FATTY OILS OF AQUATIC INVERTEBRATES

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This article contains a translation of our eight recent papers published in the Journal of the Chemical Society of Japan, Pure Chemistry Section, in Japanese. Each section of this article corresponds to one of the separate Japanese papers. It may also be mentioned here that some of our studies on related subject have been published and will continue to be published in a series of papers in the Bulletin of the Chemical Society of Japan.

I. Fatty Acids and Unsaponifiable Matter in the Oil Extracted from Salted Ovaries of Sea-urchin[1]

Salted ovaries of sea-urchin are one of fairly popular foodstuffs in this country. According to Takahashi,[2] the lipids extracted from this material are composed of lecithin, cephalin, cholesterol, pigment, fatty oil and fat-soluble vitamin. The saturated acids, which constitute approximately 26% of total fatty acids, consist chiefly of palmitic acid while the unsaturated acids contain highly unsaturated components and others. Bock and Wetter[3] found 6.65% provitamin D in the sterol mixture of the sea-urchin, *Echinus esculentus*. Bergmann and co-workers[4] indicated the presence of a sterol fraction, the acetate of which has m.p. 132°–133°C, besides cholesterol in the sterol mixture from the sea-urchin, *Centrichinus antillarum*. No other literature pertaining to the fat components of sea-urchin has come to our knowledge. This section records the results of our study on the fatty acids and unsaponifiable matter in the oil extracted from salted ovaries of sea-urchin.

Properties of oil. The salted ovaries of sea-urchin for our study were received by the courtesy of Dr. E. Nakano, Department of Biology, Nagoya University. They were commercially prepared in Shimonoseki from the sea-urchins, largely *Strongylocentrotus pulcherrimus* with some *Heliocidaris crassispina*. The material (3,550 g) received was dried at a temperature below 100°C in a vacuum oven. Extraction of the dried material (1,660 g) with ether yielded 249 g of lipid. On adding 10 parts of acetone to one part of lipid, a small amount of acetone-insoluble matter (chiefly phosphatide) was removed, and 245 g (14.8% of dried material) of acetone-soluble oil was obtained. It was reddish orange in color, and had d_4^{20} 0.9153, n_D^{20} 1.4714, acid value 57.5, saponification value 190.9, iodine value 121.2* and unsaponifiable matter 6.99%.

Fatty acids. Fatty acids and unsaponifiable matter were separated by saponification of the oil followed by extraction of the diluted soap solution with ether in

* Unless stated otherwise, all iodine values recorded in Sects. I–III were determined by the pyridine sulfate dibromide method, while in Sects. IV–VIII iodine values were determined by the Wijs method for fatty oils and fatty acid components and by the pyridine sulfate dibromide method for sterol components.

the usual way. The fatty acids (197 g) were separated into two fractions by means of the lithium salt acetone method; acetone-soluble and acetone-insoluble lithium salts. The latter were separated further into two fractions using 50% ethanol. The results of these fractionating procedures are shown in Table 1.

TABLE 1. Fractionation of the Fatty Acids

Fatty acid fraction	Neutralization V.	I.V.	Yield (%)
(1) from acetone-soluble lithium salts	176.0	282.0	17.3
(2) from 50% ethanol-soluble lithium salts	198.5	188.8	24.4
(3) from 50% ethanol-insoluble lithium salts	212.0	54.0	58.4

Saturated and mono-ethenoid acids. The fatty acid fraction from 50% ethanol-insoluble lithium salts was converted into methyl esters (S.V. 200.7 and I.V. 51.2), and 117 g of the latter were fractionated using a (7 mm in diameter and 30 cm in length) fractionating column of Whitemore-Lux type with the results shown in Table 2. The fractions 9 and 10 and the residue in Table 2 were considered to contain some highly unsaturated esters from their iodine values. These were united, and the fatty acids from the united material were separated into two fractions by treating their lithium salts with 50% ethanol. The fatty acid fraction from the 50% ethanol-insoluble lithium salts was esterified with methanol, and 13.6 g of methyl esters (S.V. 171.4 and I.V. 62.6) were fractionated as shown in Table 3.

TABLE 2. Fractionation of Methyl Esters of the Fatty Acid Fraction (3) in Table 1

Fraction	b.p. (°C/10 mm)	n_D^{20}	S.V.	I.V.	Yield	
					g	%
1	165–170	1.4402	229.1	11.3	6.6	5.6
2	170–175	1.4413	220.9	14.0	8.0	6.8
3	175–180	1.4427	212.1	25.9	9.8	8.4
4	180–185	1.4435	210.4	26.2	11.4	9.7
5	185–190	1.4443	207.4	28.2	14.2	12.1
6	190–195	1.4452	200.0	43.1	14.0	12.0
7	195–200	1.4497	192.9	66.3	14.0	12.0
8	200–205	1.4510	185.8	71.8	18.2	15.6
9	205–210	1.4545	178.7	74.8	4.0	3.4
10	210–218	1.4583	173.0	107.1	2.8	2.4
Residue	—	—	168.9	98.0	14.0	12.0

TABLE 3. Fractionation of the Methyl Esters of Less Unsaturated Acids from the Fractions 9 and 10 and the Residue in Table 2

Fraction	b.p. (°C/10 mm)	S.V.	I.V.	Yield	
				g	%
1	–210	184.3	67.1	1.6	11.8
2	210–215	180.1	65.0	2.2	16.2
3	215–220	173.4	64.1	2.0	14.7
4	220–225	168.5	62.1	2.6	19.1
5	225–235	161.3	60.3	2.8	20.6
Residue	—	158.9	58.1	2.4	17.6

On fractionating the fraction 1 in Table 2, the lowest fraction boiling below 175°C/15 mm was separated. Recrystallization of the fatty acids of this lowest fraction from ethanol gave myristic acid of m.p. 52°C and N.V. 244.6 (calcd., 245.7). Refractionation of the fraction 4 in Table 2 gave a fraction of b.p. 185°–190°C/15 mm, and the fatty acids of this fraction gave palmitic acid of m.p. 62°C and N.V. 219.5 (calcd., 218.8) after recrystallization from ethanol. The fatty acids remaining in the mother liquor of recrystallization were treated by the lead salt ethanol method yielding a liquid acid of n_D^{15} 1.4601, N.V. 220.9 and I.V. 100.1. Oxidation of this liquid acid by Hazura's method yielded dihydroxypalmitic acid of m.p. 122°C and N.V. 192.9 (calcd., 194.5). Hence the liquid acid is regarded as zoomaric acid (calcd., N.V. 220.6 and I.V. 99.8). A fraction of b.p. 210°–215°C/15 mm obtained by refractionation of the fraction 7 in Table 2 was treated in the same way as used for the fraction 4, and stearic acid of m.p. 69°C and N.V. 198.1 (calcd., 197.2) and oleic acid of n_D^{15} 1.4620, N.V. 201.3 and I.V. 89.8 (calcd., N.V. 198.6 and I.V. 89.8) were identified. Dihydroxystearic acid from this oleic acid had m.p. 134°C and N.V. 178.8 (calcd., 177.3). The fatty acids of the fraction 3 in Table 3 gave approximately 20% solid acids by the lead salt ethanol method, which showed m.p. 69°C and N.V. 180.6 after recrystallization from ethanol and appeared to consist substantially of arachidic acid (m.p. 75°C and N.V., calcd., 179.5). The remainder of fatty acids of the fraction 3 in Table 3 was also largely solid at ordinary temperature. Recrystallization from 80% ethanol gave a crystalline solid of m.p. 18°C, N.V. 183.6 and I.V. 84.0 which was considered to consist chiefly of eicosenoic acid (calcd., N.V. 180.7 and I.V. 81.7). On treating the fraction 5 in Table 3 in the same way as described above, a fatty acid fraction of m.p. 72°C and N.V. 167.1 and a fatty acid fraction of m.p. 30°C, N.V. 170.9 and I.V. 80.1 were obtained. The former was considered to consist chiefly of behenic acid (m.p. 80°C and N.V., calcd., 164.7) while the latter appeared to contain predomiantly docosenoic acid (calcd., N.V. 165.7 and I.V. 75.0).

Highly unsaturated acids. The fatty acid fraction from acetone-soluble lithium salts in Table 1 yielded 104% ether-insoluble bromide having Br-content 69.81%. Debromination of this bromide with zinc and glacial acetic acid gave a highly unsaturated acid mixture of N.V. 173.1, I.V. (pyridine sulfate dibromide method) 333.7 and I.V. (Wijs) 354.3. Its methyl ester mixture (S.V. 169.9 and I.V. 318.7) was fractionated with the results shown in Table 4.

TABLE 4. Fractionation of the Highly Unsaturated Methyl Esters

Fraction	b.p. (°C/10 mm)	n_D^{20}	S.V.	I.V.	Yield	
					g	%
1	–210	1.4841	191.6	300.1	0.8	13
2	210–215	1.4883	183.4	310.4	0.7	12
3	215–220	1.4901	174.7	314.6	1.0	17
4	220–225	1.4925	167.9	333.6	1.0	17
5	225–230	1.4944	161.7	349.1	1.1	18
Residue	—	—	149.0	289.9	1.4	23

Seeing from iodine value, the fractions 1, 3 and 5 in Table 4 contain substantially methyl esters of C_{18}, C_{20} and C_{22} acids, respectively. Saturated acids obtained

from the hydrogenation products of these three fractions 1, 3 and 5 were examined by the mixed melting point method of Twitchell[5] with the results that they contain approximately 70% stearic acid, 60% arachidic acid and 60% behenic acid, respectively. Taking into consideration that the iodine value of each fraction in Table 4 determined by the pyridine sulfate dibromide method is lower than the value corresponding to the real unsaturation by 20 or so, the highly unsaturated acid components are considered to consist chiefly of $C_{18}F_3$, $C_{18}F_4$, $C_{20}F_4$, $C_{20}F_5$ and $C_{22}F_5$ acids, among which C_{18} acids are present in a lesser amount. For references, the saponification and iodine values calculated for methyl esters of $C_{18}F_3$, $C_{18}F_4$, $C_{20}F_4$, $C_{20}F_5$ and $C_{22}F_5$ acids are given below: S.V. 191.8, 193.2, 176.2, 177.3 and 163.0, respectively, and I.V. 260.4, 349.6, 318.8, 401.1 and 368.4, respectively.

Quantitative estimation of fatty acid components. In order to estimate individual fatty acid components in total fatty acids, it is assumed that the fatty acid fractions (1) and (2) in Table 1 are composed of polyethenoid and mono-ethenoid acids while the fatty acid fraction (3) in Table 1 is composed of mono-ethenoid and saturated acids, that the total polyethenoid acids have the same iodine value as the debromination product of ether-insoluble bromide described above while the iodine value of mono-ethenoid acid mixture lies between 85 and 95, and also that the polyethenoid components in the fatty acid fractions (1) and (2) are the same with each other and the mono-ethenoid components in the fractions (1), (2) and (3) are the same with one another. If these assumptions are permitted, it is found from iodine values and yields of the fractions (1), (2) and (3) that the total fatty acids are composed of 21–25% saturated acids, 51–55% mono-ethenoid acids and 23–24% polyethenoid acids. It may also be roughly estimated from yields, saponification values and iodine values of each fraction in Table 2 that the saturated acids contain roughly 30% myristic acid, 60% palmitic acid and 10% stearic acid together with a small amount of higher members while the mono-ethenoid acids contain roughly 20% zoomaric acid, 50% oleic acid and 30% acids higher than C_{18} together with a small amount of members lower than C_{16}.

Unsaponifiable matter. The unsaponifiable matter was a crystalline solid accompanied with a reddish orange liquid. Sterol in the unsaponifiable matter was found to be 68.2% by the digitonide method. Recrystallization of the unsaponifiable matter (16.5 g) from methanol yielded 11.5 g of crystalline solid of m.p. 134°–138°C which exhibited a maximum absorption characteristic to conjugated sterol at 282 mμ. Its molecular extinction coefficient in ethanol at 282 mμ was found to be 508.6 as compared with 11,000 for a pure provitamin D. Accordingly the content of provitamin D in the crystalline solid is calculated as 4.6%. Further recrystallization of this crystalline solid from methanol and ethanol gave cholesterol; m.p. 146°–147°C and $[\alpha]_D^{13} = -38°$.* The material recovered from the mother liquors of recrystallizations, excepting that of the first recrystallization, was acetylated. Recrystallization of the acetate from methanol yielded cholesteryl acetate of m.p. 114°C and $[\alpha]_D^{13} = -37°$.

The material (5 g) recovered from the first recrystallization of unsaponifiable matter was still contaminated with solid substance. After the solid substance was removed as far as possible by crystallization from a concentrated methanol solu-

* All optical rotations were measured in chloroform.

tion, the material remaining in the mother liquor was then completely freed from sterol by using digitonin. The sterol-free unsaponifiable matter thus obtained was acetylated, and 2.4 g of acetylated product was fractionated as shown in Table 5.

TABLE 5. Fractionation of Sterol-Free Acetate

Fraction	b.p. (°C/5 mm)	S.V.	I.V.	Yield (g)
1	–230	268.6	—	0.3
2	230–235	207.0	60.2	0.5
3	235–242	191.9	57.5	0.8
Residue	—	—	—	0.8

Free alcohol obtained by saponification of the fraction 2 in Table 5 was recrystallized from petroleum ether, yielding a substance of m.p. 60°C and acetyl value 274.1, consisting possibly of a mixture of batyl alcohol with a lesser amount of chimyl alcohol. The fraction 3 in Table 5, treated in the same way as the fraction 2, yielded a substance which had m.p. 68°C and acetyl value 265.1 and showed no depression of melting point when mixed with batyl alcohol (m.p. 70°C and acetyl value, calcd., 261.8). The material remaining in the mother liquor of recrystallization of the free alcohol from the fraction 3 was recovered and dissolved in acetone, the solution was intensely cooled, and the solid crystallized out was removed by filtration. The liquid material obtained from the filtrate was hydrogenated, and the hydrogenation product was recrystallized from petroleum ether. The final product thus obtained had m.p. 60°C and acetyl value 230.1 and was deemed to consist substantially of alcohols of batyl series. Accordingly the material before hydrogenation appears to contain more or less alcohols of selachyl series which are unsaturated members corresponding to saturated alcohols of batyl series.

II. Fatty Acid Components of the Lipid of Clam[6]

Inspection of literature pertaining to the lipids of shellfish shows that although their sterol components have frequently been studied, their fatty acid components have hitherto been studied a little. Tsujimoto and Koyanagi[7][8][9][10] separated fatty acids from the lipids of over ten kinds of shellfish, and determined their neutralization and iodine values together with yields and Br-contents of ether-insoluble bromides formed by bromination of the fatty acids. As regards the fractionation and separation of fatty acid components, there are likely only a few studies including those on *Tridacna gigas* by Tsujimoto and Koyanagi,[9] *Mytilus edulis* by Lovern[11] and *Ostrea gigas* by Masumoto.[12] Referring to the fatty acids of the acetone-soluble oil separated from clam, one of the most important edible shellfish in this country, Tsujimoto and Koyanagi[7] reported m.p. 38.5°–39.5°C, N.V. 191.3, I.V. (Wijs) 182.0, yield of ether-insoluble bromide 70.5% and its Br-content 71.63%.

While sterol components of clam lipid were previously studied in this laboratory,[13][14][15] this section records the results of our studies on its fatty acid components.

The fatty acids used in this study were prepared from the same lipid which had been extracted from the dried clam with trichloroethylene and used in a previous study[15] on sterol components. The lipid contained a considerable amount of phosphatide besides fatty oil. In the course of preparation of the fatty acids from this

lipid, there were formed some substances which were difficultly soluble in both ether and water, and a complete separation of the ether solution of crude fatty acids and the aqueous washings was very difficult. For this reason, the yield of crude fatty acids from the lipid was remarkably low. The crude fatty acids were then esterified with methanol, and the methyl esters were distilled until distillation has almost ceased at a boiling point of 202°C/*ca.* 1 mm. A further raise of temperature brought about an indication of decomposition. The residue amounting to 22% of crude methyl esters was a tarry matter. It was saponified, and the soap solution was extracted with ether in order to remove some unsaponifiable matter which was contained in a small amount in the crude methyl esters and became concentrated in the residue. The soap solution was then acidified with hydrochloric acid. The material liberated was soluble in ether but difficultly soluble in petroleum ether, yielding only a small proportion of petroleum ether soluble matter. The material insoluble in petroleum ether turned into a pitch like solid on standing for a long time, in which the presence of ash, phosphorus and nitrogen was indicated by analysis. It may be inferred from these facts that the original crude methyl esters were contaminated with some products of incomplete hydrolysis of phosphatide or other non-fat substance, and these contaminations caused highly unsaturated methyl esters having high boiling points to undergo polymerization to a considerable extent in the course of distillation. In the present study, the distillate alone was examined further. Since bulk of the methyl esters of very high unsaturation and large carbon number are afraid of not being distilled due to polymerization, the possibility is not excluded that even if $C_{22}F_6$ acid or C_{24} highly unsaturated acids were really contained in a small amount in the original fatty acids, they could not be found in the distillate. So far as the results of our examination of the distillate indicate, the fatty acid components of clam lipid have no peculiar feature as compared with those of common fish oils.

Crude fatty acids and fractionation of their methyl esters. The lipid of clam was saponified, and the unsaponifiable matter was removed from the soap solution by extraction with ether in the usual way. The soap solution was acidified with hydrochloric acid, and the crude fatty acids were taken up with ether. The aqueous layer had a dark reddish orange color, and contained a small amount of dark brown insoluble matter. On washing the ether solution with water, the ether solution and the aqueous washings did not separate sharply, forming an intermediate layer contaminated with some insoluble matter. After removal of ether from the ether solution, there was obtained 200 g of residue. Petroleum ether (500 cc) was added to this residue, the mixture was refluxed for a while and then cooled, and the insoluble portion was removed. The crude fatty acids (175 g) obtained after removal of petroleum ether from the solution was a dark reddish orange liquid with some solid material. It had N.V. 183.6 and I.V. 131.7 and gave 23.6% solid acids (N.V. 189.6 and I.V. 25.5) by the lead salt ethanol method. Bromination gave 36.36% ether-insoluble bromide having Br-content 69.83%. Assuming that the iodine value of solid acids is ascribed to C_{20} and C_{22} mono-ethenoid acids, the content of saturated acids in the crude fatty acids is estimated at about 16%.

The crude fatty acids were refluxed with an equal amount of methanol containing 2% of sulfuric acid for 2 hours. The methyl esters thus obtained had S.V. 175.2 and I.V. 124.8. These were fractionally distilled, yielding seven fractions. The

distillation became almost ceased at a boiling point of 202°C/*ca.* 1 mm, and the residue began to undergo decomposition with the evolution of fumes when the temperature was raised further. Each of the seven fractions was then refractionated one after another. The fractions and residues eventually obtained from 152 g of methyl esters are shown in Table 6.

TABLE 6. Fractionation of the Methyl Esters

Fraction	b.p. (°C/mm)	n_D^{20}	S.V.	I.V.	Yield (%)
1	–185/15	1.4432	213.4	32.1	2.7
2	185–190 〃	1.4439	205.5	57.4	4.2
3	190–195 〃	1.4443	204.0	61.2	7.5
4	159–200 〃	1.4458	198.9	77.1	15.0
5	200–205 〃	1.4471	196.8	97.5	8.8
6	205–210 〃	1.4527	194.1	134.6	9.9
7	210–215 〃	1.4587	193.5	142.1	6.1
8	215–220 〃	1.4606	190.1	169.6	3.6
9	–210/10	1.4707	184.0	183.7	1.0
10	210–215 〃	1.4713	179.5	197.4	7.0
11	215–220 〃	1.4720	177.5	219.5	1.5
12	220–225 〃	1.4733	172.7	222.4	3.0
13	225–230 〃	1.4762	169.0	240.0	2.8
14	230–235 〃	1.4820	166.9	249.7	1.3
Residue of refractionation	—	—	162.2	127.5	3.3
Residue of the first fractionation	—	—	123.1	115.5	22.3

The lowest fraction. The solid acids separated by the lead salt ethanol method from the fatty acids of the fraction 1 in Table 6 had N.V. 238.8 and were considered to be a mixture of myristic acid (N.V., calcd., 245.7) and palmitic acid (N.V., calcd., 218.8). In order to separate myristic acid, the solid acids were converted into methyl esters. Fractionation of the methyl esters gave a fraction boiling below 180°C/15 mm. The fatty acids from this fraction, recrystallized from ethanol, yielded a crystalline acid which had m.p. 52°C and showed no depression of melting point when mixed with a pure specimen of myristic acid (m.p. 54°C) in various proportions.

Fraction 185°–190°C/15 mm. Refractionating the fractions 2 and 3 in Table 6, a fraction of b.p. 185°–190°C/15 mm was separated. The fatty acids of this fraction were recrystallized from ethanol, yielding palmitic acid of m.p. 62°C and N.V. 219.4. The material recovered from the mother liquor of recystallization was subjected to the lead salt ethanol method, and a liquid acid of n_D^{20} 1.4595, N.V. 220.3 and I.V. 98.5 was obtained. Oxidation of this liquid acid by Hazura's method yielded a product which had m.p. 122°C and N.V. 193.7 (calcd. for $C_{16}H_{32}O_4$, 194.5) after recrystallization from ethanol-petroleum ether.

Anal. Calcd. for $C_{16}H_{32}O_4$: C 66.63%; H 11.18%. Found: C 66.36%; H 11.29%.

Accordingly the liquid acid is identified with zoomaric acid (calcd., N.V. 220.6 and I.V. 99.8).

Fractions 205°–210°C/15 mm and 210°–215°C/15 mm. Refractionating the fractions 6, 7, 8 and 9 in Table 6, a fraction (A) of b.p. 205°–210°C/15 mm and a fraction (B) of b.p. 210°–215°C/15 mm were separated. The fatty acids of the fraction (A) were separated into solid and liquid acids. The liquid acids were

then separated into two fractions by treating their lithium salts with 50% ethanol. The fraction obtained from the 50% ethanol-insoluble lithium salts had n_D^{20} 1.4620, N.V. 199.0 and I.V. 87.8. Its elaidinization product, after purification, gave elaidic acid of m.p. 43°–44°C, N.V. 199.8 (calcd., 198.6) and I.V. 89.8 (calcd., 89.8). The acid fraction obtained from the 50% ethanol-soluble lithium salts had N.V. 200.3 and I.V. 148.3. Bromination of this acid fraction yielded an ether-insoluble bromide of Br-content 67.51%.

The solid acids obtained from the fatty acids of the fraction (B) by the lead salt ethanol method had m.p. 68°–69°C after recrystallization from ethanol, and showed no depression of melting point when mixed with a pure specimen of stearic acid (m.p. 70°C) in various proportions. The liquid acids were treated in the same way as described for the fraction (A), and oleic acid was identified in the acid fraction obtained from the 50% ethanol-insoluble lithium salts. The acid fraction (N.V. 200.8 and I.V. 158.4) from the 50% ethanol-soluble lithium salts gave an ether-insoluble bromide of Br-content 67.83%.

The ether-insoluble bromides from the fractions (A) and (B) were debrominated with zinc and sulfuric acid in methanol. The product in the form of methyl ester had I.V. 291.9. Hydrogenation of the free fatty acid from this product yielded stearic acid of m.p. 69°C. These results seem to indicate that the original fatty acid components of ether-insoluble bromides contain $C_{18}F_3$ and $C_{18}F_4$ acids. (Calcd. for $C_{18}H_{30}O_2Br_6$ and $C_{18}H_{28}O_2Br_8$: Br 63.28% and 69.83%, respectively. Calcd. for the methyl esters $C_{19}H_{32}O_2$ and $C_{19}H_{30}O_2$: I.V. 260.4 and 349.6, respectively.).

Fractions 215°-219°C/10 mm and 219°-222°C/10 mm. Refractionation of the fractions 11, 12 and 13 in Table 6 gave a fraction (C) of b.p. 215°–219°C/10 mm and a fraction (D) of b.p. 219°–222°C/10 mm.

The fatty acid mixture of the fraction (C) was treated by the lead salt ethanol method in such a way that about one tenth the total fatty acid mixture was precipitated in the form of lead salt. The solid acids obtained from the precipitate of lead salt had m.p. 60°C and N.V. 180.1 after recrystallization from ethanol, and were found to contain 51% arachidic acid by the mixed melting point method. The fatty acid fraction which was not precipitated as lead salt, was separated into two fractions by way of lithium salt in 50% ethanol. The acid fraction obtained from the 50% ethanol-insoluble lithium salt gave a solid acid of m.p. 22°C, N.V. 180.6 and I.V. 81.5 (calcd. for $C_{20}H_{38}O_2$: N.V. 180.7 and I.V. 81.7) after recrystallization from aqueous ethanol. The acid fraction obtained from the 50% ethanol-soluble lithium salt was then treated by the lithium salt acetone method, and a highly unsaturated acid rich fraction of N.V. 179.7 and I.V. 267.2 was obtained. Hydrogenation of this acid fraction gave a product which melted at 70°C and was found to contain 84% arachidic acid. The ether-insoluble bromide derived from this acid fraction had Br-content 67.33% (calcd. for $C_{20}H_{32}O_2Br_8$: Br 67.76%).

From the fraction (D), treated in the same way as for the fraction (C), a saturated acid fraction containing 76% arachidic acid and a C_{20} mono-ethenoid acid were separated.

Fraction 230°-235°C/10 mm. The fatty acid mixture of the fraction 14 in Table 6 was treated by the lithium salt acetone method by which a highly unsaturated fraction of N.V. 168.1 and I.V. 318.8 was separated. Its hydrogenation product melted at 74°C, after recrystallization from ethanol, and contained 76% behenic

acid. The ether-insoluble bromide derived from this highly unsaturated fraction had Br-content 69.91% (calcd. for $C_{22}H_{34}O_2Br_{10}$: Br 70.76%). Hence the main component of this highly unsaturated fraction is regarded as a $C_{22}F_5$ acid. The fatty acid fraction obtained from the acetone-insoluble lithium salt was treated by the lead salt ethanol method follwed by the lithium salt 50% ethanol method, giving a saturated acid fraction of m.p. 71°–72°C, N.V. 162.4 and behenic acid content 65% and a cetoleic acid fraction of m.p. 30°–31°C, N.V. 165.3 and I.V. 78.1 (calcd. for $C_{22}H_{42}O_2$: N.V. 165.7 and I.V. 75.0).

Residue. Two residues in Table 6 were united and saponified, and the soap solution was extracted with ether in order to remove unsaponifiable matter. Acidification of the soap solution with hydrochloric acid gave a product which was soluble in ether but difficultly soluble in petroleum ether, yielding about 10% of petroleum ether-soluble portion. The portion insoluble in petroleum ether was a tarry matter and became a pitch like solid on standing for a long time. It contained 1.21% ash, 1.03% phosphorus and 1.58% nitrogen. The petroleum ether-soluble portion was a dark colored liquid with some solid. Its lithium salts were treated with 50% ethanol, and the fatty acids (about 20% of the petroleum ether-soluble portion) obtained from the 50% ethanol-insoluble lithium salts were decolorized with carbon in ethanol and recrystallized. The final product obtained was a crystalline solid of N.V. 152.4 and I.V. 61.9 (calcd. for $C_{24}H_{46}O_2$: N.V. 153.0 and I.V. 69.2).

III. Fatty Acid Components of the Lipid of Corbicula[16]

This section records the results of our study on the fatty acid components of the lipid of *Corbicula leana*, one of the most popular edible shellfish in this country. No other literature pertaining to the fatty acid components of the lipid of *Corbicula leana* is known to us except the work of Tsujimoto and Koyanagi[7] who reported m.p. 28°–29°C, N.V. 194.3, I.V. (Wijs) 155.5 and ether-insoluble bromide 38.0% for its fatty acid mixture.

In this study, the boiled meat of corbicula was dried, and the dried material was extracted with trichloroethylene. The crude lipid obtained was saponified, and the unsaponifiable matter was removed by extraction of the soap solution with ether. The crude fatty acids were separated and converted into methyl esters, and the latter were fractionally distilled. Like the case with the crude methyl esters of clam lipid described in the preceding section, the amount of distillation residue was very large (25.3% of crude methyl esters) as shown in Table 7. This residue contained a considerable amount of the unsaponifiable matter which, escaping the previous extraction from the soap solution with ether, entered into the crude fatty acids in a small amount and became concentrated in the residue. It was saponified, the unsaponifiable matter was removed, and the substances obtained on acidification of the soap soluiton were reconverted into methyl esters. Distillation of the methyl esters gave a relatively small amount of distillate leaving a large amount of residue as shown in Table 8. This residue was found to contain ash, phosphorus and nitrogen by analysis. These features are presumably explicable for the same reason as noted in the preceding section that the crude fatty acids were contaminated with some compounds resulting from incomplete hydrolysis of phosphatide or non-fat material, and these contaminations caused fatty acid com-

ponents of high unsaturation and large carbon number to undergo polymerization in the course of distillation of the crude methyl esters.

Crude fatty acids and fractionation of their methyl esters. Boiled meat (37.75 kg) of *Corbicula leana* procured from the Shimono-isshiki Fishery Association, Aichi Prefecture, in June and July, 1950 was dried at 65°–75°C. The dried material (11.44 kg) was crushed into fine pieces and extracted with trichloroethylene, yielding 1.13 kg of a viscous lipid of dark greenish orange color. The lipid was saponified and the soap solution was extracted with ether in order to remove unsaponifiable matter in the usual way. The soap solution was then acidified with hydrochloric acid, and the fatty acid mixture was taken up with ether. In the course of washing of the ether solution with water, a complete separation of the ether layer and the aqueous layer was very difficult.

The crude fatty acid mixture (574 g) was a dark greenish orange solid of m.p. 30°–34°C, N.V. 185.3 and I.V. 115.1. It yielded 28.06% solid acids of I.V. 25.3 by the lead salt ethanol method. Bromination of the crude fatty acid mixture yielded 30.87% ether-insoluble bromide. Assumig that the iodine value of solid acids is ascribed to the C_{20} and C_{22} mono-ethenoid acids, the saturated acids in the crude fatty acid mixture are estimated at about 19%. The crude fatty acid mixture was converted into the methyl ester mixture, and the latter (507 g) was fractionated as shown in Table 7. Since the residue in Table 7 was found to be contaminated with unsaponifiable matter which, escaping the previous extraction from the soap solution of the initial lipid, was contained in the crude fatty acid mixture, it was saponified and the unsaponifiable matter (13.4% of the residue) was removed. The material obtained on acidfication of the soap solution was reconverted into methyl esters, and the latter were fractionated with the results shown in Table 8.

TABLE 7. Fractionation of the Methyl Ester Mixture

Fraction	b.p. (°C/mm)	Yield (%)	n_D^{20}	S.V.	I.V.
1	161–185/6	10.9	1.4466	208.5	44.3
2	185– /6–3	11.7	1.4479	203.2	46.7
3	–183/3	8.8	1.4493	201.9	58.6
4	183–193/3	19.8	1.4529	197.9	92.1
5	193–200/3	12.2	1.4597	194.3	139.8
6	200–205/3	4.8	1.4660	183.6	168.9
7	205–210/3	2.6	1.4694	178.3	172.7
8	210–215/3	3.8	1.4723	170.5	203.9
Residue	—	25.3	—	125.0	104.1

TABLE 8. Fractionation of the Residue in Table 6 after Removal of the Unsaponifiable Matter

Fraction	b.p. (°C/mm)	Yield (%)	n_D^{20}	S.V.	I.V.
R_1	194–196/1	5.2	1.4741	168.2	193.4
R_2	196–199/1	14.9	1.4758	166.2	187.8
Residue	—	79.9	—	137.1	95.3

Fractions 1, 2 and 3. The fractions 1, 2 and 3 in Table 7 were united. The fatty acids (124 g) were fractionally precipitated as lead salts from their ethanol

solution, and the following three fractions were separated and examined while the other fractions were regarded as mixed fractions and not examined.

Fraction *A* 31.3 g, N.V. 223.8, I.V. 0.4
Fraction *B* 23.2 g, N.V. 208.0, I.V. 63.9
Fraction *C* 26.5 g, N.V. 192.7, I.V. 112.9

The fraction *A* had m.p. 59°-59.5°C. Its neutralization value is slightly higher than the value for palmitic acid. This fraction was separated further into several fractions by the fractional precipitation of magnesium salt from its ethanol solution. Each fraction obtained closely approximated palmitic acid in melting point and neutralization value. The fraction from the 2nd precipitate of magnesium salt had m.p. 61°-62°C and N.V. 218.6 (calcd. for $C_{16}H_{32}O_2$, 218.8) after recrystallization from ethanol.

The methyl esters prepared from the fraction *B* were fractionally distilled, and the lowest fraction of b.p. below 175°C/6 mm, S.V. 214.8 and I.V. 45.1 was separated. The fatty acids of this fraction were recrystallized from 80% ethanol under cooling with ice, yielding a crystalline solid (a) from which a palmitic acid fraction of m.p. 59.5°-60.5°C and N.V. 221.2 and a fraction of m.p. 44°-45°C and N.V. 234.7 consisting of palmitic and myristic acids were obtained by a further fractional crystallization. The fatty acids remaining in the mother liquor separated from the crystalline solid (a) were recovered, and their lithium salts were treated with aqueous ethanol. The fatty acid fraction obtained from the lithium salts insoluble in aqueous ethanol yielded, on recrystallization from 80% ethanol under well cooling, a crystalline solid which was regarded as a mixture of palmitic and myristic acids from its m.p. 43.5-44.5°C and N.V. 231.8.

The methyl esters prepared from the fraction *C* were refractionated, and a fraction (b) of b.p. below 170°C/5.5 mm, S.V. 216.7 and I.V. 68.5 and a fraction (c) of b.p. 170°-175°C/5.5 mm, S.V. 206.6 and I.V. 80.7 were separated. The lithium salts of the fatty acids of the fraction (b) were treated with aqueous ethanol, and the fatty acid fraction from the lithium salts insoluble in aqueous ethanol was recrystallized from 80% ethanol, yielding a mixture of palmitic and myristic acids of m.p. 42°-43.5°C and N.V. 232.9. On treating the fatty acids of the fraction (c) in the same way, a liquid acid fraction of N.V. 221.3 and I.V. 88.3 (calcd. for $C_{16}H_{30}O_2$: N.V. 220.6 and I.V. 99.8) was obtained from the lithium salts soluble in aqueous ethanol. Hazura's oxidation product of this liquid acid had m.p. 124°-125°C and N.V. 196.0 (calcd. for $C_{16}H_{32}O_4$: 194.5) after recrystallization from ethanol.

Anal. Calcd. for $C_{16}H_{32}O_4$: C 66.63%; H 11.18%. Found : C 66.32%; H 10.98%.

Hence the liquid acid is found to consist mainly of zoomaric acid.

Fraction 4. Refractionation of the fraction 4 in Table 7 gave a fraction (d) of b.p. 188°-193°C/5 mm, S.V. 197.6 and I.V. 81.1 and a fraction (e) of b.p. 198°-207°C/5 mm, S.V. 181.9 and I.V. 114.1. The fatty acids of the fraction (d) were treated by the lead salt ethanol method, and the liquid acids obtained were treated by the lithium salt acetone method, yielding eventually a highly unsaturated acid rich fraction of N.V. 195.7 and I.V. 289.5. On bromination, it yielded an ether-insoluble bromide of Br-content 68.91%, which was deemed to contain chiefly bromide of C_{18} acid (calcd. for $C_{18}H_{28}O_2Br_6$: Br 69.83%). The solid acids obtained from the fraction (e) by the lead salt ethanol method gave a mixture of palmitic and stearic acids of m.p. 59°-60°C and N.V. 202.8 after recrystallization from ethanol. The

liquid acids were treated by the lithium salt acetone method, and the acetone-insoluble lithium salts were recrystallized further from 50% ethanol. Acidification of the recrystallized lithium salts gave oleic acid of n_D^{20} 1.4613, N.V. 199.1 (calcd., 198.6) and I.V. 87.3 (calcd., 89.8). Hydrogenation of this acid gave a product which melted at 66°–67°C after recrystallization from ethanol and showed no depression of melting point when mixed with a pure specimen of stearic acid (m.p. 70°C) in various proportions. Hazura's oxidation of this acid gave a product which had m.p. 135°–136°C and N.V. 178.2 (calcd. for $C_{18}H_{36}O_4$: 177.3).

Anal. Calcd. for $C_{18}H_{36}O_4$: C 68.31%; H 11.47%. Found : C 68.12%; H 11.60%.

Fractions 7 and 8. The solid acids obtained from the fatty acids of the fraction 7 in Table 7 by the lead salt ethanol method appeared to be a mixture of stearic and arachidic acids from their m.p. 61.5°–63°C and N.V. 191.0 after recrystallization from ethanol. The liquid acids were treated by the lithium salt acetone method, and the acetone-insoluble lithium salts were then recrystallized from 50% ethanol, yielding an acid fraction of N.V. 180.8 and I.V. 87.6 from the recrystallized lithium salts. The fatty acids of the fraction 8 in Table 7 were treated in the same manner. The solid acids had m.p. 64.5°–65.5°C and N.V. 174.7. The fatty acid fraction obtained from the 50% ethanol-insoluble lithium salts had N.V. 180.2 and I.V. 75.3 and was regarded as an eicosenoic acid fraction (calcd. for $C_{20}H_{38}O_2$: N.V. 180.7 and I.V. 81.7), since its hydrogenation product, after purification by recrystallization, had m.p. 72°–73°C and N.V. 178.7 and showed no depression of melting point when mixed with a pure specimen of arachidic acid (m.p. 75°C and N.V., calcd., 179.5) in various proportions. The highly unsaturated acid fraction obtained from the acetone-soluble lithium salts had N.V. 163.5 and I.V. 321.0 and yielded, on bromination, an ether-insoluble bromide of Br-content 70.29% (calcd. for $C_{22}H_{34}O_2Br_{10}$: Br 70.76%). Debromination of this bromide with zinc and glacial acetic acid gave a highly unsaturated acid fraction of N.V. 168.9 and I.V. (Wijs) 380.8. Its hydrogenation product, after recrystallization, showed m.p. 77.5°–78.5°C and N.V. 165.2 (calcd. for $C_{22}H_{44}O_2$: 164.7) and no depression of melting point when mixed with a pure specimen of behenic acid (m.p. 80°C). Hence the highly unsaturated acid component forming this bromide is regarded as docosapentaenoic acid (calcd., N.V. 169.8 and I.V. 384.0).

Fractions R_1 and R_2. The fatty acids of the fraction R_1 in Table 8 were treated in the same way as described above. The fatty acids obtained from the 50% ethanol-insoluble lithium salts had N.V. 169.2 and I.V. 76.8. Their hydrogenation product was deemed to be a mixture of behenic acid with a little arachidic acid from its m.p. 75.5°–76.5°C and N.V. 168.4 after recrystallization from ethanol. Hence the original fatty acids appear to contain docosenoic and eicosenoic acids. The solid acids obtained from the fatty acids of the fraction R_2 in Table 8 were deemed to contain tetracosanoic acid besides behenic acid from their m.p. 69°–70°C and N.V. 158.2 after recrystallization from ethanol. The highly unsaturated acid rich fraction obtained from the fatty acids of the fraction R_2 by the lithium salt acetone method had N.V. 162.3 and I.V. 222.6, and debromination of the ether-insoluble bromide therefrom gave a highly unsaturated acid fraction of N.V. 160.0 and I.V. 335.1. Its hydrogenation product, recrystallized from ethanol, had m.p. 71.5°–73°C and N.V. 158.1. Hence the fatty acid components of ether-insoluble bromide was considered to contain C_{24} acid besides C_{22} acid.

Residue. The residue in Table 8 was a dark brown viscous liquid, and contained 1.90% ash, 0.44% phosphorus and 1.51% nitrogen. A portion (44 g) of the acid substances obtained by saponification of the residue followed by acidification of the soap solution was refluxed with tenfold petroleum ether, and 12 g of petroleum ether-soluble material was obtained. On treating the lithium salts of this material with 50% ethanol, 2 g of fatty acids were obtained from the 50% ethanol-insoluble lithium salts. The fatty acids, after decolorization with carbon in ethanol, were fractionally crystallized, yielding three fractions: (1) m.p. 73°–75°C, N.V. 143.7 and I.V. 14.8; (2) m.p. 53°–55°C and N.V. 145.4; (3) m.p. 42°–44°C, N.V. 146.6 and I.V. 28.9. The properties of these three fractions seem to indicate the presence of C_{24} and C_{26} members among the saturated and mono-ethenoid acids in the residue (Calcd. for $C_{24}H_{46}O_2$: N.V. 152.2. Calcd. for $C_{26}H_{52}O_2$: N.V. 141.4).

IV. Fatty Acids and Sterols in the Fatty Oil of *Spisula sachalinensis*[17]

The shellfish, *Spisula sachalinensis*, like clam and corbicula, belongs to Eulamellibranchia. It is distributed in the northern district of this country and is used for edible purpose. No literature pertaining to fatty oil of this shellfish has been known to us. In this study, fatty oil was extracted from this shellfish, and the fatty acid and sterol components were examined. Although the separation and identification of individual fatty acid components could not be attained due to scanty of the material, the fatty acids were found to contain saturated, mono-ethenoid and highly unsaturated acids, and the unsaturation of fatty acids, on the whole, was found a little higher than the unsaturation of fatty acids of clam and corbicula.

Bromination of steryl acetate mixture in ether yielded an ether-insoluble bromide which was found to consist mainly of tetrabromide derived from di-unsaturated steryl acetate. Ether-soluble bromide was precipitated by adding ethanol to the ether solution. Since the precipitated bromide was almost completely debrominated with potassium iodide and ethanol, it contained only a little or no bromide having bromine atom in the side chain. Thus the sterol components of the precipitated bromide were mostly sterols having no ethenoid linkage in the side chain. The precipitated bromide was separated into two fractions I and II by fractional crystallization. Although the free sterol obtained by debromination of the bromide fraction I and its derivatives did not very closely agree with clionasterol and its corresponding derivatives in their properties, the benzoate of this sterol, like clionasteryl benzoate, developed a bright blue color in the course of solidification. Thus the presence of clionasterol in the sterol components of the debromination product of the fraction I was indicated. The sterol obtained from the debromi-

TABLE 9. Properties of Related Sterols

	Free sterol		Acetate		Benzoate	
	m.p. (°C)	$[\alpha]_D^{\circ}$	m.p. (°C)	$[\alpha]_D^{\circ}$	m.p. (°C)	$[\alpha]_D^{\circ}$
Sterol from the bromide I	135	−34.7	132–133	−35.1	138	—
Clionasterol[18]	137.5–138.5	−37	137	−41.9	134.5–135	−16.8
Sterol from the bromide II	135 –136	—	124	−37.5	144	—
β-Sitosterol[19]	136 –137	−36.6	125–126	−41.0	146 –147	−13.8[20]

nation product of the bromide fraction II closely approximated β-sitosterol in its properties, and was regarded as β-sitosterol. This is the first instance in which the occurrence of β-sitosterol in animal kingdom is indicated. Properties of the sterols from the fractions I and II, clionasterol and β-sitosterol together with their derivatives are shown in Table 9.

A spectrophotometric examination of the crude sterol mixture indicated the presence of 5.6% provitamin D ($\Delta^{5,7}$-sterol).

Properties of fatty oil. The material used in this study is the dried meat of shellfish, *Spisula sachalinensis*, which was received by the courtesy of Dr. M. Yamada, Faculty of Fishery, Hokkaido University in April, 1952. The dried meat was prepared by drying the fresh meat under exposure to infrared radiation in a yield of 12.1% or about 4% on the basis of the whole shellfish. Extraction of the dried meat (730 g) with ether gave 44 g (6.0%) of an extract, which was then extracted with 500 cc of acetone, yielding 32 g of acetone-soluble oil. This was a dark greenish liquid with some solid and had the following characteristics: d_4^{30} 0.9424, n_D^{30} 1.4819, A.V. 41.3, S.V. 165.6, I.V. 144.2* and unsaponifiable matter 17.04%.

Fatty acids. Fatty acids and unsaponifiable matter were separated by saponification of the acetone-soluble oil followed by extraction of the soap solution with ether in the usual way. The fatty acids were a dark greenish oil with some solid and had d_4^{30} 0.8985, n_D^{30} 1.4670, N.V. 196.0 and I.V. 168.4. Bromination of the fatty acids yielded 64.7% ether-insoluble bromide of Br-content 69.80%. The lead salt ethanol method gave 20.0% solid acids (N.V. 213.9 and I.V. 13.3) from the fatty acids. The saturated methyl esters in the methyl esters of total fatty acids were found to be 17.1% by the permanganate acetone oxidation method.

The methyl esters (S.V. 185.3 and I.V. 160.1) prepared from the fatty acids were fractionated as shown in Table 10. The residue in Table 10 was found to contain a considerable amount of the unsaponifiable matter which was contained in the initial methyl esters in a small amount and became concentrated in the residue. The fatty acid fraction (1.1 g) obtained after removal of the unsaponifiable matter from the residue had N.V. 169.8.

Saturated esters and their saponification equivalent were determined for each

TABLE 10. Fractionation of the Methyl Esters

Fraction	Yield		b.p. (°C/4 mm)	n_D^{20}	Saponif. equiv.	S.V.	I.V.
	(g)	(%)					
1	1.8	12.0	160–170	1.4477	269.1	208.5	71.4
2	2.1	14.0	170–180	1.4489	274.4	204.5	81.0
3	1.8	12.0	180–185	1.4533	283.7	197.8	105.8
4	2.8	18.7	185–190	1.4594	294.1	190.8	154.2
5	1.8	12.0	190–195	1.4678	308.0	182.2	210.3
6	2.0	13.3	195–205	1.4748	316.3	177.4	250.9
7	1.1	7.3	205–210	1.4815	329.7	170.2	293.0
Residue	1.6	10.7	—	—	415.9	134.9	165.1

* Unless stated otherwise in Sects. IV-VIII, iodine values were determined by the Wijs method for fatty oils and fatty acid components and by the pyridine sulfate dibromide method for sterol components.

fraction in Table 10, and saponification equivalent and iodine value of unsaturated esters together with carbon number and unsaturation of unsaturated acids for each fraction were deduced from the observed data by calculation. The results are shown in Table 11. Saturated esters in the residue were determined with the methyl esters after removal of unsaponifiable matter. Since the fractions 5–7 contained only a minor proportion of saturated esters, saponification equivalents of unsaturated esters in these fractions were assumed to be the same with the corresponding values for the respective fractions. Unsaturation (−H) is expressed by the atom equivalent of hydrogen required to a complete saturation of one mole of unsaturated esters.

Compositions of the saturated esters of the fractions 1–4 were calculated by assuming that each of these fractions contains two adjacent homologous esters of even acid. The saturated esters of the fractions 5–7 and the residue were regarded as those of C_{18} and higher acids. Plotting the carbon number against the unsaturation for the unsaturated acids of each fraction, a curve is obtained which shows the relation between the carbon number and the unsaturation. From this curve, each average unsaturation of the unsaturated esters of C_{16}, C_{18}, C_{20} and C_{22} acids is found by interpolation and extrapolation, and the composition of unsaturated esters of each fraction is calculated on an assumption that unsaturated acids in each fraction contain two adjacent even members, excepting that the unsaturated esters of the residue were regarded wholly as those of C_{22} acid. The composition of the total esters thus obtained by calculation are shown in Table 12. Although the figures in Table 12 denote the percentage of each methyl ester fraction in the total methyl esters, they may be regarded approximately as the percentage of each acid fraction in the total fatty acids.

TABLE 11. Saturated and Unsaturated Esters in Each Fraction

Fraction	Saturated ester		Unsaturated ester			
	Yield (%)	S.E.	S.E.	I.V.	Carbon number of fatty acid	Unsaturation (−H)
1	42.3	265.9	271.4	123.7	16.3	2.59
2	37.2	269.6	277.2	129.0	16.7	2.82
3	22.6	276 0	286.0	136 7	17.3	3.08
4	13.5	285.2	295.5	178.3	18.1	4.15
5	7.5	—	308.0	227.4	19.1	5.52
6	2.9	—	316.3	258.4	19.7	6.44
7	1.3	—	329.7	296.9	20.8	7.71
Residue	1.1	—	—	—	—	—

TABLE 12. Composition of the Methyl Esters

Carbon number of fatty acid	Saturated ester			Unsaturated ester			
	C_{14}	C_{16}	C_{18} and higher series	C_{16}	C_{18}	C_{20}	C_{22}
%	0.8	12.4	4.3	15.3	33.7	23.1	10.4
Unsaturation (−H)	—	—	—	2.4	4.0	6.8	8.7

Sterols. The unsaponifiable matter (5.3 g) was a greenish solid at ordinary temperature. Recrystallizing from 300 cc of ethanol, it gave 3.0 g of a crude sterol

mixture in which the sterol content was found to be 95.1% by the digitonide method. The crude sterol mixture exhibited ultraviolet absorption spectra characteristic to provitamin D ($\Delta^{5,7}$-sterol); $k_{282}=2.72$, $k_{277}=2.51$ and $k_{290}=1.85$. From these data, the provitamin D content in the crude sterol mixture is found to be 5.6%. Acetylation of the crude sterol mixture gave a crude steryl acetate mixture of m.p. 115°-117°C which yielded eventually 0.2 g of a fraction of m.p. 134°-135°C and I.V. 103.2 after repeated recrystallization. But the fraction recovered from the mother liquor of the final recrystallization had m.p. 129°-130°C. Thus recrystallization failed to yield a uniform steryl acetate. A steryl acetate fraction (2.0 g) of m.p. 130°-132°C was recovered from the mother liquors of recrystallizations. It was dissolved in 20 cc of ether, and brominated at −5°--15°C. A very small amount of crystalline bromide (*ca.* 30 mg) separated. This bromide was considered to consist mainly of tetrabromide from its Br-content 42.20%. The ether solution, separated from the insoluble bromide, was freed from excess bromine, and on concentration, a further quantity (0.5 g) of bromide separated. This bromide was only partially debrominated when it was treated with potassium iodide and ethanol. On adding ethanol to the ether filtrate, two bromide fractions, 1.5 g and 0.7 g, were fractionally precipitated. When a portion of these bromide fractions was treated with potassium iodide and ethanol, it was almost completely debrominated, and the product was found to contain less than 0.1% bromine. The first precipitate of bromide was recrystallized from ethanol-ether, giving a bromide (I) of m.p. 124°-125°C. The bromide recovered from the mother liquor of recrystallization and the second precipitate of bromide were united, and the united material was recrystallized from ethanol-ether, giving a bromide (II) of m.p. 120°C.

The bromide (I) had Br-content 27.17% (calcd. for $C_{31}H_{52}O_2Br_2$: Br 25.92%). Debromination of this bromide with zinc and glacial acetic acid followed by recrystallization of the product from ethanol gave a steryl acetate of m.p. 132°-133°C, $[\alpha]_D^{18}=-35.1°$ and S.V. 124.8 (calcd. for $C_{31}H_{52}O_2$: S.V. 122.8). Free sterol obtained by saponification of the acetate had m.p. 135°C and $[\alpha]_D^{18}=-34.7°$ after recrystallization from ethanol. Benzoate prepared from the free sterol melted at 138°C after recrystallization from ethanol. On cooling the melted benzoate, a bright blue color was developed in the course of solidification.

Debromination of the bromide (II) with zinc and glacial acetic acid followed by recrystallization of the product from ethanol gave a steryl acetate of m.p. 124°C, $[\alpha]_D^{18}=-37.5°$, S.V. 123.3 and I.V. 55.8 (calcd. for $C_{31}H_{52}O_2$: S.V. 122.8 and I.V. 55.6). Free sterol from this acetate had m.p. 135°-136°C after recrystallization from ethanol. Benzoate prepared from the free sterol melted at 144°C.

V. Properties of Some Japanese Shellfish Oils with Special Reference to Provitamin D Content of Their Crude Sterols[21]

In the course of our studies on fatty oils of aquatic invertebrates, it has become advisable to gain an insight into the correlation between the properties of fatty oils of aquatic invertebrates and their taxonomical locations by accumulating informations on some important properties of fatty oils from the largest possible kinds of Japanese aquatic invertebrates. It appears also desirable to know provitamin D content of individual fatty oils from a view point of finding a better utilization of fatty oil components of aquatic inverterates. For these reasons, the authors have

determined fat contents and some properties of fatty oils, fatty acids and unsaponifiable matter for sixteen kinds of Japanese shellfish. Also provitamin D content of crude sterol mixture was estimated by the spectrophotometric method for each kind of these shellfish. Of the shellfish used in this study, *Mytilus crassitesta* is kindred with *Mytilus edulis*, the fat components of which were closely studied by Lovern,[11] and *Corbicula japonica* is kindred with *Corbicula leana* and *Corbicula sandai*. Fat of *Corb. leana* was studied by Tsujimoto and Koyanagi,[9] and the present authors,[22] while fat of *Corb. sandai* was reported by Hori and Hosoda.[23] Fat of *Cellana nigrolineata* was reported by Tsujimoto and Koyanagi.[7] Fats of the other shellfish recorded in this section have not previously been studied. Among the provitamin D contents of shellfish sterol previously reported, the largest one are 35-50% for

TABLE 13. List of 16 Kinds of Japanese 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	*Anadara inflata*	—	—	3	74	18	0.64	3.6	0.43	67
2	*Pinna pectinata japonica (forma lischkeana)*	Onizaki	Middle Feb., 1954	4	289	30	1.3	4.3	0.65	50
3	*Mytilus crassitesta*	Atsumi	Late Dec., 1953	12	177	23	1.5	6.5	0.96	64
4	*Corbicula japonica*	Shimono-isshiki	(a) Middle Jan., 1954	300	700	17	1.4	8.2	0.65	46
			(b) Late Dec., 1953	117	350	9	0.86	9.6	0.42	49
5	*Sunetta menstrualis*	Atsumi	Late Oct., 1953	12	532	25	0.42	1.7	0.28	67
6	*Gomphina melanaegis*	Atsumi	Late Oct., 1953	6	465	20	0.79	3.9	0.36	46
7	*Schizothaerus mittalli*	Atsumi	Middle Dec., 1953	1	134	23	1.2	5.3	0.62	50
8	*Cellana toreuma*	Osaki-shimojima	Middle Aug., 1952	—	—	185	4.8	2.6	3.3	69
9	*Cellana nigrolineata*	Osaki-shimojima	Middle Aug., 1952	—	—	46	2.0	4.4	1.4	71
10	*Cipangopaludina japonica*	Nagoya	Middle Aug., 1953	13	—	22	0.27	1.2	0.11	41
11	*Viviparus histricus*	Nagoya	Middle Aug., 1953	110	365	47	0.68	1.4	0.31	46
12	*Neverita didyma*	Onizaki	Middle Feb., 1954	3	147	21	0.60	2.9	0.26	43
13	*Babylonia japonica*	Mikawa-isshiki	Middle Jul., 1953	30	1125	Flesh 203	1.9	0.9	0.8	42
						Viscera 61	8.5	13.9	3.8	45
14	*Thais bronni*	Osaki-shimojima	Middle Aug., 1952	—	—	37	1.5	4.1	1.2	76
15	*Thais clavigera*	Osaki-shimojima	Middle Aug., 1952	—	—	109	3.6	3.3	2.5	70
16	*Bradybaena similaris*	Nagoya	Late Aug., 1953	165	95	—	0.65	—	0.46	71

Note: Of the catching localities, Osaki-shimojima belongs to Hiroshima Prefecture, while the others belong to Aichi Prefecture. 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. Sample No. 1 was procured in Nagoya City, its catching locality being unknown.

Modiolus demissus[24] followed by 27.5% for *Buccinum undatum*,[3] while the largest provitamin D content of crude sterol mixture from the shellfish used in this study is 19.3% for *Viviparus histricus*. It is noteworthy that while *Viv. histricus* is not much important as edible shellfish, its sterol mixture contains relatively large amount of provitamin D. It may also be noted that provitamin D content of crude sterol from the viscera of *Babylonia japonica* is 4.0% whereas crude sterol from the flesh of the same shellfish contains scarcely any provitamin D. An analogous instance was previously reported for *Buccinum undatum* by Bock and Wetter[3] who found provitamin D content of 26.3% in viscera sterol and 7.5% in flesh sterol.

Preparation of fatty oil from shellfish. Name of species, catching locality and date, and some data on the yield of lipid and fatty oil for sixteen kinds of shellfish used in this study are recorded in Table 13. Some shellfish were received in fresh state, while the others had previously been sun-dried to some extent. The shellfish were shucked, and the shucked shellfish were dried at a temperature below 80°C. The dried material was reduced to powder, and extracted with ether. The ether-extract was refluxed with tenfold acetone for a while, and the mixture was cooled to room temperature. The insoluble matter (phosphatide) was removed, and the fatty oil was obtained from the acetone solution. The shuked shellfish from *Babylonia japonica* was separated into flesh and viscera, and each material was separately treated. As is seen from Table 13, flesh contains a very small amount of lipid while the lipid content of viscera is very large. In the case of *Bradybaena similaris* the whole shellfish was dried and crushed into fine pieces which were then extracted with ether.

Properties of fatty oil. Properties of acetone-soluble oils are shown in Table 14. The oils are of deep color, mostly of dark reddish orange or dark green color, excepting Nos. 6 and 16 oils which are of relatively light color. The oils have mostly high acid values, especially Nos. 8, 9, 14 and 15 oils have extremely high

TABLE 14. Properties of Fatty Oils

Sample No.	A.V.	S.V.	I.V.	Unsap. M. (%)	Fatty acids		Crude sterol			Pro-vitamin D (%)
					Neutr.V.	I.V.	Yield (%)	m.p. (°C)	m.p. of acetate	
1	—	162.4	139.8	22.95	—	—	38	134–135	—	4.8
2	38.6	163.7	196.8	29.72	—	168.0	29	118–120	—	14.2
3	26.0	170.4	155.1	20.18	—	—	33	135–137	—	3.7
4 (a)	10.4	153 4	139.4	37.24	187.8	111.7	40	132–136	—	3.2
4 (b)	14.3	166.7	154.6	—	186.9	115.4	36	123–126	—	1.7
5	30.1	161.4	137.6	38.96	—	—	32	137–139	—	0
6	25.8	162.5	132.7	—	—	—	40	133–135	—	7.7
7	28.3	168.1	157.9	26.38	—	—	34	133–136	—	3.4
8	116.6	169.1	125.8	23.97	183.5	144.1	—	142	114	0
9	128.8	156.2	132.8	24.30	185.4	158.1	—	127	117	0
10	53.1	136.8	—	48.67	—	—	15	126–133	—	7.6
11	10.3	89.5	—	52.85	—	—	22	132–140	—	19.3
12	16.5	163.5	147.0	29.17	186.9	140.4	32	128–132	—	3.7
13 Flesh	30.8	124.5	174.6	34.62	182.5	172.9	—	135–137	118	0
13 Viscera	12.9	151.1	168.9	—	181.6	185.5	35	128–130	132	4.0
14	100.3	143.9	122.4	33.19	184.1	155.2	—	142	113	1.2
15	97.8	148.2	118.2	33.92	184.3	162.3	—	142	114	2.3
16	32.8	—	115.2	33.09	—	—	18	125–133	—	9.4

acid values which have been caused by a prolonged storage of incompletely sun-dried whole shellfish till the commencement of the present study. An inspection of the data on saponification value, content of unsaponifiable matter and neutralization value of fatty acids shows that the correlations between these characteristics are diverse for each oil. This is not explicable if each oil contains only free fatty acids and free unsaponifiable matter besides glyceride and wax ester. It is presumably ascribed to the contamination of each oil with varying amounts of phosphatides or some other non-fat substances which have not been completely removed by acetone treatment. Crude sterol in Table 14 was separated in the following way: unsaponifiable matter was dissolved in fiftyfold acetone, the solution was allowed to stand over a night at room temperature, and the crystalline solid formed was separated by filtration. Ultraviolet absorption curves (in ethanol) of crude sterols are shown in Figs. 1–6. Since some crude sterols contained interferring substances having absorption at 282 mμ, the wave length of the maximum absorption corresponding to provitamin D, the content of provitamin D was calculated by the following formula:

$$\text{Provitamin D}(\%) = 12.2[k_{282} - 1/13(8\,k_{277} + 5\,k_{290})]$$

The factor 12.2 was deduced from the values, $k_{282}=30.8$, $k_{277}=26.3$ and $k_{290}=16.6$, for pure provitamin D reported by Glover and co-workers,[25] excepting that the value for $k_{281.5}$ by these authors was taken as the value for k_{282} in the above formula.

Since the occurrence of corbisterol, a $\Delta^{5,7,22}$-sterol of C_{27} series, in corbicula and a provitamin D possibly of C_{29} series in *Modiolus demissus*[26] has recently been reported, provitamin D in shellfish is not exclusively 7-dehydrocholesterol. The above formula is, however, applicable in close approximation to any provitamin D since $\Delta^{5,7}$-sterols have generally a quite similar absorption curve. It is also noteworthy that crude sterols from *Sunetta menstrualis* and *Cellana toreuma* exhibit clearly a maximum absorption at 255 mμ. As for the sterol component responsible to this absorption, a further study is needed.

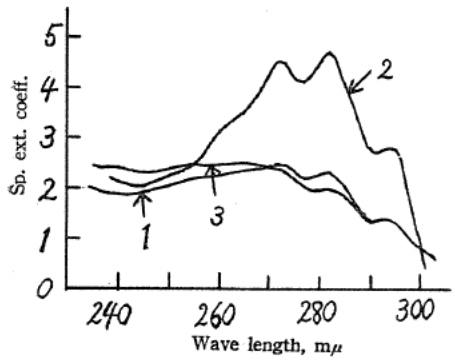


FIG. 1. Absorption curve of crude sterol.
Curve 1. *Andara inflata*
Curve 2. *Pinna pectinata japonica* (*forma lischkeana*)
Curve 3. *Mytilus crassitesta*

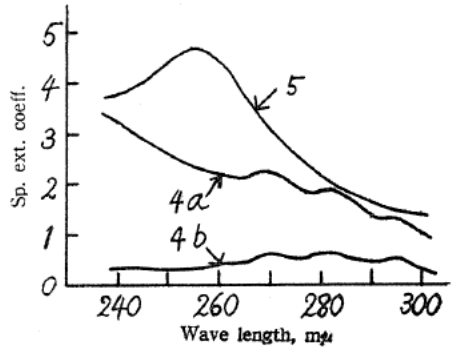


FIG. 2. Absorption curve of crude sterol.
Curve 4 a. *Corbicula japonica*
Curve 4 b. *Corbicula japonica*
Curve 5. *Sunetta menstrualis*

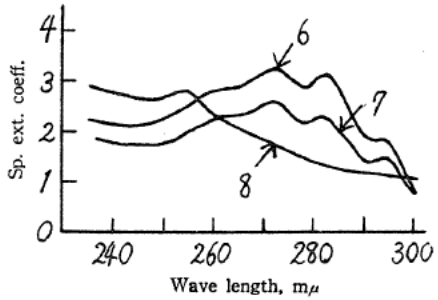


FIG. 3. Absorption curve of crude sterol.
Curve 6. *Gomphina melanaegis*
Curve 7. *Schizothaerus mittalli*
Curve 8. *Cellana toreuma*

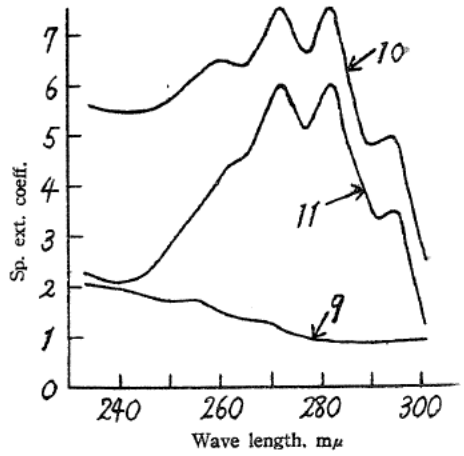


FIG. 4. Absorption curve of crude sterol.
Curve 9. *Cellana nigrolineata*
Curve 10. *Cipangopaludina japonica*
Curve 11. *Viviparus histricus*

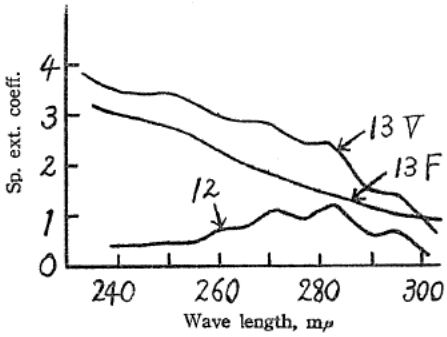


FIG. 5. Absorption curve of crude sterol.
Curve 12. *Neverita didyma*
Curve 13 F. *Babylonia japonica* (flesh)
Curve 13 V. *Babylonia japonica* (viscera)

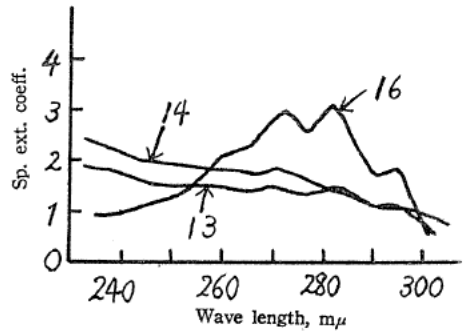


FIG. 6. Absorption curve of crude sterol.
Curve 14. *Thais bronni*
Curve 15. *Thais clavigera*
Curve 16. *Bradybaena similaris*

VI. Fatty Oils from *Gorgonocephalus caryi* and *Ophioplocus japonicus*[27]

Among the animals of the class Ophiuroidea, *Ophipholis aculeata* is the sole one whose lipid component has hitherto been studied. According to Bergmann,[28] sterol from this animal is strongly levorotatory, $[\alpha]_D = -36°$, and quite different from sterol of starfish. This section contains the results of our study on oils of *Gorgonocephalus caryi* (Lyman) and *Ophioplocus japonicus* H. L. Clark. Referring to the oil of *G. caryi*, the composition of fatty acids was estimated in approximation by the methyl ester fractionation method, and also several fatty acid components were separated and identified. The sterol mixture was found to contain chiefly Δ^5-sterol with a little or no Δ^7-sterol from its optical rotation and iodine

value. Recrystallization of the steryl acetate mixture and fractional precipitation of the brominated steryl acetate mixture afforded a sterol fraction consisting of β-sitosterol. Properties of this β-sitosterol fraction and those previously reported for β-sitosterol are shown in Table 15. This is the second instance in which the presence of β-sitosterol in sterol mixture of aquatic invertebrate is indicated, preceded by the finding of β-sitosterol in sterol mixture of *Spisula sachalinensis* (see Sect. IV).

As for the oil of *Ophio. japonicus*, fatty acid components and sterol components could not be separated due to scanty of the material. However, the sterol components were considered to consist mainly of Δ^5-sterol.

TABLE 15. Properties of β-Sitosterol

	Free sterol		Acetate		Benzoate	
	m.p. (°C)	$[\alpha]_D^\circ$	m.p. (°C)	$[\alpha]_D^\circ$	m.p. (°C)	$[\alpha]_D^\circ$
Sterol from *G. caryi*	136–137	−35.1	125–126	−38.8	144–145	−13.7
β-sitosterol [29]	139–140	−38.0	130–132	−42.5	144–145	−13.9
" [30]	140	−37.1	129.5	−42	147	−
" [19]	136–137	−36.6	125–126	−41.0	146–147	−13.8[20]

1. Oil of *Gorgonocephalus caryi*

Properties of oil. The living material used in this study was caught around Osaki-shimojima, an island in the Inland Sea of Seto, in August, 1952, and was sun-dried immediately after its catch. Arms were removed from the dried material (4,880 g; 55 in number). The central disc (1,000 g) thus obtained was crushed into small pieces and then extracted with ether. Ether-extract (60 g) was treated with 800 cc of acetone, yielding 51 g of acetone-soluble oil which was a dark reddish orange liquid with a little solid at ordinary temperature and had the following constants: d_4^{25} 0.9283, n_D^{25} 1.4775, A.V. 126.0, S.V. 162.5, I.V. 166.7 and unsaponifiable matter 16.97%.

Fatty acids. Fatty acids and unsaponifiable matter were separated by saponification of the oil followed by extraction of the soap solution with ether. The fatty acids were a solid mass at ordinary temperature and had d_4^{30} 0.9003, n_D^{30} 1.4708, A.V. 187.7 and I.V. 169.1. Methyl esters (S.V. 180.5 and I.V. 161.9) of the fatty acids were fractionated with the results shown in Table 16. Solid acids were estimated by the lead salt ethanol method for the fractions 1-9 in Table 16, and saponification equivalents and iodine values of the solid acids were determined. From these data, saturated acids, saponification equivalent of saturated acids, and saponification equivalent, iodine value, carbon number, and unsaturation of unsaturated acids in each fraction were calculated on an assumption that the unsaturated acids entered into solid acids have the same composition as the total unsaturated acids. The results are shown in Table 17, in which the saponification equivalents of unsaturated acids for the fractions 8–11 are taken as equal to the saponification equivalents of total fatty acids of respective fractions, and the fractions 10 and 11 are regarded as containing no saturated acids. Plotting unsaturation against carbon number for the unsaturated acids of each fraction in Table 17, the unsaturations corresponding to C_{16}, C_{18}, C_{20} and C_{22} unsaturated acids were obtained. From these

TABLE 16. Fraction of the Methyl Esters

Fraction	Yield		b.p. (°C/5 mm)	S.V.	S.E.	I.V.
	(g)	(%)				
1	3.1	10.3	-160	210.3	252.8	57.2
2	2.9	9.6	160-170	204.6	260.2	85.4
3	3.2	10.6	170-175	195.9	272.4	117.3
4	3.3	11.0	175-180	191.1	279.6	133.7
5	2.8	9.3	180-185	186.3	287.1	151.8
6	1.3	4.3	185-190	182.8	292.9	170.8
7	1.5	5.0	190-195	179.1	299.2	182.6
8	1.2	4.0	195-200	173.8	322.8	234.4
9	1.4	4.7	200-205	171.8	326.6	252.7
10	1.9	6.3	205-210	166.2	337.6	260.3
11	2.5	8.3	210-	163.7	342.8	270.8
Residue	5.0	16.6	—	143.8	—	177.1

TABLE 17. Solid Acids, Saturated Acids and Unsaturated Acids in Each Fraction

Fraction	Solid acids			Saturated acids		Unsaturated acids			
	(%)	S.E.	I.V.	(%)	S.E.	S.E.	I.V.	Carbon number	Unsaturation (−H)
1	48.6	248.9	6.4	46.0	248.4	256.5	111.9	16.17	2.26
2	40.1	254.7	5.6	38.6	254.3	263.9	146.2	16.75	3.04
3	31.4	261.7	6.2	30.3	261.4	277.3	176.8	17.77	3.86
4	22.3	267.9	7.9	21.3	267.2	282.9	178.2	18.17	3.97
5	16.5	275.6	11.0	15.5	274.6	289.4	188.4	18.66	4.39
6	12.3	287.4	15.3	11.4	286.8	293.7	201.8	18.99	4.67
7	11.5	290.3	19.8	10.4	289.2	300.4	213.3	19.50	5.05
8	6.8	—	27.1	6.1	—	308.8	249.6	20.18	6.07
9	4.0	—	28.6	3.6	—	312.6	273.9	20.51	6.75
10	—	—	—	—	—	323.6	—	21.30	—
11	—	—	—	—	—	328.8	281.9	21.70	7.31
Residue	—	—	—	—	—	—	—	—	—

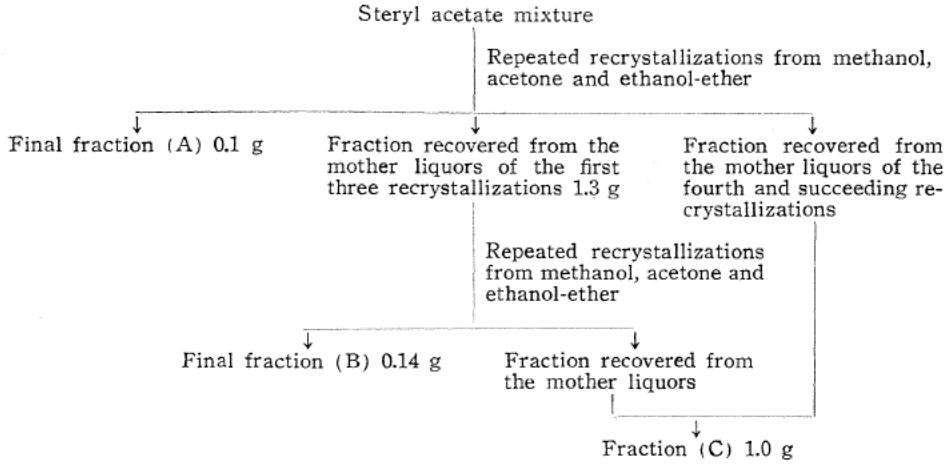
TABLE 18. Composition of the Fatty Acids

Fraction	Saturated acids (g)			Unsaturated acids (g)			
	C_{14}	C_{16}	C_{18} and higher member	C_{16}	C_{18}	C_{20}	C_{22}
1	0.39	0.96	—	1.44	0.14	—	—
2	0.10	0.96	—	1.05	0.63	—	—
3	—	0.76	0.16	0.24	1.88	—	—
4	—	0.41	0.26	—	2.25	0.21	—
5	—	0.14	0.27	—	1.50	0.75	—
6	—	—	0.14	—	0.55	0.55	—
7	—	—	0.15	—	0.32	0.96	—
8	—	—	0.07	—	—	0.98	0.10
9	—	—	0.05	—	—	0.96	0.33
10	—	—	—	—	—	0.64	1.18
11	—	—	—	—	—	0.36	2.04
Residue	—	—	—	—	—	—	0.80
Total	0.49	3.23	1.10	2.73	7.27	5.41	4.45
%, on the basis of total fatty acids	2.0	13.1	4.4	11.1	29.5	21.9	18.0
Unsaturation (−H)	—	—	—	2.0	3.9	5.8	>7.4

data, the compositions of fatty acids in each fraction were deduced by calculation with the results shown in Table 18, in which the saturated and unsaturated acids of each fraction were assumed to consist of two adjacent even acids. The fatty acids obtained from the residue after removal of unsaponifiable matter were regarded wholly as C_{22} unsaturated acids. The low yield of fatty acids from the residue is presumably ascribed to the presence of some non-fat substances in the residue which were not removed from the original fatty oil by acetone treatment and became concentrated in the residue.

The solid acids of the fraction 2 gave palmitic acid of m.p. 61°C after recrystallization from methanol. The unsaturated acids of the fraction 3 were converted into lithium salts, and the latter were recrystallized from 50% ethanol. Acidification of the lithium salts gave a liquid acid of n_D^{30} 1.4625, N.V. 199.0 and I.V. 90.4 (calcd. for oleic acid : N.V. 198.6 and I.V. 89.8). Oxidation of this acid by Hazura's method gave a dihydroxystearic acid of m.p. 134°C. The fatty acids of the fraction 11 were treated by the lithium salt acetone method, and a highly unsaturated acid fraction consisting mainly of clupandonic acid was obtained; n_D^{20} 1.5018, N.V. 171.4 and I.V. 367.5 (calcd. for clupanodonic acid: N.V. 169.8 and I.V. 384.0).

Sterols. The unsaponifiable matter was found to contain 65.9% sterol by the digitonide method. Crude sterol mixture (2.9 g) was obtained by recrystallization of the unsaponifiable matter (5.5 g) from 50 cc of ethanol. It had m.p. 130°–133°C and I.V. 75.9 by the perbenzoic acid method. Spectrophotometric measurement indicated that the crude sterol contains little or no provitamin D ($\Delta^{5,7}$-sterol). Steryl acetate mixture (2.2 g) of m.p. 125°–127°C prepared from the crude sterol was fractionated in the following way.



The fraction (A) had m.p. 137°–138°C and I.V. 84.1. The fraction (B) had m.p. 124°–126°C, $[\alpha]_D^{14} = -38.8°$, S.V. 123.5 and I.V. 63.1 (calcd. for $C_{31}H_{52}O_2$: S.V. 122.8 and I.V. 55.6). Free sterol from this acetate fraction had m.p. 136°–137°C and $[\alpha]_D^{14} = -35.1°$ after recrystallization from methanol. Benzoate prepared from the free sterol had m.p. 144°–145°C and $[\alpha]_D^{14} = -13.7°$ after recrystallization from acetone. The fraction (C) was dissolved in 15 cc of ether, and then brominated

at −10°C, yielding an ether-insoluble bromide (0.3 g). This was debrominated with zinc and glacial acetic acid, yielding a product which had m.p. 142°–145°C and I.V. 93.8 after one recrystallization from acetone and m.p. 145°–147°C after two recrystallizations. The ether solution separated from the insoluble bromide was freed from excess bromine and concentrated. Addition of ethanol to the concentrated ether solution gave a bromide precipitate (0.6 g) of m.p. 122°–123°C. Steryl acetate obtained by debromination of this bromide had m.p. 124°–126°C, $[\alpha]_D^{14} = -34.9°$ and I.V. 61.9 after recrystallization from methanol. Free sterol from this acetate melted at 136°–137°C after recystallization from methanol and was regarded as β-sitosterol like the sterol of the fraction (B).

2. Oil of *Ophioplocus japonicus*

The living material was caught around Osaki-shimojima in August, 1952 and sun-dried immediately after its catch. The sun-dried material (272 g; about 100 in number) was cut into small pieces and extracted with ether. The ether-extract (5.6 g) was treated with 70 cc of acetone, yielding 3.6 g of acetone-soluble oil which was of a dark reddish orange color and mostly solid at ordinary temperature. It had A.V. 95.5, S.V. 143.5, I.V. 89.2 and unsaponifiable matter 30.25%.

The fatty acids of this oil had N.V. 189.6 and I.V. 94.1. Such a low iodine value of fatty acids is a characteristic feature for this oil among aquatic animal oils.

The unsaponifiable matter was found to contain 35.2% sterol by the digitonide method. Recrystallizing the unsaponifiable matter (1.0 g) from 15 cc of methanol, 0.28 g of crude sterol of m.p. 122°–124°C, $[\alpha]_D^{14} = -35.2°$ and I.V. 77.8 was obtained. Steryl acetate prepared from this crude sterol melted at 124°–126°C and at 126°–128°C after recystallization.

VII. Properties of Fatty Oils from Eleven Kinds of Japanese Shellfish[31]

After the completion of our study on oils from 16 kinds of Japanese shellfish recorded in Sect. V, another oils from 11 kinds of Japanese shellfish have been submitted to our study, the results of which are recorded in this section. The shellfish used in this study contain 2 species of Gastropode, 5 species of Eulamillibranchia and 4 species of Anisomyaria. Although oils from *N. didyma* and *P. japonica* (*forma lischkeana*) were studied in the previous study in Sect. V, flesh oil and viscera oil were separately extracted from these two species in the present study. Oils from *M. arenaria japonica*,[9] *V. philippinarum*,[9] *Cris. plicata spatiosa*[7] and *Cor. sandai*[23] have already been studied by previous authors, while the other oils recorded in the present study have not yet been studied.

Shellfish used in this study. Whole shellfish was shucked. Shucked shellfish was dried at a temperature below 80° C, and dried material was refluxed with tenfold acetone and then cooled to room temperature. Insoluble matter (phosphatide) was removed, and acetone-soluble oil was obtained. In the case of *N. didyma*, *Cip. malleata* and *P. japonica* (*forma lischkeana*), flesh and viscera were separated, and each was separately treated. Name of species, catching locality, date of receipt, yield of ether-extract and acetone-soluble oil and some other data on the shellfish used in the present study are shown in Table 19, from which it is seen that yield of ether-extract is greater for viscera than for flesh of the same shellfish.

TABLE 19. List of 11 Kinds of Japanese Shellfish

Sample No.	Species	Catching locality	Date of receipt	Number	Weight (g)	Dried material of shucked shellfish (g)	Ether-extract (g)	Ether-extract (%)	Acetone soluble oil (g)	Acetone soluble oil (%)
1	*Neverita didyma*	Onizaki	Middle Mar., 1954	8	1,185	Flesh 85 Viscera 80	1.9 5.7	2.2 7.1	0.66 3.6	34.7 63.7
2	*Cipangopaludina malleata*	Rice-field at Nagashima	Late Mar., 1954	about 3,000	11,800	Flesh 568 Viscera 617	10.5 28.0	1.8 4.5	4.9 9.5	46.7 33.9
3	*Mya arenaria japonica*	Mouth of the river Nabeta	Middle Feb., 1954	36	3,710	185	9.5	5.1	8.2	86.3
4	*Sanguinolaria boeddinghausi*	Mouth of the river Nabeta	Middle Feb., 1954	16	2,012	140	7.3	5.2	4.0	54.8
5	*Venerupis philippinarum*	Fukue	Middle Jan., 1954	24	735	33	1.55	4.7	0.78	50.3
6	*Cardium muticum*	Onizaki	Middle Mar., 1954	13	—	55	3.3	6.0	2.0	61.2
7	*Corbicula sandai*	Lake Biwa	Late Dec., 1953	654	5,400	164	27.2	16.6	16.8	61.8
8	*Hyriopsis schlegelii*	Lake Biwa	Middle Jan., 1954	5	2,895	152	9.4	6.2	6.4	68.1
9	*Cristaria plicata spatiosa*	Lake Biwa	Middle Jan., 1954	6	980	79	6.3	8.0	4.2	66.7
10	*Pinna pectinata japonica*	Onizaki	Middle Feb., 1954	1	120	19	0.87	4.6	0.42	48.3
11	*Pinna pectinata japonica* (*forma lischkeana*)	Onizaki	Middle Mar., 1954	3	737	Flesh 37 Viscera 37	0.93 3.3	2.5 9.0	0.26 2.7	28.0 81.8

Notes: Catching localities, excepting Nagashima (Mie Prefecture) and Lake Biwa, belong to Aichi Prefecture. Percentage yield of ether-extract is expressed on the basis of the dried material of shucked shellfish. Percentage yield of acetone-soluble oil is expressed on the basis of ether-extract.

Properties of oil. Properties of the acetone-soluble oils are shown in Table 20. All oils are strongly colored, mostly of dark reddish orange color or dark green color. They are solidified at ordinary temperature, excepting oils from *Mya arenaria japonica* and *Car. muticum* which are mobile at ordinary temperature.

As is seen from Nos. 1, 2 and 11 oils in Table 20, viscera oil has a higher iodine value and a lower unsaponifiable matter than flesh oil of the same shellfish, while unsaponifiable matter of viscera oil has a larger provitamin D content and a smaller sterol content than unsaponifiable matter of flesh oil.

Properties of several oils recorded in the present study are compared with those reported previously, as shown in Table 21, from which it is seen that oils from the same kind of shellfish frequently show a considerable variance in their properties. Although such variance may partly be attributable to the contamination of oils with a varying amount of phosphatide, it is considered to be caused essentially by a variance in the composition of oils. The present authors,[32)33)34)] have recently noted that the content of some sterol components in total sterol mixtures from the same kind of aquatic invertebrates frequently differs widely. It appears that variance in composition of oils from the same species is more marked for aquatic invertebrate than for fish, although fish oils of one and the same kind is generally known to show a more marked variance in their composition than vegetable oils of one and the same kind.

TABLE 20. Properties of Fatty Oils

Sample No.	A.V.	S.V.	I.V.	Unsap. M. (%)	Fatty acids		Unsaponifiable matter				Pro-vitamin D (%)
					N.V.	I.V.	Sterol (%)	Crude sterol			
								Yield (%)	m.p. (°C)	I.V.	
1 Flesh	56.1	80.6	155.7	62.08	—	—	72.4	—	142–143	95.1	4.0*
1 Viscera	27.3	132.1	165.4	34.53	183.4	170.3	44.7	—	134–136	113.4	7.9*
2 Flesh	21.4	65.5	110.6	73.46	190.6	117.2	87.5	76	143–145	68.9	2.7*
2 Viscera	44.7	109.2	117.0	47.87	191.4	124.1	79.0	64	136–139	79.9	4.3*
3	24.6	130.2	145.1	34.81	186.1	158.7	34.3	—	128–133	99.0	1.5*
4	25.6	152.9	141.0	27.13	185.9	164.3	52.8	—	133–135	95.8	3.8*
5	45.6	130.1	178.6	38.08	—	175.2	59.4	53	132–136	122.4	9.9
6	81.9	172.0	174.4	17.24	183.7	179.9	38.6	—	129–133	116.2	6.2
7	3.5	162.1	139.0	22.40	191.5	147.1	63.4	52	133–136	118.1	9.3
8	8.9	150.8	130.3	27.08	187.5	136.0	61.0	54	129–133	92.9	3.1
9	10.7	152.4	135.9	25.82	186.1	128.1	—	61	129–135	115.9	15.5
10	39.2	145.9	181.8	28.76	—	185.8	46.2	—	122–125	—	6.0
11 Flesh	39.3	90.3	162.9	60.05	—	—	78.1	—	135–136	95.1	0.37*
11 Viscera	39.8	158.9	189.2	23.84	182.4	180.5	40.1	—	127–129	150.3	15.3

Notes: Sterol was determined by the digitonide method. Crude sterol was separated in the following way: the unsaponifiable matter was dissolved in twentyfold methanol or tenfold acetone, the solution was allowed to stand over a night at ordinary temperature, and the crystalline solid formed was separated. Provitamin D was estimated by measuring specific extinction coefficients at 282, 277 and 290 mμ for an ethanol solution of unsaponifiable matter or crude sterol, and by using the formula given in Sect. V. *% based on the unsaponifiable matter while the others on the crude sterol. Unsaponifiable matter from flesh oil of *N. didyma* and viscera oil of *P. japonica* (*forma lischkeana*) exhibited a maximum absorption at the region of about 260 mμ, too. Solid acids in the fatty acids were determined by the lead salt ethanol method for several oils with the following results: 28.8% (I.V. 16.2) for the flesh oil of *Cip. malleata*, 29.2% (I.V. 14.9) for the viscera oil of *Cip. malleata*, 25.4% (I.V. 11.5) for the oil of *H. schlegelii*, and 24.1% (I.V. 16.1) for the oil of *Cris. plicata spatiosa*.

TABLE 21. Properties of Oils of One and the Same Kind

Oil	S.V.	I.V.	Unsap. M. (%)	Fatty acids	
				N.V.	I.V.
Neverita didyma					
Viscera oil in Table 20	132.1	165.4	34.53	183.4	170.3
No. 12 oil in Table 14	163.5	147.0	29.17	186.9	140.4
Mya arenaria japonica					
No. 3 oil in Table 20	130.2	145.1	34.81	186.1	158.7
Tsujimoto and Koyanagi[9]	—	—	—	—	161.5*
Venerupis philippinarum					
No. 5 oil in Table 20	130.1	178.6	38.08	—	175.2
Tsujimoto and Koyanagi[7]	103.2	188.7	45.7	192.0	180.0
Corbicula sandai					
No. 7 oil in Table 20	162.1	139.0	22.40	191.5	147.2
Hori and Hosoda[23]	181.1	144.2	27.5*	197.2	142.9
Cristaria plicata spatiosa					
No. 9 oil in Table 20	152.4	135.9	25.82	186.1	128.1
Tsujimoto and Koyanagi[7]	135.0	167.9	25.9	196.3	162.3
P. japonica (*forma lischkeana*)					
Viscera oil in Table 20	158.9	189.2	23.84	182.4	180.5
No. 2 oil in Table 14	163.7	196.8	29.72	—	168.0

* Determined with ether-extract.

Sterol components of several shellfish oils. The unsaponifiable matter of flesh oil of *Cip. malleata* was recrystallized from acetone, and crude sterol obtained was acetylated, yielding a steryl acetate mixture of m.p. 121°–125°C, S.V. 131.5 and I.V. 76.7. Four recrystallizations of this acetate gave a product of m.p. 130°–131°C, I.V. 86.5 and provitamin D content 1.5%. From the unsaponifiable matter of viscera oil of the same shellfish, a steryl acetate mixture of m.p. 123°–125°C, $[\alpha]_D^{28} = -41.4°$, S.V. 130.4 and I.V. 79.9 was obtained in a similar way. Four recrystallizations of this acetate gave a product of m.p. 136°-137°C, I.V. 84.4 and provitamin D content 2.4%. Saponification of this product gave a free sterol of m.p. 137°–139°C. Differing from the sterol mixtures described below, the sterol mixture from the oils of *Cip. malleata* appears to contain predominantly monounsaturated sterol.

A steryl acetate mixture of m.p. 118°–120°C, $[\alpha]_D^{28} = -43.4°$ and S.V. 130.1 was obtained by recrystallization of the unsaponifiable matter of the oil of *Cor. sandai* from methanol followed by acetylation of the crude sterol mixture. Several recrystallizations of this acetate from acetone gave a product of m.p. 149°–151°C and provitamin D content 40.9%. By bromination of the acetate fraction recovered from the mother liquors of recrystallization, an ether-insoluble bromide of m.p. 186°C was obtained, Debromination of this bromide gave a steryl acetate which had m.p. 145°–146°C after recrystallization from ethanol and showed no depression of melting point when mixed with poriferasteryl acetate.

A steryl acetate mixture of m.p. 116°–119°C, $[\alpha]_D^{28} = -40.5°$ and S.V. 130.1 was obtained from the unsaponifiable matter of the oil of *H. schlegelii*. Three recrystallizations of this acetate from acetone gave a product of m.p. 139°–141°C and provitamin D content 6.6%. Bromination of the acetate fraction recovered from the mother liquors of recrystallization gave an ether-insoluble bromide of m.p. 175°C, which melted at 187°C after purification by recrystallization from chloroform-methanol and was regarded as tetrabromide of poriferasteryl acetate.

From the unsaponifiable matter of the oil of *Cris. plicata spatiosa*, a steryl acetate mixture of m.p. 116°–119°C, $[\alpha]_D^{25} = -38.9°$ and S.V. 129.8 was obtained in a similar way. Four recrystallizations of this acetate from methanol and acetone gave a product of m.p. 141°-143°C and provitamin D content 19.5%.

Polyethenoid acids of several shellfish oils. The fatty acids of several shellfish oils were alkali-isomerized under the condition of 21% KOH-glycol, 180°C and 15 minutes with 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 orignal fatty acids was calculated from the observed data by the formula given by Hammond and Lundberg,[35] excepting that specific extinction coefficients at the wave lengths mentioned above instead of 233, 268, 315, 346 and 374 mμ were taken, since maximum absorptions occurred at the wave lengths mentioned above in our experiment. The results are shown in Table 22. It needs a further study to ascertain that the formula given by Hammond and Lundberg is applicable to the data obtained in our measurements, since even a small variance in the condition of alkali-isomerization may affect considerably the degree of isomerization. Meanwhile it is seen from Table 22 that the fatty acids, excepting those of the flesh oil of *Cip. malleata*, contain pentaene in the largest proportion and triene in the smallest proportion. Taking pentaene as unity, triene, diene,

tetraene and hexaene are found to be 0.03–0.23, 0.17–0.53, 0.49–0.60 and 0.50–0.67, respectively. The fatty acids of the viscera oil of *Cip. malleata* form an exception, in which the proportion is largest for tetraene followed by diene, pentaene, triene and hexaene.

TABLE 22. Polyethenoid Acids Estimated by Ultraviolet Absorption Measurement of Alkali-Isomerized Fatty Acids

Oil	Specific extinc. coeff.					Polyethenoid acids									
						%					Taking pentaene as unity				
	235 (mμ)	270 (mμ)	316 (mμ)	348 (mμ)	376 (mμ)	Di	Tri	Tetra	Penta	Hexa	Di	Tri	Tetra	Penta	Hexa
Cip. malleata, viscera	15.43	12.17	10.03	3.41	0.75	6.7	3.8	10.3	5.4	2.6	1.24	0.70	1.91	1.00	0.48
Mya arenaria japonica	20.17	18.03	16.79	10.47	2.99	5.5	2.2	8.5	15.2	10.2	0.36	0.14	0.56	1.00	0.67
S. boeddinghausi	17.97	16.76	16.57	9.91	2.37	4.1	1.6	9.1	15.3	8.1	0.27	0.10	0.60	1.00	0.53
Cor. sandai	17.77	15.25	14.09	8.44	2.05	5.6	2.4	7.8	12.9	7.0	0.43	0.19	0 60	1.00	0.54
H. schlegelii	17.68	14.40	12.84	7.89	1.82	6.5	2.8	6 7	12.3	6.2	0.53	0.23	0.54	1.00	0.50
Cris. plicata spatiosa	13.70	11.76	10.48	6.40	1.48	4.5	2.3	3.5	9.9	5.0	0.45	0.23	0.56	1.00	0.51
P. japonica (forma lischkeana), viscera	19.89	20 22	20.64	13.31	3.67	2.5	0.6	9.7	19.6	12.5	0.13	0.03	0.49	1.00	0.64

VIII. Fatty Oils of *Cucumaria chronhjelmi*, *Coscinasterias acutespina* and *Comanthus japonica* with Special Reference to Their Sterol Components[36]

Of these three species of Echinodermata, *Cucumaria chronhjelmi* is the sole one whose lipid has hitherto been studied at all. A previous study in this laboratory[37] indicated the presence of batyl alcohol in unsaponifiable matter separated from the lipid of *Cucumaria chronhjelmi*. In the present study, sterol components which are contained in a relatively small amount, besides batyl alcohol were examined, and Δ^7-cholestenol was identified as the main component. Sterol components of *Coscinasterias acutespina*, like those of *Asterias amurensis*[34,39] and *Luidia quinaria*,[38] were found to consist chiefly of Δ^7-sterols (Δ^7-cholestenol and others), whereas sterol components of *Comanthus japonica* were found to contain mainly Δ^5-sterol. The compositions of polyethenoid acids of these three oils were estimated by ultraviolet absorption measurements of the alkali-isomerized fatty acids in the same way as described in the preceding section. In every case, the polyethenoid acids were found to contain pentaene in the largest proportion and triene in the smallest proportion. Proportion of diene was found relatively large as with the case in the preceding section, but it requires a further study in this point.

As for sterol components of echinoderms, several species of Echinoidea and Asteroidea have previously been studied, whereas only a few species of other classes have hitherto been studied, such as *Cucumaria chronhjelmi* and *Holothuria princepo*[4] among Holothurioidea, and *Gorgonocephalus caryi* (see Sect. VI), *Ophioplocus japonicus* (see Sect. VI), and *Ophiopholis aculeata*[28] among Ophiuroidea. Among Crinoidea, *Comanthus japonica* recorded in the present study is the sole one. Although it needs a further accumulation of information in order to come to

a decisive conclusion concerning the correlation between the location of an echinoderm in the taxonomy and its sterol components, an inspection of literature available at present seems to lead to an interesting finding. When non-conjugated sterols are divided into two series, Δ^5-series and Δ^7-series, by the location of ethenoid linkage in the sterol ring irrespective of carbon number and unsaturation in the side chain, it is found that sterols of Holothurioidea and Asteroidea contain chiefly Δ^7-series with a little or no Δ^5-series, whereas sterols of Echinoidea, Ophiuroidea and Crinoidea consist predominantly of Δ^5-series with a little or no Δ^7-series. It should, however, be mentioned that there are many individual sterols of each series having different carbon number and unsaturation in the side chain, and the content of an individual sterol in the sterol mixture of echinoderm of one and the same species often differs very markedly.

1. *Cucumaria chronhjelmi* Theel

The material used in this study was procured at Onizaki, Aichi Prefecture in the summer of 1954. It is sun-dried material prepared from the living material which was caught in the winter of the same year. The dried material (5,760 g; about 1,500 in number) was cut into small pieces, and extracted with ether. The ether-extract (144 g) was treated with tenfold acetone, and 92 g of acetone-soluble oil was obtained after removal of acetone-insoluble matter. The oil was a reddish orange liquid with some solid and had d_4^{30} 0.9053, n_D^{30} 1.4632, A.V. 136.7, S.V. 163.7, I.V. 97.4 and unsaponifiable matter 17.22%. In order to separate fatty acids and unsaponifiable matter from the oil, it was saponified in the usual way, the unsaponifiable matter was extracted from the soap solution with ether, the soap solution was then acidified with hydrochloric acid, and the fatty acids were taken up with ether. There was formed a considerable amount of dark brown resinous substances insoluble in both ether and acid water. The yield of fatty acids was very low; 41 g from 90 g of oil. This is presumably attributable to the contamination of acetone-soluble oil with a considerable amount of non-fat substances.

The fatty acids were a reddish orange liquid with some solid; d_4^{20} 0.8903, n_D^{20} 1.4612, N.V. 188.2 and I.V. 109.2. Lead salt ethanol method gave 27.5% solid acids (I.V. 15.4).

The unsaponifiable matter was a reddish orange solid with sterol content 8.4% by the digitonide method. The absorption curve of unsaponifiable matter in ethanol showed no peak at the region of 230-300 mμ. Liebermann-Burchard test for the unsaponifiable matter gave a curve (curve *A* in Fig. 7), showing absorption at 620 mμ vs. reaction period,[40)41)] by which the presence of Δ^7-sterol in the unsaponifiable matter was indicated. A portion (7.5 g) of the unsaponifiable matter and 100 cc of 90% ethanol were refluxed with 2 g of digitonin, and after standing the mixture over a night at ordinary temperature, the precipitate of digitonide was separated by filtration. The precipitate was refluxed with acetic anhydride and then diluted with water. The insoluble matter was separated from the solution and extracted with ether, yielding 0.6 g of ether-extract (crude steryl acetate). On treating the ether-extract first with acetone and then with methanol, a small amount of viscous substances insoluble in these solvents was removed, and on cooling the clear solution, 0.4 g of crystalline solid was separated. This was fractionally crystallized from methanol and acetone, and after removing a small amount of high melting

fraction (m.p. 120°–128°C) and low melting fraction, a fraction of constant m.p. 118°–119°C was eventually separated, which had $[\alpha]_D^{28}=\pm 0$ and S.V. 130.4 (calcd. for $C_{29}H_{48}O_2$: 130.8). Liebermann-Burchard reaction for this fraction gave a curve (curve *B* in Fig. 7), showing absorption at 620 mμ vs. reaction period, which lies close to the curve for Δ^7-sterol. Free sterol from the acetate fraction had m.p. 122°–123°C after recrystallization from methanol. A solution of the acetate fraction of m.p. 118°–119°C in glacial acetic acid was shaken in the presence of palladium catalyst in an atmosphere of hydrogen for 4 hours. No hydrogen absorption occurred, but the product melted at 77°–78°C after recrystallization from methanol. Liebermann-Burchard reaction for this product gave a curve (curve *C* in Fig. 7), showing absorption at 620 mμ vs. reaction period, which lies close to the curve for $\Delta^{8(14)}$-sterol. Free sterol of m.p. 122°–123°C, acetate of m.p. 118°–119°C and its isomerization product of m.p. 77°–78°C showed no depression of melting point when mixed with Δ^7-cholestenol, Δ^7-cholestenyl acetate and $\Delta^{8(14)}$-cholestenyl acetate, respectively, obtained from *Asterias amurensis* in a previous study.

The 90% ethanol filtrate separated from the precipitate of digitonide was cooled down to 0°C, and the crystalline solid was separated. Extraction of this solid with ether gave an extract of m.p. 55°–58°C, from which batyl alcohol of m.p. 71°C and mixed m.p. 71°C was obtained after recrystallization.

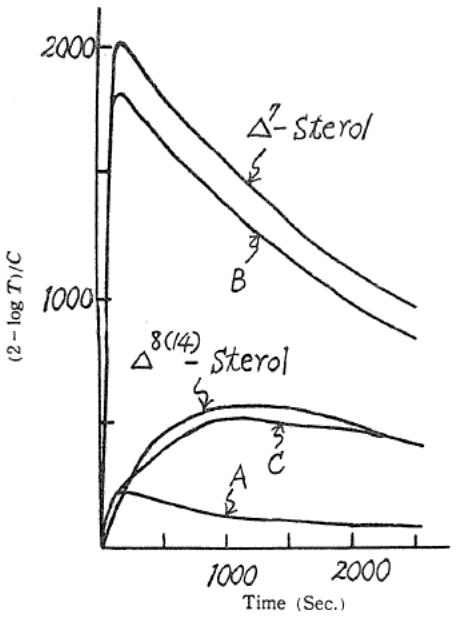


FIG. 7. Absorption at 620 mμ vs. reaction period in Liebermann-Burchard reaction for sterol components of *Cucumaria chronhjelmi*.
T: Transmittance,
C: Concentration (10^{-3} mole).

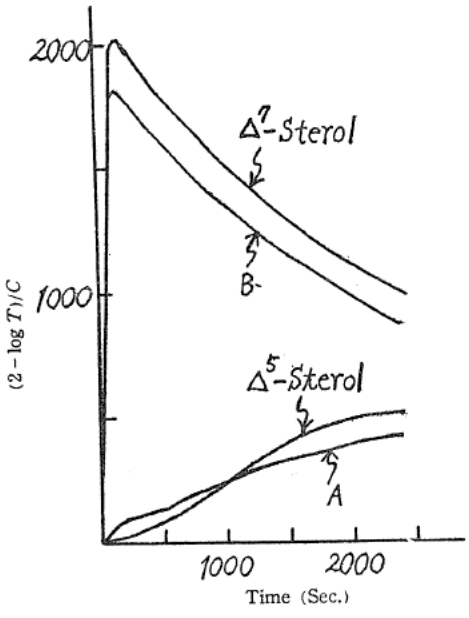


FIG. 8. Absorption at 620 mμ vs. reaction period in Liebermann-Burchard reaction for sterol components of *Coscinasterias acutespina* and *Comanthus japonica*.
T: Transmittance,
C: Concentration (10^{-3} mole).

2. *Coscinasterias acutespina* Stimpson

The starfish was caught around Osaki-shimojima, Hiroshima Prefecture, in August, 1952 and was sun-dried immediately after its catch. The sun-dried material (350 g) yielded 7.3 g of ether-extract, from which 4.5 g of acetone-soluble oil was obtained. It was a reddish orange liquid with some solid and had A.V. 114.2, S.V. 146.3, I.V. 103.6 and unsaponifiable matter 39.14%. The fatty acids showed N.V. 189.3 and I.V. 83.6, the latter being very low as compared with iodine values of common aquatic animal oils.

The unsaponifiable matter of acetone-soluble oil contained 40.6% of sterol. Recrystallization of unsaponifiable matter (1.8 g) from methanol gave a crude sterol mixture (0.7 g') which had m.p. 124°–127°C and showed no maximum absorption at the region of 230–300 mμ. Steryl acetate from the crude sterol had m.p. 133°–134°C, $[\alpha]_D^{23} = \pm 0$, S.V. 130.1 and I.V. 108.4. Liebermann-Burchard reaction for this steryl acetate gave a curve (curve *B* in Fig. 8) which lies close to the curve for Δ^7-sterol. But the melting point of this steryl acetate was raised by further recrystallizations until eventually the product obtained in a very small amount (40 mg) had m.p. 158°–159°C and I.V. by the perbenzoic acid method 69.1. Hence the steryl acetate is not a uniform compound but a mixture consisting chiefly of Δ^7-sterol.

3. *Comanthus japonica* (J. Müller)

The living material was caught around Sugashima Island, Mie Prefecture in July, 1954 and sun-dried immediately after its catch. The sun-dried material was received by the courtesy of Dr. E. Nakano, Department of Biology, Nagoya University. It was dried at a temperature below 70°C in our laboratory, and the dried material (215 g) was extracted with ether, yielding an extract (11.5 g) of dark purple color, from which 8.6 g of acetone-soluble oil was obtained. The oil was a reddish orange liquid with some solid; d_4^{30} 0.9288, n_D^{30} 1.4732, A.V. 83.7, S.V. 171.3, I.V. 154.6 and unsaponifiable matter 17.20%.

The fatty acids had N.V. 188.2 and I.V. 163.7, and gave 23.7% solid acids (I.V. 13.0) by the lead salt ethanol method.

The unsaponifiable matter contained 42.9% sterol. It exhibited in ethanol a very small absorption characteristic to provitamin D ($\Delta^{5,7}$-sterol). The provitamin D content was found to be 0.3% by the spectrophotometric estimation; $k_{277} = 0.368$, $k_{282} = 0.382$ and $k_{290} = 0.353$. Fractional crystallization of the unsaponifiable matter (0.9 g) from methanol gave two fractions of m.p. 117°–120°C and m.p. 105°–110°C, respectively. These were united, giving a crude sterol mixture (0.4 g) of I.V. 84.4. Liebermann-Burchard reaction for this crude sterol mixture gave a curve (curve *A* in Fig. 8) which lies near the curve for Δ^5-sterol. Steryl acetate mixture, m.p. 114°–119°C, from the crude sterol gave a fraction of m.p. 131°–132°C, $[\alpha]_D^{28} = -41.5°$, S.V. 125.5 and I.V. 88.3 after several recrystallizations. It exhibited no maximum absorption at the region of 230–300 mμ. Free sterol obtained by saponification of this fraction had m.p. 134°-138°C and $[\alpha]_D^{28} = -38.4°$. Benzoate prepared from the free sterol melted at 142°C. But since the melting point of the acetate fraction of m.p. 131°–132°C was raised by a further recrystallization, it was not a uniform compound but a mixture consisting chiefly of Δ^5-steryl acetate.

4. Composition of polyethenoid acids estimated by ultraviolet absorption measurement of alkali-isomerized fatty acids

The fatty acids from each oil were alkali-isomerized under the condition of 21% KOH-glycol, 180°C and 15 minutes with nitrogen, and the compositions of the polyethenoid acids were estimated from the observed data in a similar manner as described in the preceding section. The results are shown in Table 23.

TABLE 23. Composition of Polyethenoid Acids

Polyethenoid acids	*Cucum. chronhjelmi*		*Cos. acutespina*		*Com. japonica*	
	(%)	Pentaene as 1	(%)	Pentaene as 1	(%)	Pentaene as 1
Hexaene	3.7	0.47	1.7	0.37	10.3	0.64
Pentaene	7.8	1.00	4.6	1.00	16.2	1.00
Tetraene	2.4	0.31	2.1	0.46	7.6	0.47
Triene	0.3	0.04	0.6	0.13	0.2	0.01
Diene	4.4	0.56	2.8	0.61	5.5	0.34

Summary

1. The fatty acids of oil extracted from salted ovaries of sea-urchin, mainly *Strongylocentrotus pulcherrimus* with some *Heliocidaris crassispina*, contain roughly 25% saturated acids, 50% mono-ethenoid acids and 25% polyethenoid acids. The saturated acids consist roughly of 30% myristic acid, 60% palmitic acid and 10% stearic acid. Higher saturated members (possibly arachidic and behenic acids) are also present in a small proportion. The mono-ethenoid acids are composed of approximately 20% zoomaric acid, 50% oleic acid and 30% higher members, mainly eicosenoic and docosenoic acids. Mono-ethenoid acid lower than C_{16} is also present in a small amount. The polyethenoid acids contain acids of $C_{18}F_3$, $C_{18}F_4$, $C_{20}F_4$, $C_{20}F_5$ and $C_{22}F_5$, of which C_{18} acids are present only in a small amount.

The unsaponifiable matter contains 68% sterols which consist predominantly of cholesterol. Provitamin D occurs in crude sterol mixture in an amount of 4.6%. Besides sterols, the presence of batyl alcohol possibly with chimyl alcohol and some members of selachyl alcohol series is indicated.

2. The fatty acid components of lipid extracted from clam, *Meretrix meretrix*, were examined with the following results.

Among the saturated acids (approximately 16%), palmitic acid is most predominant, followed by stearic acid. Also myristic, arachidic and behenic acids are present in a lesser amount. The mono-ethenoid acids contain C_{20} and C_{22} acids besides zoomaric and oleic acids. Also C_{24} acid appears to occur in a small amount. Polyethenoid acids contain mainly C_{20} and C_{22} acids together with a lesser amount of C_{18} acids, among which $C_{22}F_5$, $C_{20}F_4$. $C_{18}F_4$ and $C_{18}F_3$ acids are main individual components. Although more highly unsaturated acids of large carbon number, such as $C_{22}F_6$ acid and C_{24} highly unsaturated acid, might be present, they could not be found in the present study since they might have undergone polymerization in the course of distillation of their methyl esters.

3. The fatty acids of lipid extracted from boiled meat of *Corbicula leana* were examined with the following results.

The saturated acids (approximately 19%) contain most predominantly palmitic acid together with C_{14}, C_{18}, C_{20} and C_{22} acids. The presence of C_{24} and C_{26} saturated acids is also indicated. The mono-ethenoid acids contain zoomaric and oleic acids together with C_{20} and C_{22} acids. Also mono-ethenoid acid higher than C_{22} appears to be present. The highly unsaturated acids regenerated from ether-insoluble bromide contain chiefly tetraenoic acids and more highly unsaturated acids among which not only $C_{22}F_5$ acid but also acid of C_{20} possibly with acid of C_{18} and of higher than C_{22} appear to be present.

4. The fatty acids and sterols of oil extracted from *Spisula sachalinensis* were studied.

The composition of the total fatty acids were calculated in approximation by the ester fractionation method from the data on saponification and iodine values, saturated ester and its saponification value for each fraction with the results that the total fatty acids contain 0.8% C_{14} acid, 12.4% C_{16} acid and 4.3% C_{18} and higher acids as saturated acids, and 15.3% $C_{16}(-H, 2.4)$ acid, 33.7% $C_{18}(-H, 4.0)$ acid, 23.1% $C_{20}(-H, 6.8)$ acid and 10.4% $C_{22}(-H, 8.7)$ acid as unsaturated acids.

The sterol components were found to contain di-unsaturated and mono-unsaturated sterols. Among the mono-unsaturated components, the presence of β-sitosterol and clionasterol was indicated. Provitamin D ($\Delta^{5,7}$-sterol) content in crude sterol mixture was estimated as 5.6% by the spectrophotometric method.

5. Fatty oils were prepared from 16 kinds of Japanese shellfish, and characteristics of each oil were determined. Provitamin D content of the crude sterol mixture separated from each oil was spectrophotometrically estimated, the largest value being 19.3% for *Viviparus histricus.*

6. Fatty oil was extracted from the central disc of *Gorgonocephalus caryi.* The compositon of fatty acids of the oil was estimated by the ester fractionation method with the following results: saturated acids —2.0% C_{14} acid, 13.1% C_{16} acid and 4.4% C_{18} and higher acids; unsaturated acids —11.1% $C_{16}(-H, 2.0)$ acid, 29.5% $C_{18}(-H, 3.9)$ acid, 21.9% $C_{20}(-H, 5.8)$ acid and 18.0% $C_{22}(-H, >7.4)$ acid. Among individual fatty acid components, palmitic and oleic acids and a highly unsaturated acid fraction containing chiefly clupanodonic acid were separated. Sterol mixture from this oil was found to contain mainly Δ^5-sterol possibly with a minor amount of di-unsaturated sterol. A Δ^5-sterol fraction was separated and identified with β-sitosterol.

The fatty acids and sterols were separated from the oil extracted from *Ophioplocus japonicus.* Sterol components were found to consist mainly of Δ^5-sterol.

7. Characteristics of oils extracted from 11 kinds of Japanese shellfish were determined. It was found for *Neverita didyma, Cipangopaludina malleata* and *Pinna pectinata japonica* (*forma lischkeana*) that the yield of ether-extract is higher for viscera than for flesh of the same shellfish. Viscera oil has higher iodine value and lower content of unsaponifiable matter than flesh oil. Unsaponifiable matter of viscera oil has smaller sterol content and larger provitamin D content than flesh oil.

It was also found that the properties of oil of one and the same kind of shellfish often show a marked variance. In this respect, shellfish oil is more conspicuous than fish oil.

Recrystallization of steryl acetate mixtures from *Corbicula sandai, Hyriopsis schlegelii* and *P. japonica* (*forma lischkeana*) brought about steady increase in

provitamin D content, while recrystallization of steryl acetate mixture from *Cip. malleata* brought about steady decrease in provitamin D content.

Polyethenoid acids of the fatty acids from *Cip. malleata, Mya arenaria japonica, Sanguinolaria boeddinghausis, Cor. sandai, H. schlegelii* and *P. japonica* (*forma lischkeana*) were examined by ultraviolet absorption measurement of alkali-isomerized fatty acids, and it was found that the proportion of pentaenoic acid is generally larger than those of tetraenoic and hexaenoic acids, excepting the fatty acids from *Cip. malleata* in which the proportion of tetraenoic acid is larger than the proportion of pentaenoic acid.

8. Oils extracted from *Cucumaria chronhjelmi, Coscinasterias acutespina* and *Comanthus japonica* were studied with the following results.

Unsaponifiable matter from *C. chronhjelmi* contains 8.4% sterol besides batyl alcohol. Sterol components consist chiefly of Δ^7-cholestenol. Sterol components from *C. acutespina* contain mainly Δ^7-sterols (Δ^7-cholestenol and others), while sterol mixture from *C. japonica* contains mainly Δ^5-sterol.

Dividing starfish sterols into two series, Δ^5-series and Δ^7-series, by the location of ethenoid linkage in sterol ring irrespective of carbon number and unsaturation in side chain, it is found that sterols of Holothurioidea and Asteroidea consist mainly of Δ^7-series whereas sterols of Echinoidea, Ophiuroidea and Crinoidea consist chiefly of Δ^5-series.

Results of the determination of polyethenoid acids in the fatty acids indicated that the proportion of pentaenoic acid is largest and the proportion of trienoic acid is smallest in every case. The proportion of dienoic acid is relatively large in every case, but it appears to require a further study in this respect.

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