RESEARCH REPORTS

FATTY OILS OF AQUATIC INVERTEBRATES

XV. FATTY OILS OF *PALAEMON NIPPONENSIS* AND *CAMBARUS CLARKII*

TORU TAKAGI and YOSHIYUKI TOYAMA

Department of Applied Chemistry

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Oils of *Crustacea* have hitherto been studied in some measure. Particularly, studies on the liver oil of *Paralithodes camtschatica* have frequently been reported in this country. Tsujimoto[1] separated kanyl alcohol, $C_{10}H_{18}O_2$, together with cholesterol, batyl alcohol and selachyl alcohol from the unsaponifiable matter of this oil. But Nakamiya[2] could not obtain a uniform component corresponding to kanyl alcohol by the fractional distillation of the liquid fraction of unsaponifiable matter. Recently Abe[3] separated two lower homologues, $C_{17}H_{36}O_3$ and $C_{15}H_{32}O_3$, of batyl and chimyl alcohols from this oil. Oils from some other species of the *Crustacea*, such as *Chionoecetes opilio*,[4] *Erimacrus isenbeckii*,[5] *Birgus latro*,[6][7] and *Leander serratis*[8] have also been reported. However, oils from fresh water *Crustacea* appear to have been little studied.

In this study, fatty oils were extracted from two species of fresh water lobster, *Palaemon nipponensis* de Haan and *Cambarus clarkii* (Girard), the characteristics of both oils were determined, and the fatty acids and unsaponifiable matter were examined with a particular reference to dienoic acids in the fatty acids and sterols in the unsaponifiable matter. Considering that these oils belong to aquatic animal oils, they had comparatively low iodine values (Table 1). Fractional distillation of the methyl esters from both oils (Tables 3 and 4) indicated that the fatty acids of both oils contain acids of C_{18} and lower than C_{18} in a relatively large proportion, amounting to over 60% in the case of *P. nipponensis* and possibly over 40% in the case of *C. clarkii*. The compositions of polyethenoid acids in both oils were calculated from the ultraviolet absorption data of alkali-isomerized fatty acids as shown in Table 2. It is seen that although the polyethenoid acids of these two oils, like those of common marine animal oils, contain various acids of different unsaturation including dienoic to hexaenoic acids, the relative proportion of these polyethenoid acids shows some peculiar features as compared with that for common marine animal oils. Namely, while the polyethenoid acids in most marine animal oils contain pentaenoic acid in the largest proportion and dienoic and trienoic acids in a remarkably smaller proportion, the polyethenoid acids of these two oils contain dienoic and trienoic acids in a relatively large proportion. Thus the polyethenoid acids of *P. nipponensis* contain dienoic acid and pentaenoic acid in nearly same

proportion. In the case of *C. clarkii*, the proportion of dienoic acid is even larger than that of pentaenoic acid.

As for dienoic acids of aquatic animal oils, linoleic acid, though in a minor amount, was identified in the fatty oil of Formosan fish, *Chanos chanos*.[9] The presence of 11, 14-eicosadienoic acid and 11, 14-docosadienoic acid in the liver oil of shark, *Carcharodon carcharias*,[10] the former also in Cambodian fish oil,[11] was reported. Also 17, 20-hexacosadienoic acid was reported to occur in the oil of sponge, *Spheciospongia vesparia*.[12] In this study, the fatty acids of a methyl ester fraction (b.p. 170°–185°C/ca. 5 mmHg) from the oil of *P. nipponensis* were fractionated by way of urea adducts, and a fatty acid fraction containing a relatively large proportion of dienoic acid was separated. This fraction was brominated, and the fractionation of the bromination product gave a crystalline bromide which, though not yet a uniform bromide, was considered to consist substantially of tetrabromostearic acid derived from linoleic acid by its melting point and Br-content. The fatty acids of a methyl ester fraction (b.p. 180°–190°C/ca. 5 mmHg) from the oil of *C. clarkii* gave, after a similar treatment, a crystalline bromide consisting chiefly of terabromostearic acid derived from linoleic acid. A fatty acid fraction rich in dienoic acid was also separated from a methyl ester fraction (b.p. above 195°C/ca. 5 mmHg) from the oil of *C. clarkii*. Since this fraction was found to consist mainly of C_{20} acid by the characteristics of its hydrogenation product, it appears that dienoic acid in this fraction contains C_{20} acid.

Sterols from both oils were found to contain cholesterol as a major component. Ultraviolet absorption spectra of the unsaponifiable matter from both oils indicated that $\Delta^{5,7}$-sterol was not present in a detectable amount.

Experimental

1. Fatty oils. The fresh water lobsters, *P. nipponensis* and *C. clarkii*, used in this study were alive and caught around Shimonoisshiki, Nagoya City in early November, 1955. The living animals were killed in boiling water and then dried at about 80°C in a vacuum oven. The dried material was crushed into small pieces and then extracted with ether. The ether-extract was refluxed with about tenfold acetone, the mixture was cooled to ordinary temperature, and the acetone-insoluble matter was removed by filtration. The acetone-soluble oil (fatty oil) was recovered

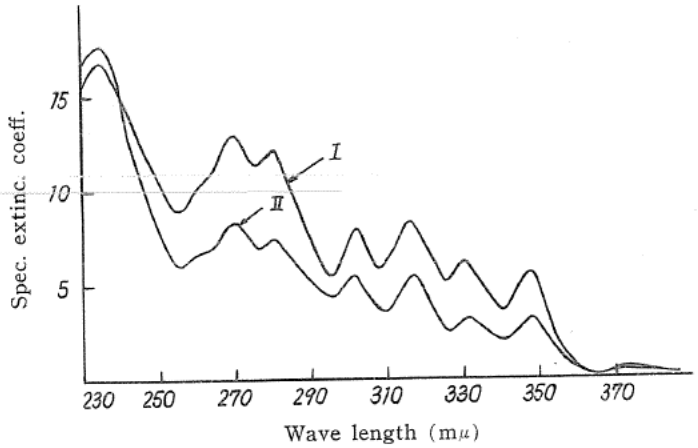
TABLE 1. Yields and Characteristics of Fatty Oils

	P. nipponensis	*C. clarkii*
Wt. of living material (g)	1,850	4,050
Number of living material	525	462
Weight of dried material (g)	439	1,150
Ether-extract (g)	37.5	44.0
Ether-extract (%)	8.5	3.8
Acetone-soluble oil (Fatty oil) (g)	29.2	36.5
Acetone-soluble oil (Fatty oil) (%)	77.9	85.0
Characteristics of fatty oil		
d_4^{30}	0.9107	0.9068
n_D^{20}	1.4723	1.4703
Acid value	27.9	60.0
Saponification value	176.6	173.3
Iodine value (Wijs)	124.0	107.9
Unsaponifiable matter (%)	9.65	11.07

Notes: Percentage yield of ether-extract is expressed on the basis of dried material. Percentage yield of acetone-soluble oil is expressed on the basis of ether-extract.

from the filtrate. The fatty oils thus obtained were reddish orange and solidified at room temperature in winter. The yields and characteristics of fatty oils are shown in Table 1.

2. Fatty acids. The fatty acids obtained by removing the unsaponifiable matter from the oils in the usual way were reddish orange at room temperature in winter. The characteristics of fatty acids are shown in Table 2. The saturated acids (%) in the total fatty acids and their neutralization value were calculated from the saturated methyl esters (%) in the total fatty acid methyl esters, determined by the acetone-permanganate method, and their saponification value. The fatty acids were isomerized under the condition of 21% KOH-glycol, 180°C and 15 minutes with a current of nitrogen, the ultraviolet absorptions (Fig. 1) of the alkali-isomerized product were measured, and the polyethenoid acids were estimated by applying the formula given by Hammond and Lundberg.[13] Since the figures showing the percentage of each polyethenoid acid, excepting hexaenoic acid, differ markedly according to whether the pentaenoic acid is assumed to be C_{20} or C_{22}, the average of both cases is given in Table 2.



I: *P. nipponensis.* II: *C. clarkii*

FIG. 1. Ultraviolet absorption curve of alkali-isomerized fatty acids.

TABLE 2. Fatty Acids

	P. nipponensis	*C. clarkii*
d_4^{30}	0.8900	0.8876
n_D^{30}	1.4612	1.4587
Neutralization value	194.0	195.8
Iodine value	127.3	112.1
Saturated acids (%)	23.8	25.4
Neutr. value of sat. acids	214.6	214.9
Polyethenoid acids (%)		
Dienoic	8.3	12.9
Trienoic	7.3	4.2
Tetraenoic	4.0	3.3
Pentaenoic	8.2	4.2
Hexaenoic	1.5	1.9

The methyl esters prepared from the fatty acids, contaminated with a little unsaponifiable matter, were fractionally distilled with the results shown in Tables 3 and 4.

TABLE 3. Fractional Distillation of the Methyl Esters of Fatty Acids of *P. nipponensis*

Fraction	Yield		b.p.	n_D^{20}	Sap. V.	Iodine V.
	(g)	(%)	(°C/ca. 5 mmHg)			
1	0.70	3.7	-160	1.4512	214.8	68.1
2	2.28	12.1	160-165	1.4524	208.2	77.3
3	2.94	15.5	165-170	1.4556	201.0	95.7
4	4.10	21.7	170-175	1.4585	195.2	101.0
5	3.05	16.1	175-180	1.4612	190.1	115.3
6	2.35	12.4	180-185	1.4667	181.4	159.1
7	0.99	5.2	185-195	1.4728	176.3	183.4
8	0.62	3.3	195-	1.4755	173.1	229.2
Residue (Diff.)	1.90	10.0	—	—	—	—

Notes: The methyl esters of fatty acids had S.V. 184.7 and I.V. 120.8. The distillation residue had S.V. 103.5, but the hexane-soluble fatty acids obtained from the residue after removal of unsaponifiable matter showed Neutr. V. 147.2.

TABLE 4. Fractional Distillation of the Methyl Esters of Fatty Acids from *C. clarkii*

Fraction	Yield		b.p.	n_D^{20}	Sap. V.	Iodine V.
	(g)	(%)	(°C/ca. 5 mmHg)			
1	0.39	1.5	-155	1.4528	216.8	72.1
2	1.04	4.1	155-160	1.4532	210.5	73.2
3	1.42	5.5	160-165	1.4541	204.1	76.5
4	2.14	8.3	165-170	1.4548	198.8	79.4
5	2.31	9.0	170-175	1.4550	197.5	81.8
6	3.09	12.1	175-180	1.4559	193.8	91.1
7	4.79	18.7	180-185	1.4582	185.8	103.3
8	3.31	12.9	185-190	1.4608	182.2	137.5
9	3.32	13.0	190-195	1.4645	178.1	161.9
10	0.88	3.4	195-	1.4720	173.2	178.1
Residue (Diff.)	2.94	11.5	—	—	—	—

Notes: The methyl esters of fatty acids had S.V. 180.9 and I.V. 106.0. The distillation residue had S.V. 102.8, but the hexane-soluble fatty acids obtained from the residue after removal of unsaponifiable matter showed Neutr. V. 159.1.

The fractions 4, 5 and 6 in Table 3 were united, and the fatty acids (7.7 g) obtained from the united fraction were fractionated into four fractions by way of urea adduct using methanol. The 2nd fatty acid fraction (0.4 g, N.V. 194.5 and I.V. 135.1) was found to contain about 15% of dienoic acid and about 8% of trienoic acid with a smaller proportion of tetraenoic and pentaenoic acids by the ultraviolet absorption data of the alkali-isomerized product. This fatty acid fraction was brominated in ether, and the ether-insoluble bromide formed was removed by filtration. The filtrate was freed from excess bromine, and the ether was distilled off. Hexane was added to the residue and the hexane-insoluble bromide was separated. Fractional crystallization of the hexane-insoluble bromide from ethanol-hexane gave a white crystalline bromide; m.p. 112°-117°C (substantially 112°-114°C with slight darkening) and Br-content 55.09%. The melting point of this bromide was not lowered below 112°C when mixed with a pure specimen of tetra-

bromostearic acid prepared from linoleic acid (m.p. 114°C; Br-content, calcd., 53.27%).

The fractions 7 and 8 in Table 4 were united, and the fatty acids (7.3 g) from the united fraction were fractionated into six fractions in the same way. The 4th fatty acid fraction (0.3 g, N.V. 191.0 and I.V. 190.7) was found to contain about 53% of dienoic acid. A white crystalline bromide of m.p. 113°–114°C and Br-content 54.71% was obtained from this fatty acid fraction by the fractionation of its bromination product. It showed no depression of melting point when mixed with a pure specimen of tetrabromostearic acid.

The fatty acids of the fraction 10 in Table 4, similarly treated, gave a fraction of N.V. 177.5 and I.V. 160.2 which contained about 31% of dienoic acid. Hydrogenation of this fraction yielded a product of m.p. 72°–73°C after recrystallization from methanol. Its melting point was not lowered below 72°C when mixed with a pure specimen of arachidic acid (m.p. 75°C).

3. Unsaponifiable matter. Unsaponifiable matter from both oils showed no absorption maximum between 220 mμ and 300 mμ. Sterol content of unsaponifiable matter was found by digitonin method to be 63.7% for *P. nipponensis* and 70.8% for *C. clarkii*. The crystalline fraction (crude sterol) obtained by recrystallization of unsaponifiable matter from methanol showed m.p. 144°–146°C and $[\alpha]_D^{15} = -40°$ in the case of *P. nipponensis* and m.p. 144°–146°C and $[\alpha]_D^{15} = -42°$ in the case of *C. clarkii*. The acetates prepared from these crystalline fractions showed m.p. 114°–115°C after recrystallization from methanol. The melting point was unaltered by a further recrystallization from methanol or acetone.

Summary

1. Fatty oils were extracted from two species of fresh water lobster, *P. nipponensis* and *C. clarkii*, and their characteristics were determined.

2. Ultraviolet absorption data of the alkali-isomerized product of the fatty acids indicated that the polyethenoid acids of these two oils, differing from those of common marine animal oils, are characterized by comparatively large proportion of dienoic and trienoic acids. Among the dienoic acids, the presence of linoleic acid was indicated in both oils, and also C_{20}-dienoic acid appeared to be present in the oil of *C. clarkii*.

3. Sterols of these two oils consist chiefly of cholesterol.

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