitative assignment could be made because of their low concentration.

Discussion

The oil contains 68.78% unsaturated fatty acids as is evident from its high iodine value which is 193. Predominant unsaturated acid is oleic with a percentage of 42.93. Palmitoleic and eicosenoic acids are 13.04 and 10.39% respectively. Poly-unsaturated acids are not present in any appreciable amount, decosenoic acid is 2.42% while linoleic and docosatetraenoic acids are present in trace amounts.

The percentage of saturated fatty acids is 31.22. This is high as compared to the typical marine fat, where the saturated fatty acid content is always around 20%. The predominant saturated constituent is palmitic acid with a percentage of 23.77. Myristic acid is 6.65%. lauric, stearic and pentadecanoic acids are present in trace amounts.

The high saturated fatty acid content of the oil brings it under the fourth group of Tsujimoto's[6] classification of the elasmobranch fish liver oils. The fourth group of the elasmobranch fish liver oils is characterised by a remarkably high concentration of the saturated fatty acids, nearly 50% in white shark, *Carcharias gangeticus*[7] and in the Chinese fan fish[8], 64%.

Lovern[9] has pointed out that the elasmobranch fish liver oil may be of diversified composition but it is possible that fats exist between the types (four groups) and that a gradual transition could be shown from one extreme to another. Such a possibility is also pointed out by Pathak and Suwal.[10] The liver oil of the shark, *Carcharias melanopterus*, studied by them has a saturated fatty acid content of 31% as in the *Mustelus monazo* shark liver oil studied by us. But whereas the unsaturated acids comprise C_{16},C_{18}, C_{20} and C_{22} acids in the former case, in the latter the main unsaturated acid is C_{18}, monoethenoid (42.93%) of the total acids) with 10.39% of C_{20} monoethenoids. The very high percentage of the oleic acid in the case of *Mustalus monazo* is noteworthy.

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References

1. S.M. Ali and S. Mahdihassan, Pakistan J. Sci. Ind. Res., **1,** 70 (1958).
2. *Official and Tentative Methods* (American Oil Chem. Soc.), 2nd edition.
3. R.T.O. Connor, J. Am. Oil Chem. Soc., **32**, 632—33 (1955).
4. *Arthur Vogel A Text Book of Practical Organic Chemistry* (Longman's Green & Co), 3rd ed., p. 973.
5. R.G. Ackman and R.D. Burgher, J. Chromatog., **11**, 185-94 (1963).
6. M. Tsujmoto, J. Soc. Chem. Ind., **51**, 317 (1932).
7. M. Tsujimoto, J. Soc. Chem. Ind. (Japan) **23**, 272 (1920).
8. T.H. Wang and C.H. Kan, J. Chinese Chem. Soc., **4**, 393 (1956).
9. J.A. Lovern, *The Composition of the Depot. Fats of Aquatic Animals*, 34 (1942).
10. S.P. Pathak and P.N. Suwal, J. Am. Oil Chem. Soc., **31**, 332—34 (1954).