FATTY OILS OF AQUATIC INVERTEBRATES

XIII. FAT FROM HORSESHOE-CRAB, *TACHYPLEUS TRIDENTATUS* LEACH

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(Received May 22, 1956)

Horseshoe-crab, *Tachypleus tridentatus* Leach (*Limulus longispina* Hoeven) is distributed in the Inland Sea of Seto and around the coast of Kyushu. Differing from common crabs, this animal belongs not to the class Crustacea but to the order Xiphosura in the class Xiphosura. No literature pertaining to the fat of Japanese horseshoe-crab has been known to us, but the presence of cholesterol and a sterol fraction of m.p. 137°–140°C (its acetate, m.p. 127°–129°C, $[\alpha]_D^{26} = -39°$) in the sterol components of *Limulus polyphenus*,[1] a species of the same genus with Japanese horseshoe-crab, was reported.

This paper records the results of our study on the fats extracted from two horseshoe-crabs, male and female. The female had a remarkably larger body size than the male, but the weights of dried material obtained from the raw material after removal of the crust and tail were nearly same for both animals, male and female. The yield of fat and the characteristics of fat were considerably different for the male and female. It is a characteristic feature of horseshoe-crab fats that they have relatively low iodine values as compared with common aquatic animal oils; the fat from the female showed an exceedingly low iodine value of 73.9. The compositions of polyethenoid acids of horseshoe-crab fats were estimated by ultraviolet absorption measurements of the alkali-isomerized fatty acids with the results that like the case with many aquatic animal oils the polyethenoid acids of horseshoe-crab fats contain pentaenoic acids in the largest proportion and some hexaenoic acids as the most highly unsaturated acids. The unsaponifiable matter of horseshoe-crab fats consisted mainly of sterols and alcohols of batyl and selachyl series. The sterol components were found to consist chiefly of Δ^5-sterols, in which the presence of cholesterol and possibly also Δ^5-sterols having two isolated double bonds was indicated.

Experimental

1. Fats

Two horseshoe-crabs, male and female, used in this study were received by the courtesy of Mr. A. Murakami, the Kasaoka Branch of the Naikai Regional Fisheries Research Laboratory, in early September, 1954. The living animals were killed in hot water. The crust and tail were removed, and the remainder of body was cut into small pieces and dried at about 70°C. The dried material was ground to fine meals and then extracted with ether. The ether-extract (lipid) was refluxed

with about tenfold acetone, the mixture was cooled to room temperature, the acetone-insoluble matter was removed, and the acetone-soluble fat was obtained after distilling off acetone from the acetone solution. Some data on the weight of animal, the yield of fat and the properties of fat are shown in Table 1.

TABLE 1. Fats of Horseshoe-Crabs

	Female	Male
Weight of living animal (g)	1,800	1,020
Dried material, after removal of crust and tail (g)	178	175
Ether-extract (g)	28.6	37.6
Ether-extract (%)	16.1	21.5
Acetone-soluble fat (g)	17.2	22.5
Acetone-soluble fat (%)	60.1	59.8
Appearance at ordinary temperature	Dark orange-yellow liquid with some solid	Dark orange-red liquid with some solid
d_4^{20}	—	0.9011
n_D^{20}	—	1.4653
Acid value	11.3	7.0
Saponification value	167.2	173.4
Iodine value	73.9	98.8
Unsaponifiable matter (%)	23.03	14.88
Sterol in Unsap. M. (%)	30.6	21.4

Notes: Percentage yield of ether-extract is expressed on the basis of dried material. Percentage yield of acetone-soluble fat is expressed on the basis of ether-extract. Unless stated otherwise, iodine values were determined by the Wijs method for fats and fatty acid components and by the pyridine sulfate dibromide method for unsaponifiable components.

2. Fatty acids

The fatty acids and unsaponifiable matter were separated by saponification of the fat followed by extraction of the diluted soap solution with ether in the usual way. The fatty acids solidified at ordinary temperature and had the characteristics shown in Table 2.

TABLE 2. Fatty Acids

	From the female	From the male
n_D^{30}	1.4562	1.4620
Neutr. value	195.1	195.7
Iodine value	83.4	108.1
Solid acids (lead salt ethanol method)(%)	32.5	30.8
Neutr. value	205.3	201.7
Iodine value	12.3	15.6

The fatty acids were alkali-isomerized under the condition of 21% KOH-glycol, 180°C and 15 minutes with nitrogen, and the compositions of polyethenoid acids[2] were estimated from the observed data as shown in Table 3.

The methyl esters (S.V. 186.6, I.V. 103.1) prepared from the fatty acids of the female were fractionated with the results shown in Table 4.

TABLE 3. Composition of Polyethenoid Acids

Polyethenoid acid	Wave length at the max. absorption (mμ)	Fatty acids			
		From the female		From the male	
		Spec. extinc. coeff.	(%)	Spec. extinc. coeff.	(%)
Hexaenoic ·····	376	0.44	1.50	0.77	2.63
Pentaenoic ·····	348	1.79	2.73	4.35	7.20
Tetraenoic ·····	316	3.24	2.06	6.55	2.79
Trienoic ·····	270	4.55	1.73	6.76	0.91
Dienoic ·····	235	4.94	1.65	6.05	0.38

TABLE 4. Fractionation of the Methyl Esters of Fatty Acids from the Female

Fraction	Yield		b.p. (°C/5 mm Hg)	n_D^{15}	S.V.	I.V.
	(g)	(%)				
1	0.60	4.5	–150	1.4419	219.2	40.1
2	1.07	6.2	150–155	1.4422	215.6	45.0
3	1.64	9.5	155–160	1.4440	207.9	50.1
4	1.40	8.1	160–165	1.4478	199.9	60.3
5	2.62	15.2	165–170	1.4487	195.6	72.5
6	1.94	11.3	170–175	1.4523	191.7	88.7
7	2.03	11.8	175–180	1.4560	185.4	109.3
8	1.38	8.0	180–185	1.4600	179.9	134.5
9	2.10	12.2	185–190	1.4608	172.9	167.0
10	1.01	5.9	190–195	1.4681	160.8	190.9
Residue (diff.)	1.41	8.2	—	—	127.8	137.6

The solid acids obtained from the fraction 3 in Table 4 by the lead salt ethanol method were recrystallized from ethanol, yielding a crystalline acid which had m.p. 62°C and showed no depression of melting point when mixed with a pure specimen of palmitic acid (m.p. 62°C).

The liquid acids obtained from the fraction 5 by the lead salt ethanol method were converted to the lithium salts, and the latter were recrystallized from 50% ethanol. Acidification of the recrystallized lithium salts gave a liquid acid fraction of n_D^{20} 1.4628, Neutr. V. 197.2 and I.V. 91.0 (calcd. for oleic acid: Neutr. V. 198.6, I.V. 89.8). Oxidation of this liquid acid fraction by Hazura's method yielded a product which, after purification by recrystallization, had m.p. 134°C and showed no depression of melting point when mixed with a pure specimen of dihydroxystearic acid (m.p. 134°C).

The fatty acids from the fractions, 9 and 10 united, were treated with urea in methanol. The urea adducts of saturated and less unsaturated acids separated from the solution were removed by filtration. From the filtrate, a highly unsaturated acid fraction of Neutr. V. 176.0 and I.V. 352.5 was obtained.

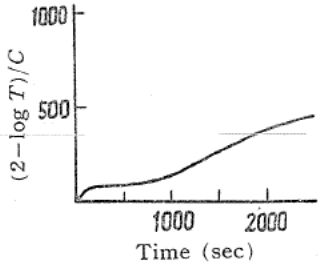
The residue in Table 4 was apparently contaminated with some unsaponifiable matter and lipid components which had not been completely removed from the original fatty acids. The content of unsaponifiable matter in the residue was found to be about 18%.

3. Unsaponifiable matter

The unsaponifiable fractions obtained from the fats of the male and female were united, and the united material (5.8 g) was fractionally crystallized from

methanol, giving three fractions: the first crop (I), the second crop (II) and the fraction from the last filtrate (III).

The fraction I (0.82 g), m.p. 135°–141°C, was acetylated, giving an acetate fraction of m.p. 118°–123°C and $[\alpha]_D^{17} = -40.3°$. Liebermann-Burchard reaction for this acetate gave a curve (Fig. 1) showing absorption at 620 mμ vs. reaction period, which lies close to the curve for a typical steryl acetate of the Δ^5-series. The melting point of this acetate fraction was raised by further recrystallizations. After three recrystallizations from methanol and acetone, it gave a fraction (0.06 g) which had m.p. 126°–127°C, $[\alpha]_D^{15} = -41.5°$ and I.V. 80.9. The ultraviolet absorption measurement indicated the absence of $\Delta^{5,7}$-dienoic component in this fraction. This fraction appears to be a mixture of steryl acetates of the Δ^5-series containing one and two isolated double bonds. The acetate mixture in the mother liquor of recrystallizations was recovered and fractionally crystallized, giving a fraction (0.26 g) of m.p. 114°–115°C, $[\alpha]_D^{17} = -41.0°$, S.V. 129.5 and I.V. 60.2 (calcd. for cholesteryl acetate: S.V. 130.9, I.V. 59.2). Saponification of this fraction gave a free sterol which, after recrystallization from methanol, had m.p. 144°–146°C and $[\alpha]_D^{18} = -38°$. Its benzoate melted at 146°C to a turbid liquid and became clear at 175°C.



T: Transmittance, C: Concentration (10^{-3} mole).

FIG. 1. Absorption at 620 mμ vs. reaction period in Liebermann-Burchard reaction for the acetate of the sterol fraction I.

The fraction II (2.38 g) was acetylated. The acetate mixture (S.V. 232.0) was recrystallized from methanol. After removal of the solid crystallized out by filtration, a soft solid mixture was recovered from the methanol filtrate. This mixture had n_D^{30} 1.4511 and S.V. 270.9 (calcd. for batyl acetate, S.V. 261.8). Saponification of this mixture and recrystallization of the saponification product from methanol-hexane yielded a crystalline substance of m.p. 70°C which showed no depression of melting point when mixed with batyl alcohol (m.p. 70°C).

The fraction III (2.38 g) was acetylated, and the acetylation product was subjected to the chromatographic fractionation using a column of silica gel, hexane as developer and hexane-ether as eluant. After the steryl acetate and batyl acetate fractions had been eluted, the last eluate gave an acetate (0.47 g) which had n_D^{20} 1.4581, S.V. 250.7 and I.V. 62.7 and was considered to consist chiefly of selachyl acetate (calcd., S.V. 263.0 and I.V. 59.5).

Summary

Characteristics of the fats extracted from two horseshoe-crabs, *Tachypleus tridentatus* Leach, male and female, were determined. Although the characteristics of fat were not alike for the male and female, both fats had low iodine values as

compared with ordinary aquatic animal oils; the fat from the female had an exceedingly low iodine value of 73.9.

Among the polyethenoid acids in fatty acid components, the proportion of pentaenoic acids was found to be the largest. The presence of hexaenoic acids as the most highly unsaturated components was indicated. Palmitic acid among saturated components and oleic acid among monoethenoid components were identified.

Sterol components consisted of cholesterol and other Δ^5-sterols including those having two isolated double bonds. Besides sterols, batyl alcohol and possibly selachyl alcohol were found in the unsaponifiable components.

References

1) W. Bergmann, M. J. McLean and D. Lester: *J. Org. Chem.* 8, 271 (1943).
2) E. G. Hammond and W. O. Lundberg: *J. Am. Oil Chem. Soc.* **30**, 433 (1953).