ABSTRACT

Willis H. Baldwin, Doctor of Philosophy, 1942.
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Menhaden fish oil has been shown to be composed of glycerides that are separable by crystallization from acetone at low temperatures. Saturated glycerides amounting to 2 percent of crude menhaden oil have been obtained. The widely spread iodine numbers of the other fractions suggest uses for these fractions. The one with the highest iodine number was used to prepare a paint suitable for use on interiors.

Distillation of the methyl esters of the fatty acids produced among others, acids with 18 and 20 carbon atoms. The monoolefinic fractions when hydroxylated by alkaline permanganate produced dihydroxy acids. The location of the double bonds in the original acids was indicated by cleavage with periodic acid to aldehydes. Evidence obtained in this manner points to the presence of 9,10 and 11,12-octadecenoic acids and 11,12-eicosenoic acid. The more highly unsaturated acids were brominated to yield bromine derivatives of an octadecatetrenoic acid and an eicosapentenoic acid.

The dihydroxystoaric acids prepared from 9,10-octadecenoic acid melt at 94° C. and 131° C. depending upon the method of hydroxylation used. The relationships of the hydroxyl groups in these two acids have been shown by measurement of the reaction velocity with lead tetraacetate

to be trans for the acid m.p. 131° C. and cis for the acid m.p. 94° C. Oleic acid, having the cis configuration, is hydroxylated with alkaline permanganate to the acid (m.p. 131° C.) having the trans configuration. This reaction differs from that between fumaric acid and alkaline permanganate to produce dl-tartaric acid. Fumaric acid has the trans configuration and the hydroxyl groups in dl-tartaric acid are placed in a position in which they are trans to one another.

PART I

CHEMICAL STUDIES ON MENHADEN FISH OIL

PART II

ISOMERISM OF 9,10-DIHYDROXYSTEARIC ACID

By

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Willis H. Baldwin

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PART II ISOMERISM OF 9,10-DIHYDROXYSTEARIC ACID

INTRODUCTION

Menhaden are small fish which rarely grow larger than a foot in length; they occur in numerous quantities along the Atlantic Coast from Nova Scotia to Brazil. The whole fish is used for the commercial production of meal (protein concentrate) and oil. Formerly, when oils of higher vitamin content were more plentiful, menhaden oil was used in the manufacture of soap, paints, and linoleum. With the present large demand for vitamins, menhaden oil is desirable for poultry feeding.

The utilization of whole menhaden oil for its vitamin content results in an uneconomic disposal of the fatty acids and their glycerides. Fractionation of the glycerides of menhaden oil has been accomplished by low temperature crystallization from acetone. The resultant fractions have desirable properties.

The precipitate obtained by cooling 1 g. of oil in 10 ml. of acetone has an iodine number of 92.9 and a saponification equivalent of 290, and thus consists of a mixture close to the theoretical for an 18 carbon acid glyceride with 1 double bond. This fraction should be suitable for hydrogenation and subsequent use as human food.

The final filtrate from the cooling with dry ice had an iodine number of 264 and seems likely to be a suitable drying oil. In a preliminary test, this oil dried as fast as linseed oil and faster than whole menhaden oil. A paint containing this oil did not last long when placed outside in the weather but after six months inside proved to be equally as good as one containing linseed oil.

Saturated glycerides were obtained from the crude oil by cooling to 10° C. and by crystallizing the precipitate from acetone. These crystalline glycerides have an iodine number of 5.9, a saponification equivalent of 268 and were obtained in a yield of 2 percent from the original oil. Fractional distillation of the methyl esters of the acids obtained from these saturated glycerides gave 19.6 percent methyl myristate, 62.0 percent methyl palmitate, 17.6 percent methyl stearate and 0.8 percent methyl arachidate.

The unsaturated fatty acids are liquids and are difficult to separate because of a similarity of properties. The methyl esters were distilled to obtain fractions of equal chain length. The components of these fractions, differing only by the number and location of double bonds, were separated by the differences in solubility exhibited by their solid derivatives obtained by addition of bromine or hydroxyl groups to the double bonds.

It was found possible to concentrate the monoethenoid acids to better than 80 percent purity (iodine value) by crystallization from acetone at low temperature (31) or by crystallization of the lithium salts from acetone. Hydroxylation of the monoethenoid acid concentrate with alkaline permanganate yielded from the 18 carbon acids, 9,10-dihydroxy stearic acid and 11,12-dihydroxy stearic acid. From the 20 carbon acid concentrate, 11,12-dihydroxy eicosanoic acid was prepared. It is thus evident that 9,10- and 11,12-octadecenoic and 11,12-eicosenoic acids were present in the distilled ester fractions.

From the respective highly unsaturated fractions, octabromostearic acid and decabromoeicosanoic acids were obtained. Thus in addition to the monoethenoid acids, there are present an 18 carbon acid with four double bonds and a 20 carbon acid with five double bonds.

In view of the above information it is evident that the fatty acids of menhaden oil vary greatly in structure and chemical properties. The component glycerides and fatty acids may provide a valuable source of raw materials for new chemical products. Since little is known concerning the unsaturated fatty acids, it was desirable to study them with the object of making them more useful.

EXPERIMENTAL

I. Fractionation of Menhaden Oil Glycerides (32)

A. 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, Baltimore, Md. which had been filter pressed at 5° C., had an iodine number of 136.

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

Alcoholysis of 120 g. of glycerides gave the methyl esters that were used for fractional distillation. Examination of the saponification equivalents of the fractions revealed 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 by saponification of the appropriate methyl fractions.

B. Low Temperature Crystallization of Destearinated Menhaden Oil. Eight hundred grams of destearinated menhaden oil supplied by the Fish Products Company, of Lewes, Del. was dissolved in the required volume of acetone (see Table I). After 48 hours at -15° C., the mixture was filtered by suction through a cooled funnel and paper. The two solutions were warmed to room temperature, dried over anhydrous calcium chloride and the acetone was evaporated in an inert atmosphere. The last traces of solvent 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 dry ice. The mixture was held at this temperature for one hour and then filtered using a filter stick. The results of these experiments are contained in Table I.

TABLE I

Analysis of Fractions Obtained by Crystallization of Menhaden Oil

Fraction	Temperature	Acetone per g. oil	Yield	Iodine number	Saponification equivalent
	°C	ml.	%		
Original Oil	. . .	. . .	. . .	181.3	300
A. Precipitate	-15°C	2.5	23.6	135.3	277
Filtrate	. . .	. . .	76.4	198.4	308
B. Precipitate	-15°C	5.0	16.0	107.7	295
Filtrate	. . .	. . .	84.0	195.3	301
C. (Precipitate	-15°C	10.0	12.4	92.9	290
(Precipitate from (Filtrate	-60°C	. . .	75.0	179.0	298
(Residue	-60°C	. . .	12.6	264.2	305
Saturated glycerides	. . .	. . .	2.0 [1]	5.9	268

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[1] from crude menhaden oil.

II. Fractionation of Unsaturated Acid Esters

A. Acids with Eighteen Carbon Atoms. Baldwin and Lanham (2) have reviewed the literature reporting the ester fractionation of menhaden oil fatty acids and have found that 25 to 31 percent of the total fatty acids present have been reported as 18 carbon unsaturated acids. One fractionation of the unsaturated methyl esters gave less than 50 percent of the theoretical yield of esters with saponification equivalent close to methyl oleate. Refractionation of mixtures, however, gave a further amount of these esters.

Distillation of the methyl esters obtained from 2 kg. of unsaturated acids yielded 220 g. of material boiling at 160-165° C. at 2 mm. The saponification equivalent was 294. Methyl oleate requires a saponification equivalent of 292. By distillation of intermediate fractions, a further 206 g. yield was obtained having a saponification equivalent of 293 and an iodine number of 123. The methyl ester of an 18 carbon acid with one double bond has a theoretical iodine number of 86.0 while that with two double bonds requires 173.

B. Acids with Twenty Carbon Atoms. The same distillation which provided the previous fraction yielded 290 g. of esters boiling from 175° to 180° C. at 2 mm. This material had a saponification equivalent of 317 and an iodine value of 228. The methyl ester of a 20 carbon acid with three double bonds has a theoretical saponification equivalent of 319 and a theoretical iodine value of 239.

III. Identification of Acids in the Ester Fractions

A. Octadecenoic Acids. A 200 g. sample of methyl esters (saponification equivalent 293 and iodine number 123) was dissolved in 2 liters of acetone. The mixture was cooled by adding carbon dioxide until an excess was present. A filter stick was used to remove 1 l. of filtrate which contained 36.0 g. of esters with an iodine number of 181.

Acetone (500 cc.) was added to the residue which was cooled and filtered as before. From this filtrate there was obtained 31 g. of esters having an iodine number of 166.

The process was repeated by adding 500 cc. 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 there was obtained 112.4 g. of material with an iodine number of 89.5. Methyl oleate requires an iodine number of 85.7.

The p-phenylphenacyl ester was prepared using the acid obtained from the above methyl ester of iodine number 89.5. The procedure of Drake and Bronitsky (10) was used to prepare a derivative which melted at 58-59° C. The p-phenylphenacyl ester prepared from an authentic specimen of oleic acid melted at 61° C. A mixed melting point using the two specimens was 60-61° C.

A 50 g. sample of esters (iodine value 89.5 was saponified and the acids were used for hydroxylation with alkaline permanganate by the method of Lapworth and Mottram (21; see Appendix 1). The acid after extraction with hot petroleum ether 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 was prepared and this melted at 131° C. A mixed melting point determination made with these samples was 130.5° C.

Analysis--$C_{18}H_{36}O_4$ requires Sap. equiv. 316

Found: Sap. equiv. 316

The more soluble fraction weighing 2.8 g. melted at 115-116° C. This was not changed by repeated crystallization from various solvents.

Analysis--$C_{18}H_{36}O_4$ requires Sap. equiv. 316

Found: Sap. equiv. 316

Periodate Cleavage of the Higher Melting Substance. Using a combination of the method of King (20) and Hsing and Chang (18) the acid (m.p. 128.5) was split and the fragments identified as semi-carbazones. 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 15 minutes standing, 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. semicarbazide 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 had a melting point of 160° C. Scanlon and Swern (25) report a melting point of 161-2° C. for azelaic acid half-aldehyde semi-carbazone.

Analysis--Calculated for $C_{10}H_{19}O_3N_3$: 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 an authentic specimen of nonanal semi-carbazone were 99° C.

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

Found: C, 60.3%; H, 10.9%

Periodate Cleavage of the Lower Melting Dihydroxystearic Acid. One gram of the acid was oxidized with potassium periodate in the same manner as described above. The semi-carbazones were prepared and separated on the basis of their solubility in alkali.

The alkali soluble portion was further fractionated from aqueous alcohol. A portion weighing 0.15 g. was obtained and melted at 155° C. The melting point when mixed with the semi-carbazone of azelaic acid half-aldehyde was 145° C.

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

Found: C, 55.6%; H, 9.17%

From the mother liquors there was obtained 0.15 g. of material melting at 149° C.

Analysis--Calculated for $C_{10}H_{19}O_3N_3$: C, 52.4%; H, 8.3%

Found: C, 53.9%; H, 8.43%

The alkali insoluble portion on crystallization from aqueous alcohol yielded 0.10 g. of a less soluble fraction melting at 99° C. This showed no depression of the melting point when mixed with an authentic specimen of nonanal semi-carbazone.

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

Found: C, 59.9%; H, 10.4%

An additional yield of 0.05 g. of a substance melting at 83° C. was obtained from the mother liquors.

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

Found: C, 58.6%; H, 10.1%

The recorded melting point of octanal semi-carbazone is 98° C. It would appear likely that the material analyzed was a mixture, the analytical results being fortuitous.

B. Octadecatetrenoic Acid. The esters with an iodine number of 181 that were obtained in the first filtrate from the acetone crystallization of the 18 carbon acid esters were examined by bromination. 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 were extracted with ether. The ether solution was washed three times with distilled water, dried over anhydrous sodium sulfate and made to 500 ml. with anhydrous ether. This solution was passed through a column (about 3 inches by 1 inch) packed with activated alumina (Grade A, mesh, minus 80, from the Aluminum Ore Company of America). The alumina removed much of the color and the brominated compounds prepared from the above purified acids were whiter than those obtained from the bromination of the more highly colored acids.

The ether filtrate from the alumina treatment was cooled to -5° C. in a 3 necked flask and cooled in chopped ice. Bromine was added dropwise to the cooled solution under mechanical stirring. Stirring was continued for an hour after an excess of bromine was indicated by a reddish color in the solution. Amylene added to the solution combined with the excess bromine. The precipitate was filtered and carefully washed four times with ether. No suitable solvent was found for crystallization. The product, weighing 1.0 g., melted at 230° C.

Analysis--Calculated for $C_{18}H_{28}O_2Br_8$: Br, 69.8%

Found: Br, 69.6%

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

A similar preparation omitting adsorption on alumina produced an ether insoluble bromo compound of melting point 230° C.

Analysis--Calculated for $C_{18}H_{28}O_2Br_8$: Br, 69.8%

Found: Br, 68.7%

Methyl esters (193 g. of saponification equivalent 293 and iodine value 142) were dissolved in 1.5 l. of petroleum ether and passed through a column of activated alumina. Bromination, as above, at 0° C. yielded 14.7 g. of petroleum ether insoluble material. Extraction with 200 ml. of ether, decolorization with charcoal, and cooling to -10° C. overnight, yielded a white powder which after two more crystallizations from ether melted at 158° C. The material weighed 0.4 g. A second crop was obtained from the mother liquors.

Analysis--Calculated for $C_{19}H_{30}O_2Br_8$: Br, 68.7%

Found: 68.2% Br.

Toyama and Tsuchiya (29) have reported an octadecatetrenoic acid melted at 220° C. and the methyl ester at 215° C.

<u>C</u>. <u>Eicosenoic Acid</u>. A 75 g. sample of the mixed 20 carbon acid esters dissolved in 750 ml. of acetone was cooled by adding solid carbon dioxide in excess and allowing the material to stand for an hour after

the evolution of carbon dioxide gas had ceased. The dried precipitate (liquid at room temperature) weighed 28.9 g. and had an iodine number of 136.7. The acids were liberated from 26.5 g. of the above esters by saponification. The lithium salts were prepared and crystallized twice from 80 percent aqueous ethanol. The iodine number was found to be 87.4 (theoretical for eicosenoic acid is 81.9) and the yield was 4.5 g.

The eicosenoic acid concentrate (4.3 g.) in 500 ml. water containing 4 g. of sodium hydroxide was cooled with 4 l. of ice water. One percent potassium permanganate (400 ml.) was added quickly, the mixture was shaken intermittently for five minutes and then decolorized by the addition of 500 ml. of 10 percent sodium bisulfite and 150 ml. of concentrated hydrochloric acid. The precipitate was filtered, air dried and extracted with warm petroleum ether. The residue (3.9 g.) after crystallization from 95 percent ethanol had a melting point of 115.5-116° C. Green, Hilditch, and Stainsby (13) reported a dihydroxyeicosanoic acid melting at 130.5° C.

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

Found: Sap. equiv. 341

<u>Periodate Oxidation of the Dihydroxyeicosanoic Acid</u>. To 0.5 g. of dihydroxyeicosanoic acid in 35 ml. of ethanol at 40° C., there was added a solution of 0.5 g. of potassium periodate in 15 ml. of normal sulfuric acid at 20° C. The mixture was allowed to stand for 15 minutes and then was cooled to 15° C. Water was added to dissolve the precipitated salts and the solution was extracted with ether. The ether was removed at the water pump and the semi-carbazones were prepared by adding 0.35 g. of semi-carbazide hydrochloride and 0.7 g. of crystalline sodium acetate in aqueous alcohol. The mixture was warmed and the precipitate obtained

by cooling the mixture was separated into a soluble and an insoluble fraction by extraction with 1 percent sodium hydroxide.

The alkaline solution was acidified with 2 percent hydrochloric acid. The precipitate was filtered, washed and crystallized three times from aqueous ethanol. The crystals (75 mg.) melted at 156° C. and showed no depression when mixed with the material of same melting point obtained 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 residue from the alkali extraction was washed twice with water and crystallized three times from aqueous ethanol. The crystals weighing 0.12 g. melted at 99° C. and showed no depression when mixed with an authentic specimen of nonanal semi-carbazone.

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

Found: C, 59.7%; H, 10.5%

D. *Eicosapentenoic Acids*. The 20 carbon acids from 13.4 g. of mixed methyl 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 result was a tan powder, weighing 1.42 g., which melted with decomposition at 258-265° C. after darkening at 230° C.

Analysis--Calculated for $C_{20}H_{30}O_2Br_{10}$: Br. 72.6%

Found: Br. 71.0%

The mother liquors after concentration and the addition of petroleum ether, yielded a material which upon crystallization from ether-petroleum ether melted at 150-155° C. This substance weighed 0.43 g.

Analysis--Calculated for $C_{20}H_{30}O_2Br_{10}$: Br. 72.6%

Found: Br. 71.0%

From the above mother liquors, a further quantity (0.09 g.) melting at 131-133° C. was obtained.

Analysis--Calculated for $C_{20}H_{30}O_2Br._{10}$: Br. 72.6%

Found: Br. 71.3%

Toyama and Tsuchiya (30) report a decabromoeicosanoic acid from sardine oil that melted around 240° C. with decomposition.

DISCUSSION

The molecular structure of the component glycerides of oils and fats is assuming greater importance. A symposium discussing the work done in this field and the methods of investigation has been published (7). In this symposium Embree presented evidence to show the fractionation of menhaden oil into five equal fractions by molecular distillation. The iodine numbers of these fractions were widely separated, indicating the presence of glycerides with greatly differing properties.

Riemenschneider, Swift and Sando (25) showed that cottonseed oil could be separated by fractional crystallization at low temperatures. If menhaden oil is composed of glycerides with differing properties, they should be separable by crystallization from acetone. This was shown to be the case and the results are listed in Table I.

By cooling the mixture of 10 volumes of acetone per gram of oil to -60° C. only 12 percent of the glycerides remain in solution and are removed in the filtrate. This fraction, however, has a high iodine number and is a quick drying oil suitable for inside paints.

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 gram of oil, the filtrate contained over 76 percent of the oil that had an iodine number of 198. A paint prepared from this fraction did not dry so well as did one prepared from linseed oil.

The saturated glycerides of menhaden oil yielded myristic, palmitic, stearic acids and a small quantity of one of higher molecular weight. While palmitic acid was present in a quantity greater than the others, the excess was not great enough to indicate the presence of tripalmitin.

The unsaturated fatty acids, with the exception of those with 18 carbon atoms found in vegetable oils, have been little investigated. Marine oils are recognized as being a good source of the more highly unsaturated fatty acids. The properties of these highly unsaturated acids make it difficult to isolate them in pure unchanged form. Farmer and van den Heuvel (12) have attempted to show that distillation of the methyl esters of the higher unsaturated fatty acids at pressures above those used in molecular distillation causes drastic changes. It must be borne in mind, therefore, that the acids with two and more double bonds which have been isolated by ester distillation are not necessarily identical with those present in the original oil. It is necessary to know the structure of the acids isolated by distillation if they are to be used as intermediates in chemical reactions even though they do differ from the natural acid.

Little has been reported about the unsaturated acids of menhaden oil. Brown and Beal (4) studied the highly unsaturated acids of menhaden oil by means of ester fractionation and bromination. They concluded that there were acids present with more than four double bonds.

The 18 carbon acid fraction has been said by Stingley (28) to be composed chiefly of oleic acid with a trace of linoleic acid, but no experimental evidence was presented in support of this.

The 18 carbon unsaturated acids that make up 25 to 31 percent of the total fatty acids in menhaden oil have been shown to consist largely of the well known 9,10-oleic acid. In addition, evidence has been obtained for the existence of another mono-olefinic 18 carbon acid. The dihydroxystearic acid melting at 115.5-116° C. is presumably an eutectic since attempted separation by crystallization had no effect upon the melting point.

When the dihydroxystearic acid of melting point 115.5-116° C. was split by potassium periodate, derivatives of the following fragments were obtained:

(1) $CH_3(CH_2)_7CH =$

(2) A mixture similar to (1) but with fewer carbons

(3) $= CH\ (CH_2)_9\ COOH$

(4) A mixture similar to (3) but with fewer carbons.

It appears likely, therefore, that the dihydroxy acid melting at 116° C. contains derivatives of the two following acids:

(1) $CH_3(CH_2)_7\ CH = CH\ (CH_2)_7\ COOH$

(2) $CH_3(CH_2)_5\ CH = CH\ (CH_2)_9\ COOH$

acid (1) being the ordinary oleic acid. The 11,12-octadecenoic acid (vaccenic acid) is reported by Bertram (3) to have been found in beef fat and butter fat and is thought by Armstrong and Hilditch (1) to exist in whale oil.

Bromination of the fraction containing 18 carbon acids has yielded only derivatives with eight bromine atoms thus indicating the existence of an octadecatetraenoic acid. Such an acid has been isolated from

Japanese sardine oil. Toyama and Tsuchiya (29) have recorded the melting of their octabromostearic acid as 220° C. and of the methyl ester as 215° C. The octabromostearic acid from menhaden oil melted at 230° C. and the methyl ester melted at 158° C. Bromination of methyl esters gave only derivatives of octabromostearic acid.

Hydroxylation of eicosenoic acid produces a dihydroxyeicosanoic acid melting at 115.5-116° C. Cleavage of this with potassium periodate gave only derivatives of the following two fragments:

(1) $CH_3\ (CH_2)_7\ CH =$

(2) $= CH\ (CH_2)_9\ \ COOH$

Therefore, the eicosenoic acid is chiefly

$$CH_3\ (CH_2)_7\ CH = CH(CH_2)_9\ COOH.$$

This 11,12-eicosenoic acid has been reported previously in the vegetable kingdom. Green, Hilditch, and Stainsby (13) report that it has been observed in a liquid seed wax where it is the chief component acid.

Bromination of the 20 carbon fraction has yielded only derivatives of decabromoeicosanoic acid. This indicates the presence of a 20 carbon acid with five double bonds.

The finding that octadecatetrenoic acid and eicosapentenoic acid are the only highly unsaturated acids in these fractions is in agreement with the observations of Farmer and Van den Heuvel (12). These authors state that marine animal oils contain only one higher unsaturated acid in each homologous group, thus the 16 carbon group has an acid with 3 double bonds, 18 carbon has 4, 20 carbon has 5 and both the 20 and 22 carbon acids have one with 6.

No attempt was made to investigate the acids of molecular weight higher than 20 carbon acids with the exception of the finding that no dihydroxy acids larger than dihydroxyeicosanoic acids could be obtained.

SUMMARY

It has been shown by low temperature crystallization from acetone that menhaden oil can be separated into components with greatly differing properties.

From the distillation of methyl esters of the unsaturated acids, derivatives have been found which indicate the presence of 9,10- and 11,12-octadecenoic acids, an octadecatetrenoic acid, 11,12-eicosenoic acid and an eicosapentenoic acid.

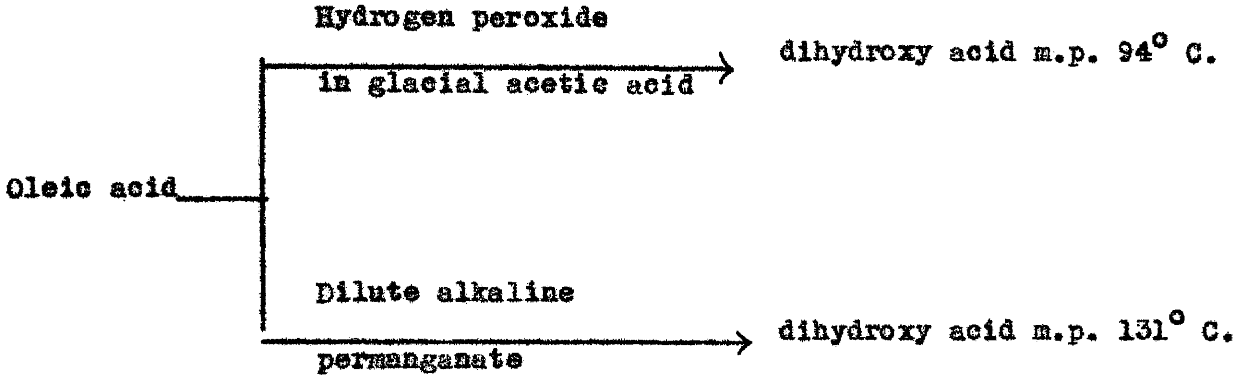
The observed and theoretical iodine numbers may be used to calculate that the 18 carbon unsaturated fraction (25 to 31 percent of the total acids) contains 86 percent octadecenoic acids and 14 percent octadecatetrenoic acids.

Similarly the 20 carbon unsaturated fraction (8 to 22 percent of the total acids) consists of 54 percent eicosenoic acid and 46 percent eicosapentenoic acid.

PART II

ISOMERISM OF 9,10-DIHYDROXYSTEARIC ACID

The 9,10-octadecenoic acid found in natural fats and oils possesses two isomeric forms by reason of geometric isomerism around the double bond. Mueller and Shearer (23) from X-ray examination and Marsden and Rideal (22) from surface film studies, assigned the cis configuration to oleic acid and the trans arrangement to the higher melting elaidic acid. Reagents which introduce hydroxyl groups at the double bond produce two dihydroxystearic acids. The relationship may well be represented by the following figure:



Starting with elaidic acid the opposite is true. Hilditch (14) reported that the higher melting dihydroxystearic acid is obtained from elaidic by the action of hydrogen peroxide in acetic acid. On the basis of the more stable compound being assigned the trans configuration, it is thus assigned to the dihydroxystearic acid melting at 131° C.

Lead tetra-acetate as developed and used by Criegee (8) and coworkers (9) offers a valuable method for the examination of the configuration of 1,2-diglycols. The reaction between dihydroxy compounds and lead tetra-acetate follows the conditions required for a bimolecular reaction. Using this information the velocity constants have been determined at 30° C. for the reaction between lead tetra-acetate and dihydroxystearic acid. Under the conditions utilized, K (higher melting acid) = 692 while for the lower melting form $K = 245 \times 10^2$.

Criegee (8b) lists the ratio K cis/K trans for several pairs of compounds. His numerical values vary from 23 up to 130,000 for the compounds he tested. The ratio for the dihydroxystearic acids is $K_{94^\circ}/K_{131^\circ} = 35.4$. By analogy the configurations of the dihydroxystearic acids are determined.

EXPERIMENTAL

Preparation of Dihydroxystearic Acid (Higher Melting Form). Commercial oleic acid was oxidized by alkaline permanganate solution by the procedure described in Appendix 1. The product was crystallized from 95 percent ethanol to a constant melting point of 131° C. The molecular weight as indicated by neutral equivalent was 316. Channon, Irving and Smith (5) have recorded 130.5-131° C. For the melting point of this acid, the calculated molecular weight is 316.

Preparation of Dihydroxystearic Acid (Lower Melting Form). The method of Scanlon and Swern as described in Appendix 2 was used for the preparation of a dihydroxystearic acid which melted at 94° C. and had a molecular weight of 315 (neutral equivalent). Scanlon and Swern (26) record 89-91° C. and Hilditch (14) reports 95° C. for the melting point. The calculated molecular weight is 316.

Determination of the Velocity Constants. To 25 ml. of a 0.02 molar solution of dihydroxystearic acid in glacial acetic acid at 30° C., there was added 25 ml. of a 0.02 molar solution of lead tetraacetate in glacial acetic acid, also at 30° C. Time was measured in minutes from the first contact of the two solutions and the reaction was allowed to proceed in a constant temperature bath at 30° C., samples (5 ml.) were removed at measured intervals. A 5 ml. aliquot was pipetted into 2 ml. of a solution made by dissolving 20 g. of potassium iodide and 500 g. of crystalline sodium acetate in one liter of water. The liberated iodine was titrated with 0.01 normal sodium thiosulfate. Calculations are based upon the equivalents of lead tetraacetate in the 5 ml. of reaction mixture.

Calculations of the velocity constant have been made using the equation for second order reactions when the concentration of both reactants are equal:

$$K_{30} = \frac{1}{ta}\left(\frac{x}{a-x}\right)$$

Where: t = time in minutes

a = original concentration of both reactants

x = concentration of both reactants at time t.

The lead tetraacetate solution was made in two ways. In one, crystalline lead tetraacetate prepared by the method of Oesper and Deasy (24) was dissolved in glacial acetic acid which had been distilled from chromium trioxide. Results obtained using this method have been listed in the first two tables. The second solution of lead tetracetate contained lead diacetate also, since red-lead oxide (Pb_3O_4) was dissolved in glacial acetic acid and used as such.

TABLE I

Reaction Velocity of Dihydroxystearic Acid (m.p. 131° C.) and Lead Tetraacetate

Time Min.	Equivalents of Lead Tetraacetate in 5 ml. Original (a) $\times 10^{-5}$	Reacted (x) $\times 10^{-5}$	(a-x) $\times 10^{-5}$	K_{30}
0	10.24			
9.0	. . .	3.91	6.33	670
14.0	. . .	5.11	5.13	695
20.0	. . .	6.00	4.24	691
32.2	. . .	7.18	3.06	711
42.2	. . .	7.67	2.57	694
			Average	692

TABLE II

Reaction Velocity of Dihydroxystearic Acid (m.p. 94° C.) and Lead Tetraacetate

Time	Equivalents of Lead Tetraacetate in 5 ml.			K_{30} x 10^2
	Original (a) x 10^{-5}	Reacted (x) x 10^{-5}	(a-x) x 10^{-5}	
0	10.24			
1.45	. . .	8.04	2.20	246
2.30	. . .	8.75	1.49	249
3.10	. . .	9.06	1.18	242
4.00	. . .	9.32	0.92	247
5.15	. . .	9.50	0.74	243

Average 245 x 10^2

Oxidation of Fumaric Acid with Alkaline Permanganate. Kekule and Anschutz (19) reported the formation of racemic tartaric acid from fumaric acid by alkaline permanganate. Fumaric acid (29 g.) was dissolved in a solution of 30 g. of potassium hydroxide in 200 c.c. of water. At room temperature, 7.5 liters of 1 percent potassium permanganate was added in five portions with cooling to keep the temperature from rising above room temperature. Three hours after the last of the solution had been added, the mixture was heated for an hour on the steam bath and filtered from the oxides of manganese. The precipitate was washed well with warm water. To the combined filtrates, acidified with acetic acid, lead acetate solution was added until precipitation was complete. The filtered and washed lead salts were decomposed with hydrogen sulfide. The lead sulfide was filtered and washed thoroughly, and the filtrates evaporated nearly to dryness, alcohol was added and mixture allowed to crystallize. The product after recrystallization from aqueous alcohol melted at 205° C. The melting point recorded for racemic tartaric acid is 204° C. (27). P-phenylphenacyl ester was prepared by method of Drake and Sweeney (11). Melting point and mixed melting point with an authentic specimen were 118° C.

DISCUSSION

The velocity constants obtained for the two acids by the two lead tetraacetate solutions are summarized:

Dihydroxystearic Acid		Lead tetraacetate solution from:	
		Crystalline tetraacetate	Red lead oxide
m.p.	131° C.	692	678
m.p.	94° C.	245×10^2	212×10^2
Ratio $K_{94^\circ}/K_{131^\circ}$		35.4	31.3

The lead tetraacetate solution prepared from red lead oxide gives velocity constants which are somewhat lower than those derived using crystalline lead tetraacetate. This is due to the presence of lead diacetate in the solution made from red lead oxide. Lead diacetate is one of the products of the reaction and the higher concentration retards the reaction.

The ratio $K_{94^\circ}/K_{131^\circ}$, which equals 35.4, falls within the range of Criegee's values for the ratio K cis/K trans. It may therefore be concluded that the dihydroxystearic acid melting at 94° C. is arranged cis while the other is trans.

Furthermore, since the cis arrangement is assigned to oleic acid, alkaline permanganate produces a trans dihydroxystearic acid. This is in contrast to the reaction of alkaline permanganate with fumaric acid, in which racemic tartaric acid is formed. The latter involves no shift since both the reagent and the product are trans acids.

Before the completion of this work, Hilditch and Jasperson (16) published a note on the oxidation of dihydroxy- and tetrahydroxystearic acids with lead tetraacetate. This information indicates that the acid melting at 95° C. is oxidized much more rapidly than is the higher melting acid. This is in agreement with the findings reported here.

SUMMARY

Chemical evidence has been presented to show that the 9,10-dihydroxystearic acid melting at 94° C. exists in the cis configuration while that melting at 131° C. possesses the trans arrangement.

In 0.01 molar concentration at 30° C. the velocity constant for the reaction of lead tetraacetate with (1) dihydroxystearic acid (m.p. 94° C.) is 245 x 10^2 and (2) dihydroxystearic acid (m.p. 131° C.) is 692.

The reaction of oleic acid with alkaline permanganate is anomalous in that a shift is produced from a cis reactant to a trans configuration of the product. In the normal reaction, as that between fumaric acid and alkaline permanganate to yield racemic tartaric acid, no shift is produced.

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APPENDIX

1. Hydroxylation of Monoolefinic Acids with Potassium Permanganate by the Method of Lapworth and Mottram (21). A clear solution of the sodium or potassium salt of the acid was made by warming 5 g. of the acid with an equivalent weight of sodium or potassium hydroxide in 500 ml. of water. This solution was cooled, 4 l. of ice water was added and the whole was shaken at 10° C. while 400 ml. of 1 percent potassium permanganate solution was added quickly. After five minutes, 200 ml. of 10 percent sodium bisulfite and then 150 ml. of concentrated hydrochloric acid were added to decolorize the solution and to precipitate the acids. After settling, the mother liquors were siphoned, the precipitate was filtered and washed with water until free of acid. The precipitate was dried, washed with light petroleum to remove saturated and unoxidized acids. The precipitate was crystallized from ethanol or ethyl acetate.

The yields with this method are excellent if one starts with a pure monoethonoid acid. If higher unsaturated acids are present in appreciable proportions the yield is reduced. Slightly lower yields were obtained when 10 times the above quantities were used.

2. Hydroxylation of Monoolefinic Acids with Hydrogen Peroxide in Acetic Acid by the Method of Scanlon and Swern (26). At room temperature, 567 g. (5 moles) of 30 percent hydrogen peroxide solution and 1800 ml. glacial acetic acid solution were mixed and heated at 80-85° C. for one hour. The mixture was cooled to about 25° C. then 706 g. (2.5 moles) of oleic acid were added. The ensuing exothermic reaction was allowed to proceed without application of heat but occasional agitation was necessary. When the temperature had decreased to 20-25° C., the mixture was filtered. The filtrate was poured into 6 l. of hot water. The aqueous layer was siphoned and rejected. The oily layer was dissolved in 4.5 l. of normal sodium hydroxide solution and heated for two hours on the steam bath. The hot solution was made acid with 6 normal hydrochloric acid and the solidified acid was separated. This product was remelted and washed thoroughly with 3 l. of hot water slightly acidified with hydrochloric acid. The acid was crystallized from ethanol.

This method of hydroxylating oleic acid works equally well with the methyl ester of oleic acid.

3. **The Determination of the Saponification Equivalent by A Modification of the Method of Chargoff (6).** A 50 to 100 mg. sample of acid or ester was refluxed with 5 ml. of 0.1 normal 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 end-point with 0.05 normal sulfuric acid. A blank determination was made under similar conditions.

The saponification equivalent was calculated using the following equation:

$$\text{Saponification equivalent} = \frac{\text{Weight of sample (mg.)}}{\text{(Blank ml. - Back titration ml.)} \times \text{Normalty of acid}}$$

It is to be noted:

(a) The back titration was made in the cold.

(b) As calculated, the saponification equivalent equals the mean molecular weight.

(c) Acids as well as esters were subjected to the same procedure.