tetraphenyl-succinonitrile. Each of these substances was identified by its melting point and the melting point of a mixture with samples of known structure.

Benzoyl-phenyl-nitrocyclopropane

Five and three-tenths g. of the cyclopropane derivative was reduced in 50 cc. of methyl alcohol in the presence of 0.25 g. of platinum black. The reduction was stopped when 740 cc. of hydrogen had been absorbed. The platinum was removed after boiling the suspension to redissolve a white solid which had separated during the reduction. On cooling the clear solution, it deposited 1.9 g. of a colorless solid which crystallized in needles and melted at 98–99°. A mixed melting point showed that this was β-phenyl-γ-nitro-butyrophenone.

Summary

1. This paper gives an account of the results obtained by reducing a series of α,β-unsaturated nitro compounds with hydrogen and platinum.

2. In a discussion of the results it is shown that the behavior of α,β-unsaturated nitro compounds on catalytic reduction is essentially the same as that of α,β-unsaturated ketones. The resulting saturated nitro compounds, however, generally undergo further reduction; the successive steps are $RCH{:}CHNO_2 \longrightarrow RCH_2CH_2NO_2 \longrightarrow [RCH_2CH_2NO] \longrightarrow RCH_2CH{:}NOH \longrightarrow RCH_2CH{:}NH \longrightarrow RCH_2CH_2NH_2$.

3. The primary reduction product of a cyclopropane derivative which has a nitro group in direct union with one of the ring carbon atoms is likewise a saturated nitro compound.

Cambridge 38, Massachusetts

[Contribution from the Chemical Laboratory of the University of Illinois]

THE HIGHLY UNSATURATED FATTY ACIDS OF FISH OILS

By J. B. Brown[1] with G. D. Beal

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Introduction

While engaged in the preparation of pure fatty acids for use in medical research during the late war under the auspices of the National Research Council, the writers had occasion to prepare pure clupanodonic acid, $C_{18}H_{28}O_2$. The information contained in the literature regarding this acid was vague, and a number of preliminary studies were made in order to divise a cheap and convenient method for its preparation. The method finally adopted was a modification of that of Riedel.[2] The methyl esters, which were prepared by debromination with zinc of the polybromides of the methyl esters of menhaden oil, had a molecular weight too high for methyl clupanodonate, and furthermore distilled over too wide a range to be a pure compound. These facts indicated that we were dealing with

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[1] Abstracted from a thesis submitted by J. B. Brown in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of the University of Illinois.

[2] Riedel, Ger. pat., 266,350.

some acids other than clupanodonic. Since practically none of the properties of clupanodonic acid has been described, the investigation was continued with the idea of determining the nature of the pure acid, and it has finally developed into an attempt at a characterization of the highly unsaturated acids of fish oils.

Hofstaedter in 1854[3] was the first to discuss in detail the presence of an unsaturated acid in a marine oil. Investigations by Fahrion,[4] Bull,[5] Tolman,[6] Twitchell[7] and others have demonstrated beyond question the presence of highly unsaturated acids in both fish and marine animal oils. By brominating the acids from Japanese sardine oil and reducing the resulting bromides with zinc and alcoholic hydrochloric acid, Tsujimoto[8] obtained an acid with an iodine number of 344, which he called clupanodonic acid. Majida and Okada[9] reported that the acids obtained in this fashion had a molecular weight too high for clupanodonic acid, and that on hydrogenation they obtained an apparent mixture of arachidic and behenic acids.

When the problem had been under way for 18 months, we read the interesting preliminary report of Tsujimoto in which he describes the separation of the acids of fish oils by means of variations in the solubility of their lithium salts in acetone.[10] From the analysis of 4 fish oils he concluded that the highly unsaturated acids were chiefly $C_{20}H_{32}O_2$ and $C_{22}H_{34}O_2$, the latter of which he proposes to call clupanodonic acid instead of that to which he had formerly given the name.[11] In view of the fact that this name has been used for the compound, $C_{18}H_{28}O_2$, for sixteen years, we prefer to retain it and are using, therefore, the following nomenclature[12] for the unsaturated acids throughout this paper.

Formula	Double bonds	Acid name
$C_{16}H_{26}O_2$	3	Hexadectrienoic
$C_{18}H_{30}O_2$	3	Linolenic
$C_{18}H_{28}C_2$	4	Clupanodonic
$C_{20}H_{32}O_2$	4	Arachidonic
$C_{20}H_{30}O_2$	5	Eicosapentenoic
$C_{22}H_{34}O_2$	5	Docosapentenoic
$C_{22}H_{32}O_2$	6	Docosahexenoic

Experimental Part

Attempted Preparation of Clupanodonic Acid from Menhaden Oil

Preparation and Bromination of the Esters of Menhaden Oil.—In order to compare the methyl, ethyl and butyl esters of menhaden oil and

[3] Hofstaedter, *Ann.*, **91**, 177 (1854).

[4] Fahrion, *Chem.-Ztg.*, **17**, 521 (1893).

[5] Bull, *ibid.*, **23**, 996 (1899); *Tidskrift Kemi, Farm. Terapi*, **14**, 1 (1917).

[6] Tolman, *J. Ind. Eng. Chem.*, **1**, 341 (1909).

[7] Twitchell, *ibid.*, **6**, 564 (1914); **9**, 581 (1917).

[8] Tsujimoto, *J. Coll. Eng. Imp. Univ. Tokyo*, **4**, 1 (1906).

[9] Majida and Okada, *Science Repts., Tôhoku Imp. Univ.*, **3**, 1 (1914).

[10] Tsujimoto, *J. Chem. Ind.* (Japan), **23**, 1007 (1920).

[11] Since the above was written, Professor Tsujimoto, writing of Japanese sardine oil, has again, given the name clupanodonic acid to the compound $C_{22}H_{34}O_2$ [*Chem. Umschau Fette, Oele, Wachse Harz*, **33**, 261 (1922)]. Since the acid $C_{18}H_{28}O_2$, to which the name was originally given, undoubtedly occurs in oils from the herring family, we can see no reason for the change in the use of the name. We feel that more confusion will be caused by a change now, since a number of authors, among others MacArthur and Burton [THIS JOURNAL, **38**, 1375 (1916)] have reported clupanodonic acid, $C_{18}H_{28}O_2$, as a constituent of animal tissues.

[12] Dr. A. M. Patterson has kindly verified the nomenclature of these acids.

their behavior towards bromination, they were prepared by a modified form of Haller's method[13] of alcoholysis.

Menhaden oil was refluxed with 1.5 times its weight of absolute methyl alcohol or an equivalent amount of the other alcohols, containing about 2% of dry hydrogen chloride, for 24 hours. The mixture was cooled and washed with strong brine to remove the excess of alcohol, the liberated glycerol and hydrochloric acid, the esters then being distilled under reduced pressure. The esters were dissolved in 9 times their weight of dry ether and bromine slowly added in 10% excess, while the mixture was kept at a temperature of —10° to —5° by a bath of ice and salt. After standing for 6 hours the precipitated bromides were washed with ether by decantation until freed from bromine, then dried in air. These methods of esterification and bromination were used generally throughout this investigation. The percentage yield of bromides based upon the original weight of ester, or polybromide number, and the percentage of bromine in the bromides were determined. The mixed butyl esters of the acids of linseed oil were carried through a like process to determine whether evidences of substitution would appear.

Determination of Bromine in the Polybromides.—The bromine content of the ester polybromides was determined by the aid of the Parr peroxide bomb, using the authors' modification of the procedure outlined by Lemp and Broderson.[14]

A sample of about 0.3 g. was placed in the fusion cup together with 1 g. of accelerator, in this case sodium nitrate, 0.5 g. of granulated sugar and a measure of sodium peroxide. After ignition, either by a Bunsen burner or the improved electrical method, the fusion mixture was cooled and dissolved in 150 cc. of water. The solution was acidified with 22 cc. of conc. nitric acid, an excess of 0.1 *N* silver nitrate solution added and the solution heated to boiling; 10 cc. of a 4% solution of hydrazine sulfate was added to reduce any bromate ions present and boiling continued until the precipitate had coagulated thoroughly. After the mixture had boiled, 5 cc. of a saturated solution of ferric alum was added and the excess of silver nitrate titrated with 0.05 *N* ammonium thiocyanate solution. A blank determination was made with the reagents and sugar alone.

TABLE I

A COMPARISON OF THE BEHAVIOR OF THE METHYL, ETHYL AND BUTYL ESTERS OF MENHADEN OIL TOWARDS BROMINATION

Ester	Boiling point at 15 mm.	Wt. ester G.	Wt. bromides G.	Polybromide No.	Bromine %	Calc. for clupanodonate
			Menhaden Oil			
Methyl	195–240°	2100	805	38.3	68.31	68.79
Ethyl[a]	195–240	600	195	32.5	67.43	67.84
Ethyl	195–240	1270	430	33.8	67.48	67.84
Ethyl	240–265	409	367	85.4	69.49	67.84
n-Butyl	190–245	305	96	31.5	67.64	65.82
			Linseed Acids			
n-Butyl	190–240	100	51	51.0	59.30	59.38[b]

[a] Two lots of ethyl esters were made and distilled into 2 fractions as shown above. The higher-boiling fractions were combined.

[b] 59.38% is the theoretical bromine content of butyl hexabromo-stearate. This compound sintered at 157° and melted at 160°. It has not been previously described.

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[13] Haller, *Compt. rend.*, **146**, 250 (1908).

[14] Lemp and Broderson, THIS JOURNAL, **39**, 2069 (1917).

It has been suggested that sodium bisulfite solution be used as a reducing agent instead of the hydrazine sulfate. This must be added and the excess oxidized with potassium permanganate solution before the addition of the silver nitrate solution. The results with this procedure were not always of the best. One of us (J. B. B.) has had an illuminating experience in the determination of iodine by this method. The peroxide reaction apparently gave only iodate since silver nitrate in the acid solution formed no precipitate. Immediately on adding the hydrazine sulfate solution silver iodide was precipitated and bubbles of nitrogen gas were evolved. There was no appearance of free iodine. When sodium bisulfite was used as the reducing agent iodine invariably appeared.

Was Pure Clupanodonic Acid Obtained?—The percentage of bromine in the polybromides obtained from the higher-boiling fraction of the ethyl esters and from the butyl esters is an indication of the presence of more highly unsaturated acids than clupanodonic. These acids are furthermore of higher molecular weight, since they are in the higher-boiling fraction. Qualitative tests indicate that the solubility of the polybromides of the esters increases with an increase in the molecular weight of the alcohol radical. The absence of more than traces of substitution in the method of bromination employed is indicated by the fact that butyl hexabromo-stearate as prepared had practically the calculated bromine content, and no evidence of formation of hydrobromic acid could be obtained. Debromination of the bromides of the fractions of the lower-boiling ethyl ester in absolute ethyl alcohol with zinc dust gave an ester with an iodine number of 333. The acid mixture as obtained by saponification had an iodine number of 342 and a molecular weight by titration of 305, which values are not in agreement with those for clupanodonic acid.

The Effect of Heat on the Fatty Acids of Menhaden Oil.—The free acids of fish oils could be distilled only with difficulty, since a thick viscous liquid remained in the distilling flasks. To test the effect of heat a quantity of mixed fatty acids from menhaden oil was placed in a Claisen flask and heated to 240°. To prevent oxidation a gentle stream of carbon dioxide was passed over the acids. Samples were withdrawn from time to time and the molecular weight was determined by titration.

TABLE II

THE EFFECT OF HEAT ON THE FATTY ACIDS OF MENHADEN OIL

Time of heating, min.	00	60	105	165
Mol. wt.	289.7	297.4	303.4	313.2

The maximum temperature to which the fractions are subjected under the conditions of fractional distillation under vacuum is 240°. A similar experiment conducted with the esters of these same acids showed practically no change in molecular weight. This apparent polymerization may explain why large quantities of tarry residue are found in the distilling flask on fractionating the free acids, while the esters may be distilled almost to dryness. All subsequent distillations, when possible, were of esters.

Comparative Analysis of Five Commercial Fish Oils

From the data given above it was evident that in the sample of menhaden oil used there were present highly unsaturated acids other than clupanodonic acid. Five fish oils which were available were analyzed to determine the probable nature of the highly unsaturated acids. The constants of the oils are given in Table III.

TABLE III

ANALYTICAL CONSTANTS OF COMMERCIAL FISH OILS

Oil	Sap. No.	Iodine No.	$n^{20°}$
Menhaden	191.2	151.7	1.4778
Salmon	185.0	137.2	1.4768
Cod	186.9	151.0	1.4770
Herring	186.5	139.8	1.4765
Sardine	187.3	158.1	1.4791

One kg. of each oil was esterified by the method mentioned previously and the methyl esters were distilled into fractions over a 10° range under 15 mm. pressure. In the case of menhaden oil 3 kg. was used and the distillation repeated thrice. The fractions were then analyzed for free acid, mean molecular weight of the acids, index of refraction, iodine number, polybromide number and percentage of bromine in the bromides, while the mean molecular weight of the acids was calculated from the molecular weight of the esters as determined by saponification.

The fish oils examined were found to be decidedly similar in character. In general, the iodine number, polybromide number and percentage of bromine in the bromides increased with the boiling point, the main divergence being in the last fraction, which might easily contain decomposed material.

The oils as a class contain acids whose esters distil at much too high a temperature for the C_{18} series. Among these higher acids are those which are more highly unsaturated than clupanodonic acid.

Separation of the Highly Unsaturated Acids

From the facts mentioned above it is quite probable that the clupanodonic acid previously described in the literature was not a pure compound, but rather a mixture of a number of acids. Accordingly a number of attempts were made to separate the fatty acids of menhaden oil, the most successful procedure being applied to cod and herring oils.

Separation by Fractional Distillation.—The esters from 3 kg. of menhaden oil obtained in the previous experiment were fractionated 6 times. The pressure during each distillation was maintained at 15 mm. and on the last distillation the fractions were collected in such a way that certain ones would represent as far as possible only acids of the same carbon content. Although the intermediate fractions had such properties as to indicate that the principal fractions were not chemical individuals, the boiling point, index of refraction, molecular weight and iodine number indicated the predominance in each of these fractions of acids of a certain group.

TABLE IV

RESULTS OF ANALYSIS OF THE FRACTIONS FROM THE SIXTH DISTILLATION OF THE METHYL ESTERS FROM THREE KILOGRAMS OF MENHADEN OIL

Fraction	15 mm. range ° C.	Wt. G.	n^{19}	M. m. wt. acids	Iodine no.	Probable series
1	156–166	21.5	1.4415	234.1	18.58	...
2	166–170	26	1.4408	233.7	14.73	C_{14}
3	170–180	129	1.4425	239.5	27.52	...
4	180–194	257	1.4470	255.2	53.2	...
5	194–198	500	1.4500	260.5	72.74	C_{16}
6	198–210	208	1.4546	268.9	101.8	...
7	210–216	191	1.4610	276.6	142.25	C_{18}
8	216–226	237	1.4670	283.2	185.2	...
9	226–230	112	1.4765	293.7	245.4	...
10	230–235	66	1.4810	300.1	277.0	C_{20}
11	235–240	94	1.4858	305.1	299.7	...
12	240–245	107	1.4895	309.9	306.5	...
13	245–250	76	1.4934	317.2	306.6	...
14	250–255	31	1.4960	324.9	304.1	C_{22}
15	255–260	22	1.4979	326.4	284.3	...

From the series of analyses just tabulated the following conclusions may be drawn: (1) It is possible by fractionation of the methyl esters to make a rough separation of acids according to molecular weight. (2) The important acids have a carbon content ranging from C_{14} to C_{22}. Those of the lower molecular weights are largely saturated. (3) The iodine numbers and mean molecular weights of the fractions rise rapidly with the boiling points. The index of refraction of the esters was also found to increase regularly. When the index of refraction was plotted against iodine numbers practically a straight line was obtained, the main divergence being with the last point. The same general relationship is seen when the index of refraction is plotted against molecular weight, the breaks at the beginning and end of the series giving the graph the form of the sign of integration. (4) The polybromide number of the esters rises with the boiling point, while the bromine content of the derivatives also increases. Unsaturation undoubtedly increases with an increase in molecular weight.

Separation by Metallic Salts.—It is apparent from the data previously given that a number of highly unsaturated acids occur in fish oils. It is furthermore probably true that no one has succeeded in isolating any of them in the free state. The clupanodonic acids of Tsujimoto and of Riedel, previously referred to, were undoubtedly complex mixtures. Various methods have been proposed for the separation of the saturated and unsaturated fatty acids. Most of the methods depend upon differences in the solubility of metallic salts of the unsaturated acids in organic solvents, those of the unsaturated acids being most soluble. Such separations are not quantitative, due to the tendency of the slightly unsaturated acids to follow the saturated acids in their behavior, and also to an apparent mutual solubility of the salts of the two types, one in the other.

The Lead-soap-ether Method.—Five hundred g. of refined menhaden oil (iodine number 183.9; saponification number 191.8) was treated according to the usual procedure for the lead-soap-ether method [15,16] using only such modifications as were necessitated by the large size of the sample. The lead salts so separated were decomposed as usual with hydrochloric acid and the solid and liquid acids examined. The liquid acids weighed 300 g., showed an average molecular weight of 328.6 (the abnormally high value is probably due to the presence of a small amount of retained ether) and an iodine number of 237.9; whereas the solid acids weighed 150 g. and showed a molecular weight of 264.6 and an iodine number of 55.3. The liquid acids were then converted into their methyl esters and distilled under 15 mm. pressure.

TABLE V

THE DISTILLATION OF THE METHYL ESTERS OF THE UNSATURATED ACIDS OF MENHADEN OIL AS OBTAINED BY THE LEAD-SOAP-ETHER METHOD

Fraction	Range ° C.	Wt. G.	Iodine No.	n
1	180–200	55	131.7	1.4560
2	200–210	36	166.5	1.4622
3	210–220	49	210.9	1.4683
4	220–230	37	266.0	1.4771
5	230–240	48	321.0	1.4860

This distillation was twice repeated, when the fraction boiling at 235–250° had an iodine number of 334.1 and a molecular weight of 326.5. Since, however, the lower fractions gave evidence of the presence of saturated acids as impurities and since the method applied to large quantities was so tedious, further investigation by this procedure was discontinued.

By the Barium-soap-benzene Method.—The application of the barium-soap-benzene method[17] was attempted. This is based on the solubility of the barium soaps of fatty acids with more than one double bond in cold benzene containing about 5% of 95% alcohol, added to bring a trace of moisture into the benzene, while the barium salts of the acids of the oleic and the saturated series will dissolve in the hot benzene-alcohol but crystallize on cooling.

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[15] Gusserow, *Ann.*, **27**, 153 (1828).

[16] Varrentrapp, *ibid.*, **35**, 197 (1840).

[17] Farnsteiner, *Z. Nahr. Genussm.*, [2] **1**, 390 (1898).

The sodium soaps of menhaden oil were neutralized with acetic acid and precipitated in the cold with an excess of barium chloride solution. The soaps so precipitated were filtered on a Büchner funnel, and when transferred to a large flask and heated on a steam-bath for a few minutes so agglutinated and shrunk that practically all of the occluded water could be decanted. The soaps were then treated with the benzene-alcohol mixture and refluxed for 30 minutes on a steam-bath. The solvent was poured off and the residual soaps were treated with a fresh portion of the solvent. This treatment was repeated until the soaps were completely disintegrated and practically all dissolved. The combined solutions were then allowed to cool overnight, and filtered from the crystalline precipitate. About 8 liters of solvent was required for 500 g. of the oil. The acids from both filtrate and precipitate were recovered by decomposing the soaps with hydrochloric acid and distilling the benzene. The last traces of benzene were removed by heating in a vacuum. The results obtained in the first trial distillation of 500 g. were so satisfactory that 3 additional distillations were made, the products being combined for analysis.

No. of distillation	Character	Wt. G.	Mol. wt.	Iodine no.	n^{20}
1	Liquid	260	306.0	267.0	1.4845
2–3–4	Liquid	790	305.9	261.4	....
1	Solid	144	281.6	53.7	....
2–3–4	Solid	420	Constants not determined		

One kg. of these liquid acids was converted into their methyl esters and distilled thrice under 15 mm. pressure. A special Claisen flask was made of Pyrex glass for this distillation by Mr. Paul Anders. The flask had an especially wide neck and side tube, to prevent the liquid from bumping over, and the side arm was inserted a short distance into the upright side tube in order to prevent esters condensing on the stopper from running into the receiver. The side arm was also especially long so that it might be surrounded by a small Pyrex condenser while it conducted the distillate directly into a Raikow receiver which permitted five fractions to be taken without breaking the vacuum. The rubber stoppers were protected from the hot ester vapors by pinning a thin sheet of cork over their lower surfaces.

Table VI

Analytical Constants of the Methyl Esters of the Liquid Acids of Menhaden Oil Obtained by the Barium Soap Separation, After Three Distillations

Fraction	Range (15 mm.) ° C.	Wt. G.	M. m. wt. acids	Iodine no. of ester	n^{18}
1	170–180	8	...	...	1.4627
2	180–200	98	255.2	111.4	1.4536
3	200–210	100	257.9	142.2	1.4580
4	210–220	147	260.7	181.0	1.4640
5	220–225	49	...	231.2	1.4718
6	225–230	86	...	249.3	1.4740
7	230–235	66	...	280.1	1.4791
8	235–240	95	304.0	316.5	1.4845
9	240–245	65	308.5	334.6	1.4890
10	245–250	43	312.6	347.7	1.4930
11	250–255	58	321.6	348.4	1.4960
12	255–260	34	336.7	330.9	1.4980

Fractions 8–12, boiling over 5° ranges from 235–260°, had mean molecular weights ranging from 304 to 336 and iodine numbers for the esters

ranging from 316 to 348. Fraction 8 gave values corresponding to the theoretical values for the methyl ester of arachidonic acid. Probably the higher-boiling fractions contain docosapentenoic and docosahexenoic acids.

The polybromide numbers of Fractions 8 and 11 were determined by the following procedure.

Place 1–2 g. of the ester in a weighed 50cc. centrifuge tube and add 35 cc. of anhydrous ether. Place the tube in a cooling bath kept below 0° and add bromine, with vigorous stirring to a distinct excess or until the solution is colored red. Allow the tube and contents to stand in an icebox overnight in the dark, centrifuge, decant the ether, and wash the precipitate 4 times by centrifuging with 40cc. portions of ether. Dry the tube and contents at 60° for 2 hours and weigh. The weight of bromides, divided by the weight of ester and multiplied by 100, gives the "*polybromide number.*" In order to determine the nature of the residual acids in the ether filtrates, they were shaken with sodium thiosulfate solution to remove the excess of bromine, then dried with calcium chloride, the ether was allowed to evaporate and the residue finally dried in a vacuum oven at 60°.

Fraction 8 showed a polybromide number of 97.90, giving 69.61% of bromine in the bromides, and 56.56% of bromine in the ether-soluble portions, while for Fraction 11 the polybromide number was 101.13 and the bromine percentages 70.07 and 56.05 respectively.

Fraction 8, above, could not be pure methyl arachidonate since this would yield a bromine derivative with 66.78% of bromine, whereas the derivative formed actually contained 69.61%. If this fraction contained a considerable quantity of methyl docosapentenoate, which gives a polybromide with 71.66% of bromine, such results might be obtained. However, the substitution of this acid in part or entirely would give iodine numbers which would be much higher than those obtained. It is more probable that the fraction is a mixture of methyl arachidonate and methyl docosapentenoate with small quantities of the esters of less unsaturated acids.

An examination of the ether-soluble bromides leads to one of two conclusions: either the liquid bromides are not completely saturated with bromine, because of some sort of steric hindrance, or they are isomeric liquid bromides mixed with esters of the saturated acids or with bromides of less unsaturated acids.

Separation by Reduction of the Polybromides.—The method used in the preparation of these polybromides was essentially the same as that which had previously been employed.

A sample of commercial menhaden oil, dark reddish-amber in color, was available. Two portions of 3 kg. each were converted into their methyl esters. The first lot was refluxed with the acid-alcohol mixture for 12 hours and the second for 24 hours. The yields of esters on distillation, between the usual range of 190–250° under 15 mm. pressure were 2000 g. of ester for the first trial and 2700 g. for the second. The methyl esters were brominated in kilogram quantities in ether solution at a temperature which was kept below 0°, as has been previously described. In all, 1772 g. of polybromides was obtained. Three portions of the esters were brominated in ether containing 10% of glacial acetic acid, but no appreciable difference could be observed in either the yield or the percentage of bromine taken up.

Previous experiments have been rewarded with low yields when the polybromides were reduced.[2] This was thought to be due possibly to the insolubility of the reacting substances. Accordingly *normal* butyl alcohol and benzyl alcohol were tried as solvents, since the polybromides appeared to be much more soluble in these liquids. Boiling benzyl alcohol dissolves the polybromides to the extent of 100 g. per liter, giving a dark colored solution from which the white polybromides deposit on cooling.

Reduction in Benzyl Alcohol.—One hundred g. of the polybromides and an excess of zinc dust were placed in a Pyrex flask with about 200 cc. of benzyl alcohol. The mixture was carefully heated, but as soon as the reaction had begun it progressed violently, and the heat developed was sufficient to break the flask. A second 100g. portion was mixed in the dry condition with 100 g. of zinc dust and added in small portions to 250 cc. of benzyl alcohol boiling in a 500 cc. flask under an air condenser. No heat was applied after the addition of the first portions of the mixture, the heat of the reaction being sufficient to keep the mixture refluxing vigorously. After cooling, the mixture was a very thick viscous liquid from which nothing could be separated. It was thought that the benzyl alcohol had possibly entered into the reaction.

Reduction in Butyl Alcohol.—A mixture of the polybromides with zinc dust was refluxed for 10 hours in the presence of *normal* butyl alcohol, the alcohol filtered off and the residue washed twice with fresh portions of butyl alcohol. After the alcohol was removed an attempt was made to distil the residue, but nothing passed over even under 15 mm. pressure. After the distillation flask had been removed from the heat for 5 minutes a strongly exothermic reaction set in which resulted in the formation of a mass of tar in the flask.

Reduction in Methyl Alcohol.—Equal parts of the polybromides and zinc dust were refluxed in methyl alcohol for 48 hours, the alcoholic layer was filtered off and the solid residue washed thrice with alcohol, the alcoholic filtrates being combined and the alcohol distilled under atmospheric pressure. The ester-alcohol mixture remaining was agitated with warm dil. hydrochloric acid to remove the remaining alcohol and to decompose any zinc soaps present. The ester layer was then separated and refluxed for 12 hours with twice its weight of absolute methyl alcohol and hydrochloric acid, under the usual conditions for methanolysis, this operation being made necessary by the formation of some free acid during the debromination. The esters which were finally recovered were distilled under 15 mm. pressure. The first two runs of 500 g. each gave a total of 137 g. of reduced ester; yield, 45%. A third run of 1050 g. of bromides gave 163 g. of esters; yield, 50.9%; iodine number of esters, 368.5; mean molecular weight of acids, 307.9; n^{20}, 1.4910.

The esters distilled between 215° and 250° under 15 mm. pressure. There was practically no residue in the distilling flask after these esters were distilled, a fact accounted for by the absence of free acid carried into the alcoholic solution as zinc salt.

Comparison of the Acids of Cod and Herring Oils

The highly unsaturated esters from cod and herring oils were prepared in a similar manner; 1620 g. of the methyl esters from 2000 g. of cod oil gave 480 g. of bromides, containing 70.75% of bromine. These when debrominated gave 93 g. of esters; yield, 67.65%. 1875 g. of esters from 2200 g. of herring oil gave 517 g. of bromides, with a bromine content of 69.29%. These yielded 83 g. of esters, or 52.3%.

The highly unsaturated esters of menhaden oil were distilled thrice under diminished pressure, while those of cod and herring oils were distilled only once, on account of the small quantity available. For the second and third distillations of the menhaden oil esters, the fractions were introduced in order into the distilling flask by means of a separatory funnel, the stem of which had been drawn out to a fine tip. All of the temperatures were corrected to 15 mm. pressure, including the stem correction for the thermometer. The last 4 fractions of the esters of cod oil showed abnormal values for all but molecular weight, due to accidental overheating. The analyses follow.

TABLE VII

THE CONSTANTS OF THE FRACTIONATED METHYL ESTERS OF THE HIGHLY UNSATURATED FATTY ACIDS OF MENHADEN, COD AND HERRING OILS, PREPARED BY REDUCTION OF THE POLYBROMIDES

Menhaden oil, distilled thrice

Fraction	Range (15 mm.)	Wt. G.	M. m. wt. acids	Iodine no.	n^{20}	Poly-bromide. no.	Br %
Mixed esters (215–250°)			307.9	368.5	1.4910	105.33	69.40
1	below 203°	9.5	322.0	260.6	1.4753	....	...
2	203–213	30.0	274.1	334.8	1.4800	....	...
3	213–218	16.0	280.8	348.8	1.4860	94.67	68.62
4	218–223	22.0	284.7	359.4	1.4888	96.62	69.71
5	223–228	47.0	292.2	363.8	1.4907	101.9	69.78
6	228–233	35.5	298.1	372.8	1.4919	101.0	69.69
7	233–238	33.0	307.5	376.3	1.4950	101.21	70.45
8	238–243	31.0	315.8	379.2	1.4970	97.56	70.46
9	243–248	17.0	317.4	373.2	1.4980	76.02	70.48
10	248–255	11.0	324.7	357.9	1.4987	64.90	70.99
Cod oil, distilled once							
Mixed esters (210–247°)			298.0	383.5	1.4912	125.8	70.4
1	below 213°	11.0	279.4	362.2	1.4865	104.0	68.70
2	213–218	11.0	286.7	369.8	1.4881	109.8	69.21
3	218–223	18.0	293.0	380.0	1.4895	113.0	69.56
4	223–228	11.5	303.3	305.9	1.4940	....	...
5	228–233	4.0	307.3	277.9	1.4985	....	...
6	233–250	5.5	313.0	257.5	1.4992	....	...
7	250–270	10.0	323.5	232.5	1.4993	....	...
Herring oil, distilled once							
Mixed esters (215–250°)			299.2	373.9	1.4910	113.25	69.66
1	below 213°	6.0	277.8	354.0	1.4860	97.7	68.99
2	213–218	7.0	283.4	361.8	1.4873	101.1	68.81
3	218–223	9.0	286.0	366.3	1.4888	103.0	69.05
4	223–228	11.5	294.0	369.5	1.4900	111.0	69.45
5	228–233	16.5	299.0	376.0	1.4928	106.5	69.69
6	233–238	11.0	309.3	379.4	1.4960	102.0	70.12
7	238–245	4.5	315.0	372.0	1.4992	94.0	70.61
Menhaden oil, faction 2, redistilled							
2a	180–195	9.0	266.8	316.5	1.4800	72.78	68.59
2b	195–205	7.5	274.5	333.7	1.4829	....	...
2c	205–212	5.5	282.7	344.7	1.4896	....	...

The Nature of the Fatty Acid

The following conclusions may be drawn from the examination of the ester fractions of the various oils in the preceding table.

The highly unsaturated acids would seem to contain 16, 18, 20 and 22 carbon atoms, the molecular weights of the tetra-unsaturated acids being 248, 276, 304 and 332, respectively. The lowest mean molecular weight determined was 266.8 (see below), which is 9 points lower than that for clupanodonic acid. The highest value found was 324.7, which is only 7 units below that for the C_{22} acid.

From the examination of the iodine numbers it will be seen that up to the last two fractions the unsaturation increases with the boiling point and molecular weight. The calculated iodine numbers of the methyl esters of the acids which may be present are: hexadecatrienoate, 288.5; clupanodonate, 350.1; arachidonate, 319.3; eicosapentenoate, 401.7; docosatetrenoate, 293.5; docosapentenoate, 369.0; docosahexenoate, 445.2. Fraction 2 when slowly distilled into 3 sub-fractions gave a fraction having an iodine number of 316.5 and a molecular weight for its acids of 266.8, which data indicate a mixture composed of $^2/_3$ methyl clupanodonate and $^1/_3$ methyl hexadecatrienoate. The data for Fraction 3 are very close to the theoretical values for methyl clupanodonate. The data for Fractions 6 and 7 indicate mixtures of methyl arachidonate and eicosapentenoate, while those for Fraction 9 indicate methyl docosatetrenoate, docosapentenoate and docosahexenoate.

The percentages of bromine in the polybromides of the various fraction show that the degree of unsaturation increases with an increase in the boiling point of the fraction and, therefore, with the molecular weight. One of the sub-fractions obtained from Fraction 2 and also Fraction 3 yielded bromides with 68.59 and 68.6% of bromine which are almost in exact agreement with the calculated value for methyl octobromostearate. This compound has not been previously prepared. It is a white amorphous solid which melts at 240°, uncorr.

The following percentage values for bromine content have been calculated: octobromostearate, 68.79; octobromo-arachidate, 66.78; decabromoarachidate, 71.66; octobromobehenate, 64.90; decabromobehenate, 69.90; dodecabromobehenate, 73.72. Fractions 6 and 7 would seem from this also to consist of methyl arachidonate and eicosapentenoate, while Fractions 9 and 10 are probably chiefly methyl docosapentenoate. Since Fraction 10 yields a bromide which contains more bromine than Fraction 9, the former undoubtedly contains some of the methyl docosahexenoate. This is the principal evidence which we have of the presence of this acid.

The polybromide numbers of the fractions furnish little evidence of the nature of the fatty acids. The very important fact which should be observed here is that they are only about $^1/_3$ of the theoretical value, which is always in the vicinity of 300.

On the basis of the analyses of Fraction 3, it is believed that this fraction is almost pure methyl clupanodonate. It is further believed that this represents the first time that a derivative of clupanodonic acid has been prepared in a reasonably pure state. The constants of methyl clupanodonate based on this sample are, iodine number (Wijs) 348.8; b. p. (15 mm.) 215°; n^{20}, 1.4860. The octobromide is described above.

The data in Table VII for cod and herring oils offer no evidence to contradict our conclusions based on the more complete distillation of the esters of menhaden oil, but rather confirm most of these data. The polybromide numbers of the mixed esters of the 3 oils are 105.3, 125.8 and 113.3, values which are about $^1/_3$ of those calculated.

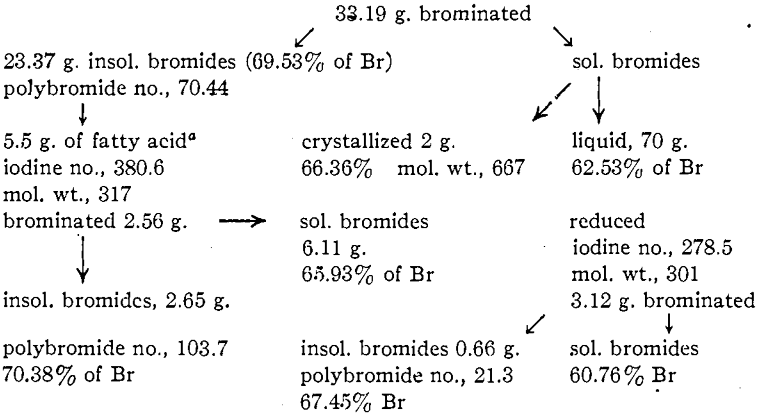
The Nature of the Bromination Reaction

A low yield of polybromides upon bromination of the pure, highly unsaturated esters of linolenic and linolic acids has been reported by Rollett[18] and Erdmann.[19] The most satisfactory explanation of the liquid bromides is that perhaps they are isomeric soluble bromides with correspondingly different properties. To furnish further light on this problem the following experiment was carried out. Starting with menhaden oil, the methyl esters were prepared and these reduced to the esters of the unsaturated acids. Then, under quantitative conditions, the supposedly pure unsaturated acids were prepared by saponification and brominated, and both the liquid and solid bromides weighed, reduced and again brominated. Suitable analyses were made at each step. The diagram, Table VIII, shows the result of this experiment. Although no final conclusion may be drawn from these data, the following suggestions may be made.

TABLE VIII

QUANTITATIVE BROMINATION AND REDUCTION OF MENHADEN OIL ESTERS

1900 g. of menhaden oil → 1337 g. of methyl esters → 500 g. of polybromides (68.62% of bromine) → 47 g. of methyl esters → 40 g. of fatty acids. Iodine number, 360.5. Molecular weight, 304.2



[a] The acid obtained here represents that from an additional 7 g. of solid bromides from the same point in a parallel experiment.

The liquid bromides seem to contain in general considerably less bromine than the solid. When reduced, the former give a product with an iodine

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[18] Rollett, *Z. physiol. Chem.*, **62**, 410, 421 (1909).

[19] Erdmann and Bedford, *Ber.*, **42**, 1324 (1909).

number considerably less than the original acid, while the molecular weight is only slightly less. These in turn yield only a small amount of solid bromides on bromination. The low bromine content of the liquid bromides and the decrease in iodine number suggest a change in the degree of unsaturation.

There seem to be four possibilities of change here. (1) One double bond may have been saturated during the reduction. (2) There may have been a rearrangement leaving one of the unsaturations in a position where it cannot be brominated, since it is known that an α-β unsaturation causes a reduction in the iodine number of an acid. (3) Oxidation may have occurred. (4) A double bond may have disappeared by ring formation as from a 1–5 di-ene grouping. The second and fourth possibilities are the most reasonable. In support of the second it has been noticed repeatedly in this Laboratory that at 0° bromine is not added to the unsaturated acids with the same ease as iodine monochloride or monobromide in attempts at the determination of the bromine absorption values of drying vegetable and fish oils. In support of the possibility of ring formation we may recall the fact that when the highly unsaturated esters of cod oil were accidentally overheated, there was a rapid lowering of the iodine and polybromide numbers with no change in molecular weight. Bromination might catalyze a similar change.

Whatever may be the answer to the question of the liquid bromides, apparently $^1/_3$ or less of the ester brominated goes to form the normal ether-insoluble bromides, while the other $^2/_3$ forms a product with 4 to 8% less bromine, which is soluble in ether. The liquid bromides on debromination yield a product from which a smaller proportion of polybromides may be obtained on rebromination in ether solution.

Presumably the material soluble in ether after bromination consists of two portions: the solid bromides which are dissolved in the ether solution of the liquid bromides, and the true liquid bromides, either isomeric with the others or formed from altered unsaturated acids.

Summary

The methyl, ethyl and *normal* butyl esters of the acids of menhaden oil and their bromination products have been prepared and analyzed. The presence of more highly unsaturated acids than clupanodonic is indicated.

The analysis of the methyl ester fractions from 5 fish oils indicates the presence of acids more highly unsaturated than clupanodonic.

By esterifying menhaden oil and fractionating the esters 6 times by distillation under diminished pressure, collecting 16 fractions, the presence of acids with 16, 18, 20 and 22 atoms of carbon is indicated.

The unsaturated acids obtained by the lead soap-ether and barium soap-

benzene separations have been esterified and fractionated and their bromine addition products analyzed.

The ether-insoluble bromine addition products of the unsaturated acids have been debrominated and fractionated and their bromine addition products analyzed.

We believe that we have demonstrated the presence in fish oils of myristic, palmitic, palmitolic (hexadecatrienoic), clupanodonic (octodecatetrenoic), arachidonic, eicosapentenoic, docosapentenoic and docosahexenoic acids.

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A NEW METHOD OF PREPARING DIBENZOYL ETHYLENE AND RELATED COMPOUNDS

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Unsaturated 1,4 diketones are of interest because they contain a very reactive grouping similar in many respects to that which is present in *para* quinones. In connection with our studies of the reversible and irreversible reductions of unsaturated compounds we desired to prepare dibenzoyl ethylene and some of its derivatives. Dibenzoyl ethylene has hitherto been prepared either by heating dibenzoyl malic acid[1] or by condensing benzoylformaldehyde and acetophenone.[2] Both of these methods involve expensive and somewhat difficult preparative steps and are not convenient for preparing such substances as ditoluyl ethylene. We endeavored, therefore, to find a more convenient and general method of preparation.

We were also led to a study of this question by our interest in a convenient method of preparing substituted 1,4 saturated diketones and from them pyrroles and furans. We are now investigating the addition reactions of dibenzoyl ethylene with such substances as acid chlorides and anhydrides and sodium malonic ester, and hope at some future time to report on the synthesis of pyrroles and furans through these reactions.

Dibenzoyl ethane was prepared some years ago by the action of succinyl chloride on benzene in the presence of aluminum chloride. By the use of fumaryl chloride in a similar Friedel and Crafts reaction we have been able to prepare dibenzoyl ethylene with good yields (60–75%).

$$\mathrm{ClOCHC{=}CHCOCl} + 2\mathrm{C_6H_6} \xrightarrow[(\mathrm{AlCl_3})]{} \mathrm{C_6H_5COCH{=}CHCOC_6} + 2\mathrm{HCl}.$$

By replacing benzene by chlorobenzene, toluene and mesitylene we have also prepared the corresponding derivatives of dibenzoyl ethylene. The

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[1] Paal and Schulze, *Ber.*, **33**, 3800 (1900).

[2] Smedley, *J. Chem. Soc.*, **95**, 219 (1909).