

THE CHEMISTRY OF MENHADEN OIL

By

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Thesis submitted to the Faculty of the Graduate School
of the University of Maryland in partial
fulfillment of the requirements for the
degree of Doctor of Philosophy

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Abstract

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A procedure of low-temperature crystallization can be used to isolate hexadecenoic acid in a reasonably pure form from a mixture of sixteen carbon atom acids and to concentrate the hexadecatrienoic acids of the same fraction.

The methyl esters prepared from the unsaturated fatty acids of menhaden oil were fractionated at a low pressure, and the fraction containing the esters of the sixteen carbon atom acids was subjected to low-temperature crystallization from various solvents.

The original commercial destearinated menhaden oil was found to contain 7.7 per cent hexadecenoic acid and 1.3 per cent of hexadecatrienoic acids. On the basis of the total unsaturated acids (lead soaps soluble in ethanol), these values become 10.0 per cent and 1.8 per cent, respectively.

Hydroxylation of the hexadecenoic acid with alkaline permanganate and with hydrogen peroxide, followed by periodate cleavage of the hydroxylated compounds and subsequent identification of the oxidation fragments as the semi-carbazones, showed it to be Δ 9,10-hexadecenoic acid.

Bromination studies conducted on the concentrated solution containing the highly unsaturated methyl esters resulted in the isolation of methyl hexadecatrienoates.

Ozonolysis of these esters was unsatisfactory, but it did indicate the presence of an acid or acids containing the groups, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}^=$, $=\text{CHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}^=$, and possibly $=\text{CHCH}_2\text{CH}^=$. The yield of decomposition products was far from quantitative.

Unsaturated acids with less than sixteen carbon atoms may be present in negligible quantities in menhaden oil.

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INTRODUCTION

Menhaden (*Brevoortia tyrannus*) are small fish which rarely grow larger than a foot in length. They occur in large quantities along the Atlantic Coast from Nova Scotia to Brazil, and have been utilized commercially for a long time. The whole fish has been used in the production of meal and oil. Formerly, menhaden oil was used in the manufacture of soap, paint, varnish, and linoleum; with the increased scarcity of oils of a high vitamin content, it is desirable for such uses as poultry feeding (6).

The utilization of whole menhaden oil for its vitamin content results in an uneconomical disposal of the fatty acids and their glycerides, so a study of menhaden fish oil was undertaken with the belief that a knowledge of its chemical composition would make it a more valuable commodity.

For some time, the method of low-temperature crystallization from various solvents has been employed to separate glycerides into fractions with varying iodine numbers; it also has been extended to the separation of fatty acids of varying degrees of unsaturation. Most of this work has been done on the eighteen carbon atom series. Baldwin (1) employed it in the concentration and isolation of the eighteen and twenty carbon atom unsaturated fatty acids of menhaden oil.

The method was used in this work to isolate an acid with one double bond, and to concentrate a more highly unsaturated fraction from the sixteen carbon atom acid methyl ester fraction.

The former acid was converted into 9,10-dihydroxypalmitic acid. Bromination of the highly unsaturated fraction gave methyl hexabromopalmitates, from which methyl esters of hexadecatrienoic acids were prepared by debromination. Attempts to locate the double bonds were unsatisfactory.

The sixteen carbon atom fraction is composed chiefly of Δ 9,10-hexadecenoic acid and a hexadecatrienoic acid or acids.

If unsaturated acids with fewer than sixteen carbon atoms are present, they exist in negligible quantities.

EXPERIMENTAL

Preparation of the Methyl Esters of the Fatty Acids

A commercial sample of destearinated menhaden oil (saponification equivalent 303 and iodine number 173),* supplied by Fish Products Company, Lewes, Delaware, was saponified with alcoholic potassium hydroxide according to the method of Hilditch (7). After the solution had refluxed gently for two hours on a steam bath, most of the alcohol was removed by distillation at reduced pressure in an atmosphere of nitrogen. The soaps were dissolved in water and the unsaponifiable matter was extracted with ether. The solution was acidified with dilute sulfuric acid to liberate the free fatty acids which were taken up in ether. The solution was washed with water, dried with anhydrous sodium sulfate, and the ether was removed under reduced pressure in an inert atmosphere.

The mixed acids were dissolved in 95 per cent ethanol and converted into their lead soaps by use of an alcoholic solution of lead acetate containing a small amount of glacial acetic acid (8). The lead salts which deposited after cooling to 15°C over night were removed by filtration. The filtrate was concentrated under reduced pressure and acidified to congo red with dilute nitric acid. Water was added

*During the course of this work, the saponification equivalents were determined by a modification of Chargoff's method (Appendix I), and the iodine numbers were determined by a modification of the method of Hoffman and Green (Appendix II).

and the free unsaturated fatty acids were extracted with ether and recovered in the usual manner.

The methyl esters of the unsaturated acids were prepared by treating the acids with an excess of dry methanol and dry hydrochloric acid. Water was added and the esters were extracted with ether. The ethereal solution was washed with potassium carbonate solution, dried with anhydrous sodium sulfate, and the esters were recovered.

A 2 kg. sample of the mixed unsaturated methyl esters was fractionated at a pressure of 1 mm. through an electrically heated column 36 inches in length packed with helices. The column was equipped with a head designed for total reflux with partial take off.

Hexadecenoic Acid

The first distillation gave a 136 g. fraction of esters (boiling range 126-130°C, saponification equivalent 270, and iodine number 115). By redistillation of the neighboring fractions (123-126° and 130-133°), an additional 125 g. of material (saponification equivalent 271) was obtained.

The theoretical saponification equivalent for the methyl ester of a 16 carbon atom acid with one double bond is 268 and the theoretical iodine number is 94.5.

A. Low temperature crystallization of methyl hexadecenoate.- A 100 g. sample of methyl esters (saponification equivalent 270 and iodine number 115) was dissolved in one

liter of acetone and allowed to stand in the refrigerator at -15°C for 24 hours. No crystals were formed, so the solution was cooled in a thermos flask by adding dry ice (solid carbon dioxide) until an excess was present. After one hour, the mixture was filtered as quickly as possible, using a Buchner funnel which had been precooled with dry ice in acetone, and the white residue was washed three times with precooled acetone.

The material, after being allowed to melt, was dissolved in acetone and dried with anhydrous sodium sulfate to get rid of traces of water which had condensed from the atmosphere during the low-temperature operation. The acetone was distilled in an inert atmosphere, the last traces being removed at the aspirator. The product was a straw-colored oil:

Yield	62 g.
Saponification equivalent	270
Iodine number	82

In a similar manner, a second fraction of esters was recovered from the filtrate of the low-temperature crystallization:

Yield	37 g.
Saponification equivalent	269
Iodine number	167

Fifty grams of the first fraction (saponification equivalent 270 and iodine number 82) was dissolved in low-boiling petroleum ether (13.5 ml. of petroleum ether per gram of ester) and cooled to -40°C with dry ice in a thermos flask. After an hour at this temperature, the mixture was filtered using an inverted filter stick and suction from the aspirator.

The residue was taken up in petroleum ether, dried, and the ether removed:

Yield	5.5 g.
Iodine number	23.2

The filtrate was cooled again with an excess of dry ice, allowed to stand for an hour, and filtered as before.

A second residue was obtained:

Yield	7.1 g.
Iodine number	42.1

A third fraction was obtained when the filtrate was dried and the petroleum ether removed:

Yield	33.4 g.
Iodine number	99.0

A portion (25 g.) of fraction three (iodine number 99) was distilled at a pressure of 8 mm. and a sample was taken at 173°C:

Yield	12.4 g.
Saponification equivalent	269.5
Iodine number	96.0

Theoretical values for a 16 carbon acid methyl ester with one double bond are:

Saponification equivalent	268
Iodine number	94.5

B. Identification of hexadecenoic acid.- The methyl ester of hexadecenoic acid was oxidized with hydrogen peroxide and saponified to give a low-melting hydroxylated product; hexadecenoic acid was oxidized with alkaline permanganate to give a high-melting hydroxylated product. These products were split with periodic acid and the fragments were identified.

1. Hydroxylation with 30 per cent hydrogen peroxide.-

The method of Scanlan and Swern (15) was employed in the hydroxylation of methyl hexadecenoate.

At room temperature, 8.39 g. of 30 per cent hydrogen peroxide was mixed with 33 ml. of glacial acetic acid; the solution was heated to 80-85°C for one hour. After cooling to about 25°C, it was mixed with 10 g. of the methyl ester of saponification equivalent 269.5 and iodine number 96. The heat of reaction was not sufficient to raise the temperature to 65°C as was indicated in the literature, so the mixture was heated to 65°C on a water bath, whereupon it became homogeneous immediately.

The reaction mixture was allowed to cool and poured into 100 ml. of hot water. The oily layer was dissolved in normal sodium hydroxide solution and heated for two hours on the steam bath. The hot solution was acidified with 6 N hydrochloric acid and cooled in a refrigerator.

The crude white precipitate was melted and washed thoroughly with hot water slightly acidified with hydrochloric acid. The product was dried, extracted with cold petroleum ether to remove unreacted acid, and then recrystallized four times from 95 per cent ethanol. The final product, 2.6 g. of white crystals, melted at 85°C:

Analysis:

Saponification equivalent for $C_{16}H_{32}O_4$	
Calculated:	288
Found	: 287

Green and Hilditch (5) reported 84°C as the melting point of one form of 9,10-dihydroxypalmitic acid.

2. Periodate cleavage of the low-melting dihydroxypalmitic acid.- The method of King (10) was employed to split the hydroxy acid (m.p. 85°C) and to separate the fragments, which were identified as semi-carbazones.

Potassium periodate (2.3 g.) in 76.8 ml. of normal sulfuric acid at 20°C was added to a solution of 2 g. of dihydroxypalmitic acid in 153.6 ml. of 95 per cent ethanol at 40°C. After ten minutes, the clear solution was cooled to 15°C, diluted with sufficient water to dissolve the precipitated potassium sulfate, and extracted with ether.

The ether was removed under reduced pressure and the product was steam distilled. The distillate was extracted with ether and washed with a sodium bicarbonate solution followed by water until the washings were neutral. When the ether was removed at reduced pressure, a small amount of colorless oil remained.

Semi-carbazide hydrochloride (0.85 g.) and 1.5 g. of sodium acetate in aqueous alcohol were added to the residue and the semi-carbazone was removed by filtration. The yield was 0.70 g. of white powder. After several crystallizations from aqueous ethanol, the melting point and mixed melting point with authentic heptaldehyde semi-carbazone were 105°C:

Analysis:

	Per cent carbon and hydrogen in $C_8H_{17}ON_3$
Calculated:	C, 56.12%; H, 10.01%
Found	: C, 56.33%; H, 10.12%

The aqueous solution of the non-volatile product of the periodate cleavage after being cooled was extracted with ether. The ether was removed and the semi-carbazone was

prepared as outlined for the volatile fraction. The yield was 0.94 g. and the product melted at 161°C:

Analysis:

Per cent carbon and hydrogen in $C_{10}H_{19}O_3N_3$
 Calculated: C, 52.35%; H, 8.35%
 Found : C, 52.17%; H, 8.30%

Scanlan and Swern (15) reported a melting point of 161-162°C for azelaic acid half-aldehyde semi-carbazono. It is evident that the compound in question was the 9,10-dihydroxypalmitic acid.

3. Hydroxylation with alkaline permanganate.- A 4.5 g. sample of methyl ester (saponification equivalent 270 and iodine number 102) was saponified with alcoholic potassium hydroxide according to the rapid method of Olcott and Mattill (13). The free acid was hydroxylated with alkaline permanganate according to the procedure of Lapworth and Mottram (11).

A clear solution was obtained by warming the 4.26 g. of acid (calculated from the ester) with an equivalent weight of sodium hydroxide in 500 ml. of water. The solution was cooled and 4 liters of ice water was added. The mixture was then shaken at 10°C while 400 ml. of 1 per cent potassium permanganate solution was added rapidly. After five minutes, the mixture was decolorized by adding sodium bisulfite and 150 ml. of concentrated hydrochloric acid. The white flocculent precipitate was filtered, dried, and extracted with light petroleum ether to remove the unreacted fatty acid.

The white crystalline product (3.0 g.) was fractionated from aqueous ethanol to a constant melting point of 125°C.

Analysis:Saponification equivalent for $C_{16}H_{32}O_4$

Calculated: 288

Found : 288

Green and Hilditch (5) reported 123-124°C as the melting point of one form of 9,10-dihydroxypalmitic acid.

Extensive fractionation of this product and of another 11 g. sample, prepared in the same manner, gave no evidence of more than one hydroxylated product.

4. Periodate cleavage of the high-melting dihydroxypalmitic acid.- One gram of the high-melting form of the dihydroxypalmitic acid was split with periodic acid and the fragments were separated by steam distillation according to the procedure of King (10). The procedure was exactly like that used for the low-melting isomer and the fragments of oxidation were identified as the semi-carbazones.

The volatile fragment yielded 0.23 g. of semi-carbazone which melted at 105°C. The melting point was unchanged when the compound was mixed with the semi-carbazone of the corresponding oxidation fragment of the low-melting dihydroxypalmitic acid:

Analysis:Per cent of carbon and hydrogen in $C_8H_{17}ON_3$

Calculated: C, 56.12%; H, 10.01%

Found : C, 55.64%; H, 9.92%

The non-volatile fragment yielded 0.36 g. of semi-carbazone which melted at 161°C. This melting point was not lowered when the compound was mixed with the semi-carbazone of the corresponding oxidation fragment of the low-melting dihydroxypalmitic acid:

Analysis:Per cent of carbon and hydrogen in $C_{10}H_{19}O_3N_3$

Calculated: C, 52.35%; H, 8.35%

Found : C, 52.06%; H, 8.43%

It is evident that the high-melting hydroxy acid is also 9,10-dihydroxypalmitic acid.

Hexadecatrienoic Acid

A. Preliminary study by low-temperature crystallization.- The ester fraction with a saponification equivalent of 269 and an iodine number of 167 was studied.

A 25 g. sample was dissolved in 90 ml. of dry acetone, cooled with an excess of dry ice, and filtered in the usual manner. The residue yielded 12.1 g. of ester with an iodine number of 122, and from the filtrate there was obtained a 11.2 g. fraction with an iodine number of 203.

The theoretical iodine number for a 16 carbon acid methyl ester with two double bonds is 191.

Attempts to raise the iodine number above 203 by use of different concentrations of acetone and with mixtures of methyl alcohol and acetone were unsuccessful. The ester fraction was saponified and further attempts were made to raise the iodine number of the acids without success.

This work indicated the presence of a 16 carbon acid with more than one double bond and the possibility of an acid with more than two double bonds, therefore a larger sample of menhaden oil was worked up.

B. Isolation of the methyl esters of hexadecatrienoic acids.- A highly unsaturated fraction of 16 carbon acid methyl ester was obtained by the usual low-pressure fractional

distillation and low-temperature crystallization.

1. Preliminary separation and distillation of the methyl esters.- A sample of 11 kg. of mixed fatty acid methyl esters from commercial destearinated menhaden oil (saponification equivalent 303 and iodine number 173) was distilled rapidly at a pressure of 2-3 mm. until all the 16 carbon acid ester had passed over.

These esters were dissolved in acetone (10 ml. acetone per gram of ester) and cooled at -15°C for 24 hours. The mixture was filtered by suction through a precooled Buchner funnel and the residue was washed with precooled acetone. A second acetone extraction was made (5 g. acetone per gram of ester); the residue had an iodine value of 4.5.

The combined filtrate was dried with anhydrous sodium sulfate recovered from the solvent and redistilled at a pressure of 1 mm. through the electrically heated column described previously. The intermediate fractions were combined and re-fractionated.

Table I

The Fractionation of the Unsaturated Fatty Acid Esters					
Fraction	Boiling range ($^{\circ}\text{C}$)	Yield (g.)	Saponification equivalent	Iodine number	Comment
1	80-110	51.0	---	---	Very dark
2	110-115	465.0	244	10.3	
3	126-130	1250.0	271	78	

2. Low-temperature crystallization.- A 600 g. sample of Fraction 3 (saponification equivalent 271 and iodine number 78) was dissolved in 6 liters of acetone and cooled with an excess of dry ice. After one hour, a filter stick was used to remove two liters of filtrate which gave fraction number one:

Yield	48.3 g.
Iodine number	155.0

The residue was extracted with another liter of acetone in the presence of excess dry ice and the filtrate yielded fraction number two:

Yield	23.0 g.
Iodine number	147.0

A third extraction with one liter gave a fraction number three:

Yield	11.5 g.
Iodine number	138.0

The residue was recrystallized from light petroleum ether in the presence of excess dry ice and a fraction number four was obtained from the filtrate:

Yield	220 g.
Iodine number	109

The above process was repeated with another 590 g. sample of esters. The three extractions with acetone in the presence of excess dry ice gave 51.7 g. of ester with an iodine number of 159, 27.6 g. with an iodine number of 157, and 22.5 g. with an iodine number of 143.

3. Bromination of the methyl esters.- The highly unsaturated methyl esters from the first acetone extraction of each of the above samples were combined and studied by

bromination.

The sample of ester (100 g. with a saponification equivalent of 268 and an iodine number of 157) was dissolved in one liter of ethyl ether and passed through a three inch column of activated alumina (Grade A, mesh, minus 80, from the Aluminum Ore Company of America). This procedure removed some of the color from the solution. The ethereal solution in a three-necked flask was cooled with cracked ice to 0°C and bromine was added dropwise from a dropping funnel. During this operation the mixture was stirred mechanically, the stirring being continued for one hour after a reddish coloration, indicating an excess of bromine, appeared in the flask. The excess bromine was destroyed with amylene and the mixture allowed to stand for twelve hours at -15°C. The mixture was filtered and the product (42 g.) was washed three times with large amounts of cold ether. Nothing else could be crystallized by concentration of the filtrate.

The product was recrystallized four times by dissolving it in hot dioxane and allowing it to stand at room temperature for twelve hours. The final product, 8.3 g. of white powder, melted at 190-191°C:

Analysis:

Per cent bromine in $C_{17}H_{28}O_2Br_6$

Calculated: 64.46%

Found : 64.10%

Petroleum ether was added to the combined dioxane mother liquor from the above crystallization. After standing for 24 hours at 12-15°C, a second crop of material was obtained.

This light brown residue was washed carefully with a mixture of ether and petroleum ether. The residue, which was slightly soluble in ethyl ether but insoluble in a mixture of ethyl and petroleum ether, was extracted by refluxing with the ether mixture for a few minutes and finally was recrystallized from benzene:

Yield	6.2 g.
Melting Point	169-170°C

Analysis:

Per cent bromine in $C_{17}H_{28}O_2Br_6$	
Calculated:	64.46%
Found :	64.23%

The dioxane-petroleum ether mother liquor was concentrated to 75 ml., and 200 ml. of petroleum ether was added. After standing for 24 hours at 0°C, a third crop of dark material was obtained by filtration.

This residue, after four extractions by refluxing with ether and petroleum ether, and after recrystallization from benzene, weighed 4.4 g. The white powder melted at 165-166°C. This melting point was not lowered by the low-melting fraction obtained previously:

Analysis:

Per cent bromine in $C_{17}H_{28}O_2Br_6$	
Calculated:	64.46%
Found :	63.29%

4. Debromination studies.- The sample (8.2 g.) of the high melting bromide was added gradually to 100 cc. of hot methanol and 15 g. of zinc powder in a 500 ml. flask. A few drops of concentrated hydrochloric acid was added from time to time to catalyze the reaction. After all the bromide was

added, the mixture was refluxed for one hour and filtered. About 75 per cent of the methanol was removed under pressure, water was added and the mixture extracted with ether. The ethereal solution was dried with anhydrous sodium sulfate for several days and the ether was removed in an atmosphere of nitrogen. The last traces were removed at the aspirator:

Yield	2.2 g.
Saponification equivalent	265
Iodine number	280

The theoretical values for a 16 carbon acid methyl ester with three double bonds are:

Saponification equivalent	264
Iodine number	288

The lower melting fractions were combined and 10 g. of the bromide was debrominated with zinc dust in the same manner:

Yield	2.8 g.
Saponification equivalent	266
Iodine number	236

The presence of a hexadecatrienoic acid was found in Japanese sardine oil in 1929 by Toyama and Tsuchiya (18), and identified by the same investigators in 1935 (19) as the Δ 6,7; 10,11; 14,15-hexadecatrienoic acid which was called, hirogonic acid.

5. Bromination of the free acids.- The free fatty acid was also concentrated and brominated, but in this case the yields were very low. A 128 g. sample of unsaturated methyl ester with an iodine number of 123 was saponified with 33 g. of potassium hydroxide in 26 g. of water and 112 ml. of 95 per cent ethanol according to the mild method of Oleott and

Mettlil (13).

The mixture was shaken for a few minutes until it became homogeneous, and 600 ml. of water was added. The solution was acidified with hydrochloric acid, cooled, and the acids were extracted with ether.

The acids (117 g.) were recovered, dissolved in 351 ml. of acetone, and cooled with an excess of dry ice. The filtrate yielded 19.1 g. of dark oil which had a saponification equivalent of 255 and an iodine number of 173.

The material was dissolved in 300 ml. of petroleum ether and passed through a three-inch column of activated alumina. The slightly colored solution was brominated at 0°C to yield 9.8 g. of dark material insoluble in petroleum ether. Nothing else could be crystallized from the mother liquor.

By extensive fractional crystallization from large volumes of ethyl ether and benzene, two products were isolated from the dark residue.

One fraction (0.5 g. m.p. 185°C) was slightly soluble in ether and soluble in benzene:

Analysis:

Per cent bromine in $C_{16}H_{26}O_2Br_6$
 Calculated: 65.7%
 Found : 65.3%

The other fraction was insoluble in ether and only slightly soluble in benzene. It melted at 215-218°C:

Analysis:

Per cent bromine in $C_{16}H_{26}O_2Br_6$
 Calculated: 65.7%
 Found : 66.1%

C. Ozonolysis of methyl hexadecatrienoate.- A combination of methods suggested by Toyama and Tsuchiya (19), Farmer and Van den Heuvel (4), and Spadola and Riemenschneider (16) was used to ozonize 2 g. of the ester (saponification equivalent 265, iodine number 280) in 20 ml. of chloroform at -5°C .

Ozonized oxygen (2.07 per cent ozone) was passed through the solution until it was saturated with ozone. The gaseous mixture flowed in at the rate of 350 ml. per minute; three hours were required for the operation.

The solvent was removed under reduced pressure and 2.5 g. of light yellow viscous material was left. The calculated yield for normal ozonide, $\text{C}_{17}\text{H}_{28}\text{O}_{11}$ is 3.09 g. About 150 ml. of water was added to the ozonide and the mixture was heated on a hot-water bath for 30 minutes at 90°C , while a gentle current of hydrogen was passed through the flask in order to sweep out any volatile decomposition products. The decomposition flask was attached by a delivery tube to three other flasks connected in succession. Flask number one contained 50 ml. of water and was cooled with ice. Each of the next two flasks contained 100 ml. of one-third normal barium hydroxide to catch any carbon dioxide given off by decomposition.

A water layer and a viscous yellow residue remained in the flask after thirty minutes on the hot-water bath.

The first wash bottle gave a positive test with Schiff's reagent and had an odor of aldehyde. When the liquid was added to a luke-warm alcoholic solution of 0.5 g. of 2,4-dinitrophenylhydrazine containing a small amount of hydrochloric acid,

an orange colored precipitate settled. This was recrystallized from alcohol several times to yield 0.21 g. of material melting at 92-93°C:

Analysis:

C, 48.43%; H, 5.13 %

The 2,4-dinitrophenylhydrazone of valeraldehyde melts at 98°C and contains 49.61 per cent carbon and 5.3 per cent hydrogen.

The barium hydroxide solution was found to contain 0.31 g. of barium carbonate, which is equivalent to 0.00157 g. mol. of carbon dioxide. This was obtained from 0.00758 g. mol. of methyl ester.

The aqueous layer in the decomposition flask was extracted with two liters of ether (five 400 ml. portions). Anhydrous sodium sulfate was used to dry the ethereal solution and the ether was removed at a reduced pressure.

The trace of liquid residue remaining was refluxed over night with a dilute solution of hydrogen peroxide according to the method suggested by Farmer and Van den Heuvel (4). The solution after being allowed to cool was extracted with a large volume of ether. When the ether was removed, a trace of acidic liquid residue remained. It was not studied further since there was such a small amount present. No solid material was isolated.

The residue from the decomposition flask was oxidized by refluxing over night with a dilute solution of hydrogen peroxide. Then 25 ml. of 35 per cent potassium hydroxide

solution was added and refluxing was continued for another hour to saponify any esters. The mixture was acidified with hydrochloric acid and extracted with four 250 ml. portions of ether. The solution was dried and the ether was removed. The oily semi-crystalline residue was extracted with petroleum ether to give an insoluble solid. This material was recrystallized several times from ethyl acetate to yield 0.22 g. of white crystals which had a melting point of 149°C which was not lowered when mixed with authentic adipic acid. The melting point of adipic acid is listed in the literature as 151-153°C. The material had a saponification equivalent of 71.6 as compared with a theoretical value of 73.1 for adipic acid. No other acid was isolated.

A 1.3 g. sample of the methyl ester (saponification equivalent 266 and iodine number 236) was ozonized to give 2.3 g. of ozonide.

On decomposition, the volatile aldehyde again gave a small amount of 2,4-dinitrophenylhydrazone melting at 94°C. The second absorption flask was found to contain 0.52 g. of barium carbonate. The only dibasic acid that could be isolated was 0.13 g. of material melting at 148°C. This melting point was not lowered when the sample was mixed with the material isolated in the previous run.

Investigation of The Fraction With a Saponification
Equivalent Near That of The Methyl Ester of a Four-
teen Carbon Atom Acid

A. Isolation of the fraction.- A methyl ester fraction (450 g., Table I, page 12) with a saponification equivalent

of 244 and iodine number 10.3 was dissolved in 4.5 liters of acetone, cooled with excess dry ice and filtered as usual with a filter stick. One and one-half liters of filtrate gave a fraction number one:

Yield	20.2 g.
Iodine number	81.1

A second extraction of the residue with a liter of acetone in the presence of excess dry ice gave a fraction number two:

Yield	11 g.
Iodine number	80.7

A third and similar extraction gave a fraction number three:

Yield	4.5 g.
Iodine number	81.0

The residue (394 g.) was recrystallized from light petroleum ether (13.5 ml. petroleum ether per gram of ester) and excess dry ice. A fraction number four was obtained from the filtrate:

Yield	7.7 g.
Iodine number	48.8

The residue (385 g.), fraction number five, had an iodine number of 1.45.

The first three fractions (20.2 g. with an iodine number of 81.1, 11 g. with an iodine number of 80.7, and 4.5 g. and iodine number of 81.0) were combined and recrystallized from acetone in the usual manner, and the filtrate gave fraction six:

Yield	23 g.
Saponification equivalent	250
Iodine number	104

A fraction number seven was obtained from the residue:

Yield	12 g.
Iodine number	44.5

Theoretical values for a 14 carbon atom acid ester with one double bond are:

Saponification equivalent	240
Iodine number	106

A mixture of 6 g. of fraction four (iodine number 48.8) and 10 g. of fraction number seven (iodine number 44.5) was recrystallized from petroleum ether and from the filtrate a fraction number eight was obtained:

Yield	9.2
Saponification equivalent	245
Iodine number	60.2

Fraction number six (23 g.) with a saponification equivalent of 250 and an iodine number of 104 was fractionated at a pressure of 0.5-1 mm. through a 12-inch electrically heated column packed with helices.

Table II

Fractionation of Methyl Esters					
Fraction	Boiling range (°C)	Yield (g.)	Saponification equivalent	Iodine number	Comment
1	102-105	5.5	243	102	Dark
2	105-106	3.2	244	95.7	Dark
3	106-136	8.5	253		

The dark residue and column hold-up were not considered.

B. Hydroxylation of the distilled Fraction 1.- Hydroxylation of this fraction gave a small amount of product which was evidently dihydroxypalmitic acid.

The sample of 5 g. was saponified in the usual manner with potassium hydroxide in water and alcohol. The soaps were extracted with ether and a small amount of dark unsaponifiable matter was removed (iodine number was not determined). The soap solution was neutralized with dilute hydrochloric acid, and the acids were extracted with ether.

The somewhat viscous material was hydroxylated by the alkaline permanganate method, and after the product was extracted with petroleum ether to remove unreacted material, only a very small amount of white crystalline material remained. This product was recrystallized from alcohol and water to give 0.07 grams of final product which melted at 123-124°C, and had a saponification equivalent of 285. When this product was mixed with the high-melting dihydroxypalmitic acid, the melting point was not lowered.

The hydroxylation was repeated using 4.06 g. of Fraction 3 (saponification equivalent 253) and again a small amount of material which melted at 124°C was isolated. This melting point was not altered by the high-melting dihydroxypalmitic acid.

By concentrating the aqueous alcoholic mother liquors from the above fractionations, it was shown that no appreciable amount of any other hydroxylated product could have been present.

A small amount of substance was obtained from the hydroxylation mother liquor but it could not be crystallized to a constant melting point.

The hydroxylation procedure works well for hexadecenoic and oleic acids, and according to Toyama and Tsuchiya (20), tetradecenoic acid can be hydroxylated by the same method and recrystallized from ethanol in good yields.

C. Bromination studies.- A 3 g. sample of Fraction 2 (saponification equivalent 244 and iodine number 95.7) was brominated in petroleum ether at 0°C. A very small amount of dark insoluble material was formed. The product was extracted with a mixture of ether and petroleum ether, and decolorized with activated charcoal in benzene. Petroleum ether was added to the benzene solution and a small amount of material crystallized when the mixture was allowed to cool. This material was recrystallized to yield about 0.02 g. of tan powder which melted at 158-159°C:

Analysis:

Per cent bromine in $C_{17}H_{28}O_2Br_6$
 Calculated: 64.46%
 Found : 63.7%

The material was evidently a mixture of hexabromopalmitic acid methyl esters; this indicated that a small amount of hexadecatrienoic acid methyl ester was still present in the fraction.

During the course of this work, the low-boiling fractions from a number of ester distillations were redistilled,

and that portion which was soluble in petroleum ether (13.5 ml. of petroleum ether per gram of ester) in the presence of excess dry ice was brominated.

The small amount of ether-insoluble solid was removed by filtration, and a very dark viscous liquid residue was obtained from the filtrate when the ether was removed at a reduced pressure.

A dark material was distilled at a pressure of 1 mm., and a large portion of it, chiefly methyl myristate, came over below 110°C. No higher-boiling material could be distilled. The flask residue was a dark, resinous material which was insoluble in ether.

According to Riemenschneider and Ellis (14), methyl dibromomyristate distills easily at 140-145°C and 0.5 mm. pressure.

If tetradecenoic acid is present, it exists in negligible quantities.

Investigation of The Low-boiling Methyl Ester Fraction

A 25 g. sample of the dark fraction (Table I, page 12, boiling range 80-110°C, pressure 1 mm.) was saponified with potassium hydroxide. The soaps were extracted five times with ether and acidified with hydrochloric acid. The free acids were extracted with ether, dried, and recovered. The residue of 16.3 g. had a saponification equivalent of 194 and an iodine number of 4.3.

Since this fraction represents the methyl esters distilling below methyl myristate, it is evident that unsaturated acids with less than fourteen carbons are absent, or present only in negligible quantities.

DISCUSSION

It has been shown by earlier investigators (2, 17) that the 16 carbon unsaturated acids of commercial menhaden oil represent 15.5-23.4 per cent of the total fatty acids.

On the basis of data obtained in the isolation of hexadecenoic acid during the course of this work, it was found that hexadecenoic acid represents 7.72 per cent and the hexadecatrienoic acids 1.31 per cent of the total fatty acids.

These low figures may be attributed to several factors. First, the fatty acid composition of marine oils is known to vary slightly with the time and location of the catch. Second, in the distillation of the methyl esters, it was of interest to separate the 16 carbon acid fraction as cleanly as possible from the lower and higher fractions. The intermediate fractions were not included in the figures reported. In earlier work where the amount of acid was of major importance, the composition of the intermediates was calculated from mean molecular weights. Third, after distillation, the esters were concentrated into one fraction with an iodine number of 82 and another with iodine number of 167 by low-temperature crystallization. The amount of each acid was calculated on the assumptions that the first fraction was composed of palmitic and hexadecenoic acids, and that the second fraction was composed of hexadecenoic and hexadecatrienoic acids. In this manner the small amount of saturated acid that passed the lead soap separation was eliminated. Without a knowledge of the presence of the higher unsaturated acids, the iodine number of the mixed

acids would not necessarily show the presence of saturated acid.

The hexadecenoic acid was isolated and hydroxylated with hydrogen peroxide to give a dihydroxy derivative melting at 85°C, and with alkaline permanganate to give a dihydroxy derivative which melted at 125°C. Periodate oxidation of these compounds gave semi-carbazones indicating that the following fragments were products of each oxidation:

1. $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{O}$
2. $=\text{CH}(\text{CH}_2)_7\text{COOH}$

It appears that the acid was Δ 9,10-hexadecenoic acid (palmi-toleic acid), and that the two dihydroxypalmitic acids were geometric isomers.

Bromination of the methyl esters of the highly unsaturated acid fraction yielded two different solid hexabromo derivatives melting at 169-171°C and 190-191°C.

These compounds were difficult to separate and it is likely that neither was completely free from the other. The bromo-compounds were debrominated to give methyl esters comparing very favorably with methyl hexadecatrienoate.

Ozonolysis of these esters was unsatisfactory, so it was not determined whether the hexabromo derivatives were geometric isomers representing only one hexadecatrienoic acid, or if the bromines were actually in different positions representing two or more hexadecatrienoic acids.

A derivative which compared favorably with the 2,4-dinitrophenylhydrazone of valeraldehyde was obtained when the

contents of the first decomposition absorption flask was treated with 2,4-dinitrophenylhydrazine. The melting point as well as the carbon and hydrogen content was low, and the yield (0.21 g.) was far from quantitative; but it seems probable that the material swept into the first flask was valeraldehyde and possibly some lower aldehyde. Since valeraldehyde boils at 103°C, the method used to isolate it was not the best, but it is feasible that a small amount would be swept over by hydrogen at 90°C.

Adipic acid (0.22 g.) was isolated from the water insoluble fraction left in the ozonide decomposition flask. Here again the yield was far below theoretical, but it indicates the presence of an acid containing the group,



The material was not isolated before saponification, so it can not be said whether or not this was the terminal group.

Toyama and Tsuchiya (19) showed the presence of this group in the hexadecatrienoic acid from sardine oil and proved it to be the terminal one, $=CH-CH_2CH_2CH_2CH_2COOH$. They isolated only methyl adipate from the insoluble fraction left after decomposition.

No dibasic acid was isolated from the water soluble fraction left in the decomposition flask. A small amount of acidic liquid remained which could have been derived from some mono-aldehyde left in the solution or from the decomposition of malonic acid if the group, $=CHCH_2CH=$, was present in any of the acids.

It is known (12) that the presence of the group, $=CHCH_2CH=$, gives rise to acetic acid and carbon dioxide on ozonolysis and subsequent decomposition. The amount of carbon dioxide given off in the decomposition of the first ozonide was equivalent to 21.0 per cent the theoretical amount for one $=CHCH_2CH=$ group, and the amount given off in the decomposition of the second ozonide was slightly greater. These yields are not quantitative, but they are large enough to indicate the possible presence of an acid containing a $=CHCH_2CH=$ group. This is especially true since the ozonolysis and decomposition was not complete. Only a trace of carbon dioxide is given off when the group, $=CHCH_2CH=$, is absent (12, 19).

Since the results of this part of the work were indefinite and far from the theoretical, it can not be said whether one hexadecatrienoic acid or a mixture of acids was present.

If it is assumed that only one acid was present, there is a faint indication that it might contain the following groups:

1. $CH_3CH_2CH_2CH_2CH=$
2. $=CHCH_2CH_2CH_2CH_2CH=$
3. $=CHCH_2CH=$
4. $=CHCH=$

The $=CH-CH=$ group would be necessary to make sixteen carbons.

No -COOH group is indicated since it can not be said which of the last three groups occurs on the end of the molecule.

It is regretted that a large sample of material was not available so that the work could have been repeated in the light of the above information.

Low-temperature crystallization of the methyl esters of the 14 carbon acids resulted in the isolation of an ester, with a saponification equivalent and iodine number that corresponded favorably with methyl tetradecenoate. Hydroxylation and bromination of this fraction showed the presence of myristic acid and traces of hexadecenoic and hexadecatrienoic acids, but failed to show the presence of a tetradecenoic acid.

It is evident that low-temperature crystallization as employed in this work would not be sufficient to isolate small amounts of methyl tetradecenoate. It might act to concentrate unsaponifiable decomposition products from higher unsaturated acids or traces of unsaponifiable matter that escaped extraction from the original large batch of soaps. These compounds might possess unsaturation and tend to give high iodine values.

The concentrated esters should be saponified, the unsaponifiable matter removed, and the lead soap method applied to further purify the fraction.

Such a procedure would have been advisable in this work. There would have been no doubt about the presence of unsaponifiable matter, and most likely the myristic acid, and

unsaturated 16 carbon acids could have been separated more completely. This might have shown the absence of tetradecenoic acid without further work.

BIBLIOGRAPHY

1. Baldwin, E. H., "Chemical Studies on Menhaden Fish Oil", Unpublished Ph. D. Thesis, University of Maryland, College Park, Maryland, 1942.
2. Baldwin, E. H., and Lauham, W. B., Jr., Ind. Eng. Chem., Anal. Ed., 13, 615 (1941)
3. Chargoff, E., Z. Physiol. Chem., 199, 221 (1931)
4. Farmer, E. H., and Van den Heuvel, F. A., J. Chem. Soc., 427 (1938)
5. Green, T. G., and Hilditch, T. P., J. Chem. Soc., 764 (1937)
6. Halverson, J. O., Smith, F. H., Sherwood, F. W., and Darstynne, R. S., North Carolina Agr. Expt. Sta. Tech. Bull., 57, 31 (1936)
7. Hilditch, T. P., "The Chemical Composition of Natural Fats", p. 367, New York, John Wiley and Sons, 1940.
8. Ibid., p. 371.
9. Hoffmann, D. H., and Green, C. E., Oil and Soap, Dec. 1939.
10. King, C., J. Chem. Soc., 1826 (1938)
11. Lepworth, A., and Mottram, E. M., J. Chem. Soc., 127, 1628 (1925)
12. Long, Louis, Jr., Chem. Rev., 27, 437 (1940)
13. Olcott, H. S., and Mattill, H. A., J. Am. Chem. Soc., 58, 1628 (1936)
14. Riemenschneider, E. W., and Ellis, H. R., J. Biol. Chem., 113, 219 (1936)
15. Scanlan, J. T., and Swern, D., J. Am. Chem. Soc., 62, 2306 (1940)
16. Spadola, J. H., and Riemenschneider, E. W., J. Biol. Chem., 121, 787 (1937)

BIBLIOGRAPHY

17. Stingley, D. V., Ind. Eng. Chem., 32, 1214 (1940)
18. Toyama, Y., and Tsuchiya, T., Bull. Chem. Soc. Japan,
4, 83 (1929)
19. Ibid., 10, 192 (1935)
20. Ibid., 10, 563 (1935)

APPENDIX I

Determination of Saponification Equivalents

A modified method of Chargoff (3) was used for these determinations. A 50 mg. to 100 mg. sample of acid or ester was refluxed with 5 ml. of 0.1 N sodium propoxide under an air-cooled condenser for 30 minutes. The ground glass joint was washed with a few ml. of distilled water and the solution was titrated to a phenolphthalein endpoint with 0.05 N sulfuric acid. A blank determination was made under similar conditions, and the mean molecular weight or saponification equivalent was calculated.

$$\text{Sap. equiv.}^{\circ} = \frac{\text{Wt. of sample (mg.)}}{(\text{ml. of Blank} - \text{ml. of Back tit.}) \times N \text{ of acid.}}$$

APPENDIX II

Determination of the Iodine Number

A modification of the Hoffman and Green method (9) was employed in these determinations.

About 100 mg. of sample, or enough to use 50 to 60 per cent of the blank titration, was weighed in a small vial and transferred to a glass stoppered flask of 200 ml. capacity. The sample was completely dissolved in 20 ml. of chloroform, then 25 ml. of approximately 0.2 N iodide monochloride solution and 3 ml. of 2.5 per cent mercuric acetate in glacial acetic acid was added with shaking.

After three minutes at room temperature, 20 ml. of 15 per cent potassium iodide and 50 ml. of distilled water was added. The excess iodine was titrated with 0.1 normal sodium thiosulfate to an end-point with starch. The calculation was made in the usual manner.