FISH OIL RESEARCH AT THE HORMEL INSTITUTE

By W. O. Lundberg*

The Hormel Institute is a research branch of the Graduate School of the University of Minnesota, established at Austin, Minn., under an agreement between the University and the Hormel Foundation. It is staffed by academic and civil service personnel of the University. A major portion of the program at the Hormel Institute consists of researches on lipid materials.

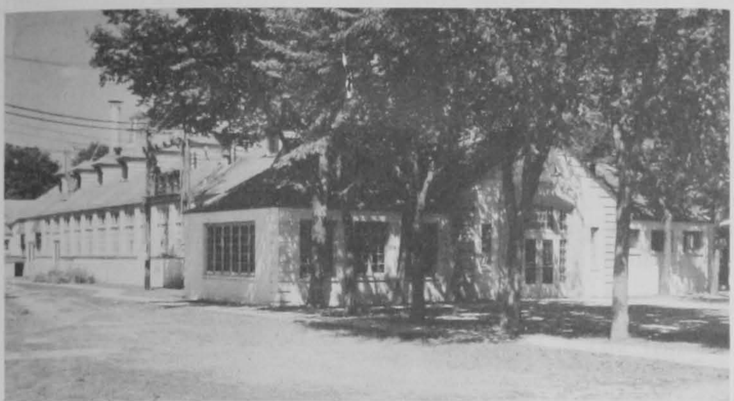

Fig. 1 - Main building Hormel Institute of University of Minnesota, Austin, Minn.

Currently, the Hormel Institute is carrying on several projects in the research program started by the U. S. Fish and Wildlife Service with Saltonstall-Kennedy funds. The three original projects begun in 1955 were concerned with (1) determining the structures of fish-oil fatty acids and developing an analytical method for the determination of the fatty acid composition of fish oils, (2) studying the chemistry of the odor problem in fish oils, and (3) studying the fractionation of fish-oil fatty acids by means of "inclusion" compounds. The third one of these projects has been completed and now has been replaced by a fourth project: A study of chemical reactions of fish-oil fatty acids. Although all of the projects fall in the category of what is commonly called fundamental research, it is anticipated that the results will have important practical applications. The following describes each of the projects briefly:

STRUCTURE AND ANALYSIS OF FISH-OIL FATTY ACIDS

For the development of new derivatives and new applications of fish oils, it is obviously important to know the composition and structure of fish-oil fatty acids. Relatively complete information is available about common animal and vegetable fats and oils, but much remains to be learned about fish oils. It is well known that the commercially-important fish oils contain appreciable amounts of highly unsaturated fatty acids and that the carbon skeletons of some of the fatty acids are longer than are those in ordinary animal and vegetable fats, but the locations of the unsaturated centers in the fatty acids have not been established unequivocally. Knowledge of the locations of the double bonds is essential to the development of new and useful derivatives and to the development of analytical methods whereby the composition of fish oils may be determined accurately.

It has been established that the more unsaturated fatty acids of land-animal and vegetable fats have what is called a methylene-interrupted type of unsaturation; that is, a single $-CH_2-$ group lies between successive double bonds in the carbon chain. It is a well-known fact in organic chemistry that a $-CH_2-$ group between two double bonds is highly reactive. The reactivity of the $-CH_2-$ group in unsaturated fatty acids of this type is exploited in the manufacture of commercially-important derivatives of vegetable oils, particularly in the field of protective coatings.

In various past studies of the structure of fish-oil fatty acids, it has been reported that ethylene groups, $-CH_2-CH_2-$, rather than methylene groups, separate the double-bond systems. This type of structure, if it predominates in fish oils, is

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decidedly disadvantageous insofar as developing certain potentially useful derivatives is concerned, because the ethylene-interrupted type of structure is far less reactive than is the methylene-interrupted type. Further, the existence of any appreciable amount of ethylene-interrupted structures would greatly complicate the problem of analyzing fish oils to determine the proportions of the various types of fatty acids. There was, however, some reason to doubt the reports of the ethylene-interrupted type of structure for fish-oil fatty acids, and thus a critical study of structure was undertaken.

A complete determination of the structure of the more unsaturated fish-oil fatty acids involves the isolation of the individual fatty acids in highly purified form. This has not been accomplished yet in the project at the Hormel Institute, but good progress is being made. Various methods--including solvent segregation, urea-complex fractionation, distillation, and chromatography on silica-gel columns--are being applied in the isolation of individual pure acids.

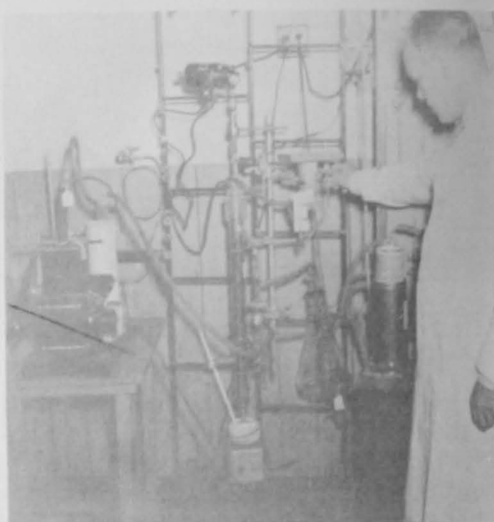

Fig. 2 - Low-pressure fractional distillation of methyl esters of menhaden oil fatty acids being performed on a Podbielniak Whirling Heli-Band Column at Hormel Institute.

Even though the pure acids have not yet been isolated, the studies to date have shown that, in the common types of commercially-important fish oils, the more favorable methylene-interrupted type of unsaturation predominates. That at least some of this type of unsaturation is present was evident from the ultraviolet absorption spectra of various fish oils following isomerization with alkali. Alkali-isomerization followed by spectral measurements has served as a basis for the analysis of vegetable oils, and may also be applied to fish oils if methylene-interrupted unsaturation exists.

The predominance of methylene-interrupted unsaturation was unequivocally established in studies using soybean lipoxidase. It is well known that this enzyme catalyzes the oxidation only of substances that contain the 1,4 pentadiene grouping, that is, methylene-interrupted unsaturation. In the work at the Hormel Institute, it was found that at least 97 percent (and possibly more) of the polyunsaturated fatty acids of menhaden oil have their double bonds separated by methylene rather than by ethylene groups. On this basis, it appears highly probable that the alkali-isomerization spectrophotometric technique can be adapted to the analysis of common fish oils.

The investigators in this project, Dr. O. S. Privett and his coworkers, are now concentrating on the isolation of individual polyunsaturated fatty acids from menhaden oil for complete structural determination and to establish analytical constants to be used in the analysis of all common fish oils.

CHEMISTRY OF THE ODOR PROBLEM

It is well known that the typical odor of fish oils is a deterrent to their use in many applications. Even though in the production of fish oils the objectionable odor may be removed almost entirely by steam deodorization, an objectionable odor returns in a relatively short time. Evidence indicates that the returning odor may stem from two sources: (1) a liberation of nitrogeneous compounds of low molecular weight from proteins or other nitrogeneous materials of higher molecular weight, and (2) the development of substances of low molecular weight by reaction of atmospheric oxygen with highly unsaturated fatty acids. Obviously it is important to find out what these compounds are and how they are formed, if one is to endeavor intelligently to prevent their formation or to neutralize them as they are formed.

In a first approach to the problem, Dr. J. R. Chipault and his coworkers are studying the products formed in the oxidation of fish oils by air. The volatile odor components are being removed from oxidized oil and analyzed. This is not easy because the offensive volatile components, although possessing powerful odors, ordinarily are present only in very small amounts.

Fig. 3 - Paper chromatography of fatty acids derived from fish oils and their quantitative measurement with a densitometer at Hormel Institute.

Nevertheless, by prolonged and repeated oxidations of relatively large quantities of oils, appreciable amounts of the odor components have been collected. Paper chromatography now is being used to separate various derivatives of the volatile products, and it is hoped that later they may be analyzed more completely by gas-phase chromatography.

The studies to date indicate that the odor and flavor components to a considerable extent consist of unsaturated carbonyl and dicarbonyl compounds derived primarily from the more unsaturated fatty acids. An effort will be made to establish whether the undesirable odors can be attributed predominantly to one category or one type of compound. If this should be the case, there is a good possibility that additives can be found that will react with and destroy the odor components.

SEPARATION OF FATTY ACIDS BY MEANS OF INCLUSION COMPOUNDS

The development of new uses for fish oils depends largely on an exploitation of those structural characteristics of fish oils that distinguish them from common land-animal and vegetable fats and oils. The principal distinguishing characteristics are that fish oils contain appreciable quantities of fatty acids having relatively long carbon-chain lengths and high degrees of unsaturation. For some applications, therefore, it will be important to have economical methods for separating the longer and more unsaturated acids.

Fatty acids have the peculiar property of forming solid complexes or "inclusion" compounds with materials like urea. The extent to which such solid complexes are formed, however, depends upon the structure of the fatty acid. Differences in the tendencies of various fatty acids to form such inclusion compounds may be employed in their separation. Dr. Hermann Schlenk and his coworkers, who have had much experience with inclusion compounds, devoted their efforts to finding materials other than urea that might be employed in such separations. In particular, they studied thiourea and deoxycholic acid. Neither of these "host" compounds would be practical at present because of their cost, even if they were found to give better separations than does urea, but it was important to study them in order to obtain additional information about types of molecules that might be employed effectively.

As is often the case in fundamental research, certain findings were made that were incidental but, nevertheless, important. First, it was found that thiourea was effective in removing peroxides from fish oils, apparently by reaction of the hydroperoxide group with thiourea. This reaction will be explored further in connection with the odor problem, inasmuch as hydroperoxides are probably the precursors of the odor components that develop by oxidation of fish oil. Second, it was found that deoxycholic acid inclusion compounds of fish-oil fatty acids can be separated readily by paper chromatography. This finding will be useful in establishing the purity of the individual fish-oil fatty acids that are being prepared in the first project described above, and it also has been applied by Dr. Schlenk to show that the content of fatty acids of different chain lengths in menhaden oil are quite different from the value published heretofore.

CHEMICAL REACTIONS OF FISH-OIL FATTY ACIDS

Following completion of the project on inclusion compounds of fish-oil fatty acids, Dr. Schlenk and his coworkers recently have undertaken a new project, the purpose of which is to investigate various types of reactions of fish-oil fatty acids, with a view to developing new derivatives. The project aims to exploit those characteristics of fish oils that cause them to be different from common vegetable and animal fats, notably their content of long-chain highly unsaturated fatty acids.

Reactions of the carboxyl group will not be explored to any appreciable extent because such reactions, and the derivatives obtainable from them, already are well known. Instead, attention will be devoted particularly to reactions of the double bonds. A number of promising reactions have been formulated on paper, and experiments to determine the feasibility of them now are well under way.

SEA LAMPREY

The sea lamprey, Petromyzon marinus, is found along the Atlantic coast of Europe and North America; from the west coast of Greenland to Florida in the western side of the Atlantic; from northern Norway to the Mediterranean and West Africa in the eastern Atlantic. This species runs up fresh rivers to breed and is found in certain American lakes.

Lampreys have on occasion been found fastened to sea fish, and judging from the activities of their landlocked relatives they probably are destructive to some marine species. They have been found preying on mackerel, various herrings, cod, haddock, pollock, salmon, basking sharks, swordfish, hake, sturgeons, and eels. Sometimes as many as 3 or 4 are fast at one time to a single shad.

In the past, considerable numbers of lampreys were caught for food in the rivers of New England, and prior to that in Europe. The lamprey fishery has declined and now only small numbers are taken for consumption.

Another use of the lamprey is to supply biological laboratories with specimens for student use. The larvae are taken in large numbers for bait in certain localities along the Atlantic coast.

--"Sea Secrets," July 17, 1956
The Marine Laboratory,
University of Miami,
Coral Gables, Fla.