$$CH_3\ (CH_2)_7\ \underset{\diagdown\ \diagup}{\overset{CH_2}{C = C}}\ (CH_2)_6\ COOH \qquad (II)$$

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Phospholipids of Menhaden Muscle

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Abstract

The phospholipids of menhaden fish muscle represent about 0.5% of the wet weight. They were separated by fractionation on a silica gel column. Choline glycerophosphatides were the major components, about 60%. Ethanolamine and serine glycerophosphatides accounted for about 20%, and sphingomyelin and less well-characterized components for the rest. The fatty acid composition of the CGP,[1] EGP[1] + SGP,[1] and triglyceride preparations were compared. The EGP + SGP fraction contained 20% stearic acid whereas the neutral or CGP fractions had only 2–4%. The phospholipids contained about 30% of docosa-hexaenoic acids compared to about 10% in the neutral fraction.

Introduction

THE MENHADEN (*Brevoortia tyrranus*) is the most important United States fishery in terms of tonnage. Over a million tons are caught each year, essentially all of which are used for oil and meal manufacture (1). Information is available concerning the fatty acid composition of pressed menhaden oil (2) but the nature of the phospholipid components in menhaden muscle has not previously been described. This paper presents the results of such a study. The methods were those used in a parallel study of the phospholipids of tuna muscle (3).

Experimental

Raw Material

Menhaden fish weighing about 500 gm each were obtained as follows: They were caught Aug. 7, 1961, in Delaware Bay, eviscerated while still alive, and placed on dry ice within an hour after having been caught. They were then shipped with dry ice packaging and held at −18C.

TABLE I

Analyses of Menhaden Samples (Wet Weight)

Batch No.	No. fish	Wgt. muscle tissue gm.	Total lipid %	Phospholipid %	Residue %
1	15	372	3.53	0.55	16.0
2	18	433	3.56	0.45	16.2

[1] CGP refers to total choline diacylglycerophosphatides plus choline plasmalogens, EGP to ethanolamine glycerophosphatides and SGP to serine glycerophosphatides.

Preparation and Handling of Lipid Extracts

The fish were allowed to thaw, filleted, and the muscle tissue was separated and extracted with chloroform:methanol by the Bligh and Dyer (4) procedure as previously described (3). Yields are shown in Table I. The total lipids were stored in chloroform at −18C under nitrogen.

Fractionation and Analysis Procedures

A preliminary separation of phospholipids was achieved by passing solutions of the total lipids in chloroform through a silica gel column and washing with chloroform. The silicic acid had been carefully prepared by washing, deoxygenation and dehydration according to Rouser et al. (5). The portion that was not retained contained fatty acids, triglycerides, cholesterol and cholesterol esters. The phospholipids remaining on the column were eluted with chloroform: methanol (1:1), concentrated, and fractionated on another silica gel column. By this method, the amount of material to be fractionated was reduced; separations of the individual phospholipids were then more readily reproducible. Fractions were obtained by gradually increasing the amount of methanol in chloroform.

TABLE II

Tentative Identification of Menhaden Muscle Lipid Fractions

Eluant[a]	Identity[b]	Yield[c] %
0.5% CH_3OH	Pigment, oxid. glycerides, unknown	4
7% CH_3OH	White flaky solid, unknown	2
12% CH_3OH	EGP + SGP	17
17% CH_3OH	Same plus unknown white solid	3
23% CH_3OH	CGP	63
27% CH_3OH	Sphingomyelin	3
above 27% CH_3OH	Lysolecithin plus unknown	8

[a] Chloroform plus the amounts of methanol noted.
[b] Based on infrared absorption and analytical data. See footnote 1.
[c] Separations in duplicate runs occurred in the same order but were not always equally sharp or at the same methanol level. The data are from a single run.

TABLE III

Composition of Menhaden Muscle Lipid Fractions

Fraction[a]	Eluant methanol in chloroform %	Plasmalogen %	Phosphorus %	Phosphorus μM P/100 mg	Ester μeg/100 mg	Ester/phosphorus ratio
Last part of forerun	8	5.6	..	.. .	2.87	....
EGP + SGP (Run I)	15	10.8	3.06	.98	2.10	2.1
EGP + SGP (Run II)	25	4.9	3.71	1.20	2.42	2.0
CGP (Run I)	30	4.3	3.66	1.18	2.38	2.0
CGP (Run II)	30	4.0	3.86	1.25	2.35	1.9
Sphingomyelin and CGP	50		2.88	.93	1.44	1.6

[a] See footnote 1.

Each fraction was examined for homogeneity by thin-layer chromatography and identified by infrared absorption spectra, by color tests such as Liebermann-Burchard for sterols, and ninhydrin for free amino groups, and by its content of plasmalogen, phosphorus and ester bonds. Fractions with similar properties were combined. Care was taken at all stages to prevent oxidation both by the liberal use of purified nitrogen in flushing containers and by the addition of small amounts of hydroquinone to each preparation and fraction. Finally aliquots of the fractions were esterified with acidified methanol for determination of the fatty acid composition by gas chromatography.

Results and Discussion

The estimated yields of the major fractions are presented in Table II. The CGP fraction occurred in largest amount. EGP plus SGP accounted for approximately 20% of the total, and smaller amounts of sphingomyelin and unidentified fractions were also present. Analytical data for plasmalogen, phosphorus and ester contents are shown in Table III and fatty acid analyses in Table IV. Plasmalogen contents were generally lower than those observed with corresponding fractions from tuna.

The fatty acid analyses of the neutral fraction are within the range of those obtained by Gruger et al. (2) on a number of samples of commercial crude menhaden oils from different areas and at different seasons. Each figure is the mean of two or more runs. The differences between the two batches illustrate the difficulties in the methodology. Each sample was hydrogenated and rerun in order to identify $C_{15:0}$ and $C_{17:0}$ with certainty.

TABLE IV

Fatty Acids from Neutral, Cephalin (PE + PS) and Lecithin (PC) Fractions from Two Preparations of Menhaden Muscle Lipids

Fatty acids[a]	Neutral		EGP + SGP[b]		CGP[b]	
	I	II	I	II	I	II
8:0	....	....	0.4	Tr	....	....
9:0	....	....	Tr	Tr	....	....
10:0	....	....	Tr	Tr	....	....
?	....	....	Tr	Tr	....	....
12:0	Tr	....	0.3	Tr	....	....
13:0	....	....	0.4	0.5	....	....
14:0	8.9	10.9	2.3	1.6	0.7	0.8
14:1	Tr	Tr	....	1.2	....	....
15:0	0.5	0.6	1.4	....	Tr	Tr
16:0	29.7	25.1	25.4	20.7	40.4	43.7
16:1	10.3	13.1	0.9	2.6	0.7	1.7
16:2	0.8	1.8	0.4	0.4	....	....
17:0	....	....	0.6	0.7	....	....
18:0	2.1	3.9	22.0	16.0	2.2	4.3
18:1	13.3	11.5	6.1	6.6	6.4	8.2
18:2	1.6	1.7	0.2	1.6	Tr	0.6
18:3	0.2	Tr	....	....	....	0.3
20:1	1.8	1.9	....	1.1	....	....
20:2	2.4	3.2	....	1.7	....	0.3
20:4	1.6	1.2	2.7	2.3	1.4	5.4
20:5	10.9	11.7	4.6	10.3	14.2	10.5
22:1	....	0.2	....	....	....	....
22:2	1.2	1.0	....	....	....	....
22:4	1.7	1.6	Tr	Tr	Tr	1.0
22:5	2.0	1.8	....	Tr	....	1.0
22:6	10.7	8.8	31.2	32.7	33.7	22.9

[a] The first number is the carbon chain length, the second is the number of double bonds.
[b] See footnote 1.

The results with the cephalin and lecithin fractions also illustrate the difficulties in the duplication of the chromatography of complex mixtures of fatty acids. Despite several unresolved discrepancies, some patterns seem to be clear. The EGP plus SGP fractions always contained small but detectable amounts of several fatty acids with chain lengths of C_{13} and lower not found in either the neutral or CGP fractions. This was also noted in the tuna phospholipid fatty acids (3). Similarly also to the tuna phospholipid results, the EGP plus SGP fractions contained considerably more stearic acid than did either the neutral or CGP fractions. These combined observations are difficult to reconcile with the theory that the several phospholipids have a common diglyceride precursor (6). Another noteworthy observation was the high concentration of $C_{22:6}$ fatty acid in the phospholipid as compared with the neutral fraction.

Since this work was completed, Davenport (7) has reported that the major ninhydrin-positive component in pigeon and ox muscle differs from phosphatidyl ethanolamine. The methods used in the present study would not have differentiated this component, if it occurs in menhaden muscle, from the remainder of the EGP plus SGP moiety.

In general, one may conclude that fish muscle phospholipids differ from those of other animals in the complex nature of their fatty acids but in other respects show many resemblances (8,9). It should be emphasized that the results of this paper were obtained with one catch of menhaden fish and that different data might well be obtained from menhaden caught in a different area, in a different season, or on different feed. Gruger's study shows considerable variation in the composition of the total fatty acids from different batches of crude menhaden oils (2). It is possible that glycerides might reflect such variations more than do phospholipids but much work remains to be done to determine the magnitude of the expected differences.

Subsequent to this study, Menzel and Olcott (10) compared the fatty acid composition at the α' and β positions of menhaden lecithin and showed that most of the unsaturated fatty acids occurred at the β position.

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