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The Body Oil From Menhaden (*Brevoortia tyrannus*)¹

A. Low Temperature Crystallization of Glycerides B. Some of the 18 and 20 Carbon Atom Acids

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Introduction

The body oil of menhaden (*Brevoortia tyrannus*) has long been available as an industrial product. Stingley (15) has published information on the commercial availability of fatty acid concentrates from fish oils including menhaden.

The study of menhaden fish oil was undertaken with the belief that fundamental knowledge of the chemical composition would make it a more valuable intermediate in the manufacture of chemical products.

Embrece (6) has reported that molecular distillation of menhaden oil yields glycerides of widely separated iodine numbers. Riemenschneider, Swift, and Sando (13) have shown that cottonseed oil glycerides can be separated by low temperature crystallization from acetone. Menhaden oil was separated into three fractions in this study: 12.4 percent with an iodine number of 92.9, 75.0 percent with an iodine number of 179.0, and 12.6 percent with an iodine number of 264.2. The last fraction remained in solution at the temperature of dry ice. The attempted use of less acetone at higher temperatures failed to give a soluble fraction with an iodine number above 198.4.

The precipitate obtained from winterizing crude menhaden oil was crystallized several times from acetone to give a crystalline solid. Methanolysis and fractional distillation indicated 19.6 percent methyl myristate, 62.0 percent methyl palmitate, 17.6 percent methyl stearate and 0.8 percent of an ester of higher molecular weight.

The unsaturated acids with eighteen carbon atoms obtained from menhaden oil were found to give dihydroxystearic acids from which 9, 10-dihydroxystearic acid was isolated. Evidence was found also for an 11, 12-dihydroxystearic acid. Bromination of concentrates with high iodine numbers gave octabromostearic acids. The eighteen carbon fraction contains chiefly Δ 9, 10-octadecenoic acid with some Δ 11, 12-octadecenoic and octadecatetrenoic acid.

The twenty carbon unsaturated acids produced 11, 12-dihydroxy eicosanoic acid and decabromoicosanoic acid. This fraction is composed mainly of Δ 11, 12-eicosenoic and eicosapentenoic acids.

Experimental

Saponification equivalents were determined by a modification of the method of Cbargoff (5). A modification of the mercuric acetate catalyzed Wij's

method described by Hoffman and Green (9) was used for the determination of the iodine number.

A. LOW TEMPERATURE CRYSTALLIZATION OF GLYCERIDES

Saturated Glycerides. Crude menhaden oil after standing at 15° C. for several days was filtered by suction to remove as much of the adhering oil from the precipitate as possible. A sample of crude oil weighing 809 g. deposited 81 g. of paste with an iodine number of 139. A commercial sample of similar material (supplied by Menhaden Fish Products Company of Baltimore, Md.) that had been filter pressed at 5° C. had an iodine number of 136.

The paste was crystallized several times from acetone in the usual manner and the white crystals, having an iodine number of 5.9 and saponification equivalent of 268, melted at 53-54° C.

Alcoholysis of 120 g. of glycerides gave methyl esters that were used for fractional distillation. The fractions obtained and their properties are listed in Table 1. Assuming that each fraction contained two esters, it has been calculated that the composition was 19.6 percent methyl myristate, 62.0 percent methyl palmitate, 17.6 percent methyl stearate and 0.8 percent methyl arachidate. Pure myristic, palmitic and stearic acids were obtained from fractions 1, 7, and 11 respectively.

TABLE 1
 Fractionation of the Methyl Esters From the Saturated Glycerides

Fraction	Weight gms.	B. pt. at 8 mm.	Saponification Equivalent
		°C.	
1	10.29	143-155	242
2	6.40	145-150	253
3	5.54	150-155	254
4	4.78	155-157	255
5	3.08	157	255
6	14.48	157-162	272
7	35.76	162-165	273
8	12.79	165	274
9	2.81	165-170	280
10	3.96	170-179	289
11	5.61	179-183	300
12	3.64	183-184	298
13	1.76	Holdup	313
14	5.32	Residue	Dark Red

Crystallization of Destearinated Menhaden Oil. Eight hundred g. of destearinated oil obtained from Fish Products Company, of Lewes, Delaware, was dissolved in the required volume of acetone (see Table 2). After 48 hours at -15° C., the mixture was filtered by suction through a previously cooled funnel and paper. The two solutions were allowed to reach room temperature, dried over anhydrous calcium chloride and the acetone was distilled in an

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inert atmosphere. The last traces were removed at the water pump.

In one instance, when 10 ml. of acetone was used per g. of oil, the filtrate was cooled to -60°C . with solid carbon dioxide. The mixture was held at this temperature for one hour, then the supernatant liquors were removed with a filter stick.

TABLE 2
Analysis of Fraction Obtained by Crystallization of Menhaden Oil

Fraction	Temperature	Acetone per g. oil	Yield %	Iodine number	Saponification equivalent
Original oil.....	$^{\circ}\text{C}$.	ml.	181.3	800
A. Precipitate.....	-15	2.5	23.6	135.3	277
Filtrate.....			76.4	148.4	308
B. Precipitate.....	-15	5.0	16.0	107.7	295
Filtrate.....			84.0	195.3	301
C. Precipitate.....	-15	10.0		92.9	290
Filtrate recooled:			12.4		
Precipitate.....	-60	75.0	179.0	298
Filtrate.....	-60	12.6	264.2	305
Saturated glycerides.....	2.0 ¹	5.9	268

¹ From crude menhaden oil.

B. SOME OF THE 18 AND 20 CARBON ACIDS (W.H.B.)

Separation of the Unsaturated Acids With 18 Carbon Atoms. Distillation of the methyl esters obtained from 2 kg. of unsaturated acids (lead soap soluble in ethanol) yielded 220 g. of material boiling at $160-165^{\circ}\text{C}$. at 2 mm. The saponification equivalent was 294. Redistillation of some of the intermediate fractions gave a further 206 g. yield having a saponification equivalent of 293 and an iodine number of 123. Methyl oleate requires a saponification equivalent of 296 and an iodine number of 86.0; methyl linoleate requires a saponification equivalent of 294 and an iodine number of 173.

Octadecenoic Acids. A 200 g. sample of methyl esters (sap. equiv. 293 and I.V. 123) was dissolved in 2 liters of acetone. The mixture was cooled by adding solid carbon dioxide until an excess was present. A filter stick was used with vacuum to remove 1 l. (iter), of filtrate that contained 36.0 g. of esters having an iodine number of 181.

Acetone (500 ml.) was added to the residue that was cooled and filtered as before. From this filtrate there was obtained 31.0 g. of esters having an iodine number of 166.

The process was repeated by adding 500 ml. of acetone to the residue and by cooling and filtering as before. The filtrate yielded 19.4 g. of esters having an iodine number of 153. From the precipitate was obtained 112.4 g. having an iodine number of 89.5. Methyl oleate requires an iodine number of 86.0.

The acid was liberated by saponification from the ester of iodine number 89.5 and used for the preparation of p-phenylphenacyl ester by the method of Drake and Bronitsky (7). The compound isolated melted at $58-59^{\circ}\text{C}$.; authentic p-phenylphenacyl oleate melted at 61°C . A mixture of the two substances melted at $60-61^{\circ}\text{C}$.

A 50 g. sample of esters (iodine value 89.5) was saponified and the acids were hydroxylated with alkaline permanganate by the method of Lapworth and Mottram (12). After extraction with petroleum ether, the product was fractionated from 95 percent

ethanol. The less soluble fraction consisted of 32 g. of crystals melting at 128.5°C . An authentic specimen of 9, 10-dihydroxystearic acid melted at 131°C . A mixture of the two melted at 130.5°C .

Analysis—Calculated for $\text{C}_{18}\text{H}_{36}\text{O}_4$: Sap. equiv., 316.

Found: Sap. equiv., 316.

The more soluble fraction weighing 2.8 g. melted at $115-116^{\circ}\text{C}$.

Analysis—Calculated for $\text{C}_{18}\text{H}_{36}\text{O}_4$: Sap. equiv., 316.

Found: Sap. equiv., 316.

Periodate Cleavage of the Higher Melting Substance. Using a combination of methods suggested by King (11) and Hsing and Chang (10) the acid (m.p. 128.5) was split and the fragments identified as semicarbazones. Potassium periodate (1 g.) in 30 ml. of normal sulfuric acid at 20°C . was added to a solution of 1 g. of the dihydroxystearic acid in 65 ml. of 95 percent ethanol at 40°C . After standing 15 minutes, the mixture was cooled to 15°C ., diluted with water to dissolve precipitated salts and extracted with ether. The ether was removed under reduced pressure and 0.7 g. semi-carbazide hydrochloride and 1.5 g. sodium acetate in aqueous alcohol were added. The precipitated semi-carbazones were filtered and extracted with 1 percent sodium hydroxide.

The alkaline filtrate was acidified with 2 percent hydrochloric acid and the precipitate (0.45 g.) when crystallized from aqueous alcohol melted at 160°C . Scanlon and Swern (14) reported a melting point of $161-2^{\circ}\text{C}$. for azelaic acid half-aldehyde semicarbazone.

Analysis—Calculated for $\text{C}_{10}\text{H}_{18}\text{O}_3\text{N}_3$: C, 52.4%; H, 8.3%.

Found: C, 52.8%; H, 8.2%.

The alkali insoluble portion weighing 0.25 g. was crystallized from aqueous alcohol. The melting point and mixed melting point with authentic nonanal semicarbazone was 99°C .

Analysis—Calculated for $\text{C}_{10}\text{H}_{21}\text{ON}_3$: C, 60.3%; H, 10.6%.

Found: C, 60.3%; H, 10.9%.

Periodate Cleavage of the Lower Melting Substance. One g. of acid (m.p. $115-116^{\circ}\text{C}$.) was oxidized with potassium periodate in the same manner as described above. The semicarbazones were prepared and separated on the basis of their solubility in alkali.

The alkali soluble portion, after acidification, was fractionated from aqueous alcohol. A portion weighing 0.15 g. melted at 155°C . Mixed m.p. with the semicarbazone of azelaic acid half-aldehyde was 145°C .

Analysis—Calculated for $\text{C}_{12}\text{H}_{23}\text{O}_3\text{N}_3$: C, 56.0%; H, 8.95%.

Found: C, 55.6%; H, 9.17%.

The mother liquors yielded 0.15 g. of material, m.p. 149°C .

Analysis—Calculated for $\text{C}_{10}\text{H}_{19}\text{O}_3\text{N}_3$: C, 52.4%; H, 8.3%.

Found: C, 53.9%; H, 8.4%.

The alkali insoluble portion on fractionation from aqueous ethanol yielded 0.10 g. m.p. and mixed m.p. with authentic nonanal semicarbazone, 99° C.

Analysis—Calculated for $C_{10}H_{21}ON_3$: C, 60.3%; H, 10.6%.

Found: C, 59.9%; H, 10.4%.

From the mother liquors were obtained 0.05 g., m.p. 83° C.

Analysis—Calculated for $C_9H_{19}ON_3$: C, 58.4%; H, 10.3%.

Found: C, 58.6%; H, 10.1%.

The m.p. recorded for octanal semicarbazone is 98° C.

Octadecatetrenoic Acid. The esters (iodine number 181) obtained in the first filtrate from the acetone crystallization of the 18 carbon acid esters were saponified and the liberated acids brominated. The esters (35.0 g.) were saponified by means of a hot solution containing 9 g. of potassium hydroxide in 7 ml. of water and 30 ml. of alcohol. After complete saponification the mixture was acidified, cooled, and the acids extracted with ether. The ether solution was washed three times with distilled water, the volume was brought to 500 ml. by the addition of ether and dried over anhydrous sodium sulfate. This solution was passed through a column (about 75 x 25 mm.) of activated alumina (Grade A, mesh 80 to 200 from the Aluminum Ore Company of America) to remove colored material.

The ether solution that had passed through the alumina was cooled to -5° C. in a 3-necked flask and surrounded by chopped ice. Bromine was added dropwise with mechanical stirring. Stirring was continued for an hour after an excess of bromine was indicated by the color of the solution. Amylene added to the mixture reacted with the excess bromine. The precipitate was filtered and carefully washed 4 times with ether. No solvent was found suitable for the recrystallization of this material. The yield was 1.0 g., m.p. 230° C.

Analysis—Calculated for $C_{18}H_{35}O_2Br_3$: Br, 69.8%.
Found: Br, 69.6%.

A second crop of 1.0 g. was obtained from the ethereal mother liquors by evaporating to a small volume and adding petroleum ether.

Separation of the Unsaturated Acids with 20 Carbon Atoms. The same distillation which provided the 18 carbon fraction yielded 290 g. of esters boiling 175-180° C. at 2 mm. having saponification equivalent of 317 and an iodine value of 228. The methyl ester of a 20 carbon acid with 3 double bonds requires a saponification equivalent of 320 and an iodine value of 235.

Eicosenoic Acid. A 75 g. sample of the mixed esters of 20 carbon acids dissolved in 750 ml. of acetone was cooled by adding solid carbon dioxide in excess and was allowed to stand for 1 hour. The dried precipitate (liquid at room temperature) weighed 28.9 g. and had an iodine number of 136.7. The acids liberated by saponification of 26.5 g. of esters, were converted to their lithium salts and crystallized twice from 80 percent aqueous ethanol. The liberated acid

(4.5 g.) had an iodine number of 87.4 (theoretical for eicosenoic acid is 81.9).

A sample (4.3 g.) of the eicosenoic acid was converted to dihydroxyeicosanoic acid by alkaline permanganate according to the method of Lapworth and Mottram (12). The crude product after extraction with petroleum ether (3.9 g.) was crystallized from 95 percent ethanol m.p. 115.5-116° C.

Analysis—Calculated for $C_{20}H_{40}O_4$: Sap. equiv., 344.

Found: Sap. equiv., 341.

Periodate Cleavage of the Dihydroxyeicosanoic Acid. A 0.5 g. sample of the dihydroxyeicosanoic acid was oxidized with potassium periodate using half the quantities used before. The semicarbazones were prepared and separated with alkali.

The alkaline extract, after acidification and washing, was crystallized 3 times from aqueous ethanol. The crystals (75 mg.) melted at 156° C. and showed no depression of the melting point when mixed with the similar semicarbazone from the lower melting dihydroxystearic acid.

Analysis—Calculated for $C_{12}H_{23}O_3N_3$: C, 56.0%; H, 8.95%.

Found: C, 55.7%; H, 8.91%.

The alkali insoluble semicarbazone was washed with water and crystallized from aqueous ethanol. The crystals weighed 0.12 g., m.p. and mixed m.p. with authentic nonanal semicarbazone 99° C.

Analysis—Calculated for $C_{10}H_{21}ON_3$: C, 60.3%; H, 10.6%.

Found: C, 59.7%; H, 10.5%.

Eicosapentenoic Acid. The 20 carbon acids from 13.4 g. of mixed esters were brominated in ether. The ether insoluble portion was extracted with 30 ml. quantities of hot solvent, first with toluene, then four times with benzene. The resulting tan powder, m.p. 258-265° C., after darkening at 230° C., weighed 1.42 g.

Analysis—Calculated for $C_{20}H_{30}O_2Br_{10}$: Br, 72.6%.
Found: Br, 71.0%.

The mother liquors after concentration and addition of petroleum ether yielded crystals which were crystallized from ether-petroleum ether mixtures. This substance weighed 0.43 g., m.p. 150-155° C.

Analysis—Calculated for $C_{20}H_{30}O_2Br_{10}$: Br, 72.6%.
Found: Br, 71.0%.

Discussion

The saturated glycerides recrystallized from acetone yielded myristic acid, palmitic acid, stearic acid, and one with higher molecular weight. While palmitic acid was present in the greatest quantity, the proportion was not large enough to indicate the existence of tripalmitin.

Cooling menhaden oil in acetone (10 ml. of acetone per g. of oil) to -15° C. precipitated 12.4 percent of the oil having an iodine number of 92.9. The filtrate from the above when further cooled to -60° C. with carbon dioxide ice precipitated 75.0 percent of the glycerides with an iodine number of 179.0. The fil-

trate still contained 12.6 percent of the glycerides and these had an iodine number of 264.2. The iodine numbers of these fractions suggest possible uses to which they might be put.

An attempt was made, by reducing the volume of acetone used and by using a higher temperature, to concentrate the highly unsaturated glycerides in the filtrate. At -15°C ., using 2.5 ml. of acetone per g. of oil, the filtrate contained over 76 percent of the oil and this had an iodine number of 198.

The separation of unsaturated fatty acids in pure form is a long process. The dihydroxy derivatives prepared from mono-olefinic acids are crystalline and after purification can be split either by lead tetraacetate or periodic acid into aldehydes that locate the original position of the double bond. By this procedure, it has been demonstrated that the 18 carbon acids that represent 25 to 31 percent (2) of the total fatty acids are mostly Δ 9, 10-octadecenoic acid.

Fractional crystallization of the mixed dihydroxy derivatives produced a mixture melting at $115.5-116^{\circ}\text{C}$. Periodate oxidation of this mixture gave semicarbazones indicating the following fragments:

1. $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{}$
2. A mixture similar to 1 but with fewer carbons
3. $=\text{CH}(\text{CH}_2)_9\text{COOH}$
4. A mixture similar to 3 but with fewer carbons.

It appears likely that the mixture contains both Δ 9, 10, and Δ 11, 12-octadecenoic acids. The Δ 11, 12-octadecenoic acid (vaccenic acid) has been reported by Bertram in beef and butter fats and by Armstrong and Hilditch (1) in whale oil.

Bromination of the 18 carbon acids has yielded solid derivatives with 8 Br only. A similar octadecatrenoic acid has been isolated from Japanese sardine oil. Toyama and Tsuchiya (16) obtained an octabromostearic acid that melted at 220°C . Brown and Beal (17) also reported the isolation of the ester of octadecatrenoic acid from menhaden oil.

The methyl esters of the 18 carbon acids that were obtained by fractional distillation had an iodine number of 123. It can be calculated from theoretical iodine numbers that this fraction is composed of 86 percent octadecenoic and 14 percent octadecatrenoic acids.

The dihydroxyeicosanoic acid melted at $115.5-116^{\circ}\text{C}$. but was probably not homogeneous. Green, Hilditch and Stainsby (8) obtained an 11, 12-dihydroxyeicosanoic acid from a seed wax that melted at 130.5°C . The product obtained here was shown to be mainly 11, 12-dihydroxyeicosanoic acid by periodate cleavage to the following two fragments:

1. $\text{CH}_3(\text{CH}_2)_9\text{CH}=\text{}$
2. $=\text{CH}(\text{CH}_2)_9\text{COOH}$.

The isolation of decabromoeicosanoic acids as the only solid bromo derivatives indicates the presence of

at least one 20 carbon acid with 5 double bonds. It does not rule out the possibility of other acids in small amounts. Toyama and Tsuchiya (16) have reported a decabromoeicosanoic acid from sardine oil melting around 240°C .

The mixed 20 carbon acid esters obtained from the distillation had an iodine number of 228. Calculations based on theoretical iodine numbers show that this fraction contains 54 percent eicosenoic and 46 percent eicosapentenoic acids.

Summary

A saturated glyceride from crude menhaden oil contained myristic, palmitic, stearic, and a small quantity of higher molecular weight acids.

The glycerides of destearinated menhaden oil have been fractionated by crystallization from acetone at low temperatures.

Distillation of methyl esters has given in the distillate Δ 9, 10 and Δ 11, 12-octadecenoic acids, an octadecatrenoic acid, Δ 11, 12-eicosenoic acid, and an eicosapentenoic acid.

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