Ethane	Product	M. p., °C.	M. p. of oxime, °C.
Diphenyltribromo	Benzophenone	45	139
Ditolyltribromo	*p*-Dimethylbenzophenone	94[18]	162
Dianisyltribromo	*p*-Dimethoxybenzophenone	144[19]	
Diphenetyltribromo	Diethoxybenzophenone	130[20]	

Summary

1. Halogen-substituted aldehydes can be made to condense almost quantitatively with aromatic hydrocarbon or phenolic ethers in the presence of anhydrous aluminum chloride.

2. These condensation products in the presence of alcoholic potassium-hydroxide lose a molecule of halogen acid to form the ethylenic compound.

3. When the hydrocarbon-substituted dihalogen ethylene compounds are heated with sodium alcoholate in a sealed tube they are converted to disubstituted acetic acids.

Dianisyl- and diphenetyl-ethylenic compounds give substituted tolanes.

4. Moist silver oxide oxidizes the condensation products to benzoic acid or substituted benzoic acids.

5. Metallic sodium in boiling benzene converts the trihalogen condensation products into stilbenes and the ethylenic derivatives into tolanes.

6. Chromic acid oxidizes all compounds of this series to benzophenone or its derivatives.

Minneapolis, Minnesota

[Contribution from the Laboratory of Analytical Chemistry of the University of Illinois]

THE HIGHLY UNSATURATED FATTY ACIDS OF FISH OILS. II THE LIMIT OF UNSATURATION IN MENHADEN OIL[1]

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Introduction

Although the study of unsaturated fatty acids has interested investigators with many different ends in view, there has always been a difficulty in definitely characterizing the unsaturated acids present in a mixture. This difficulty becomes more and more marked as the unsaturation increases. The mutual solubilities of these acids, one in the other, and the similarity of chemical properties make them difficult, or impossible, of

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[18] Weiler, *Ber.*, **7**, 1183 (1874). Odor, *Ber.*, **12**, 2303 (1878). Goldschmidt, *Ber.*, **23**, 2747 (1889).

[19] Bösler, *Ber.*, **14**, 328 (1880).

[20] Gattermann, *Ber.*, **28**, 2871 (1894).

[1] Abstracted from a thesis presented by Rob Roy McGregor in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of the University of Illinois, 1926.

separation, and the best that can be done is to assume from the results of analysis the composition of the substance examined.

Hofstädter[2] has furnished the first indication of unsaturation in marine oils, reporting the presence of physetoleic acid, $C_{16}H_{30}O_2$, in walrus oil. Since then Ljubarsky,[3] Bull,[4] Tsujimoto,[5] Majima and Okada,[6] Brown and Beal,[7] Toyama[8] and many others have reported the presence of different unsaturated acids in fish oils. All the data point to the fact that the amount and number of double bonds increase with an increasing number of carbon atoms. The results obtained, however, are more or less desultory and provide little or no grounds for determining how far we may expect natural products to go in the matter of unsaturation. An attempt has been made to determine the highest unsaturation in each of the different series of fatty acids of the same number of carbon atoms in menhaden oil.

Method of Investigation

In the fractionation of the esters of the naturally occurring fatty acids it is impossible to separate sharply the compounds of differing carbon content. Thus the analysis of any particular fraction gives merely the mean molecular weight of all the acids contained therein, and any accurate statement as to the quantities of the acids of the different series present has been regarded as impossible. To avoid this difficulty it was decided to attempt to make use of Twitchell's mixed-melting-point method.[9] This method is based on the fact that when a fatty acid is mixed with one of higher melting point (in some definite proportion up to 15 or 20%) there is a drop in the melting point which is approximately the same in degree regardless of the kind of acid, the extent of the drop depending only on the amount of foreign acid added. Thus, by hydrogenating the acids of any particular fraction and submitting them to this method of analysis, the proportions of the different acids formed could be determined.

A somewhat similar difficulty arises in the determination of the extent of the unsaturation. The iodine number gives only the mean unsaturation of all the acids in the mixture and thus the presence of acids of very high unsaturation might be completely masked by the less unsaturated acids. To show the presence of acids of very high unsaturation the bromides were extracted with solvents that exerted a stronger solvent action on the compounds of lower bromine content. Analysis of the residue gave evidence of greater unsaturation than could be definitely proved otherwise.

[2] Hofstädter, *Ann.*, **91**, 177 (1854).

[3] Ljubarsky, *J. prakt. Chem.*, **57**, 26 (1898).

[4] (a) Bull, *Chem.-Ztg.*, **23**, 996 (1899); (b) *Ber.*, **39**, 3570 (1906).

[5] Tsujimoto, *J. Soc. Chem. Ind.*, **25**, 818 (1906).

[6] Majima and Okada, *Sci. Repts.*, Tohoku Imperial University, **3**, 1 (1914).

[7] Brown with Beal, THIS JOURNAL, **45**, 1289 (1923).

[8] Toyama, *C. A.*, **19**, 410 (1925).

[9] (a) Twitchell, *J. Ind. Eng. Chem.*, **6**, 564 (1914); (b) **9**, 581 (1917).

By the use of the two foregoing methods it was possible to determine the series present and the maximum unsaturation of that series.

Determination of Mixed Melting Points

For the mixed-melting-point determination behenic and arachidic acids were prepared as follows.

Behenic Acid.—Rapeseed oil was saponified with sodium hydroxide, the fatty acids liberated and washed and dissolved in hot alcohol. The erucic acid which separated upon cooling was esterified with absolute methyl alcohol and hydrogen chloride and the esters fractioned under 3 mm. pressure. The fraction which was collected between 194° and 196° was hydrogenated as described by Beal and Katti,[10] as follows:

Four hundred g. of nickel sulfate was dissolved in water and 250 g. of finely powdered diatomaceous earth stirred in. While the mixture was being vigorously stirred a solution of 200 g. of sodium carbonate was added. The precipitate was filtered off, washed with water until free from sulfates, dried at 105° and passed through a 100-mesh sieve.

Thirty-five g. of this catalyst was placed in a 3-necked flask fitted with a stirrer and while being constantly stirred was heated at 285–295° for four hours, a stream of hydrogen being passed through the flask meanwhile. The flask was cooled to 150° and 293 g. of the methyl erucate fraction described above was added. The temperature was raised to 190–200° and the passage of hydrogen and the stirring continued for six hours. The molten ester was filtered while hot and saponified with alcoholic potassium hydroxide. The alcohol was evaporated, the soap dissolved in water and decomposed with hydrochloric acid. The liberated acid was crystallized five times from equal parts of alcohol and acetone; m. p., 79.87°; mol. wt. by titration, 346.4 (calcd., 340).

Arachidic Acid.—Methyl erucate prepared as described in the preparation of behenic acid was used in this experiment.

Twenty and five-tenths g. of methyl erucate was saponified with alcoholic potassium hydroxide and the solution evaporated to dryness; 60 gms. of stick potassium hydroxide was fused in a nickel crucible in a Hoskins crucible furnace and the potassium erucate added. For a stirring rod was used a copper tube sealed at one end, in which was placed a thermometer and some paraffin to furnish thermal contact.

The melt was held at 260° for 90 minutes, during which time it became dark brown and considerable gas was evolved. The temperature then rose to 325°, when the material showed signs of charring. The crucible was removed from the furnace and the melt dissolved in water and acidified with sulfuric acid, then boiled to decompose the soaps, and chilled. The cake of acid was dissolved in chloroform and decolorized with Norit, then evaporated to dryness and dissolved in hot alcohol and allowed to crystallize. The first yield of crystals amounted to 64% and melted at 62°. After four crystallizations the product was again saponified to insure the absence of esters, again decomposed with sulfuric acid and the acid finally crystallized from acetone. The final product melted at 74.54° and had a molecular weight, by titration, of 312.1 (calcd., 312).

Palmitic acid obtained from Kahlbaum (m. p., 62.14°) was once recrystallized from 75% alcohol. It melted at 62.42° and had a neutral equivalent of 254.6 (calculated, 256).

Stearic acid obtained from Kahlbaum (m. p., 68.87°) was once recrystallized from 80% alcohol. Thus purified, the acid melted at 69.42° and had a neutral equivalent of 283.2 (calcd., 284).

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[10] Beal and Katti, *J. Am. Pharm. Assoc.*, **14,** 1094 (1925).

For the determination of the mixed melting points a thermometer having a range of 40° (40° to 80°) and calibrated in 0.1° was fixed by means of a cork into a test-tube of 3 cm. diameter. The test-tube was almost completely immersed in a beaker of water which was kept constantly stirred by a mechanical stirrer. For melting-point tubes, capillary tubes of 0.5 to 1 mm. diameter and open at both ends were used. The tubes were dipped into the melted fatty acids and afterwards by a violent jar the column of liquid in the tube was broken into a number of short segments which were allowed to solidify. The solidified acids were allowed to stand for at least 12 hours to remove strain before any melting points were taken. The tubes were then fixed to the thermometer by means of a rubber band and the temperature of the water-bath was raised at such a rate that for the last degree or two the increase of temperature in the air-bath was between 0.1° and 0.15° per minute. The melting-point tube was observed with a small hand lens and the point of disappearance of the last particle of solid material taken as the melting point. The thermometer reading was taken with the aid of a buret magnifier. A temperature correction was made for the emergent stem of the thermometer.

The four acids prepared were mixed with one another in two proportions, namely, 1 to 5 and 1 to 10. The acids were weighed into a small beaker, melted together and thoroughly mixed before being poured into a small test-tube. Melting-point samples were taken from the mixtures so obtained. The melting-point depressions obtained are recorded in Table I.

It will be noted that mixtures containing more than 10% of foreign acid show considerable variation when arachidic or behenic acid is mixed with stearic or palmitic. Accordingly, for purposes of calculation, the values obtained from the admixtures of smaller proportions of foreign acids were used. It was further found that arachidic acid gave abnormal depressions when mixed with unknown acids, but when the arachidic

TABLE I

MELTING-POINT DEPRESSIONS OBTAINED WITH MIXTURES OF PURE FATTY ACIDS IN KNOWN PROPORTIONS

0.1 g. of		0.5 g. of palmitic	Stearic	Arachidic	Behenic
Palmitic	M. p., °C.	62.40	66.18	71.25	76.46
	Lowering, °C.		3.24	3.29	3.41
Stearic	M. p., °C.	59.14	69.42	71.66	76.88
	Lowering, °C.	3.26		2.88	2.99
Arachidic	M. p., °C.	57.11	66.23	74.54	77.19
	Lowering, °C.	5.29	3.19		2.68
Behenic	M. p., °C.	57.61	64.09	71.07	79.87
	Lowering, °C.	4.79	5.33	3.47	

0.1 g. of		1.0 g. of palmitic	Stearic	Arachidic	Behenic
Palmitic	M. p., °C.	62.40	67.63	72.84	78.24
	Lowering, °C.		1.79	1.70	1.63
Stearic	M. p., °C.	60.68	69.42	73.09	78.23
	Lowering, °C.	1.72		1.45	1.64
Arachidic	M. p., °C.	60.48	67.83	74.54	78.49
	Lowering, °C.	1.92	1.59		1.38
Behenic	M. p., °C.	60.65	67.60	72.92	79.87
	Lowering, °C.	1.75	1.82	1.62	

acid was determined by difference there was a good agreement between the neutral equivalent found for the unknown acid by titration and that calculated from the amount of acids present as shown by the melting-point depression.

Preparation and Analysis of Esters

About 12 liters of menhaden oil was esterified according to the method of Haller as modified by Brown and Beal[7] and fractionally distilled. The distillation was carried out twice at 5 mm. pressure. Fractions 5, 6, 7, and 8 were distilled a third time, the pressure on the final distillation dropping to 3 mm. On account of this change of pressure the constants of these fractions showed a sharp break from those of the lower fractions. On the preliminary distillations all runs were carried to 210°, after which the small residues and high temperature required for distillation made it seem advisable to collect these residues and distil them together. Distillation was carried to about 240°, but above 230° the distillate proved to be a heavy, reddish liquid solidifying in the receiver, evidently a polymer of some description. The analytical constants for these fractions are given in Table II.

TABLE II

CONSTANTS OF THE ESTER FRACTIONS FROM MENHADEN OIL

Fraction	Pressure, mm.	B. p., °C.	I No. (Wijs)	Mean mol. wt.[a]	$n^{19.5}$	Free acid as stearic, %
1	5	-130	40.35	270.0	1.4493	0.00
2	5	130–140	32.74	232.4	1.4455	.47
3	5	140–150	56.70	245.7	1.4491	.30
4	5	150–160	80.33	257.2	1.4530	.38
5	3	160–170	146.3	277.2	1.4630	.75
6	3	170–180	244.9	291.8	1.4761	.78
7	3	180–190	325.0	305.6	1.4894	.66
8	3	190–200	348.8	319.5	1.4962	2.45
9	5	200–210	298.1	307.7	1.4895	..
10	5	210–220	316.2	321.5	1.4942	..
11	5	220–230	288.1	339.2	1.4964	..

[a] Calculated from the saponification number.

Hydrogenation of Esters

Esters from three different fractions were hydrogenated in alcoholic solution with the aid of a platinum catalyst as described in many papers by Adams and co-workers at Illinois. After the catalyst had been filtered off, potassium hydroxide was added and saponification of the esters effected while about two-thirds of the alcohol was being evaporated. The remaining solution was poured into warm 10% hydrochloric acid, upon which the acids rose to the top and were solidified by cooling. The solid acids were removed and washed with hot water to remove any mineral acid. They were then crystallized from a mixture of water and acetone, and the precaution was taken to cool the solution in an ice-bath before filtration. They were sucked dry on a Büchner funnel and finally dried in a vacuum desiccator over sulfuric acid for 24 hours. The hydrogenated acids had the following iodine numbers: fraction No. 5, 6.2; No. 7, 1.5; No. 8, 1.9.

The acids were mixed in definite proportions with the pure acids as previously described, all being melted together before making up the melting-point tubes. The melting-point depressions obtained are given in Table III.

TABLE III

MELTING-POINT DEPRESSIONS OBTAINED BY THE ADMIXTURE OF HYDROGENATED ACIDS FROM MENHADEN OIL WITH PURE SATURATED FATTY ACIDS

0.1 g. of	0.5 g. of palmitic	Stearic	Arachidic	Behenic
Fraction 5; m. p., °C.	59.68	68.36	72.02	...
Fraction 7; m. p., °C.	...	66.45	73.99	77.92
Fraction 8; m. p., °C.	...	64.56	71.99	79.65

0.1 g. of	1.0 g. of palmitic	Stearic	Arachidic	Behenic
Fraction 5; m. p., °C.	60.88	68.99	73.13	...
Fraction 7; m. p., °C.	...	67.85	74.29	78.88
Fraction 8; m. p., °C.	...	67.54	73.29	79.63

The method of calculation was as follows. In Fraction 5 the presence of 9.1% of the unknown in pure palmitic acid produced a lowering in the melting point of 1.52°. From Table I it is found that 1.72° lowering is produced in palmitic acid by 9.1% of stearic acid (of which this fraction is mostly composed). Thus a lowering of 1.52° indicates the presence of 8.0% of stearic acid (or foreign acid); therefore, acids *other than* palmitic in the added acids = (8.0 × 100)/9.1 = 87.9%. Therefore, palmitic acid = 100 − 87.9 = 12.1%.

In the same fraction the presence of 9.1% of the unknown in pure stearic acid produced a lowering in the melting point of 0.43°. From Table I it was found that 1.59° lowering is produced in stearic acid by 9.1% of arachidic acid (which is present in larger quantity than any of the other acids foreign to stearic). Then a lowering of 0.43° indicates the presence of 2.5% of foreign acid.

Therefore, acids *other than* stearic in the added acids = (2.5 × 1000)/9.1 = 27.5%. Therefore stearic acid = 100 − 27.5 = 72.5%. Attributing the difference, 15.4%, to arachidic acid we have a mixture of palmitic, 12.1%; stearic, 72.5%; arachidic, 15.4%. Such a mixture should give a neutral equivalent of 284.9. The value found by titration is 287.6.

Fraction 7 is apparently a mixture of stearic, 1.1%; arachidic, 70.3%; behenic, 28.6%. Such a mixture should give a neutral equivalent of 319.7. The value found by titration is 319.4.

Fraction 8 is apparently a mixture of arachidic, 8.6%; behenic, 91.4%. Such a mixture should give a neutral equivalent of 337.6. The value found by titration is 337.5. (In this fraction the lowering of the melting point was so small that it was considered that that obtained with the larger amount of unknown, although still containing little else than behenic acid, was more indicative of the actual content.)

The examination of the lower fractions proved to be useless as they had been fractionated only twice and contained, as shown by the bromine content of their bromides, quite appreciable quantities of stearic acid.

Differential Solvents

Having obtained a knowledge of the proportion of acids of the different series in the three fractions mentioned, the next problem was to separate the most highly unsaturated acids from those of lower unsaturation. The preparation of the insoluble bromides provides a very satisfactory preliminary separation, as the di- and tetrabromides are soluble in ether. Hartley[11] has shown that prolonged extraction with benzene will remove a certain amount of the lower bromides, but it was hoped to obtain a solvent that would be speedier and more effective. Four different solvents were tried—benzene, *n*-butyl alcohol, bromobenzene and benzyl alcohol.

One hundred g. of bromides from esters boiling at 200–210° (5 mm.) brominated in ether solution at —10° and thoroughly washed with ether, was refluxed with four successive 500cc. portions of ether and the solvent decanted each time. The colors of the extracts were, respectively, dark brown, light brown, light yellow and colorless. (Failure to make this preliminary extraction with ether resulted in the blackening of the product when it was extracted with the higher boiling solvents.) Twenty-g. portions of the residue from the ether extraction were then extracted with the different solvents, refluxing with successive portions of the solvents until no more color was extracted and the residue was white. Three extractions were generally sufficient. Only one portion of benzyl alcohol was used, as one digestion with this solvent left only enough residue to insure a proper analysis. The digestions were filtered hot in a hot water funnel, the filtrates cooled and the resulting precipitates filtered off and washed with ether to remove the heavier solvent. The residues were also freed from the heavier solvent by the use of ether. The products were then dried overnight at 100°. The product obtained by the cooling of the filtrate is designated "soluble," and the residue from the digestion "insoluble." Analysis of these different products gave the results shown in Table IV.

TABLE IV

ANALYSES FOR BROMINE

Solvent	Bromine in[a] "soluble," %	Bromine in "insoluble," %[a]
Ether-washed bromides	...	70.77
Benzene	70.92	71.16
n-Butyl alcohol	70.18	71.19
Bromobenzene	71.04	71.86
Benzyl alcohol	70.57	71.83

[a] For the determination of bromine see Brown and Beal.[7]

From Table IV it will be seen that although all four solvents effect a certain separation, bromobenzene and benzyl alcohol are the most effective.

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[11] Hartley, *J. Physiol.*, **38**, 353 (1909).

The latter two give very similar products, but bromobenzene was selected as the more desirable because the larger yield of insoluble bromide indicated a sharper separation and because of the fact that the presence of a considerable quantity of benzyl chloride in the benzyl alcohol made the latter a very unpleasant reagent to handle.

Accordingly an experiment similar to the above was run upon bromides obtained from the esters of Fraction 8, bromobenzene being used as the solvent. The following results were obtained: ether-washed bromides, 70.33% of bromine; bromobenzene insoluble, 71.99% of bromine. The product insoluble in bromobenzene appeared white when washed with ether, but on standing in the Büchner funnel it became gray and finally quite dark colored. However, upon evaporation of all the ether the material became light colored again and when ground in a mortar it appeared white with a light grayish cast.

Bromides from the esters of Fraction 7 were treated similarly with the following results: ether-washed bromides, 69.58% of bromine; bromobenzene insoluble, 71.46% bromine.

Fraction 6 was not brominated as it was evident that it would contain large amounts of both the stearic and arachidic acid series and no satisfactory conclusions would be possible through the analysis.

The bromides obtained from the esters of Fraction 5 were not extracted with bromobenzene as the solvent action of this compound was much too complete for the product on hand. The bromides contained 66.46% of bromine.

The three lower fractions were brominated but the polybromide number[12] was very low (9%, 4% and 1%) undoubtedly due to contamination by the highly unsaturated esters of the C_{18} series. The bromine values obtained were: Fraction 4, 67.19%; Fraction 3, 67.36%; Fraction 2, 67.05%.

Discussion

The very low yield of bromides obtained from the three lowest fractions makes it very improbable that the acids of the C_{14} and C_{16} series contain any acids of sufficiently high unsaturation to account for the percentage of bromine shown. The more probable explanation is that the bromides obtained are the result of contamination of the fraction with other acids. Esters that had been prepared before and that had been fractionated four times showed a much lower bromine content for the bromides. Esters boiling at 140–150° (5 mm.) furnished bromides with 63.85% of bromine. These figures point to the probability that the unsaturation in the C_{16} series does not surpass three double bonds. (Table V shows the maximum bromine content of the bromides that could possibly be obtained from the esters examined.) The bromides of the three lower fractions, containing

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[12] For a determination of the polybromide number see Brown and Beal, Ref. 7.

67.19, 67.36 and 67.05% bromine, if due to unsaturated acids of the C_{14} and C_{16} series, would require the presence of methyl octobromomyristate or methyl octobromopalmitate in the mixture. The low bromine content of the bromides from the more completely fractionated esters shows that no such acids can be present, and as far as the C_{16} series is concerned there remains only the possibility of a maximum unsaturation of three double bonds.

Again referring to the bromides prepared earlier, a bromine content of 63.85% in the lowest fraction precludes the possibility of the presence of acids of three or four double bonds, the bromides of which would give 67.02 and 73.21% of bromine, respectively. The bromides of one and two double bonds would be soluble in ether. The bromine content of 63,85% can then be most readily explained on the basis of contamination by acids of higher unsaturation from the C_{16} and C_{18} series. The polybromide number of this fraction was negligible, thus adding to the probability that the C_{14} series contains no acids of higher unsaturation than two double bonds, and the C_{16} series no acids of higher unsaturation than three double bonds.

An examination of the results obtained with Fraction 5 shows that it is composed of acids of the following series: C_{16}, 12.1%; C_{18}, 72.5%; C_{20}, 15.4%. The bromine content of the insoluble bromides was 66.46%. As by far the major part of the product belongs to the C_{18} series the only reasonable explanation is that the bromine content is due to a mixture of hexa- and octobromostearates, probably contaminated with a small amount of hexabromopalmitate and hexa-, octo- and decabromo-arachidate. (The proof for the absence of methyl dodecabromo-arachidate will be given later.) It is readily seen from Table V that there can be no possibility of the presence of any methyl decabromostearate.

TABLE V

BROMINE CONTENT OF BROMO-SUBSTITUTED FATTY ACIDS

Methyl ester	Br, %	Methyl ester	Br, %
Hexabromomyristate	67.02	Octobromo-arachidate	66.78
Octobromomyristate	73.21	Decabromo-arachidate	71.67
Hexabromopalmitate	64.49	Dodecabromo-arachidate	75.34
Octobromopalmitate	70.93	Hexabromobehenate	57.95
Hexabromostearate	62.15	Octobromobehenate	64.89
Octobromostearate	68.79	Decabromobehenate	69.91
Decabromostearate	73.51	Dodecabromobehenate	73.72
Hexabromo-arachidate	59.98		

The presence of this latter compound in any appreciable quantity, having a bromine content of 73.51%, would be bound to raise the bromine content of the mixed bromides higher than 66.46%. As this fraction contains both arachidic and palmitic acids it may be reasonably assumed that the C_{18} acids present show all the different degrees of unsaturation to be found in that series. Such a bromine content as has been found then points definitely to the presence of methyl hexa- and octobromostearates and the absence of methyl decabromostearate. In the C_{18} series then, the highest unsaturation is that of four double bonds.

Fraction 7 has been shown to consist of C_{18} acids, 1.1%; C_{20} acids, 70.3%; C_{22} acids, 28.6%. The bromine content of the insoluble bromides was 69.58%. This high bromine content, showing the presence of acids of more than four double bonds, suggested the possibility that unsaturation even higher than five double bonds might be present but masked by the preponderance of acids of less unsaturation. To decide this the bromides were extracted with bromobenzene as previously described. The residue was found to have a bromine content of 71.46%. Clearly, no methyl dodecabromo-arachidate could be present, as after the extraction of the lower bromides the bromine content would be expected to exceed that of the decabromo compound. Therefore, the highest unsaturation in the C_{20} series is 5 double bonds.

Fraction 8 has been shown to consist of C_{20} acids, 8.6%; C_{22} acids, 91.4%. The bromine content of the bromides was 70.33%. Extraction with bromobenzene raised the bromine content to 71.99%. Such a bromine content in this fraction is conclusive proof of the presence of methyl dodecabromobehenate.

We may conclude, therefore, that the maximum unsaturation to be found in menhaden oil is as follows: C_{14} acids, 2 double bonds; C_{16} acids, 3 double bonds; C_{18} acids, 4 double bonds; C_{20} acids, 5 double bonds; C_{22} acids, 6 double bonds.

Such a regular increase in the maximum unsaturation must have some underlying cause, and a reasonable explanation was looked for. As has been pointed out previously, the number of naturally occurring unsaturated acids that have been fully described is small and the exact configuration of many of these is still to be established. However, the natural acids of the C_{18} series having one, two, or three double bonds have been studied rather closely and it is interesting to note that in each instance the first double bond occurs in the 9,10 position, and that subsequent double bonds are added in that part of the molecule terminated by the methyl group. Should such a condition hold rigidly it would be impossible to conceive of a stable compound of the C_{18} series containing more than four double bonds, that is, assuming that no double bond occurs next to the terminal carbon atom, a condition which has not been observed in any natural fatty acid. The formula would appear,

$$HOOC—C—C—C—C—C—C—C—C{=}C—C{=}C—C{=}C—C{=}C—C—CH_3$$

Similarly, should this chain of nine carbon atoms hold intact in the C_{20} and C_{22} series the former could not contain more than five double bonds, nor the latter more than six. This is exactly what has been found. Bull's claim[13] to have prepared a bromide which indicates the presence in cod-liver oil of an acid of the C_{20} series having 6 double bonds would, of course, require a revision of the above hypothesis, but his statement has never been confirmed and should therefore be accepted with caution.

Attempts at Ozonation

In order to test the validity of the hypothesis just outlined it was decided to attempt to ozonize the unsaturated esters of one of the fractions. Decomposition of the ozonides should not provide aldehyde esters of less than ten carbon atoms, $CH_3OOC—(CH_2)_7—CHO$. It was planned to distil fractionally the decomposed ozonides and identify the fractions by the use of semicarbazine. Unfortunately, the very high unsaturation made it impossible to ozonize more than 50 g. in one run. As a result the yield was so small and the products were so numerous that three successive attempts failed to yield any satisfactory results. Armstrong's report[14] on the determination of the constitution of the natural unsaturated fatty acids described a method of determination of the position of the double bond by means of oxidation with potassium permanganate in acetone or acetic acid. He reports that examination of the C_{14}, C_{16} and C_{18} series of acids of whale oil by this method showed that the first double bond occurred at the 9,10 position in all cases. Oxidation of the acids of the C_{20} and C_{22} series showed no dibasic acid lower than azelaic. "In neither group (C_{20} or C_{22}) was there the slightest evidence of the occurrence of an ethylene linkage between the COOH group and the ninth carbon atom of the chain. . . .The present results established the fact that in whale oil acids, as in the majority of the vegetable oil acids, unsaturation does not commence before the 9,10 position except in special circumstances."

These findings tend to bear out the original assumption that the unsaturation of fatty acids from natural sources tends towards a definite maximum, that maximum being governed by the length of the fatty acid chain.

Summary

1. The methyl esters of menhaden oil have been fractionated, their constants determined, and the amounts of the different carbon chains present in some of the different fractions determined by Twitchell's method of hydrogenation and mixed-melting-point depression.
2. The ether-insoluble bromides of the highly unsaturated acids have been prepared and analyzed.
3. The bromides of the higher series have been extracted with bromobenzene and a partial separation has thus been made of the bromides of very high halogen content.

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[13] Bull, *Tid. Kemi Farm, Terapi*, **14**, 1 (1917).

[14] Armstrong and Hilditch, *J. Soc. Chem. Ind.*, **44**, 43T (1925); **44**, 180T (1925).

4. As a result of these determinations it has been established that in menhaden oil the highest unsaturation to be expected in the C_{14} series is 2 double bonds, in the C_{16} series, 3 double bonds, in the C_{18}, 4, in the C_{20}, 5 and in the C_{22}, 6.

5. In natural products, acids of higher unsaturation than indicated above are not likely to be found.

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[CONTRIBUTION FROM THE LABORATORY OF FOODS AND SANITATION, UNIVERSITY OF WISCONSIN]

MONACETIN[1]

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An attempt at experimental verification of the statement[2] that a water-soluble mixture of monacetin and diacetin is obtained when glycerol is esterified in the presence of sulfuric acid and sodium acetate has resulted in the development of a modification of that method to the end that the stage of esterification is confined to the formation of monacetin. Previously published statements on the preparation of the acetins are contradictory,[3-10] but Geitel[11] claims to have isolated all three of them, by means of solvent extraction and fractional distillation, from a reaction mixture which contained only glycerol and acetic acid, and Guédras[12] is reported to have obtained monacetin with the aid of casein as catalyst. By the substitution of phosphoric acid or its anhydride for sulfuric acid, we have been able to prepare in one operation practically pure monacetin, the experimental development of which is here outlined.

Experimental Part

Esterification in the Presence of Sulfuric Acid.—A mixture consisting of 180 g. of glycerol, 1080 g. of glacial acetic acid and 1.8 g. of sulfuric acid (66° Bé.) was heated for one and a half hours at 110°. About 10

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[1] This paper was constructed from a thesis submitted by Peter P. T. Sah to the Faculty of the Graduate School of the University of Wisconsin in partial fulfilment of the requirements for the degree of Master of Science, August 8, 1924.

[2] G. J. Esselen, Jr., U. S. Pat. 1,378,099, June 5, 1921.

[3] Berthelot, *Ann.*, **88**, 310 (1853).

[4] Würtz, *Ann.*, **102**, 339 (1857).

[5] Hübner and Müller, *Z. Chem.*, [II] **6**, 343 (1870); *Chem. Zentr.*, **41**, 450 (1870).

[6] Schmidt, *Ann.*, **200**, 99 (1879).

[7] Markownikoff, *Ber.*, **13**, 1842 (1880).

[8] Kabloukoff, *Bull. soc. chim.*, [II] **34**, 347 (1880).

[9] Böttinger, *Ann.*, **263**, 359 (1891).

[10] Seelig, *Ber.*, **24**, 3466 (1891).

[11] Geitel, *J. prakt. Chem.*, **163**, 417 (1897).

[12] Guédras, *Chem.-Ztg.*, **29**, 523 (1905).