The Chemical composition of tunny liver oil.

By

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Introduction.

In regard to the fatty acids of aquatic animal oils, much study has been made on their body fats, but comparatively little done on their liver oils. Concerning the study of the body fats of tunny (*Thunnus orientalis*) there is I. Okada's work, but no researches have been done yet on the fatty acids of its liver oil. Again, with reference to the vitamin-A of tunny liver oil, H. Sekine's work came to our attention years ago, but no determination has yet been made regarding its potency. Lately, Y. Kawakami has estimated by color reaction the quantities of vitamin-A contained in the liver oils of many kinds of fish, and reported that tunny liver oil contained 213.6 units, while cod liver oil, 2.7~7.9 units. The author has made researches on the fatty acids of tunny liver oil and estimated the vitamin-A content by means of biological test. Of course the quantities of these fatty acids vary owing to the factors, such as the seasons of the year, the ages and the sexes of the fish, but it would seem possible to obtain the general inference regarding the contents.

Experiment.

(I) The Preparations of Sample Oil and Mixed Fatty Acids, and Their General Properties.

The oil was obtained by grinding the liver which has passed a day in an ice box after catching the fish in spring time, making it dehydrated powder by mixing with anhydrous Na_2SO_4 , then extracting with petroleum ether (b.p. < 50°). The extract was made to be a constant weight, removing the solvent under the current of CO_2 . The oil thus obtained was of liquid form having yellowish brown color, and in winter, it separated a little quantity of solid fat. Its general properties are as shown in Table 1 below. In spring time, the liver contains a large quantity of oil, and the quantity obtained amounted to 26% of the fresh one. As its degree of unsaturation was high and its oxidation during the chemical treatment was considerable, special efforts have been made to perform the following treatments in dried CO_2 .

Table 1

Acid value	28.0	Reichert-Meissl value	2.2
Saponification value	177.6	Polenski value	0.5
Ester value	149.6	Specific gravity (15°C.)	0.924
Iodine value (Wijs' method)	175.6	Unsaponifiable matter	1.6%
Acetyl value	31.5	Melting point	28~29°C.
Hehner value	89.8		

The fatty oil was saponified by the usual method for about 40 minutes at 60°C., adding to it alcoholic caustic potash. After eliminating the unsaponifiable matter by ether, the saponified liquid was decomposed by H_2SO_4 , and the liberated fatty acids were transferred into ether and washed several times with water until free from mineral acid; then, they were dehydrated by anhydrous Na_2SO_4 and the solvent was removed under diminished pressure. The mixed fatty acids thus obtained were of red brown color, and at ordinary temperature they were solid. The general properties of mixed fatty acids and of the divisions separated into the liquid and the solid by the lead salt ether method are as given in the following Table 2.

Table 2

	Mixed fatty acid	Liquid fatty acid	Solid fatty acid
Solidification point	29.0°C.	—	
Melting point	33~35°C.	—	51~54°C.
Iodine value (Wijs)	191.5	240	12.7
Neutralization value	188.8	182.3	210.3
Mean molecular weight	297.0	307	266.8
Rhodan value		122	
Ether insoluble bromide		84.8%	

(II) Isolation of the Fatty Acids.

First of all, according to the Toyama's method, sodium salt of the highly unsaturated acid, which was soluble in acetone, was filtered from the insoluble salt of the mixed fatty acids; the insoluble part was dissolved by warming in a little quantity of 50% alcohol, and after neutralizing the excess of NaOH by acetic acid, the sodium salt was changed to the lead salt by pouring into the boiling 10% lead acetate; and was separated into two parts—the soluble and the insoluble in ether at ordinary temperature. Then, these parts were decomposed by 20% HCl and transferred as free fatty acids into the petroleum ether (b. p. <50°) and freeing from the solvent at low temperature under diminished pressure, each free fatty acid was prepared.

The quantities obtained from the mixed fatty acids were as follows:

(1) The highly unsaturated part, 36%, (2) The lowly unsaturated part, 33%, and (3) The saturated part, 31%. The degrees of the unsaturation and the average molecular weights, of the highly and the lowly unsaturated parts of the acids were as the following:

Table 3

	Highly unsaturated group	Lowly unsaturated group
Neutralization value	178.1	191.0
Mean molecular weight	315.2	293.8
Iodine value (Rosenmund und Kuhnheim's method)	356.0	155.5

(A) Researches of the Highly Unsaturated Part.

25 g. of the fatty acid were changed to its methyl ester under CO_2 atmosphere according to the Haller method, and it was fractionated by distilling in vacuo, and the results obtained were as given in Table 4.

Table 4

No. of fraction	Temp. of air bath	Distilling point		Yield	Iodine value	Ester value
		Temp. °C	Press. mm.			
1	190~200	120~144	0.4	2.2	169.6	200.8
2	200~215	160~173	1.0	4.2	272.4	184.5
3	220	185~193	1.5~2.0	2.8	339.0	176.4
4	227~230	197~205	2.0	6.0	362.1	168.5
5	235~240	205~210	2.0~2.5	7.0	274.0	164.7
Residue				small quantity		

In comparing the constants of each division in the foregoing table with the theoretical ones:—

	Iodine value	Ester value
$\text{C}_{18}\text{H}_{38-4}\text{O}_2\cdot\text{CH}_3$	172.7	190.9
$\text{C}_{18}\text{H}_{36-6}\text{O}_2\cdot\text{CH}_3$	260.8	192.2
$\text{C}_{20}\text{H}_{38-8}\text{O}_2\cdot\text{CH}_3$	319.3	176.4
$\text{C}_{22}\text{H}_{43-10}\text{O}_2\cdot\text{CH}_3$	369.0	163.1

It will be noted that about 60% of the highly unsaturated acids was clupanodonic acid $\text{C}_{22}\text{H}_{44-10}\text{O}_2$, and it is possible to infer that the rest was composed of the fatty acids, namely, $\text{C}_{20}\text{H}_{40-8}\text{O}_2$, $\text{C}_{18}\text{H}_{36-4}\text{O}_2$, ($\text{C}_{18}\text{H}_{38-4}\text{O}_2$?).

Furthermore, to ascertain these facts each division was changed to the reduced methyl ester ($C_nH_{2n-1}O_2-CH_3$), having them reduced in ether by hydrogen, using platinum black as the catalyser; the liberated acids ($C_nH_{2n}O_2$) were separated, the constants estimated, and the results obtained are as shown in Table 5.

Table 5

No. of fraction	Reduced methyl-esters				Freed reduced fatty acids			
	Saponification value		Melting point °C.		Saponification value		Melting point	
	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.
(1)	198.3	188.3 ($C_{18}H_{35}O_2-CH_3$)	26~27	38	195.2	197.3	58	69.3
(2)	180.3		36~37	46~47	178.0		67~68	
(3)	172.5	172.0 ($C_{20}H_{41}O_2-CH_3$)	41~42		173.5	179.8	69~70	75
(4)	167.0		45~46		167.5		74~75	
(5)	160.9	158.5 ($C_{22}H_{43}O_2-CH_3$)	47~48	53~54	194.0	165.0	79~80	79~80

To elucidate—it is inferable that the division (1), the divisions (2) and (3), and the divisions (4) and (5) were composed of the unsaturated acids corresponding to stearic acid, arachidic acid, and behenic acid respectively.

(B) Researches of the Lowly Unsaturated Part.

(a) By the method of oxidation.

20 g. of the fatty acids were changed by Hazura's oxidizing method to oxidized acids using 3/4 % $KMnO_4$. These acids were extracted with petroleum ether and the unchanged fatty acids eliminated, and the quantity of the oxidized acids thus obtained was found to be about 10 g. This was again extracted for 20 hours with ether and the soluble part was separated from the insoluble.

(1) The soluble part in ether:— The quantity obtained was 4.5 g.; recrystallized twice from 90% alcohol and once from 95 % alcohol; mp. ~~148~~¹⁴³°~119°C.; no change of m. p. in recrystallizing from ethyl acetate; the crystal was flat irregular hexagonal; the neutralization value of the acids were 175.7~177.6 (The value as dihydroxy-stearic acid was 177.3).

(2) The insoluble part in ether:— Boiled 8 times in a liter of water for 1~3 hours each. The quantity of the soluble part in hot water was 1.7g., and that of the insoluble part 1.0 g. The former contracted at 120°C., and melted at 166~170°C., and a large part of it dissolved in 90 % alcohol. In crystallizing it formed flat hexagonal crystals; mp. 129~130°C.; the neu-

tralizing point 175.0. The latter is soluble in hot 90 % alcohol and when recrystallized from 95% alcohol, ethyl acetate, m.p. became definite at 119° C; the neutralization value was 174.6. Viz. even though the insoluble part had been extracted for 20 hours with ether, dihydroxy stearic acid still remaining was a large part of it, and tetra hydroxy and hexahydroxy stearic acids might have been present there but the quantities of these acids were so small that their further examinations could not be carried out.

(b) By the method of bromination.

4.2 g. of the fatty acids were treated by the usual method and the bromides were obtained.

(1) The insoluble bromides in ether:— The quantity obtained was 0.5 g.; the content of bromine in the insoluble part in hot benzene was 76.0 % and in the soluble part 71.0 % (The theoretical percentage as $C_{22}H_{34}O_2Br_{10}$ was 70.76%). Thus, it is readily seen that it was the decabromide of clupanodonic acid.

(2) The soluble bromides in ether:— Eliminating the excess of bromine and ether, and in extracting it with petroleum ether, a part became white precipitate. Besides, there was the coating of gelatinous substance on the vessel. The quantity obtained was small; the precipitate white, mp. 113° ~ 115°C.; perhaps, this part might have been the tetrabromides of linolic acid and isolinolic acid.

(3) The soluble part in ether and petroleum ether:— The quantity obtained was 4.2 g. The bromine content was 39 %; the neutralization value was 120; (the theoretical value as the dibromide of oleic acid $C_{18}H_{32}O_2Br_2$ —36.18 % Br; the neutralization value 126.9). It is clear that the greater part was the dibromide, though still mixed with tetrabromide.

From the foregoing results, it is seen that a large part of the division of this lowly unsaturated fatty acids was composed of oleic acid, small quantities of linolic acid and the highly unsaturated acids.

(c) Researches of the Saturated Fatty Acids.

20 g. of the fatty acids were made to be their methyl esters by the same method as that used for the division (A), and by distilling twice at the different temperatures under diminished pressure, the divisions obtained are as given in Table 6.

Table 6

The 1 st distillation	The 2 nd distillation					
	Temp. of air bath	Distilling point		Yield	Melting point°C.	Neutralization value
		Temp.	Press. mm.			
I fraction { 0.8 mm. 136~147°C.	(1) 160	<140	2.0	0.4	16~19	228.5
	(2) 170	146~150	2.0	1.4	18~20	223.7
	(3) 173~190	150~160	2.6	1.0	19~22	216.6
	(4) >190	160	3.0	1.7	26~27	206.7

Myristic acid
theor. 231.8

II fraction 0.8 mm, 150~155°C.	(1) 175	130	1.0	1.3	23~24	208.7	} Palmitic acid theor. 207.8
	(2) 180~190	143~148	1.0	4.0	23~24	204.0	
	(3) 230	155	1.0	0.3	24	207.6	
	(4)	Residue		0.8		195.1	
III fraction 1.0 mm, 160~168°C.	(1) 183	<140	2.0	0.6	24	202.3	} Stearic acid theor. 188.3
	(2) 190	145~150	2.0	0.5	24	205.3	
	(3) 190~200	155~160	2.0	0.4	27~28	199.1	
	(4) 205	165~168	2.0	1.2	27~32	194.2	
	(5)	Residue		1.7	31~33	186.1	
IV fraction				Small quantity			

From these divisions the liberated fatty acids were prepared, and the results determined of their melting points and neutralization values were as follows:

Table 7

No. of fraction	Melting point °C.		Neutralization value	
	Exper.	theor.	Exper.	theor.
I {	(1) 45~50	53.7	242.6	} 245.8 Myristic acid C ₁₄ H ₂₈ O ₂
	(2) 47		235.5	
	(3) 61~62		220.8	
	(4) 58~59		217.5	
II {	(1) 61~62	62.5	217.3	} 218.9 Palmitic acid C ₁₆ H ₃₂ O ₂
	(2) 62~63		215.1	
	(3) 57~58		213.5	
	(4) 57~60		198.0	
III {	(1) —	72.0	—	197.3 Stearic acid C ₁₈ H ₃₆ O ₂
	(2) 55~56		211.5	
	(3) 55~56		206.5	
	(4) 68~69		199.0	
	(5) 68~69		195.0	

(III) The Vitamin-A Potency of Tunny Liver Oil.

Albino rats weighing about 40 g. were fed with the vitamin-A free diet and, when their weight diminished and xerophthalmia appeared, the tunny liver oil mixed at the rate of 20 % with olive oil was given to them through the mouth. The sample oil was prepared in the manner as follows; the fresh liver of tunny caught in February was dehydrated by anhydrous Na₂SO₄ and was extracted with ether at low temperature. As shown in the diagram in case 0.2 mg. or more of the Biosterin was given to each of the animals per day, they were perfectly cured, while the tunny liver oil showed its complete curative power when 9 mg. or more of it was given.

Considering from the fact that relatively large amount of the Biosterin was required at the present test, it is inferrable that the curative power of the tunny liver oil is approximate to that of a commercial cod liver oil. This may also be verified from the fact that its color reaction by antimony

trichloride is about the same as that of the Shimoda's cod liver oil on market.

Summary

(1) The isolation and determination of fatty acids of tunny liver oil were performed, and further the examination of the curative value of its vitamin-A was carried out.

(2) The fatty acids contents of the liver oil, collected in spring, were about as follows:

Oleic acid	30%	Clupanodonic acid	22%
Arachidonic acid	20%	Palmitic acid	19%
Stearic acid	7%	Myristic acid	5%
Small amount of Linolic acid,			

(3) The curative power of the vitamin of this oil was determined by biological test, and 9 mg. of the oil per day for one albino rat was found to be sufficient.

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