FATTY OILS OF AQUATIC INVERTEBRATES. IX. PROPERTIES OF FATTY OILS FROM THIRTEEN KINDS OF JAPANESE SHELLFISH*

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The 5th and 7th reports of this series recorded some properties of oils extracted from a number of kinds of Japanese shellfish with particular reference to the provitamin D ($\Delta^{5,7}$-sterol) content of unsaponifiable matter or crude sterol mixture of these oils. In the present study, thirteen additional species of Japanese shellfish are examined for some properties of their oils and the provitamin D content of their sterol mixture. Also some noteworthy features pertaining to shellfish oils are set forth in this paper by inspecting the results of our studies hitherto carried out.

The shellfish used in the present study (Table 1) are eight species of Gastropoda and five species of Bivalvia. Lipid (ether-extract) of *Rapana thomasiana*,[1] fatty oil[2] and lipid (petroleum ether soluble portion of ether-extract)[3] of *Turbo cornutus*, and fatty oil of *Haliotis gigantea*[2] have been more or less studied by previous authors, whereas there seems no literature on oils of the other kinds of shellfish used in this study.

Experimental and Discussion of the Results

1. Shellfish used in this study. Excepting four species mentioned below, shellfish used in this study were living whole shellfish. The shell was removed, and the meat was dried in an oven at a temperature below 80°C. The dried meat was reduced to powder and extracted with ether. The ether-extract (lipid) was refluxed with tenfold acetone for a while, the solution was cooled to ordinary temperature and the insoluble portion (phosphatide) was removed by filtration. The fatty oil was recovered from the acetone filtrate. In the case of *T. cornutus* and *H. gigantea*, the shucked shellfish were separated into the flesh and the viscera, and both parts were separately extracted with ether. The shellfish, *N. albicilla*, *L. coronata coreensis* and *M. labio*, were sun-dried immediately after their catch, and the sun-dried material was then treated as described above. The shellfish, *C. farreri nipponensis*, was received in the form of dried meat by the courtesy of Dr. Yamada, Faculty of Fisheries, Hokkaido University. This dried meat was prepared by drying the fresh meat under exposure to infrared radiation. Name of species, catching locality, date of receipt, and some data on the yield of lipid and fatty oil for the thirteen kinds of shellfish used in this study are recorded in Table 1. In the 5th and 7th reports of this series, it was noted for four kinds of shellfish that the

* The 1st to 7th reports of this series were published in a single article in *Memoirs of the Faculty of Engineering, Nagoya University* 7, No. 1, 1–35 (1955).

TABLE 1. List of Thirteen Kinds of Shellfish

Sample No.	Species	Catching locality	Catching date	Number	Weight (g)	Dried material of shucked shellfish (g)	Ether-extract		Acetone-soluble oil	
							(g)	(%)	(g)	(%)
1	*Hemifusus ternatanus*	South-east off Atsumi	Late Aug., 1954	2	1,537	119	5.8	4.9	3.6	62
2	*Rapana thomasiana*	South-east off Atsumi	Late Aug., 1954	2	544	57	2.3	4.0	1.4	61
3	*Tonna luteostoma*	South-east off Atsumi	Late Aug., 1954	4	581	78	5.0	6.4	3.4	68
4	*Nerita albicilla*	Osaki-shimojima	Middle Aug., 1952	—	129	20	0.40	2.0	0.20	50
5	*Turbo cornutus*	Toba	Late Jul., 1954	60	3,937	Flesh 122 Viscera 118	3.3 12.1	2.7 10.3	1.9 8.8	58 73
6	*Lunella coronata coreensis*	Osaki-shimojima	(a) Middle Aug., 1952	—	1,245	—	5.8	—	5.2	90
			(b) Middle Aug., 1954	—	7,905	1,632	25	1.5	19	76
7	*Monodonta labio*	Osaki-shimojima	(a) Middle Aug., 1952	—	1,481	136	4.6	3.4	3.1	67
			(b) Middle Aug., 1954	—	2,080	260	6.9	2.7	5.6	81
8	*Haliotis gigantea*	Toba	Late Jul., 1954	3	290	Flesh 36 Viscera 10	0.84 4.4	2.3 44	0.60 4.0	71 91
9	*Solen gouldi*	Mouth of the river Nabeta	Early Feb., 1954	306	3,326	259	9.5	3.7	5.3	56
10	*Sanguinolaria olivacea*	Mouth of the river Nabeta	Early Feb., 1954	672	4,188	253	8.5	3.4	6.0	71
11	*Dosinia japonica*	Mouth of the river Nabeta	Early Feb., 1954	68	4,038	120	2.6	2.2	1.8	69
12	A variety of *Chlamys farreri nipponensis*	Hokkaido	Early Nov., 1954	—	—	665	33	5.0	12	36
13	*Anadara subcrenata*	Mouth of the river Nabeta	Late Jan., 1954	202	3,600	236	11.0	4.7	7.6	69

Notes: Of the catching localities, Atsumi and the river Nabeta belong to Aichi Prefecture, and Osaki-Shimojima to Hiroshima Prefecture. Weight of sample Nos. 4, 6 and 7 denotes the weight of sun-dried shellfish. In the case of sample No. 6 (a), sun-dried whole shellfish were reduced to powder and extracted with ether. Percentage yield of ether-extract is expressed on the basis of dried material of shucked shellfish. Percentage yield of acetone-soluble oil is expressed on the basis of ether-extract.

viscera gave always a higher yield of ether-extract than the flesh of the same shellfish. As is seen from Table 1, such is the case also with *T. cornutus* and *H. gigantea*. It is also found from Table 1 that the ether-extract of viscera gives a higher yield of acetone-soluble oil than the ether-extract of flesh of the same shellfish. Such was the case also with three of the four species of shellfish previously studied; only the ether-extract of the shellfish, *Cipangopaludina malleata*, made an exception in which the yield of acetone-soluble oil from the ether-extract was lower for the viscera than for the flesh.

2. Properties of oils. Properties of acetone-soluble oils are shown in Table 2. The sterol content of unsaponifiable matter was determined by the digitonide method. For the separation of the sterol in Table 2, the digitonide prepared from the unsaponifiable matter was refluxed with tenfold acetic anhydride for one hour, the product was poured into hot water, and the mixture was agitated. The insoluble matter was separated by filtration and then dried. Extraction of the dried matter with ether gave an ether-extract consisting of steryl acetate. This was saponified, and the free sterol formed was separated by extraction of the saponification product with ether. In the case of *N. albicilla*, *L. coronata coreensis* (b), *M. labio* (b) and *C. farreri nipponensis*, however, the crude sterol mixture was separated by simply recrystallizing unsaponifiable matter from about thirtyfold methanol. The content of provitamin D in sterol or crude sterol mixture was calculated from the specific extinction coefficients (in ethanol) at 277 mμ, 282 mμ and 290 mμ by applying the formula given in the 5th report of this series.

All of the oils deposit more or less solid at ordinary temperature, and some of them become a soft solid at ordinary temperature. The color of oil is not alike for each oil, but it is mostly dark reddish orange wtih or without a dash of green. Oils from *H. ternatanus*, *R. thomasiana*, *T. luteostoma* and *A. subcrenata* are devoid of green tinge, while oils from *S. gouldi*, *S. olivacea*, *D. japonica* and *C. farreri nipponensis* are strongly greenish. The oils have mostly high acid values; especially oils from *L. coronata coreensis* and *M. labio* have abnormally high acid values which have been possibly caused by a previous sun-drying of the whole shellfish. Comparing the flesh oil with the viscera oil of the same shellfish, *T. cornutus* and *H. gigantea*, the latter has a higher iodine value, a lower content of unsaponifiable

TABLE 2. Properties of Oils

Sample No.	n_D^{40}	A.V.	S.V.	I.V.	Unsap. M. (%)	Fatty acids: Neutr. V.	Fatty acids: I.V.	Unsaponif. matter: Sterol (%)	Unsaponif. matter: Sterol: m.p. (°C)	Unsaponif. matter: Sterol: Pro-vitamin D (%)
1	1.4808	12.7	145.4	155.9	25.51	181.9	155.4	38.40	131–133	7.5
2	1.4847	13.8	144.0	156.7	35.34	179.7	135.6	32.95	129–131	1.6
3	1.4781	52.1	151.3	133.8	23.22	193.3	134.8	43.03	114–117	8.6
4	1.4782	—	152.0	133.2	22.95	—	—	—	125–127*	0.11
5 Flesh	1.4865	70.1	111.9	117.6	48.47	186.1	111.8	68.63	134–136	0.50
5 Viscera	1.4719	74.7	194.9	131.4	12.69	203.7	141.5	57.35	135–137	0.56
6 (a)	1.4750	113.3	159.8	116.9	18.95	201.9	—	52.16	132–135	0.65
6 (b)	—	106.6	156.3	118.1	22.60	196.6	124.1	68.70	145–146*	—
7 (a)	1.4795	114.0	144.0	127.9	26.41	203.4	—	54.21	126–128	0.23
7 (b)	—	108.2	147.6	114.5	25.54	199.5	128.8	72.40	144–145*	—
8 Flesh	1.4868	80.1	126.2	108.1	39.09	198.1	110.9	52.80	132–134	0.21
8 Viscera	1.4725	29.0	190.5	122.8	6.19	210.0	114.6	42.82	130–133	0.34
9	1.5008	47.8	123.5	158.6	42.27	178.7	170.4	52.88	127–130	5.2
10	1.4906	39.0	136.8	136.7	32.53	183.8	154.8	51.75	125–127	2.3
11	1.5037	31.3	119.6	148.2	44.82	177.1	130.3	46.40	134–136	0.22
12	1.4835	23.5	139.7	114.4	29.03	203.0	143.9	71.40	140–142*	0.73
13	1.4877	26.3	145.9	157.4	25.52	190.3	167.6	62.77	122–124	1.9

Notes: * Crude sterol. Nos. 9, 10 and 13 have d_4^{40} 0.9723, 0.9543 and 0.9521, respectively.

matter and a lower sterol-content in unsaponifiable matter than the former, as previously noted in the 7th report of this series for several shellfish oils. Provitamin D content in sterol, as is seen from Table 2, is higher for the viscera oil than for the flesh oil of the same shellfish. Although it was noted in the 7th report of this series that the unsaponifiable matter of viscera oil has a larger content of provitamin D than the unsaponifiable matter of flesh oil of the same shellfish, the oil from *T. cornutus* in Table 2 shows, however, that if provitamin D content in unsaponifiable matter instead of sterol is taken, the viscera oil has a slightly lower value than the flesh oil.

3. Sterols. The unsaponifiable matter of each oil, excepting Nos. 2, 4 and 8, was recrystallized from methanol, the crude sterol thus obtained was acetylated, and the acetyl ester was recrystallized once to several times from methanol, ethanol or acetone. Table 3 shows some properties of the acetyl ester obtained by the final recrystallization and the free sterol obtained by saponification, the latter being mostly once recrystallzed from methanol.

As is seen from Table 3, sterols from three species of Gastropoda, *T. cornutus*, *L. coronata coreensis* and *M. labio*, consist exclusively or substantially of cholesterol (calcd. for cholesteryl acetate: S. V. 130. 9 and I. V. 59. 2; calcd. for cholesterol: I. V. 65. 6). Since, however, the melting point of the acetates from the viscera oil of *T. cornutus* and from the oil (a) of *M. labio* are slightly higher than the melting point of cholesteryl acetate, these acetates seem to contain more or less F_2-steryl acetate besides cholesteryl acetate. The acetate fractions recovered from the mother liquors of recrystallizations of the steryl acetates from the oils (a) of *L. coronata coreensis* and *M. labio* were dissolved in ether, and brominated at about −10°C with a solution of bromine in glacial acetic acid. The insoluble bromides formed were separated and identified with dibromocholesteryl acetate. The bromide from *L. coronata coreensis* had m.p. 115°–117°C and Br-content 27.35%, and the bromide from *M. labio* had m.p. 115°–117°C and Br-content 27.30% (calcd. for $C_{29}H_{48}O_2Br_2$: Br, 27.16%).

TABLE 3. Steryl Acetate and Free Sterol after Recrystallization

Sample No.		Acetate				Free sterol		
		m.p. (°C)	$[\alpha]_D^\circ$	S.V.	I.V.	m.p. (°C)	$[\alpha]_D^\circ$	I.V.
1		124.5–125.5	—	131.5	—	139 –140	−36.4	89.8
3		113 –115	—	130.5	—	116 –117.5	−32.5	178.8
5	Flesh	114 –115	−42.7	130.4	60.3	145 –146	−37.4	—
	Viscera	115 –116	−44.2	131.2	62.4	145 –146	−38.8	—
6	(a)	114 –115	—	129.8	—	141 –142	−40.0	66.2
	(b)	114 –115	−41.4	128.1	60.1	145 –146	−38.7	—
7	(a)	115.5–117	—	—	—	144 –145	—	—
	(b)	114 –115	−40.2	131.7	60.4	144 –145	−40.1	—
9		143 –144	—	127.1	—	138 –139	−44.2	111.2
10		137 –138	—	128.5	—	138.5–140	−43.0	97.4
11		135 –136.5	—	127.5	—	137 –138	−39.5	96.9
12		133 –135	−44.4	—	92.8	—	—	—
13		139 –140	—	128.1	—	136 –137	−49.5	108.6

Notes: Iodine values recorded in Table 3 were determined by the pyridine sulfate dibromide method.

The sterol from the oil of *H. ternatanus seems* to contain a considerable amount of F_2-sterol together with cholesterol. Its provitamin D content was found to be 6.7% compared with 7.5% for the total sterol before recrystallizaton. Thus the provitamin D was more or less removed by the recrystallization of steryl acetate.

The sterol from the oil of *T. luteostoma* has a very low melting point, though the melting point of its acetate is close to that of cholesteryl acetate. Also it has an abnormally high iodine value. The ultraviolet absorption curve of this sterol (Fig. 1) is of a typical $\Delta^{5,7}$-sterol type, exhibiting $k_{277}=4.424$, $k_{282}=5.593$ and $k_{290}=2.825$, from which provitamin D content is found to be 21.8%. This sterol is almost quantitatively precipitated with digitonin, and so the possibility of the presence of any substance other than sterol is excluded. This sterol is worth a closer study.

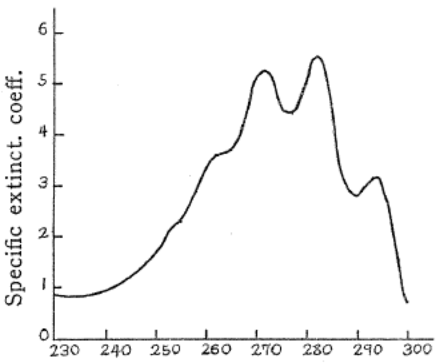

FIG. 1. Ultraviolet absorption curve of sterol from *Tonna luteostoma.*

Sterols from Bivalvia (sample Nos. 9–13) are considered to contain substantially F_2-sterol rather than F_1-sterol from their iodine values. The provitamin D contents of sterols from *S. gouldi*, *S. olivacea* and *A. subcrenata* were found to be 2.2%, 2.5% and 1.9% compared with 5.2%, 2.3% and 1.9%, respectively, for the sterols before recrystallization. Thus recrystallization of steryl acetate is not effective to concentrate provitamin D in sterol, bringing about a slight increase in provitamin D content only in the case of *S. olivacea.*

4. Fatty acids of oils of *L. coronata coreensis*, *M. labio* and *C. farreri nipponensis*. The fatty acids from these oils were alkali-isomerized under the condition of 21% KOH-glycol, 180°C and 15 minutes with a current of nitrogen, and the specific extinction coefficients of the isomerized fatty acids in ethanol at 235, 270, 316, 348 and 376 mμ were measured. Each polyethenoid acid in the original fatty acids was calculated from the observed data in the same way as described in the 7th report of this series. Ether-insoluble bromide and solid acids by the lead salt ethanol method were also determined with these fatty acids. The results are shown in Table 4. While the fatty acids of the oil (b) from *L. coronata coreensis*

TABLE 4. Fatty Acids of the Oils from *Lunella coronata coreensis*, *Monodonta labio* and *Chlamys farreri nipponensis*

Fatty acids from	Ether-insol. bromide (%)	Solid acids			Polyethenoid acids (%)				
		Yield (%)	N.V.	I.V. (Wijs)	Hexa-ene	Penta-ene	Tetra-ene	Tri-ene	Di-ene
L. coronata coreensis (b)	30.8	27.6	213.0	7.7	2.5	8.5	11.0	5.0	6.4
M. labio (b)	36.4	23.7	220.0	7.3	3.4	11.0	8.6	0.2	6.0
C. farreri nipponensis	42.6	22.9	217.6	18.8	9.2	16.4	7.3	1.7	5.9

contain tetraene in a larger proportion than pentaene, and triene in the smallest proportion, the fatty acids of the oil (b) from *M. labio* and the oil from *C. farreri nipponensis* contain pentaene in the largest proportion and triene in the smallest proportion. Among ten kinds of oils recorded in the 7th and 8th reports of this series, the fatty acids of the viscera oil from *Cipangopaldina malleata* formed an exception, in which the proportion of tetraene is largest, whereas the fatty acids of the other oils contain pentaene in the largest proportion. In this respect, the fatty acids of the oil (b) from *L. coronata coreensis* is similar to those of the viscera oil from *C. malleata*. It should also be mentioned that although the proportion of diene is relatively large in all cases, it appears to need a further study to ascertain whether the figures in Table 4 denote correctly the real content of each polyethenoid acid.

Summary

1. Fatty oils were extracted from thirteen kinds of Japanese shellfish, and their properties were determined. Comparing the flesh with the viscera of the same shellfish, the viscera gives a higher yield of ether-extract (lipid) than the flesh. The yield of acetone-soluble oil (fatty oil) from the ether-extract of the viscera is mostly higher than that of the flesh. The viscera oil has a higher iodine value and a smaller content of unsaponifiable matter than the flesh oil. The unsaponifiable matter of viscera oil contains a smaller proportion of sterol than the unsaponifiable matter of flesh oil, while the provitamin D content in sterol is larger for viscera oil than for flesh oil.

2. Among the oils from the Gastropoda, the oils from *Turbo cornutus*, *Lunella coronata coreensis* and *Monodonta labio* contain cholesterol as the chief component of sterols. Sterol from *Hemifusus ternatanus* seems to contain a considerable amount of F_2-sterol together with cholesterol. Sterol from *Tonna luteostoma* is characterized by its very low melting point. Provitamin D in this sterol was concentrated up to 21.8% by recrystallization of its acetate. Sterols from the Bivalvia seem to consist substantially of F_2-sterol rather than F_1-sterol.

3. Polyethenoid acids of the fatty acids from *Lunella coronata coreensis*, *Monodonta labio* and *Chlamys farreri nipponensis* were examined by ultraviolet absorption measurement of alkali-isomerized fatty acids, and it was found that the fatty acids from *M. labio* and *C. farreri nipponensis* contain pentaene in the largest proportion and triene in the smallest proportion, whereas the fatty acids from *L. coronata coreensis* contain tetraene in a larger proportion than pentaene.

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