FATTY OILS OF AQUATIC INVERTEBRATES

XVII. FATTY OILS FROM ONE SPECIES OF THE *OPHIUROIDEA*, THREE SPECIES OF THE *ASTEROIDEA* AND THREE SPECIES OF THE *LORICATA* WITH A PARTICULAR REFERENCE TO THEIR STEROL COMPONENTS

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(Received October 24, 1956)

The present paper is concerned with the fatty oils from the following seven species of invertebrates: *Gorgonocephalus caryi* of the *Ophiuroidea*; *Luidia quinaria, Astropecten scoparius* and *Astropecten polyacanthus* of the *Asteroidea*; *Acanthochiton rubrolineatus, Cryptoplax japonica* and *Liolophura japonica* of the *Loricata*. Although oils of *G. caryi, L. quinaria, A. scoparius* and *L. japonica* among these seven oils have been repoted in previous papers from this laboratory, another samples of these oils obtained thereafter are examined in this study with a view to accumulate the records pertaining to the properties of these oils. Regarding *G. caryi* oil, two oil samples, one sample (I)[1] from the animals caught around Osaki-shimojima Island in the Inland Sea of Seto and the other sample (II)[2] from the animals caught around Himagajima Island, Aichi Prefecture, were examined in previous studies, and the former (iodine value 166.7) was found to have a remarkably higher iodine value than the latter (iodine value 91.6). Two oil samples (Table 1) examined in this study, like the sample II, were extracted from the animals caught around Himagajima Island, but their iodine values were found remarkably higher than the iodine value of the sample II and rather close to that of the sample I. It may worth special mention that *G. caryi* oils, extracted from the animals of the same catching locality, show a considerable variance in iodine value. The previously reported oil sample (iodine value 132.4)[3] from *L. quinaria*, extracted from the animals caught around Osaki-shimojima and the oil sample (iodine value 136.0) in this study, extracted from the animals caught around Himagajima, were found similar with each other in their properties. The oil (iodine value 91.7) of *A. scoparius* recorded in this paper, extracted from the animals caught around Himagajima, showed a similar iodine value but a noticeable difference in content of unsaponifiable matter and in some other properties as compared with the previously reported oil sample (iodine value 92.5; contaminated with a little phosphatide)[4] extracted from the animals caught around Sugashima Island, Mie Prefecture. As for the oil of *L. japonica*, two samples, one from the animals caught around Osaki-shimojima and the other from the animals caught around Sugashima, were examined in a previous paper,[5] and it was noted that while Δ^7-cholestenyl acetate was easily obtained by recrystallization of the steryl acetate mixture from the former oil sample, recrystallization of the steryl acetate mixture from the latter oil sample yielded eventually a fraction which had a higher melting point than Δ^7-cholestenyl acetate. The oil sample in this study, extracted from the animals caught at Nishiura and Noma, Aichi Prefecture, was found to resemble the previously reported sample extracted from the animals caught around Sugashima.

Sterols from *G. caryi* were fouud to consist chiefly of Δ^5-sterols, from which a β-sitosterol fraction, m.p. 135°–137°C and $[\alpha]_D^{24} = -36.7$, was separated. Each of the crude sterol mixtures obtained by recrystallization of the unsaponifiable matters from *L. quinaria*, *A. scoparius* and *A. polyacanthus* showed a higher melting point than Δ^7-cholestenol (m.p. 122°–123°C). The crude sterol mixture from *A. rubrolineatus* showed a melting point which is close to the melting point of Δ^7-cholestenol, but its acetylation product showed after two recrystallizations m.p. 122°–124°C which is a little higher than the melting point of Δ^7-cholestenyl acetate (m.p. 118°–119°C). The sterol mixture from *C. japonica* could not be fractionated due to the scarcity of material. Recrystallization of the crude steryl acetate mixture prepared from *L. japonica* oil yielded a fraction of m.p. 126°–128°C, while a higher alcohol, possibly of the selachyl alcohol series, having a high acetyl value (256.8) was separated from the liquid unsaponifiable fraction of this oil.

In order to know the approximate content of Δ^7-sterols in total sterols from the three species of the *Loricata*, the sterol content of unsaponifiable matter was determined for each oil, and on the other hand, the Δ^7-sterol content of unsaponifiable matter was approximately estimated by measuring the absorption maximum at 620 mμ for the unsaponifiable matter in the course of Liebermann-Burchard reaction. The results are shown in Table 2. It is thus indicated that sterol components, for the most part, are Δ^7-sterols in every case. While it has been known by previous studies that sterol components from the *Cephalopoda*, *Gastropoda* and *Bivalvia* among the *Mollusca* consist chiefly of Δ^5-sterols (and $\Delta^{5,7}$-sterols), the results of this study indicated that the sterol components from the *Loricata* among the same class consist chiefly of Δ^7-sterols.

Regarding the sterol components of oils from the *Echinoderma*, it was previously pointed out by the authors[6] that the sterol components from the *Holothurioidea* and *Asteroidea* consist chiefly of Δ^7-sterols, while those from the *Echinoidea*, *Ophiuroidea* and *Crinoidea* consist chiefly of Δ^5-sterols (and $\Delta^{5,7}$-sterols). These remarks are quite consistent with the results of this study, since the sterol components from the three species of the *Asteroidea* were all found to consist chiefly of Δ^7-sterols by estimating the approximate contents of total sterols and of Δ^7-sterols in the unsaponifiable matter.

While the presence of $\Delta^{5,7}$-sterols in some kinds of mollusks and echinoderms has frequently been reported, an inspection of previous literature shows that while $\Delta^{5,7}$-sterols are generally present in company with Δ^5-sterols, the occurrence of $\Delta^{5,7}$-sterols in company with Δ^7-sterols has not been known with the exception that the presence of a very minor amount (0.09–0.36%) of $\Delta^{5,7}$-sterols in starfish was reported.[7] In this study, ultraviolet absorption spectra for the unsaponifiable matters of oils from the three species of the *Asteroidea* and the three species of the *Loricata* were found to exhibit no absorption maxima characteristic to $\Delta^{5,7}$-sterols. Although a further study with a number of oil samples is desirable, the authors' experience hitherto encountered seems to indicate that when the sterols from mollusks and echinoderms contain Δ^7-sterol as a chief component, $\Delta^{5,7}$-sterol is found absent or present, at most, in a very minute amount in these sterols.

The composition of polyethenoid acids in the fatty acids from *L. japonica* was estimated from the ultraviolet absorption measurement of the alkali-isomerized fatty acids with the results that the proportion of the content of diethenoid and triethenoid acids to the content of tetraethenoid and pentaethenoid acids is comparatively large as compared with the case for the fatty acids of common aquatic animal oils.

Experimental

1. Invertebrates and fatty oils. Among the invertebrates used in this study, only the species *A. rubrolineatus* and *L. japonica* were alive. These were killed in boiling water and then dried in an electric oven at a temperature of 70°–80°C. Invertebrates of all other species were sun-dried material which was dried further in an electric oven in this laboratory. The dried material was extracted with ether, and the ether-extract was treated with acetone in the same way as described in previous papers of this series to give acetone-soluble oil (fatty oil). Catching locality, date of receipt, and yield and characteristics of fatty oil are given in Table 1.

TABLE 1. List of Materials and Characteristics of Fatty Oil

Species	*Gorgonocephalus caryi*		*Luidia quinaria*	*Astropecten scoparius*	*Astropecten polyacanthus*	*Acanthochiton rubrolineatus*	*Cryptoplax japonica*	*Liolophura japonica*
Catching locality	Himagajima		Himagajima	Himagajima	Himagajima	Noma	Nishiura and Noma	Nishiura and Noma
Date of receipt (1955)	Middle Sept.		Middle Sept.	Middle Sept.	Middle Sept.	Middle Aug.	Middle Aug.	Middle Aug.
Number of individual	No. 1 1	No. 2 1	37	40	36	19	4	412
Weight (g)	—	—	—	—	—	20	—	2,157
Wt. of dried material (g)	62	120	572	106	249	11.5	4.0	1,300
Ether extract (g)	0.99	2.74	32.0	2.5	12.1	0.48	0.12	20.0
Ether extract (%)	1.6	2.3	5.6	2.4	4.9	4.2	3.0	1.5
Acetone sol. oil (fatty oil) (g)	0.59	2.24	15.0	1.8	7.5	0.37	0.082	10.2
Acetone sol. oil (fatty oil) (%)	0.95	1.9	2.6	1.7	3.0	3.2	2.05	7.8
n_D^{30}	1.4824	1.4839	1.4757	1.4697	1.4818	1.4777	—	1.4782
Acid value	107.1	116.4	124.9	95.2	137.0	35.0	—	43.9
Saponif. value	147.7	143.4	146.8	141.3	147.3	144.1	—	151.1
Iodine value (Wijs)	156.4	183.3	136.0	91.7	152.0	130.2	—	136.7
Unsaponif. matter (%)	25.46	29.86	25.94	28.77	26.52	22.52	18.80	23.89
Neutr. V. of fatty acids	186.8	183.2	186.1	193.5	184.0	188.9	—	190.2
Iodine V. of fatty acids	160.1	182.6	138.0	108.8	163.7	132.6	—	135.8

Notes: Percentage yields of ether-extract and acetone-soluble oil are expressed on the basis of dried material. All catching localities belong to Aichi Prefecture. Oils from the three species of the *Loricata* are dark reddish orange with a dash of green, while the other oils are reddish orange or dark reddish orange. All oils deposit some solid at ordinary temperature.

2. Sterol. Unsaponifiable matters from both samples of *G. caryi* oil were united. Recrystallization of the united material (0.9 g) from methanol gave a crude sterol mixture (0.48 g). The acetylation product of crude sterol mixture gave a steryl acetate fraction of m.p. 125°–127°C after recrystallization first from methanol and then from acetone. Free sterol from this acetate, recrystallized from methanol, had m.p. 135°–137°C, $[\alpha]_D^{24} = -36.7°$ and iodine value (perbenzoic acid method) 60.8. Benzoate of this sterol had m.p. 143°–145°C.

Crude sterol mixtures were obtained from *L. quinaria* oil, *A. scoparius* oil and *A. polyacanthus* oil by recrystallization of unsaponifiable matters from methanol. They had the following characteristics, respectively; m.p.: 128°–130°C, 128°–130°C, 131°–133°C; $[\alpha]_D^{25} = +3°$, 0°, 0°; iodine value (perbenzoic acid method): 65.1, 68.2, 69.2.

Crude sterol mixture obtained from *A. rubrolineatus* oil by recrystallization of the unsaponifiable matter from methanol had m.p. 119°–122°C and $[\alpha]_D^{29} = +3°$, and its acetylation product had m.p. 122°–124°C after recrystallization from methanol.

The unsaponifiable matter (2.3 g) from *L. japonica* oil was recrystallized from methanol, yielding crystalline substance (0.9 g; crude sterol mixture) and oily fraction recovered from the mother liquor. The acetylation product of crystalline substance was recrystallized first from methanol and then from acetone to give a steryl acetate of m.p. 126°–128°C, $[\alpha]_D^{28} = 0°$ and iodine value (perbenzoic acid method) 61.7. The oily fraction was dissolved in acetone, the solution was cooled at −20°C and the solid formed was removed by filtration. The filtrate yielded, after removal of acetone, an oily substance, the acetylation product of which had n_D^{20} 1.4637, saponification value 256.8 and iodine value (Wijs) 73.2.

3. The contents of total sterols and Δ^7-sterols in unsaponifiable matter. The total sterol content was determined using digitonin for the unsaponifiable matter of each oil except *G. caryi* oil which contains Δ^5-sterols as the major component of total sterols. On the other hand, the Δ^7-sterol content was approximately estimated

TABLE 2. The Contents of Total Sterols and Δ^7-Sterols in Unsaponifiable Matter*

Species	Maximum absorption value at 620 mμ in the L.B. reaction	Δ^7-Sterols calculated from the maximum absorption value (%)		Total sterols
		A	*B*	(%)
L. quinaria	752	37.6	39.6	40.4
A. scoparius	814	40.7	42.8	42.0
A. polyacanthus	794	39.7	41.8	38.5
A. rubrolineatus	880	44.0	46.3	43.8
C. japonica	891	44.6	46.9	42.9
L. japonica	958	47.9	50.4	51.5

Notes: Absorption value is expressed by $(2-\log T)/C$, where T and C denote transmittance (%) and concentration (10^{-3} mole), respectively. The maximum absorption value at 620 mμ for a pure Δ^7-sterol was taken as 2,000 for the figures in the column *A* and as 1,900 for the figures in the column *B*.

* The sterol content of unsaponifiable matter from oils of the *Holothurioidea* is relatively small. Results of the determination of the contents of total sterols and Δ^7-sterols for the unsaponifiable matters from *Cucumaria chronhjelmi* oil[6] and *Stichopus japonicus* oil[2] indicated that Δ^7-sterols constituted the major component of total sterols.

by measuring the absorption maximum at 620 mμ for the unsaponifiable matter in the course of Liebermann-Burchard reaction. The results are shown in Table 2. According to Idler and Baumann,[8] the absorption value (see notes on Table 2) at 620 mμ reaches the maximum after the lapse of 90 sec., the maximum being 2,010 for Δ^7-cholestenol and 1,870 for α-spinasterol. In Table 2, the Δ^7-sterol content was calculated by taking the maximum absorption value for a pure Δ^7-sterol as 2,000 or 1,900.

4. $\Delta^{5,7}$-Sterol content. Absorption spectrum in the region of 250 mμ to 300 mμ was taken in ethanol for the unsaponifiable matter of each sample in Table 2, which was found to contain Δ^7-sterols as the main sterol component. In every case, the unsaponifiable matter showed a small absorption which decreases with increasing wave length from 250 mμ to 300 mμ; for instance, the unsaponifiable matter from *A. polyacanthus* showed $k_{250} = 3.48$ and $k_{300} = 1.18$, and the unsaponifiable matter from *L. japonica* $k_{250} = 1.13$ and $k_{300} = 0.23$. However, the absorption maxima characteristic to $\Delta^{5,7}$-sterol were not exhibited in every case.*

5. Fatty acids from *L. japonica* oil. Fatty acids from *L. japonica* oil were found by the lead salt ethanol method to contain 27.4% of solid acids which had neutralization value 205.7 and iodine value (Wijs) 12.7. Composition of polyethenoid acids in the fatty acids was estimated by measuring the ultraviolet absorption of the alkali-isomerized fatty acids in the same way as described in the preceding paper for the fatty acids from *Cynthia roretzi* oil. The results are shown in Table 3.

TABLE 3. Polyethenoid Acids in the Fatty Acids of *L. japonica* Oil

	Wave length (mμ)	Spec. extinc. coeff.	Polyethenoid acids (%)	
			Pentaethenoid acid taken as C_{22}	Pentaethenoid acid taken as C_{20}
Hexaethenoid	376	0.62	2.2	2.2
Pentaethenoid	347	5.66	10.1	5.8
Tetraethenoid	316	10.90	7.5	9.1
Triethenoid	270	14.26	5.4	7.0
Diethenoid	235	18.82	8.8	9.5

Notes: The iodine value of total fatty acids can be calculated from the data given in Table 3 by assuming all monoethenoid acids to be oleic acid and the iodine value of solid acids to be attributed to the presence of oleic acid in solid acids. The calculated iodine value thus obtainable is 142.9 when pentaethenoid acid is taken as C_{22} and 139.8 when pentaethenoid acid is taken as C_{20}, while the observed iodine value (Table 1) is 135.8.

Summary

1. Characteristics of fatty oils extracted from *Gorgonocephalus caryi* of the *Ophiuroidea*; *Luidia quinaria, Astropecten scoparius* and *Astropecten polyacanthus* of the *Asteroidea*; *Acanthochiton rubrolineatus, Cryptoplax japonica* and *Liolophura japonica* of the *Loricata* have been determined.

* Besides unsaponifiable matters from the oil samples in Table 2, unsaponifiable matters from oils of *Cucumaria chronhjelmi*,[6] *Stichopus japonicus, Coscinasterias acutespina*,[6] *Asterias amurensis*,[4] and *Asterina pectinifera*[10] also showed no absorption maxima characteristic to $\Delta^{5,7}$-sterol.

2. Sterol components of *G. caryi* oil were found to consist chiefly of Δ^5-sterols, from which β-sitosterol was separated, while sterol components of the other oils consisted chiefly of Δ^7-sterols.

3. While it is known that sterol components from the *Cephalopoda*, *Gastropoda* and *Bivalvia* among the *Mollusca* consist chiefly of Δ^5-sterols (and $\Delta^{5,7}$-sterols), the results of the present study indicated that the sterol components from the *Loricata* among the same class consist chiefly of Δ^7-sterols.

4. It is concluded from the results of the present study that $\Delta^{5,7}$-sterols are absent or present, at most, in an extremely minor amount in the sterols from the *Holothurioidea* and *Asteroidea* among the *Echinoderma* and from the *Loricata* among the *Mollusca*, all of which contain Δ^7-sterols as the main sterol component.

5. The fatty acids of *L. japonica* oil were found to contain 27.4% of solid acids (iodine value 12.7). The polyethenoid acids of this oil contained different members ranging from diethenoid to hexaethenoid acids. The proportion of diethenoid and triethenoid acids to tetraethenoid and pentaethenoid acids in the fatty acids of this oil was found relatively large as compared with the case for common aquatic animal oils.

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