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NEW OBSERVATIONS ON THE LIPIDS OF AQUATIC ORIGIN

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Contents

1. Introduction

1.1 Historical survey on the origin of fish lipids

Marine lipids have two characteristics in their fatty acid composition, one is the wide spread distribution of chain length ranging from 12 to 24 carbon atoms, and the other is its especially high unsaturation. Even the acids of C_{14} and C_{16} contain unsaturated bonds, simultaneously the acids of C_{20} and C_{22} have 4, 5 or 6 double bonds in their molecules. As to the saturated acids their dominant component is C_{16} acid, and the unsaturated acids are composed of C_{14} to C_{22} acids.[1),5)]

Cod liver oil is the model example of fish lipids having a typical fatty acid composition close to the average of all those so far examined. The fatty acid composition of cod liver oil is shown in Table 1.[2),3)]

On the other hand, the fatty acid compositions of the lipids of terrestrial mammals are mainly composed of palmitic, stearic and oleic acids. The fatty acid compositions of the lipids of some mammals are shown in Table 2.[4)] In mammals, the fatty acids consist of 35–56% saturated acids and 44–65% unsaturated acids. Besides the above mentioned three acids, saturated C_{14} and unsaturated C_{16}, C_{18} (diene) and C_{20-22} acids are also distributed in small amounts.

Furthermore, the fatty acid composition of the lipids of marine fishes is different from that of fresh-water fishes. There are two types of lipids of aquatic

Table 1. Fatty acid composition of cod liver oil (per cent wt)

Investigator

J.A. Lovern[2]

Saturated acid

C_{14}	C_{16}	C_{18}	Total
4.0	11.0	1.2	16.2

Unsaturated acid

C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	Total
0.1	11.1	27.2	26.8	18.6	83.8
(−2.0H)	(−2.0H)	(−2.5H)	(−5.0H)	(−7.2H)*	

* The mean unsaturation of this group — e.g. −7.2H would indicate that 7.2 hydrogen atoms are needed to saturate an 'average' molecule of the mixture.

R.G. Ackman et al[3]

Saturated acid

14:0	15:0	16:0	17:0	18:0	19:0	20:0	21:0	22:0	24:0	Total
3.3	0.6	9.0	0.6	3.2	0.1	0.1	NSA**	—	NSA**	16.9

Unsaturated acid

14:1	16:1	16:2	16:3	16:4	17:1	18:1	18:2	18:3	18:4
0.1	10.7	0.2	0.4	0.3	0.3	21.9	1.8	0.8	1.5
	11.6					26.0			

19:1	20:1	20:2	20:3	20:4	20:5	21:5	21 6
0.1	13.2	0.3	0.3	0.7	9.2	0.2	0.1
	23.7					0.3	

22:1	22:4	22:5	22:6	24:1	Total
7·9	0.1	2.1	10.6	0.3	83.1
20.7					

** NSA=No significant amount

Table 2. Fatty acid composition of depot fats of mammals (per cent wt)

Class	Animal	Depot fat	I.V.	Saturated			Unsaturated			
				C_{14}	C_{16}	C_{18}	C_{16}	Oleic	C_{18} Diene	C_{20-22}
Herbivora	Horse	Mesentric	95. 6	5	26	5	7	34	5*	2
	Pig	Back, outer	60. 0	1	28	12	3	48	6	2
		Back, inner	54. 3	1	30	16	3	41	7	2
	Ox	Perinephric	43. 2	3	29	21	3	41	2	trace
	Sheep	Perinephric	43. 4	3	25	28	1	37	5	1
Omnivora	Human	Male, 74 years	67. 4	6	25	6	7	45	8	2
		Female,53 years	68. 9	3	24	8	5	47	10	3
Carnivora	Cat	Body	43. 6	4	29	17	4	41	2	—

* Also 16 per cent linolenic acid

origin, one of which is marine fish type and the other is fresh-water fish type. The average compositions of unsaturated acids of the two types of fishes are shown in Table 3.[5] (In saturated acid the difference between the two types is very small.)

Table 3. Unsaturated fatty acid compositions of marine and fresh water fishes[5] (per cent wt)*

type	C_{16}	C_{18}	C_{20}	C_{22}
Fresh water fish	20	40	13	2.5
Marine fish	10	25	25	15

* These values were obtained from the results of 17 fresh-water fishes and 44 marine fishes.

Comparing marine fishes with fresh-water fishes and terrestrial mammals, it is evident that the fatty acid composition of the lipids of fresh-water fishes is intermediate between those of terrestrial mammals and marine fishes.

In the case of algae, regardless whether it belongs to marine or fresh-water species, it falls into groups according to the fatty acid composition of lipids under the influence of their botanical relationships. The lipids of Chlorophyceae belong to the fresh-water fish type, those of Rhodophyceae to the marine fish type, and those of Phaeophyceae are inclined to the fresh-water fish type rather than the marine fish type.[6] With the crustacean plankton, the lipids of marine and fresh-water species belong to the marine fish and the fresh-water fish types respectively.[7]

It was indicated that the factors influencing the properties and composition of fish lipids are diet, sexual maturity, temperature of sea water and species of fishes and others,[8] and that among those factors the most important one is diet. The following dispute is indicated as the basis of argument.[5]

(1) The fatty acid composition of the lipids of the marine species of crustacean plankton shows the same class distinction when contrasted with the acids of fresh-water species as is observed with the fatty acids of fish from the respective habitats. The close correspondence in the types of the lipids from crustacea and fish suggests that lipids ingested by the fish are largely deposited and unchanged, and that the class differences observed between the lipids from marine and fresh-water fish as a whole have a dietary orign.[7]

(2) When the fishes living in fresh-water have been fed with oily marine fishes, for instance, eel living in fresh-water having been fed with herrings, the lipids of eels change from the lipids of the fresh-water fish type to the marine fish type.[9]

(3) Most fishes are carnivorous and almost do not ingest carbohydrate as nutrient with some expection, but only ingest lipid and protein. Fishes, differently from mammals and birds, grow continually throughout their life corresponding to the quantity of the ingested nutrients. Consequently, almost all the lipids of fish are derived from the dietary lipid as nutrient.

Already Hilditch and Lovern[1] had pointed out that many close parallelisms exist between the composition of fatty acids combined as triglycerides in living animal organisms, and their places in the evolutionary scale of development.

Furthermore, Shorland$^{10)}$ indicated the possibility to divide animal fats into two types:

(a) Fats, the fatty acid composition of which is substantially unaffected by the nature of the dietary fat, as in beef and mutton tallow. It was proposed to designate such fats by the term 'heterolipid' to indicate lack of resemblance to the dietary fat.

(b) Fats which readily incorporate the fatty acids present in the dietary fat. For such fats it was proposed to use the term 'homolipid' to designate similarity in composition to that of the ingested fat.

As we pass from depot fats of aquatic to those of land animals we find marked simplification in the mixed fatty acids. Continuously Shorland$^{11)}$ suggested that the observed increasing simplicity of animal depot fats, as one proceeds from the lower to the more highly organized form of animal life, is largely due to the fortuitous influence of the diet and only in part to the animal themselves in the former and the faculty of synthesizing the endogenous fat *in vivo* in the latter.

He also suggested that the major trend of evolutionary development of fats is discernible with respect to the fat metabolism of animals as follows:

(a) In the early stages of evolutionary development of fats in animals, the organism, as examplified by fishes, is able to assimilate the dietary fat and so deposit it in its tissues more or less in the present form of the diet, subject, of course, to minor modification induced by hydrogenation, dehydrogenation and in some instances to the lengthening or shortening of the carbon chains. At this stage of development, the organism is apparently unable to make fat (here termed synthetic fat) from the non-fatty (protein and carbohydrate) constituents of the diet. (On the capacity of synthesis of fats *in vivo*, it was known that fishes synthesize saturated and monounsaturated acids from acetate as diet.$^{12)}$ But, fishes in nature do not ingest carbohydrates as diet, with exception of some species.)

(b) In the next stage, as examplified by amphibians, reptiles and most mammals, there is acquired, in addition to the ability to deposit the dietary fat mainly in its original form in the depot fat, an ability to make 'synthetic' fat. Such 'synthetic' fat is more or less of a uniformly simple composition and contains, as its main components, palmitic, stearic and oleic acids.

(c) In the final stage, the organism, as examplified by certain ruminants, largely loses the power to deposit the fat from the diet, the constituents of which are broken down by the bacteria of the rumen before assimilation by the animal. The dietary fat no longer has any appreciable influence on the composition of the depot fat, which now consists almost entirely of 'synthetic' fat.

Hilditch$^{13)}$ has appreciated Shorland's conclusions above mentioned as constructive and broadly accorded proposals with the general trend of the facts so far elicited, and moreover recognized them in the nature of a preliminary statement which will require in due course further elaboration or modification.

The formation of characteristic wax esters of sperm whale (Physeteridae) oil and singular isovaleric acid containing glycerides of common dolphin (Delphinidae) oil may be accomplished on the assumption of the considerable change of dietary fats in the animal body. Even the micro-fauna as crustacean plankton has peculiarly unique fats according to species.[7] It is the same with some species of fishes.

Therefore, concerning the influence of the dietary fats on the depot fats, we must clarify the influence of species of organism on the depot fats.

1.2 The history of the studies on the lipids of plankton

It has been well known that plankton play an important role as a diet of fishes, and all species of fishes grow by preying upon the plankton in the young stage. Among important aquatic animals, many species of fishes are zooplankton feeders, to which belong the species of baleen whale (Mystacoceti) such as right whale and blue whale, and the species of fishes such as mackerel, herring and sardine.[14] We can easily suppose that there is close relationship between the lipids of plankton, which is importantly significant as a diet of aquatic animals, and the lipids of them. It has been also known that the depot fat of fishes is especially influenced by the dietary fat of them.[11] The elucidation of the lipids of plankton is very interesting on the clarification of the relationship between the lipids of plankton and those of fishes fed with plankton.

Studies on the lipids of plankton have been done from of old. Brandt,[15] Brandt and Raben[16] reported the lipid content of mixed plankton. Moberg[17] and Wimpeny[18] reported seasonal variations of the lipid content of mixed plankton.

About the lipids of single species of plankton, Klem[19] reported those of copepods and euphausiids. The lipid content of copepods was 8.7–10.2% on dry weight, and the lipids of *Calanus finmarchicus* (Gunnerus) had a saponification value of 107.1–134.0, an iodine value of 157.5–158.1, an unsaponifiable matter of 2.5% and d^{15}_{15} 0.9108. The fatty acids of *Meganyctiphanes norvegica* (Euphausiid) had a neutralization value of 179.0, an iodine value of 167.5, contained myristic, palmitic, hexadecenoic and oleic acids. The bromination of the fatty acid yielded 10% of octabromide, and this suggested the existence of a highly unsaturated fatty acid.

Orr[20] found the lipid content of *Euchaeta norvegica* (Calanoida) being 23% in adult male, 21% in non-ovigerous adult female, 36% in ovigerous adult female, 30% in Stage V male and 21% in Stage V female. The lipid contents of important marine plankton in the adjacent seas of Japan were investigated by Nakai,[21] and the results were as follows:

Euphausia pacifica (Euphausiacea) 2.7%, *Calanus helgolandicus* (Calanoida) 5.2–11.0%, *Calanus cristatus* (Calanoida) 36.1%, *Calanus plumchrus* (Calanoida)

31.4–53.9%, *Parathemisto sp.* (Amphipoda) 39.6%, *Euchaeta japonica* (Calanoida) 33.8%, *Pseudocalanus elongatus* (Calanoida) 17.3%, *Paracalanus parvus* (Calanoida) 19.1%, *Acartia clausi* (Calanoida) 5.8%.

Orr,[22] and Marshall and Orr[23] reported that the maximum lipid contents of *Calanus finmarchicus* were reached in March at Loch Fyne and in April at Clyde Sea. Tsujimoto[24] found that the color reaction by antimony trichloride was rather obscure about the lipid of mixed plankton of the diatoms and copepods collected in the sea off Asamushi, and that the lipid had even a high iodine value, which contained some of highly unsaturated fatty acids.

Collin, Drummond, Hilditch and Gunther[25] investigated the characteristics of the lipid of phytoplankton (mainly consisting of *Chaetoceros spp.* and *Lauderia borealis*) and zooplankton (mainly consisting of *Calanus finmarchicus*), and found that the lipid of phytoplankton had a saponification value of 182, an iodine value of 141, an unsaponifiable matter of 17.9%, an ether-insoluble bromide of 7.6%; on the other hand, that of zooplankton had a saponification value of 123, an iodine value of 126, an ether-insoluble bromide of 30.2%, and a highly unsaturated acid by the lithium salt acetone method occupied 43.6% of total acids. The unsaponifiable matter of the zooplankton lipid contained 9.3% of sterol (mainly consisting of cholesterol), squalene and related compounds, cetyl alcohol and unsaturated C_{20} alcohol.

Lovern[7] examined the lipids from three fresh-water and one marine species of plankton crustacea. The mixed fatty acids of the fresh-water species showed the same class distinction when contrasted with the acids of the marine species as it was observed with the fatty acids of fishes from their respective habitats.

The fatty acids of fresh-water species were characterized by higher contents of C_{16} and C_{18} unsaturated acids than those of marine species, and an almost entire lack of C_{22} unsaturated acids. On the other hand, the lower contents of C_{16} and C_{18} unsaturated acids and the abundance of C_{22} unsaturated acids distinguished the fatty acids of marine species. It would seem that the lipids of crustacean plankton from both marine and fresh-water correspond so closely to those of the marine and fresh-water fish respectively that a simple deposition of ingested fat, largely unchanged in composition, would account for the observed characteristics of marine and fresh-water fish lipids. It is also observed that all the lipids contained large amounts of unsaponifiable matter.

Saiki and Mori[25] examined the lipids of *Calanus cristatus*, collected in the stomach of a fin whale caught in the Northern Pacific Ocean, which showed a lipid content of 58.6% in dry matter, a saponification value of 103, an iodine value of the lipids of 159, an unsaponifiable matter of 43.1%. The mixed fatty acids of this lipid were composed of 16% saturated acids (main components were C_{14} and C_{16} acids) and 84% unsaturated acids (containing large amounts C_{20} and C_{22} acids).

Saiki, Fang and Mori27) examined the lipids of krills, *Euphausia* from the Antarctic Ocean and Northern Pacific Ocean. The lipid content of the former was 18.4%, and the latter 22.7%, and the unsaponifiable matter was 4.3% in the former and 16.8% in the latter. The fatty acids of the former were composed of 28.2% saturated acids (main components were palmitic and myristic acid, accompanied with small amounts of stearic and a trace of lauric acid) and 71.8% unsaturated acids (main components were C_{18}, followed by C_{16}, C_{20} and C_{14} acids), whereas the fatty acids of the latter were composed of 16.1% of saturated acids (main components were palmitic, and traces of stearic and mysistic acids) and 83.9% unsaturated acids (containing large amounts of C_{18}, followed by C_{20}, C_{16} and C_{14} acids). It was indicated that the difference of fatty acid composition between Euphausiacea gathered in the Antarctic and the Northern Pacific Ocean resembles that observed between fin whale blubber oils from both oceans.

Yamada examined the lipid of *Neomysis Nakazawai*28) and *Neomysis intermedia*,29) and reported about the properties and fatty acid composotions of both plankton. He indicated that the fatty acid compositions of the lipids of each species of plankton respectively resemble those of marine and fresh-water species of fishes.

Klenk and Eberhagen30) observed the presence of 16:4ω1*, 16:3ω4*, 16:2ω7*, 16:2ω4* and 16:1ω7* in the lipids of plankton, especially of phytoplankton, and also found that considerable large amounts of C_{20} and C_{22} polyenoic acids were contained in zooplankton rather than in phytoplankton.

Tsuyuki, Naruse, Mochizuki and Ito31,32) reported about the acetone-soluble lipid and the phospholipids contained in *Euphausia superba*. Acetone-soluble lipid was obtained from a cold sample, the yield of which in wet matter was 2.41% having an iodine value of 136.1, an unsaponifiable matter of 5.5%. The mixed fatty acids of the acetone soluble lipid were composed of 31.8% saturated acid (5.8% myristic, 11.4% palmitic, 10.2% stearic, 4.2% arachidic and 0.2% behenic) and of 68.2% unsaturated (0.4% C_{14} (-2.0H), 14.5% C_{16} (-2.3H), 25.0% C_{18} (-3.3H), 20.4% C_{20} (-6.2H) and 8.0% C_{22} (-5.4H)). The paper chromatography indicated the presence of myristic, palmitic, stearic, arachidic and behenic acids as saturated fatty acids, and zoomaric, oleic, eicosenoic, erucic, linoleic and linolenic acids as unsaturated fatty acids. The component fatty acids of phospholipids were similar to that of acetone-soluble lipid, but, in the former, behenic acid was absent and the unsaturated acids other than the one presented in the latter might be

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* Abbreviation of particular fatty acid
The first number (before the colon) designates the number of carbon atoms in the fatty acid molecule and the second number (after the colon) designates the number of methylene-interrupted *cis* ethylenic bonds. The greek letter ω, and a number that refers to the number of carbon atoms separating the terminal methyl group from the ethylenic bond nearest to the terminal methyl, designates the terminal structure of an unsaturated fatty acid.

present. Furthermore, the presence of polyglycerophosphoric acid, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl choline, sphingomyelin and monophosphoinositide in the phospholipids could be observed.

Nonaka and Koizumi[33] examined the lipid of *Euphausia superba* collected from the stomach of an Antarctic finback whale. The results indicated that *Euphausia superba* contained on dry matter 19.4% lipid of which 5.5% is unsaponifiable matter. The predominant components of fatty acids were 14:0 and 16:0 in saturated acids, and 18:1 and 16:1 in unsaturated acids. In the unsaturated acids of above C_{20}, 20:1, 20:5 or 6, 22:1, 22:3 and 22:6 were members in a relatively high proportion. The unsaponifiable matter contained smaller quantity of higher alcohol, of which the presence of C_{14} and C_{16} saturated alcohols and C_{18} and C_{20} unsaturated alcohols were observed.

Yamada[34,95] examined the lipids of zooplankton collected in the adjacent seas of Japan and the sea area of N40°–52°, E147°–176°. It was found that especially the lipids of plankton of genus *Calanus* had a lower saponification value and contained large amounts of unsaponifiable matter composed of high content of fatty alcohol. These properties of the plankton lipid were discussed in relation to the ecology of the plankton moving vigorously and especially vertically with depth. Moreover, the important role of plankton as a carrier of polyunsaturated fatty acids in aquatic food chain was pointed out.

Yamada[36], and Yamada and Ota[35] also reported the components of the unsaponifiable matters of plankton lipids.

In regard to the biochemical studies on the plankton lipid, Kelly, Reiser and Hood,[37] in the study on the origin of the marine polyunsaturated fatty acids, observed that phyto- and zooplankton contained polyunsaturated fatty acids and phytoplankton produce a high level of polyunsaturated fatty acids.

Comparing the lipid of the plankton of Arctic species with that of temperate water species offshore California, Lewis[38] observed that a lower temperature led to an increase in unsaturation and an elongation in the carbon chain length of fatty acids of the lipids.

DeWitt[39] observed that the iodine value of liver oil of cod caught in White Sea and Iceland varied regularly in an annual cycle over a range of 20–30 units and that these changes in iodine value correspond to complementary changes in the total polyunsaturated fatty acids and the total monoenoic acids, the saturated fatty acids of the oil remaining virtually constant throughout the cycle. He also observed these changes being in proportion to the increase and decrease of plankton on which the fish feed in the fishing area.

In a model experiment of aquatic food chain (phytoplankton (*Chaetoceros simplex*) → zooplankton (*Artemia salina*) → fish (*Lebistes reticulatus*)) with special significance in fatty acid conversion, Kayama, Tsuchiya and Mead[40] confirmed that the highly unsaturated acids were not detected in phytoplankton (*Chaetoceros*) oil, but in zooplankton (*Artemia*) oil, arachidonic and eicosapentaenoic acids were found and, in guppy oil added to these, docosatetraenoic, docosapentaenoic and docosahexaenoic acids were also found.

Farkas and Herodek[41] found that the proportions of C_{20}-C_{22} polyunsaturated acids in the fresh-water planktonic crustaceans increased with decreasing temperature and in some species exceeded the characteristic values of marine animals and, in fresh-water fish, fed on freshly collected fresh-water plankton, the lipids formed in winter resembled marine fish oil. He concluded that the environmental temperature had an effect indirectly on the fatty acid compositions of aquatic animals through aquatic food chains.

Brockerhoff, Yurkowski, Hoyle and Ackman[42] reported that the polyunsaturated fatty acids of the triglyceride in the diatom, as well as in a zooplankton sample, were found accumulated in the β-position of the glycerol, the fatty acid distribution of which is typical for animal fats, in particular for fish oil, and the findings of their study showed that the typical structure of marine triglycerides originates in phytoplankton and is to a large degree retained through the marine food chain. They also examined the fatty acid composition of several other lipid fractions — lecithin and phosphatidylethanolamine — of the plankton samples and determined larger contents of 20:5 and 22:6 in those lipid fractions than in glyceride.

Ackman, Jangaard, Hoyle and Brockerhoff[43] reported that the analysis of the fatty aicd composition of a marine diatom showed that the longer chain highly unsaturated fatty acid characteristic of fish lipids can be produced by some phytoplankton as well as through intermediary zooplankton.

Ackman and Sipos[44] examined the saturated fraction of some of the fish oil fatty acids. They reported that the normal odd-numbered fatty acids found in marine lipids were originated in phytoplankton and that there was little evidence of a remarkable presence of branched chain fatty acids in phytoplankton. They presumed that these branched chain fatty acids are formed to some degree in zooplankton.

Ackman and Eaton[46] reported that the lipid of the zooplankton, *Meganyctiphanes norvegica* (M. Sars), predominant in Nova Scotia Waters, had a fatty acid composition resembling that of herring oil, which is rich in 20:1 and 22:1 acid and reflect to the lipid of fin whales living in the same sea area.

In regard to the hydrocarbon in the lipid of plankton, several studies were carried out by Blumer and his co-workers. Blumer, Mullin and Thomas[46] found that the hydrocarbon pristane (2,6,10,14-tetramethypentadecane) occurred in unusually high concentration (1 to 3% of the body fat) in these three copepods, *Calanus finamarchicus*, *C. glacialis* and *C. hyperboreus*, and other species in the same zooplankton samples contain much less pristane, or none at all. They considered that the copepods of the genus *Calanus* are among the major producers of pristane. The structural similarity between pristane and phytol suggested that the hydrocarbon may be derived in *Calanus* from the chlorophyll of its phytoplankton diet. Further, Blumer and Thomas47) isolated four isomeric phytadienes from mixed zooplankton, namely neophytadiene (2,6,10-trimethyl-14-methylene-1-pentadecene), 1,3-phytadiene (*cis and trans*), and 2,4-phytadiene. The chemical structures suggested that the mixture is derived by the dehydration of phytol (presumably by acid catalysis).

Blumer and Thomas,48), subsequently isolated three isomeric C_{19} monoolefins with the carbon skeleton of pristane from mixed zooplankton and from liver oils of various marine fishes and mammals. Those were 2,6,14-trimethyl-10-methylene-pentadecane, 2,6,10,14-tetramethyl-1-pentadecene (norphytene) and 2,6,10,14-tetramethyl-2-pentadecene, which is the predominant one in all cases. The isomeric C_{19} monoolefins is derived from phytol fed by zooplankton as well as pristane and phytadiene.

1.3 The purpose of this investigation

To the elucidation of the question how the fatty acids characteristic of marine fish lipid may be produced, through what pathway the lipid which contains large amounts of wax esters as in sperm whale oil, large amounts of higher hydrocarbon as in some of the shark liver oil, and large amounts of acyl glyceryl ethers as in the lipid of some marine organisms may be produced, and what the functions of these classes of lipid in organisms are, the author noticed the necessity of clarifying the general properties, fatty acid composition and the unsaponifiable matter of the lipids of plankton on which the fish feed.

The author examined the lipids of plankton and fishes, and pursued the relationship between the two.

2. Experimental and results

2.1 Squid oil and mackerel oil

Squid oil is obtained from the viscera as a byproduct of the manufacture of dried squid (Japanese SURUME) and other processed goods from squid, for which the species *Ommastrephes sloani pacificus* Steenstrup is chiefly used, but the resulting oil is derived mainly from livers.

The production of this oil has so rapidly increased after the war with Hokkaido as the production center that this oil has replaced sardine and herring oils which were formerly the most important fish oils in Japan. In addition to squid, YARI-IKA, MA-IKA and HOTARU-IKA are caught off the coast of Japan, and the viscera of YARI-IKA are used in some degree as raw material for oil rendering.

Mackerel oil is prepared either from the offals or sometimes from the whole body of mackerel, *Scomber japonicus* Houttuyn. The production of this oil has also greatly increased after the war chiefly in Hokkaido.

Squid oil and mackerel oil were formerly studied by Tsujimoto and his co-workers. The squid oil examined by Tsujimoto[49] showed d_4^{15} 0.9316, n_D^{20} 1.4806, A.V. 3.9, S.V. 189.6, I.V. 177.0, unsaponifiable matter 1.14%, and polybromides of fatty acids 57.45% with Br content 70.91%.

The unsaponifiable matter of another sample examined by the same author contained 48% of cholesterol together with cetyl, oleyl, batyl, chimyl and selachyl alcohols. The oil reported by Tsujimoto and Kimura[50] had d_4^{15} 0.9300, n_D^{20} 1.4833, A.V. 18.2, S.V. 176.9, I.V. (pyridine sulfate dibromide method) 179.5, unsaponifiable matter 4.20%, solid acids by lead salt alcohol method 22.6% with I.V. 14.1, and highly unsaturated acids by lithium salt acetone method 41.3% with I.V. 372.1. The saturated acids contained palmitic acid, and the unsaponifiable matter contained 56% of cholesterol. The oil contained also vitamin A. Kitabayashi, Nakamura and Shuto[51] have reported 3 samples of squid oil having d_4^{15} 0.9365–0.9393, A.V. 10.0–15.8, S.V. 180.5–186.2, I.V. 175.0–182.6 and unsaponifiable matter 3.5–5.5%. One sample contained 25.4% of solid acids with I.V. 8.8.

Suzuki[52] reported the presence of zoomaric, linolenic, stearidonic, isomer of stearidonic, and arachidonic acids as the fatty acid constituents of the glycerides of squid oil by the solvent fractionation of bromide of the oil.

After the war, on the fatty acid composition of squid oil, Takao and Tomiyama[53] reported the presence of myristic, palmitic, stearic, arachidic, zoomaric, oleic, gadoleic, docosenoic, $C_{20}H_{32}O_2$, $C_{20}H_{30}O_2$, $C_{22}H_{34}O_2$, $C_{22}H_{32}O_2$, $C_{24}H_{38}O_2$, capric, lauric, tetradecenoic, $C_{18}H_{28}O_2$, $C_{18}H_{30}O_2$, and $C_{22}H_{36}O_2$. Tsujino[54] reported the presence of $C_{14}H_{28}O_2$, $C_{16}H_{32}O_2$, $C_{18}H_{36}O_2$, $C_{20}H_{40}O_2$ and $C_{22}H_{44}O_2$ as saturated acids, $C_{16}H_{30}O_2$, $C_{18}H_{34}O_2$, $C_{20}H_{38}O_2$, $C_{22}H_{42}O_2$ as monoenoic acids, $C_{18}H_{30}O_2$, $C_{18}H_{28}O_2$, $C_{20}H_{32}O_2$, $C_{22}H_{36}O_2$, $C_{22}H_{34}O_2$ as polyenoic acids. Tsujimoto[55] examined also an oil obtained from another kind of squid HOTARU-IKA, *Watasenia scintillans* (Berry). André and Canal[56] reported the properties of the European squid oil.

The mackerel oil reported by Tsujimoto[57] showed d_4^{15} 0.9301, n_D^{20} 1.4811, A.V. 1.7, S.V. 191.6, I.V. 167.4 and polybromide of fatty acids insoluble in acetic acid 36.18% with Br content 69.38%. Spanish mackerel oils[58] were reported to have S.V. 182.5–204.9 and I.V. 115.3–136.7.

In this chapter are recorded the more important properties of the squid and mackerel oils which were produced in 1951.

2.1.1 Squid oil[59,60]

The properties of 26 oils examined are given in Table 4. All the oils were prepared by rendering under the condition which facilitated much autolysis in the course of rendering. They have a reddish orange color, though the color of each

Table 4. Characteristics of squid oil

Sample No.	Catching		d_4^{20}	n_D^{20}	A.V.	S.V.	I.V. (Wijs)	Unsap. matter (%)	Ether-insol. brominated fatty acids (%)
	Locality*	Date (1951)							
1	Matsumae	Dec. 4	0.9289	1.4838	11.3	182.4	206.5	4.04	79.27
2	Ōhata	Late Oct.	0.9273	1.4838	7.0	187.2	203.2	2.45	75.27
3	Tokai District	Late Nov.	0.9277	1.4835	22.8	187.3	197.9	3.62	74.68
4	Kayabe	Late Aug.	0.9267	1.4820	16.8	184.9	195.9	4.28	77.81
5	Ōhata	Early Oct.	0.9274	1.4832	10.2	185.0	193.3	3.01	76.19
6	Muroran	Aug. 30	0.9263	1.4817	12.5	186.8	193.2	4.43	75.00
7	Hakodate	Dec. 1	0.9279	1.4832	7.3	185.1	193.0	2.59	73.90
8	Hachinohe	Late Oct.	0.9263	1.4832	17.8	185.2	192.9	3.45	76.07
9	Hakodate	Sept. 19	0.9257	1.4834	14.2	186.7	191.8	4.47	76 32
10	Hakodate	Aug. 11	0.9263	1.4820	5.3	187.7	190.8	3.72	75.60
11	Fukushima	Aug. 17	0.9259	1.4820	12.2	183.1	190.2	4.83	72.34
12	Fukushima	Aug. 17	0.9267	1.4820	12.4	183.6	189.4	4.84	75.51
13	Aonae	Aug. 10	0.9268	1.4830	12.3	183.0	188.5	5.31	74.29
14	Hachinohe	Middle Oct.	0.9270	1.4822	16.3	179.8	188.5	3.64	74.36
15	Hakodate	Dec. 15	0.9272	1.4831	8.3	181.5	188.1	6.94	72.52
16	Mitsuishi	—	0.9248	1.4820	17.3	182.8	186.7	4.87	74.88
17	Matsumae	Oct. 23	0.9267	1.4825	5.2	179.1	186.4	4.02	73.79
18	Hakodate	Oct. 3	0.9249	1.4820	10.3	186.2	185.1	3.65	71.94
19	Hakodate	Sept. 20	0.9244	1.4828	6.4	187.0	185.0	2.94	71.99
20	Urakawa	—	0.9254	1.4821	14.9	183.8	183.8	4.32	73.32
21	Urakawa	—	0.9253	1.4820	11.5	185.4	183.8	3.26	72.99
22	Hakodate	Aug. 2	0.9240	1.4809	14.4	186.8	183.2	4.16	70.33
23	Urakawa	—	0.9247	1.4818	11.2	185.3	181.5	3.56	71.39
24	Urakawa	—	0.9249	1.4820	12.5	186.7	180.1	3.79	71.37
25	Urakawa	—	0.9254	1.4820	13.9	186.9	179.9	4.11	73.32
26	Hakodate	Jul. 24	0.9230	1.4800	17.0	184.9	178.8	4.58	64.71
Highest			0.9289	1.4838	22.8	187.7	206.5	6.94	79.27
Lowest			0.9230	1.4800	5.2	179.1	178.8	2.45	64.71
Average			0.9261	1.4824	12.4	184.8	189.1	4.03	73.93

* The localities of catching of Nos. 2, 5, 8 and 14 belong to Aomori Prefecture, and that of No. 3 to Tokai District while those of other samples belong to Hokkaido. Oils from No.s 1, 3, 7, and 15 were prepared in our laboratories.

oil varies somewhat in depth. They are liquid at ordinary temperature, and deposit a very small amount of stearine even in winter. Since a few oils were turbid due to the presence of moisture, they were filtered through a dry filter paper before they were used for the determination of their characteristics.

It is known for some fish oils that there is a definite relationship between the season of fish catching and the properties of fish oils, but no such relationship is found in the case of squid oil, so far as the results of the present experiments recorded in Table 4 are concerned. The yields of ether-insoluble brominated fatty acids are very high, exceeding 70% except oil No. 26, and hence squid oils are found to contain a very large amount of highly unsaturated acids.

Determination of saturated acids

When the lead salt alcohol method is applied for the separation of solid acids from mixed fatty acids of fish oils, it gives solid acids having a relatively high iodine value in most cases, while lower members of saturated acids, such as lauric and myristic acids, are not completely precipitated as lead salts insoluble in alcohol. If the procedure is carried out so as to obtain solid acids having a very low iodine value, a considerable proportion of saturated acids tends to remain in liquid acids and the estimation of saturated acids in mixed fatty acids becomes difficult. In these experiments, mixed fatty acids were converted into methyl esters which were then subjected to the oxidation with potassium permanganate in acetone. The acidic scission product from unsaturated esters was then separated from the neutral saturated ester.

The acetone permanganate method was first devised by Hilditch[61] for the purpose of determining tri-saturated glycerides in oils and has been applied to various kinds of oils, but it has been little used for the determination of saturated acids in mixed fatty acids. The procedures taken in these experiments are as follows: Mixed fatty acids freed from unsaponifiable matter were esterified with methanol in the usual way. Two g of methyl esters (A.V. below 2) were dissolved in 40 ml of acetone, and 20 g of powdered potassium permanganate was added in small portions (little by little). After the mixture was refluxed for 1–1.5 hours in order to complete the oxidation, the acetone was distilled off.

The residue was added with 120–150 ml of water and then treated with a concentrated solution of sodium bisulfite in order to reduce the oxides of manganese and the excess of potassium permanganate. The solution then was extracted with 700 ml of ether. The extraction was repeated using 400–500 ml of ether. The ether solution was washed several times with water and then with a solution of sodium carbonate in order to remove the acidic substances as sodium salts. After washing with water and dehydration with anhydrous sodium sulfate, the ether solution was distilled, and the residue consisting of saturated esters was weighed. Four oils were examined by this method with the results given in Table 5. Thus the contents of saturated acids in mixed fatty acids of these oils are estimated at 25–27%.

Determination of tri-saturated glycerides.

The same four oils which were used for the determination of saturated acids were subjected to the acetone permanganate oxidation for the determination of

Table 5. Saturated acids (Squid oil)

Sample No.	6	16	25	26
Saturated methyl esters (%)	26.53	25.79	25.54	27.41
I.V. of saturated methyl esters	0.82	1.4	0.73	0.92

tri-saturated glycerides. The oxidation of glycerides was performed in a similar way as described above for the oxidation of methyl esters.

The oxidation product was extracted with ether, and the ether solution was washed with a solution of sodium carbonate and then with water. After dehydration and distillation of ether, the residue obtained was again oxidized with potassium permanganate in acetone. The oxidation product was treated as before.

On distilling off ether from the ether solution, a solid residue was obtained. It had, however, an exceedingly high acid value indicating the presence of a considerably large proportion of some acidic substances, possibly the oxidation product from di-saturated glycerides, besides tri-saturated glycerides. By washing a petroleum ether solution of the residue obtained above with a solution of sodium carbonate, the acid value of the substances remaining in the petroleum ether solution decreased, but the substances obtained finally from the petroleum ether solution, designated as tri-saturated glycerides in Table 6, still showed relatively high acid values, and the complete removal of acidic oxidation product was not attained in these experiments. Based on the data given in Table 6, the amount of tri-saturated glycerides in the examined oils are estimated at about 1–2%.

Table 6. Tri-saturated glycerides (Squid oil)

Sample No.	6	16	25	26
Trisaturated glycerides (%)	2.1	2.5	2.6	2.5
A.V. of trisaturated glycerides	19.0	9.1	7.6	15.7

Sterols

The amounts of sterols in the unsaponifiable matter of oils No. 19 and No. 21 were found to be 70.55% and 63.41%, respectively, by the digitonide method. The unsaponifiable matter from several oils recorded in Table 4 was combined, and recrystallized from alcohol. The product obtained after three recrystallizations had mp 142–143°C, which was raised to 145°C by further two recrystallizations. These results indicate that the main component of sterols is cholesterol.

Vitamin A

Oils No. 6, No. 25 and No. 26 in hexane were examined by the spectrophtometric method, and values $E^{1\%}_{1cm}$ at 328 mμ were found to be 0.418, 0.585 and 0.511, respectively.

Fatty acids

The fatty acids of oils No. 4 were examined. The properties of mixed fatty acids obtained from the oil are shown in Table 7. The mixed fatty acids were separated into saturated and unsaturated acids by the lead salt alcohol method, and the results are shown in Table 8. The determination of saturated acids by the oxidation of the methyl esters of mixed fatty acids gave the results shown in

Table 7. Mixed fatty acids (Squid oil)

Color	Yellow inclining slightly to orange
Melting point	27–31°C
Neut. V.	188.8
I.V	193.3

Table 8. Solid and liquid acids (Squid oil)

	Solid acids	Liquid acids
Yield (%)	23.5 (diff.)	76.5
Neut. V.	203.6	185.5
I.V.	18.2	241.7
Ref. index n_D^{20}	—	1.4815
Melting point	53–55°C	—

Table 9. Saturated acids (Squid oil)

Exp. No.	1	2
Saturated methyl esters (%)	27.21	26.78
I.V. of saturated methyl esters	8.4	2.7

Table 9. The unsaturated acid not subjected to the oxidation present in neutral esters was assumed to be methyl oleate (I.V. 85.7), and the amount of saturated methyl esters were calculated as 24.5–26.0%. The amount of highly unsaturated fatty acid (N.V. 168.5, I.V. 338.2, n_D^{20} 1.4966) by the litium salt acetone method was 38.4% in mixed acids.

(Analysis of fatty acid composition by gas-liquid chromatography)

Samples of squid were purchased at Hakodate, in November, 1966. The viscera obtained from 10 squids supplied an extracted lipid with $CHCl_3$-CH_3OH mixture by the method of Bligh and Dyer.[62] The lipid yield was 28.3%. The lipid was then treated with tenfold acetone, and the acetone-soluble lipid (97.9% of total lipid) was obtained, which had A.V. 6.9, S.V. 176.8, I.V. 178.9 and unsaponifiable matter 4.5%. The acetone-soluble lipid was saponified and the fatty acids obtained were converted to the methyl esters by the BF_3-methanol method. The methyl esters were analyzed on GLC.

The gas chromatograms obtained are shown in Fig. 1, and the fatty acid composition calculated from peak areas is shown in Table 10. The peaks were identified by comparison with standard mixtures and from a semilogarithmic plot of retention times versus the number of carbon atoms. The peak areas were measured by the half value width method, and the composition of each component was shown by the weight percentage of methyl esters.

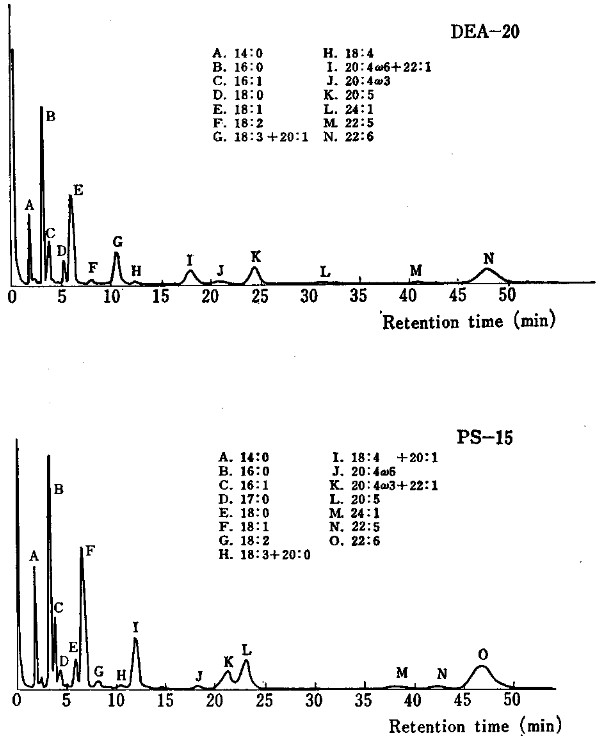

Fig. 1. Gas-liquid chromatograms of methyl esters of squid oil fatty acids.

Operating conditions:

Instrument .... Yanagimoto GCG-5DH
Column .... 1) Diethylene glycol adipate polyester on Neosorb NC (60–80 mesh), 1/5wt/wt, 1.5m×3 mm i.d., 200°C (DEA-20)
2) Polypropylene glycol succinate ester on Neosorb NC (60–80 mesh), 3/20wt/wt, 1.5×3mm i.d., 205°C (PS-15)
Detector ...... FID, H_2 17 ml/min, Air 600 ml/min, Attenu. 1/8, Sens. 10^9
Carrier gas .... He 15 ml/min (DEA) He 25 ml/min (PS)
Chart speed .... 10 mm/min
Sample size .... 0.5–2.2μl (5% petroleum ether solution)

Table 10. Fatty acid compositon by GLC (Squid oil)

Fatty acid	Wt. %	Fatty acid	Wt. %	Fatty acid	Wt. %
12:0	trace	13:1	trace	18:2	1.6
13:0	trace	14:1	0.8	18:3	0.9
14:0	4.2	15:1	0.5	18:4	1.6
15:0	0.9	16:1	6.3	20:4ω6	1.8
16:0	15.1	17:1	1.1	20:4ω3	1.1
17:0	1.6	18:1	15.9	20:5	9.6
18:0	3.4	20:1	9.1	22:4	1.1
19:0	0.6	22:1	5.8	22:5	1.1
		24:1	1.5	22:6	13.8
Total	25.8	Total	41.0	Total	32.6

The results obtained relatively resemble those of Ito and Fukuzumi.[63] The saturated acids contained large amounts of palmitic acid, followed by myristic and stearic acids. The unsaturated acids contained monoenoic acids, which were the chief components in unsaturated acids, and a large amount of highly unsaturated fatty acids. In the monoenoic acids, 18:1 was the component of the largest amounts, followed by 20:1, 16:1 and 22:1. In the polyenoic acids 22:6 and 20:5 were the members rich in amounts.

2.1.2 Mackerel oil[59]

General characteristics

Sixteen samples of mackerel oil prepared by boiling out process were examined. The characteristics of these oils are given in Table 11. Oils No. 8, No. 13 and No. 15 are light orange yellow, while the other oils are reddish orange; especially oils No. 4 and No. 12 are dark in color. All the oils deposit more or less stearine at

Table 11. Characteristics of mackerel oil

Sample No.	Catching		d_4^{20}	n_D^{20}	A.V.	S.V.	I.V.	Unsap. m. (%)	Ether-insol. brominated fatty acids (%)	Source of oil
	Locality*	Date (1951)								
1	Kushiro	Early Sept.	0.9246	1.4794	3.5	188.9	166.1	1.45	58.11	Whole body
2	Hiro-o	Aug. 21	0.9217	1.4780	3.2	190.4	157.9	0.41	44.30	Viscera and heads
3	Hiro-o	Aug. 29	0.9216	1.4780	6.6	191.8	157.9	1.24	52.74	Viscera
4	Kushiro	Aug. 5	0.9215	1.4782	26.8	187.0	156.2	3.02	50.46	Whole body
5	Kushiro	—	0.9220	1.4766	7.7	187.6	155.2	1.53	45.40	Whole body
6	Kushiro	Early Sept.	0.9208	1.4782	0.38	193.4	153.0	1.11	43.12	Viscera
7	Kushiro	—	0.9199	1.4770	26.7	196.6	153.0	0.64	48.92	—
8	Hiro-o	Aug. 26	0.9212	1.4780	4.0	192.3	152.2	1.06	49.67	—
9	Kushiro	—	0.9210	1.4780	7.7	196.0	152.2	1.43	49.66	—
10	Kushiro	Early Sept.	0.9217	1.4781	0.31	189.1	152.0	1.31	46.91	Viscera
11	Kushiro	Middle Aug.	0.9219	1.4772	24.8	191.5	151.1	1.32	40.49	Viscera and heads
12	Otaru	Early Jun.	0.9226	1.4783	9.3	193.7	148.9	1.30	48.67	—
13	Hiro-o	Aug. 26	0.9206	1.4780	0.64	193.7	148.6	0.47	49.14	—
14	Hiro-o	Aug. 27	0.9215	1.4780	7.7	190.9	148.4	0.88	49.56	—
15	Otaru	Early Jun.	0.9210	1.4780	1.0	191.7	143.9	1.17	49.71	—
16	Otaru	Early Jul.	0.9178	1.4764	10.3	194.4	139.7	1.38	39.84	—
Highest			0.9246	1.4794	26.8	196.6	166.1	3.02	58.11	
Lowest			0.9178	1.4764	0.31	187.0	139.7	0.41	39.84	
Average			0.9213	1.4778	8.79	191.8	152.3	1.23	47.92	

* All localities of catching belong to Hokkaido.

Table 12. Saturated acids and others (Mackerel oil)

Sample No.	3	4	12	15	16
Saturated methyl esters (%)	24.85	24.11	22.39	22.82	22.17
I.V. of saturated methyl esters	0.4	0.6	2.0	0.3	1.0
Trisaturated glycerides (%)	2.84	1.87	—	—	3.26
A.V. of trisaturated glycerides	7.4	14.8	—	—	8.3
$E^{1\%}_{1cm}$ (328 mμ)	0.144	—	—	0.288	0.328

Table 13. Acetone-soluble oils (Mackerel oil)

Sample	A.V.	S.V.	I.V.	Unsap. matter (%)	n_D^{20}
Body oil	1.8	188.0	154.4	0.74	1.4784
Viscera oil	17.3	180.6	159.0	3.08	1.4774

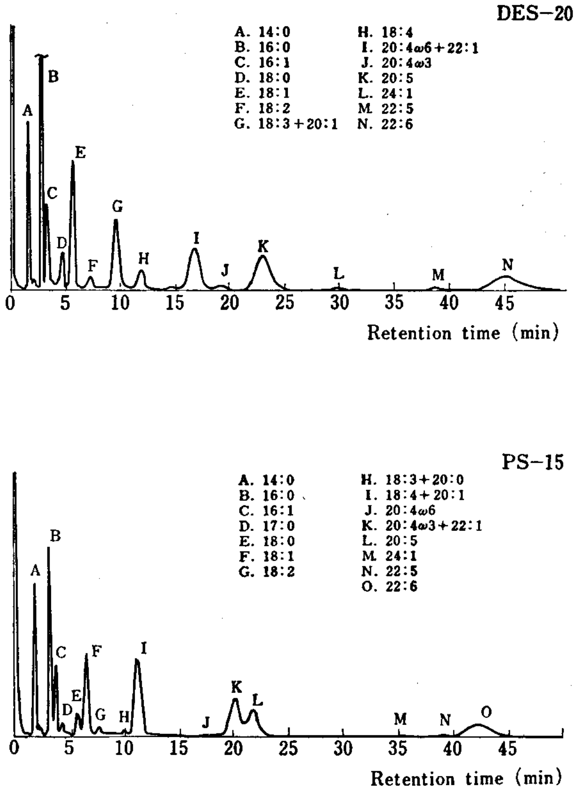

Fig. 2. Gas-liquid chromatograms of methyl esters of mackerel fatty acids
Operating conditions are the same as given in Fig. 1.

Table 14. Fatty acid composition by GLC (Mackerel oil)

Tatty acid	Wt. %	
	Body oil	Viscera oil
14:0	6.2	6.1
15:0	0.6	0.5
16:0	14.1	15.1
17:0	1.3	1.1
18:0	2.9	2.9
20:0	1.0	0.7
Total	26.1	26.4
14:1	—	—
15:1	0.3	0.3
16:1	6.4	6.7
17:1	0.4	0.5
18:1	13.7	14.1
20:1	11.8	10.7
22:1	14.4	12.4
24:1	1.1	1.4
Total	48.1	46.1
18:2	1.6	1.7
18:3	0.3	0.6
18:4	3.9	3.8
20:4ω6	0.5	1.0
20:4ω3	1.1	0.8
20:5	10.2	11.2
22:5	0.9	1.0
22:6	7.3	7.4
Total	25.8	27.5

ordinary temperature. In winter, they deposit large amounts of stearine, and oils No. 3, No. 4 and No. 8 are not mobile.

Determination of saturated acids, tri-saturated glycerides, and vitamin A were carried out with several oils in the same way as described in the case of squid oil. The results are shown in Table 12, from which the saturated acids in mixed fatty acids are found to be 22–25%, while the tri-saturated glycerides in oils are estimated at roughly 1–3%.

Fatty acid composition

(Analysis of fatty acid composition by GLC)

The samples of mackerel was caught off Kushiro, Hokkaido, in August, 1966. Three mackerels (1,540 g) were divided into the body part (1,020 g) and the viscera part (540 g). From both parts the oil with $CHCl_3$-CH_3OH mixture was extracted by the method of Bligh and Dyer.[62] From the body and the viscera parts 143.8 g (14.1%) and 17.1 g (3.2%) of lipids were extracted respectively.

The lipids were then treated with tenfold acetone, and acetone-soluble lipids were obtained, the properties of which are given in Table 13. The fatty acids were obtained by the saponification of the acetone-soluble lipids, then converted to the methyl esters by the BF_3-methanol method. The methyl esters were analyzed on gas-liquid chromatography. The gas chromatograms obtained are shown in Fig. 2, and the fatty acid compositions calculated from the peak areas are given in Table 14. The peak areas were measured by the half value width method, and the composition of each component was shown by the weight percentage of methyl esters. The results showed that there is practically no difference between the body and the viscera lipids. The fatty acids were composed of saturated acids ca. 26%, monoenoic acids 46–48% and polyenoic acids 26–28%, monoenoic acids being the main components. Saturated acids contained large amounts of palmitic acid, followed by myristic and stearic acids. In the monoenoic acids, 18:1, 22:1 and 20:1 were the components in large amounts. In the polyenoic acids, large amounts of 20:5 and 22:6 were present.

2.2 The lipids of twenty species of fishes and shell-fish

The lipids of 18 species of fishes, one species of cephalopod and one species of shell-fish were examined.

2.2.1 Materials and extraction of lipids

The species used as materials were as follows:

1) YOSHIKIRI-ZAME, great blue shark. *Glyphis glaucus* (Linné)
2) SODE-IKA, squid. *Thysanoteuthis rhombus* Troschel
3) CHIKA, surf smelt. *Hypomesus japonicus* (Brevoort)
4) HATAHATA, a kind of sandfish. *Arctoscopus japonicus* (Steindachner)
5) IBODAI, butterfish. *Psenopsis anomala* (Temminck and Schlegel)
6) MAGAREI, flounder. *Limanda herzensteini* Jordan and Snyder
7) MA-AJI, horse mackerel. *Trachurus japonicus* (Temminck and Schlegel)
8) SHISHAMO, longfin smelt. *Spirinchus lanceolatus* (Hikita)
9) ISHIGAREI, stone flounder. *Kareius bicoloratus* (Basilewsky)
10) AYU, sweet smelt. *Plecoglossus altivelis* Temminck and Schlegel
11) WAKASAGI, pond smelt. *Hypomesus olidus* (Pallas)
12) NISHIN, herring. *Clupea pallasii* Cuvier and Valenciennes
13) BABAGAREI, slime flounder. *Microstomus achne* (Jorgan and Starks)
14) BENIMASU, sockeye salmon. *Oncorhyncus nerka* (Walbaum)
15) ANKOU, goosefish. *Lophius litulon* (Jordan)
16) FUNA, crucian carp. *Carassius carassius* (Linné)
17) KICHIJI, spiny-cheek. *Sebastolobus macrochir* (Günther)
18) SUKETODARA, Alaskan pollack. *Theragra chalcogramma* (Pallas)
19) MADARA, cod. *Gadus macrocephalus* Tilesius
20) EZOAWABI, abalone. *Haliotis discus hannai* Ino

Among these materials, "SHISHAMO", longfin smelt (*Spirinchus lanceolatus*) was dried, "YOSHIKIRI-ZAME", great blue shark (*Glyphis glaucus*) was refrigerated, "WAKASAGI", pond smelt (*Hypomesus olidus*) was immersed in twofold acetone, and the others were fresh.

About these materials, the lipids were extracted respectively from flesh, liver, viscera, testis and ovary by the Procedure A or B.

Procedure A. The hashed materials were treated with twofold weight of

Table 15. Fishes and shellfish

Kind of fishes	Catching		Source of lipid	Procedure of obtaining lipid	Lipid content (%)
	Locality***	Date			
1. YOSHIKIRI-SAME			Liver	A	22.7
2. SODE-IKA	Kami-iso	Dec. '64	Liver	A	3.9
3. CHIKA	Kushiro	Nov. '64	Flesh	A	1.2
4. HATAHATA		Dec. '64	Flesh	A	3.3
5. IBO-DAI	Kami-iso	Oct. '64	Flesh	A	1.8
6. MA-GAREI	Kikonai	Jan. '65	Flesh	A	2.4
7. MA-AJI		Jan. '65	Flesh	A	1.2
8. SHISHAMO			Flesh	A	9.9
9. ISHI-GAREI	Kikonai	Sept. '64	Flesh	A	2.2
10. AYU		July '64	Flesh	A	4.8
11. WAKASAGI	Lake Tōro	Nov. '66	Whole body	B	4.3
12. NISHIN	Funka Bay	Oct. '66	Flesh	B	8.3
			Viscera	B	9.0
13. BABA-GAREI	Funka Bay	Oct. '66	Flesh	B	11.8
			Viscera	B	15.4
14. BENI-MASU	N 54°18′	June '66	Flesh-1*	B	8.1
	W 176°00′		Flesh-2*	B	6.1
			Ventral flesh	B	15.0
			Head	B	20.7
15. ANKOU	Hakodate	June '67	Flesh	B	0.4
			Liver	B	37.5
			Viscera**	B	1.5
16. FUNA	Lake Ōnuma	July '67	Flesh	B	1.1
			Viscera	B	4.9
17. KICHIJI	Esashi	May '67	Flesh	B	11.9
			Viscera	B	20.2
18. SUKETO-DARA	Kushiro	Nov. '67	Liver	B	46.2
			Viscera**	B	1.8
			Testis	B	2.5
			Ovary	B	1.0
19. MA-DARA	Kushiro	Nov. '67	Liver	B	45.9
			Viscera**	B	1.2
			Testis	B	0.3
20. EZO-AWABI	Hakodate	Feb. '68	Liver	B	17.7

* *The fish body was divided into two by a line starting from the back joint of dorsal fin* and crossing the lateral line of fish vertically, then flesh-1 and flesh-2 were obtained respectively from the forepart and the hind part.

** Excluded liver

*** Localities are all round Hokkaido, No. 14 excluded.

acetone, then the lipid extraction and dehydration from the materials could be proceeded. This procedure was repeated three times and the resulting solutions after filtration by means of the Buchner funnel were distilled to remove acetone. The residual solution was shaken with ether using a separatory funnel. The residual material obtained by filering off the acetone solution was dried at 80°C for one hour, then the dried powder was extracted by ether for about 20 hours in a Soxhlet apparatus. After the removal of the solvent from all of the ether solutions the lipids were obtained.

Procedure B. The lipids were obtained by the method of Bligh and Dyer.[62] Catching locality and date, source of lipid, and yield of lipids of the materials are shown in Table 15.

2.2.2 Characteristics of the lipids

The lipids No. 11 to No. 20 were treated with tenfold acetone, after that the

Table 16. Characteristics of lipids of fishes and shellfish

Kind of fishes	Source of lipid	Acetone-soluble fr.	n_D^{20}	A.V.	S.V.	I.V.	Unsap. matter (%)
1. YOSHIKIRI-SAME	Liver			27.3	179.7	185.9	
2. SODE-IKA	Liver				144.3	186.4	
3. CHIKA	Flesh			20.8	187.2	182.3	
4. HATAHATA	Flesh			3.9	183.1	161.8	
5. IBO-DAI	Flesh			3.8	182.9	136.9	
6. MA-GAREI	Flesh			5.4	189.6	165.0	
7. MA-AJI	Flesh			4.6	187.0	131.1	
8. SHISHAMO	Flesh			59.8	192.8	120.4	
9. ISHI-GAREI	Flesh			3.4	185.4	185.1	
10. AYU	Flesh			20.4	201.5	145.9	
11. WAKASAGI	Whole body		1.4816	24.8	175.8	153.0	7.06
12. NISHIN	Flesh		1.4768	2.2	186.4	139.3	0.79
	Viscera		1.4767	17.0	186.4	129.4	3.43
13. BABA-GAREI	Flesh		1.4818	4.0	186.6	177.9	1.81
	Viscera		1.4808	43.5	163.6	143.0	10.30
14. BENI-MASU	Flesh-1		1.4762	7.3	181.6	121.7	1.22
	Flesh-2		1.4768	10.3	183.1	125.2	1.75
	Ventral flesh		1.4749	4.4	201.5	115.6	0.84
	Head		1.4767	3.2	176.7	132.2	0.85
15. ANKOU	Liver	99.0	1.4816	2.0	206.1	164.5	1.93
	Viscera	79.1	1.4832	56.3		139.0	
16. FUNA	Flesh	84.0	1.4835	26.5	199.6	159.3	5.46
	Viscera	80.6	1.4806	34.7	190.8	150.7	5.42
17. KICHIJI	Flesh	96.5	1.4761	1.4	184.7	114.8	1.17
	Viscera	96.8	1.4779	2.4	188.1	121.6	5.27
18. SUKETO-DARA	Liver	99.1	1.4842	2.2	175.4	160.2	2.90
	Ovary	50.0	1.4875				
19. MA-DARA	Liver	98.6	1.4812	2.5	185.9	180.9	1.12
	Viscera	76.0	1.4875				
20. EZO-AWABI	Liver	87.0					

acetone-insoluble matter was removed, then the acetone-soluble lipids were obtained.

Characteristics of the lipids No. 1 to No. 10 and the acetone-soluble lipids No. 11 to No. 20 are shown in Table 16. The acid values tended to be higher in viscera lipids than in flesh lipids. The saponification values lay between 170 and 200 in a large majority of lipids. The iodine values were ca. 180, relatively higher in the lipid of the liver of "YOSHIKIRI-ZAME", great blue shark (*Glyphis glaucus*), the liver of "SODE-IKA", squid (*Thysanoteuthis rohmbus*), the flesh of "CHIKA", surf smelt (*Hypomesus japonicus*), the flesh of "ISHIGAREI", stone flounder (*Kareius bicoloratus*), the liver of "MADARA", cod (*Gadus macrocephalus*) and the flesh of "BABAGAREI", slime flounder (*Microstomus achne*), and were below 130, relatively lower, in the lipid of the flesh of "SHISHAMO", longfin smelt (*Spirinchus lanceolatus*), the viscera of "NISHIN, herring (*Clupea pallasii*), the flesh of "BENI-MASU", sockeye salmon (*Oncorhyncus nerka*) and flesh and viscera of "KICHIJI", spiny-cheek (*Sebastolobus macrochir*). Unsaponifiable matter contents were relatively higher in the lipid of the viscera of "BABAGAREI", slime flounder (*Microstomus achne*) and the whole body of "WAKASAGI", pond smelt (*Hypomesus olidus*), 10.30% and 7.06% respectively. Generally, no definite relationship was found between the taxonomical situations of the fishes and the characteristics of fish lipids.

2.2.3 Analysis of fatty acid compositions of the lipids by gas-liquid chromatography

The fatty acids were liberated from the lipid samples by the usual way and converted to methyl esters by the BF_3-methanol method, then analyzed on gas-liquid chromatography. The gas chromatograms obtained are shown in Fig. 3, Fig. 4 and Fig. 5, and the fatty acid compositions calculated from the peak areas are given in Table 17. The peak areas were measured by the half value width method, and the composition of each component was shown by the weight percentage of methyl esters.

2.2.4 Summary of the results

(1) Generally no clearly definite relationship was found between the taxonomical situations of the fishes and the fatty acid compositions of the fish lipids.

(2) The fatty acids of the shark liver lipid and "SODE-IKA" liver lipid contained large amounts of 20:5 and 22:6 acids, and the fatty acids of the sockeye salmon flesh lipid contained small amounts of 20:5 and 22:6 acids, but large amounts of monoenoic acid. The abalone liver lipid contained large amounts of 14:0 and 16:0 acids but small amounts of 20:5 and 22:6 acids.

They showed distinctive fatty acid composition respectively.

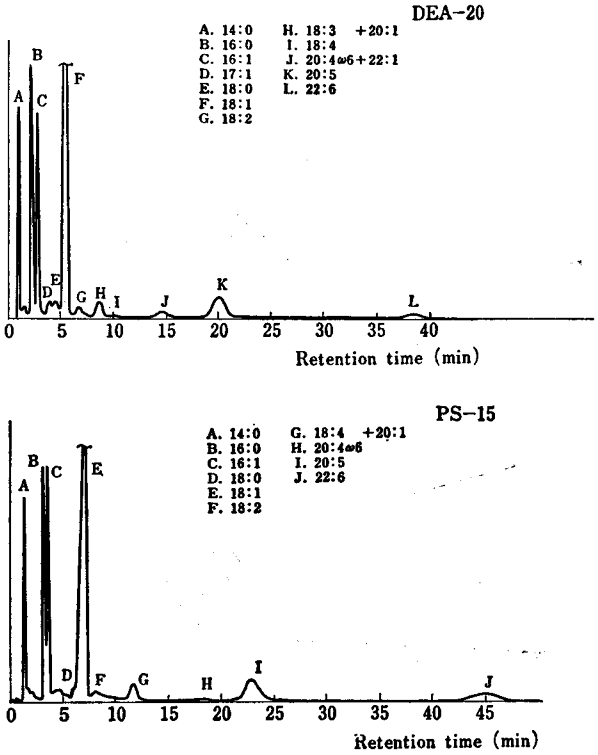

Fig. 3. Gas-liquid chromatograms of methyl esters of "SHISHAMO" (longfin smelt) flesh lipid fatty acids

Operating conditions:

Instrument	Yanagimoto GCG-2
Column	1) Diethylene glycol succinate polyester on Celite 545 (60–80 mesh), 1/5wt/wt, 2m×5m i.d., 210°C (DES-20) 2) Propylene glycol succinate polyester on Celite 545 (60–80 mesh), 3/20wt/wt, 2m×5m i.d. 205°C (PS-15)
Detector	TCD, Bridge current 170 mA, Sens. 2mV
Carrier gas	He 60 ml/min (DES), 50 ml/min (PS)
Chart speed	10 mm/min
Sample size	3.8–5.0μl

(3) "KICHIJI" (*Sebastolobus macrochir*) had large amount of 18:1 and small amounts of 20:5 and 22:6 as the fatty acids of its lipid, which is unusual to the marine fishes. The chracteristics of this fatty acid composition may be derived from that "KICHIJI" is a mesopelagic fish. (depth of habitat is 300–400 m or more)

(4) Comparing the main fatty acid components of flesh lipids of marine fishes with those of fresh-water fishes, in the former, the saturated fatty acids were composed of large amounts of 16:0 accompanied by smaller amounts of 14:0 and 18:0, and a very few or trace amounts of 20:0 and 12:0. The unsaturated acids

Table 17. Fatty acid compositions of the lipids of fishes and shell-fish

Class or Subclass	Teleostomi										Teleostomi											Teleostomi										Elasmobranchii	Cephalopoda	Gastropoda
Order	Lophiida			Gadida							Cottida		Percida			Pleuronectida				Clupeida										Cyprinida		Selachii	Decapoda	Archaeo-gastropoda
Suborder	Lophiina			Gadina							Cottina		Percina	Carangina	Stromateina	Pleuronectina				Clupeina		Salmonina										Squali		
Family	Lophiidae			Gadidae							Scorpaenidae		Trichodontidae	Carangidae	Stromateidae	Pleuronectidae				Clupeidae		Salmonidae				Plecoglossidae	Osmeridae			Cyprinidae		Galeorhinidae	Thysanoteuthidae	Haliotidae
Species	ANKOU			MADARA			SUKETO-DARA				KICHIJI		HATAHATA	MA-AJI	IBO-DAI	MA-GAREI	BABA-GAREI		ISHI-GAREI	NISHIN		BENI-MASU				AYU	WAKA-SAGI	SHISHA-MO	CHIKA	FUNA		YOSHIKIRI-ZAME	SODE-IKA	EZO-AWABI
Source of lipid / Fatty acid	Liver	Viscera	Flesh	Liver	Viscera	Testis	Liver	Viscera	Testis	Ovary	Flesh	Viscera	Flesh	Flesh	Flesh	Flesh	Flesh	Viscera	Flesh	Flesh	Viscera	Felsh-1	Flesh-2	Ventral flesh	Head	Flesh	Whole body	Flesh	Flesh	Flesh	Viscera	Liver	Liver	Liver
12:0	tr*	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr	-	—	tr
13:0	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr	—	—	tr
14:0	4.7	5.3	4.2	3.1	3.7	2.1	3.6	3.2	2.5	3.2	4.7	4.1	2.8	8.0	3.5	6.5	3.6	3.4	6.0	6.6	7.1	4.7	3.9	4.5	4.3	7.4	4.8	8.1	5.1	5.4	5.4	4.1	2.2	12.6
15:0	0.5	0.6	0.5	0.6	0.6	0.5	0.4	0.4	0.4	0.7	0.4	0.6	0.6	1.0	1.6	1.2	0.8	0.5	1.1	0.4	0.4	0.6	0.5	0.5	0.6	0.8	2.0	0.8	2.1	2.0	1.9	1.3	0.8	0.7
16:0	18.8	17.6	20.7	15.1	21.5	16.1	11.2	11.5	21.3	15.1	13.0	11.0	17.6	18.7	21.6	14.7	15.1	17.5	16.6	12.5	15.3	15.1	14.4	14.9	15.4	20.7	15.4	14.8	19.9	17.2	15.9	21.7	15.5	24.7
17:0	1.9	1.3	2.6	1.3	1.8	1.5	0.6	1.2	1.5	1.3	1.1	1.4	1.3	2.1	3.7	2.1	3.3	1.6	1.7	1.3	1.2	1.2	0.9	1.1	1.1	4.9	3.3	1.4	1.9	1.9	2.3	3.3	3.9	0.4
18:0	2.1	3.4	4.3	3.3	6.4	3.5	1.8	3.1	2.7	1.6	2.6	2.4	2.9	4.7	5.4	3.9	2.8	3.7	2.6	1.7	1.2	1.8	1.7	1.8	2.5	2.9	3.1	2.0	3.7	4.8	4.5	5.6	10.2	2.8
19:0	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr	tr	tr	—	—	tr	—	—	—	—	—	—	tr		tr	tr	—	—	1.4	1.6	—
20:0	tr	tr	tr	tr	0.2	0.6	tr	tr	tr	tr	0.1	0.2	tr	0.6	1.1	tr	0.4	0.2	1.5	0.5	0.3	0.5	0.5	tr	tr	1.0	0.6	tr	tr	0.4	0.7	0.9	1.5	0.6
Total	28.0	28.2	32.3	23.4	34.2	24.3	17.6	19.4	28.4	21.9	21.9	19.7	25.2	35.1	36.9	28.4	26.0	26.9	29.5	23.0	25.5	23.9	21.9	22.8	23.9	37.7	29.2	27.1	32.7	31.7	30.7	38.3	35.7	41.8
12:1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr	—	—	tr
13:1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr	—	—	tr
14:1	0.8	0 8	0.9	0.2	0.6	0.3	0.3	0.3	0.4	0.5	0.7	0.9	0.7	0.7	0.5	1.2	—	0.5	1.0	0.4	0.5	0.4	0.6	0.6	0.7	0.7	2.4	1.1	1.8	1.2	1.3	tr	tr	1.8
15:1	0.2	0.8	0.4	0.1	0.2	0.2	0.2	0.2	0.1	0.3	0.2	0.4	tr	0.4	tr	0.5	0.5	0.3	tr	0.4	0.4	0.4	0.3	0.6	0.4	tr	0.9	tr	0.7	1.3	1.0	tr	tr	tr
16:1	8.9	9.3	9.8	6.8	6.0	5.3	7.7	6.3	5.0	9.8	12.2	14.7	9.6	7.8	9.2	12.0	12.1	10.8	11.5	8.1	9.1	8.1	7.4	7.8	7.4	21.7	12.3	13.6	10.3	14.3	17.7	6.1	3.4	8.8
17:1	1.1	0.6	0.5	tr	tr	0.4	0.1	0.3	tr	0.8	0.6	tr	1.1	1.1	1.5	1.3	0.8	0.8	1.6	0.3	0.3	1.1	1.0	1.1	1.0	3.7	2.5	1.2	1.2	2.2	2.8	1.6	0.9	tr
18:1	18.3	17.6	17.9	27.8	22.8	22.4	21.6	21.2	22.7	19.7	34.1	41.4	29.0	14.4	23.3	14.9	23.7	32.1	13.5	25.7	21.1	22.7	22.6	23.2	23.4	11.3	16.0	37.0	17.5	13.9	13.6	10.8	15.0	22.0
19:1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	1.3	1.2	—	—	tr	—	—	—	—	—	—	1.2	1.1	tr	tr	tr	tr	tr	1.7	tr
20:1	6.6	8.9	4.3	7.4	4.9	5.9	12 2	9.8	5.5	6.6	9.6	6.1	6.3	6.5	3.0	4.4	5.5	6.6	3.0	11.9	12.2	17.0	17.0	16.3	14.1	2.1	1.6	0.1	2.5	1.4	1.4	2.7	11.2	5.5
22:1	8.0	8.3	3.7	3.8	3.0	1.4	12.5	9.8	2.1	6.1	5.9	3.1	1.4	7.8	0.3	1.2	1.2	1.0	0.8	11.8	12.4	10.3	11.3	11.0	10.2	tr	0.4	2.0	2.6	5.3	5.5	0.9	2.6	1.9
24:1	tr	0.6	tr	tr	tr	0.5	1.0	1.1	tr	tr	0.6	tr	tr	2.3	2.2	2.7	1.2	0.4	3.3	0.8	0.3	0.8	1.0	0.8	0.7	tr	0.6	tr	tr	0.6	tr	2.7	tr	tr
Total	43.9	46.9	37.5	46.1	37.5	36.4	55.6	49.0	35.8	43.8	63.9	66.6	48.1	41.0	41.3	39.4	45.0	52.5	34.7	59.4	56.3	60.8	61.2	61.4	57.9	40.7	37.8	55.0	36.6	40.2	43.3	24.8	34.8	40.0
16:2	1.7	1.6	0.8	0.8	0.8	tr	0.2	tr	tr	0.6	0.3	1.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.7	2.8	—	—	tr
16:3	0.6	0.5	0.3	tr	tr	tr	0.1	0.1	0.7	0.7	0.1	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.7	2.0	—	—	tr
18:2	1.5	0.9	1.0	1.2	0.8	1.0	0.7	1.5	0.9	1.5	1.3	0.9	1.3	1.3	1.3	1.7	1.2	0.7	1.8	0.9	1.2	1.6	1.6	1.8	1.7	2.7	4.9	1.7	1.8	2.8	2.7	2.4	0.9	3.3
18:3	0.7	1.4	1.8	0.3	0.6	0.2	0.7	0.7	0.6	0.8	0.4	0.7	0.8	1.0	0.3	1.2	0.2	tr	1.4	0.3	0.2	0.4	0.6	1.2	0.8	3.6	8.0	2.7	1.2	2.6	1.8	0.7	tr	0.6
18:4	1.3	1.7	1.2	1.1	0.3	1.6	2.5	1.8	0.5	1.5	0.8	0.7	1.4	1.0	tr	1.8	2.3	1.0	2.3	1.1	1.1	1.4	1.2	1.4	1.9	2.1	0.6	1.7	2.1	1.6	1.9	tr	tr	3.8
20:2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	—	—	—	—	—	0.2	—	—	—	0.4	0.4	0.5	0.4	—	0.9	—	—	tr	tr	—	—	tr
20:3	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr	—	—	tr
20:4ω6	1.4	1.9	2.7	0.7	2.7	2.6	0.6	1.4	1.3	0.9	1.0	0.8	1.2	0.8	3.2	2.5	0.4	1.9	1.5	0.8	0.6	0.3	0.4	0.6	0.4	1.4	2.9	tr	2.2	3.8	3.2	4.0	3.3	2.2
20:4ω3	tr	tr	tr	0.5	tr	0.9	1.4	1.7	0.6	0.7	tr	0.8	tr	tr	tr	2.4	1.1	0.4	2.3	0.3	tr	0.9	0.8	0.5	0.8	tr	1.2	tr	tr	1.0	0.5	tr	tr	0.7
20:5	11.9	8.8	9.7	9.5	10.6	12.2	11.3	11.4	13.5	13.0	6.1	5.0	9.4	9.4	4.9	13.0	14.9	8.7	10.1	8.7	10.6	4.6	4.9	4.6	6.7	6.1	5.4	5.7	8.3	6.1	6.0	8.8	8.6	3.8
21:5	0.9	0.7	tr	tr	tr	tr	0.3	1.0	0.6	0.5	tr	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr	—	tr	tr
22:5	1.4	1.2	1.5	1.0	tr	2.1	1.5	1.5	1.5	1.8	0.9	1.2	tr	2.6	1.7	4.9	4.0	3.2	2.7	1.4	0.5	0.5	0.5	0.5	0.6	2.5	1.1	0.8	tr	1.6	1.7	2.5	tr	2.6
22:6	6.6	6.1	11.1	15.5	12.4	18.7	7.5	10.5	15.6	12.1	3.3	2.6	12.7	7.8	8.1	4.5	4.8	4.5	13.7	4.1	3.8	5.0	6.4	4.8	4.7	3.1	4.6	3.5	14.9	4.3	3.5	16.2	15.1	1.0
Total	28.0	24.8	30.1	30.6	28.2	39.3	26.8	31.6	35.8	34.1	14.2	13.7	26.8	23.9	19.5	32.0	28.9	20.6	35.8	17.6	18.0	15.1	16.8	15.9	18.0	21.5	29.6	16.1	30.5	28.2	26.1	34.6	27.9	18.0
?														0.1	2.2												3.4	1.9	0.2				1.7	

* Trace

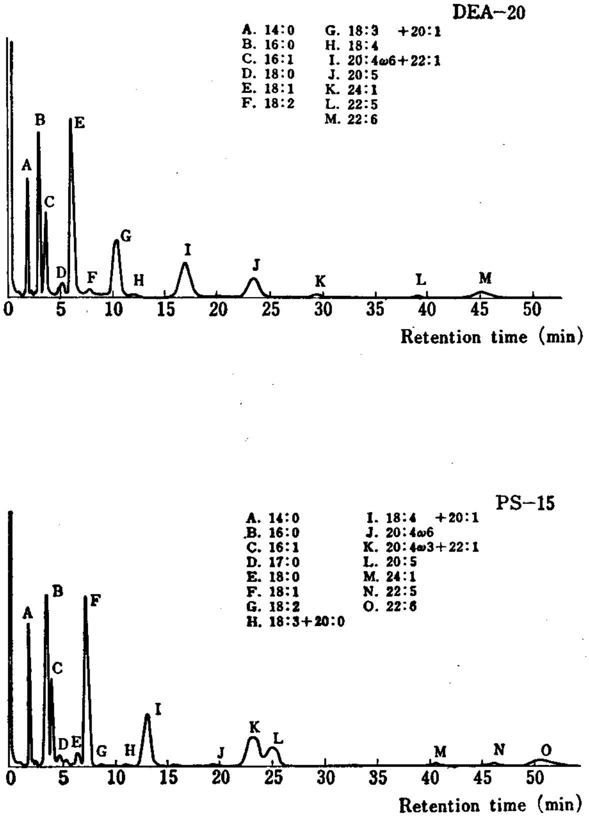

Fig. 4. Gas-liquid chromatograms of methyl esters of herring flesh lipid fatty acids
Operating conditions:
Instrument .... Yanagimoto GCG–5DH
Column .... 1) Diethylene glycol adipate polyester on Neosorb NC (60–80 mesh), 1/5wt/wt, 1.5m×3mm i.d., 200°C (DEA–20)
2) Polypropylene glycol succinate ester on Neosorb NC (60–80 mesh), 3/20wt/wt, 1.5 m×3 mm i.d., 205°C (PS–15)
Detector ...... FID, H_2 17 ml/min, Air 600 ml/min, Attenu. 1/8, Sens. 10^9
Chart speed .... 10 mm/min
Sample size .... 0.5–2.2 μl (5% acetone solution)

contained the largest amount of monoenoic acids which are the main components of marine fish fatty acids. Among the monoenoic acids, 18:1 was the most abundant, followed by relatively large amounts of 16:1, 20:1 and 22:1. The main components of polyenoic acids, 20:5 and 22:6 were also contained in large amounts.

Fresh-water fishes contained smaller amounts of 20:1, 22:1, 20:5 and 22:6, and larger amounts of 14:0, 16:1, 18:2 and 18:3 than marine fishes did.

(5) The difference between the fatty acid composition of flesh lipid and of liver lipid was not significant, but the former was somewhat predominant over

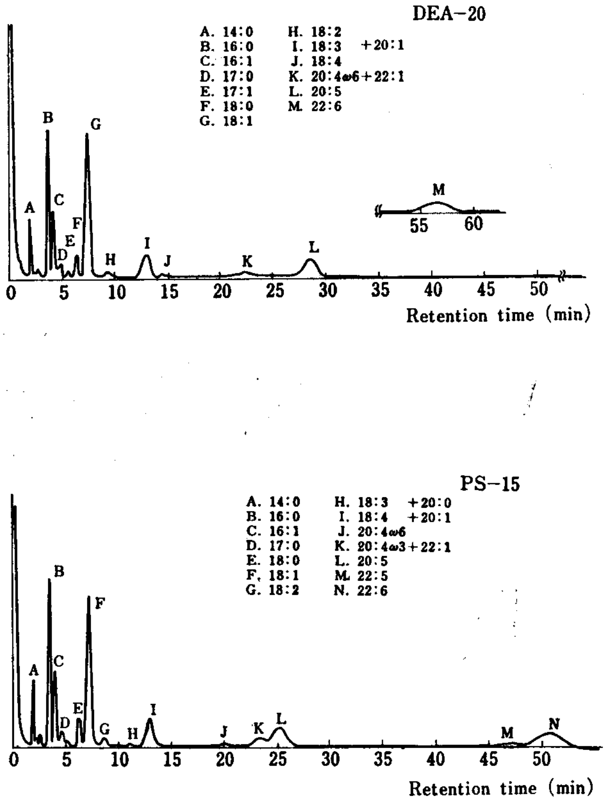

Fig. 5. Gas-liquid chromatograms of methyl esters of cod liver lipid fatty acids
Operating conditions are the same as given in Fig. 4, excluded column temperature: 190°C, carrier gas: He 0.8–0.9 kg/cm^2, and sample size: 0.6–0.7 μl (5% petroleum ether solution).

the latter in the contents of 20:5 and 22:6.

(6) In cod and Alaskan pollack the lipids of testis and ovary contained smaller amounts of monoenoic acid, larger amounts of 20:5 and 22:6 than the lipids of liver.

2.3 The lipids of plankton

2.3.1 Properties of the lipids of plankton

Properties of the lipid of plankton, *Calanus finmarchicus*7),19),25), *Calanus cristatus*,26) *Euphausia pacifica*,27) *Euphausia superba*,27),31),32),33) *Neomysis nakazawai*,28) *Neomysis intermedia*,29) *Cyclops strenuus*,7) *Daphnia galeata*,7) *Diaptomus gracilis*,7) *Artemia sarina*,40) *Chaetoceros simplex*,40) *Skeletonema costatum*,43) *Meganyctiphanes norvegica*45) were reported.

Among these, the six species of the first half (from *Calanus finmarchicus* to *Neomysis intermedia*) were reported relatively in detail, but the seven species of the latter half (from *Cyclops strenuus* to *Meganyctiphanes norvegica*) were reported relatively in brief.

The author examined the lipids of the fourteen species of plankton collected at adjacent seas of Japan and the Northern Pacific Ocean.

2.3.1.1 Materials

The fourteen species of plankton examined with their body length, distribution and classification are as follows:64),65),66),67)

(1) *Calanus plumchrus* Marukawa
Body length: 4.4–6.3 mm
Distribution: the Japan Sea, Sea of Okhotsk, Bering Sea, (cold sea type)
Crustacea, Copepoda, Calanoida, Calanoidae

(2) *Calanus cristatus* Kröyer
Body length: 7.8–9 mm
Distribution: Northern Pacific Ocean, Northern Antarctic Ocean, Sea of Okhotsk, Bering Sea, Sagami Bay (deep zone), (cold sea type)
Crustacea, Copepoda, Calanoida, Calanoidae

(3) *Calanus finmarchicus* (Gunnerus)
Body length: 3.5–4.1 mm
Distribution: Northern Pacific Ocean, Northern part of Japan Sea, (cold sea type)
Crustacea, Copepoda, Calanoida, Calanoidae

(4) *Metridia longa* Lubbock
Body length: 3.5–4.7 mm
Distribution: Northern Pacific Ocean, Northern Antarctic Ocean, Sea of Okhotsk, the Japan Sea, (cold sea type)
Crustacea, Copepoda, Calanoida, Metridiidae

(5) *Parathemisto japonica* Bovallius
Body length: 10–15 mm
Distribution: Oyashio waters
Crustacea, Malacostraca, Amphipoda, Hyperiidae

(6) *Actes japonicus* Kishinouye
Body length: 10–14 mm
Distribution: Sea of Ariake, Kojima Bay, Toyama Bay
Crustacea, Malacostraca, Amphipoda, Sergestidae

(7) *Neomysis nakazawai* Ii
Body length: 30 mm
Distribution: Adjacent seas of Hokkaido and Sakhalin
Crustacea, Malacostraca, Mysidacea, Mysidae

(8) *Neomysis intermedia* (Czerniavsky)
Body length: 8–10 mm
Distribution: Area of brackish water, Lake Kasumiga-ura
Crustacea, Malacostraca, Mysidacea, Mysidae

(9) *Neomysis spinosa* Nakazawa
Body length: 11 mm
Distribution: Adjacent seas of the Miura Peninsula and the Izu Peninsula
Crustacea, Malacostraca, Mysidacea, Mysidae

(10) *Neomysis awatschensis* (Brandt)
Body length: 10 mm
Distribution: Area of brackish water, Lake Hamana
Crustacea, Malacostraca, Mysidacea, Mysidae

(11) *Acanthomysis sp.*
Distribution: Ariake Bay, Pacific Ocean (warm sea type)
Crustacea, Malacostraca, Mysidacea, Mysidae

(12) *Proneomysis sp.*
Crustacea, Malacostraca, Mysidacea, Mysidae

(13) *Euphausia pacifica* Hanzen
Body length: 22–25 mm
Distribution: Northern Pacific Ocean, the Japan Sea, Sea of Okhotsk
Crustacea, Malacostraca, Euphausiacea, Euphausidae

(14) *Thysanoessa longipes* Brandt
Body length: 25–30 mm
Distribution: Northern Pacific Ocean, Northern part of Japan Sea, Sea of Okhotsk
Crustacea, Malacostraca, Euphausiacea, Euphausidae

The plankton were obtained as (a) fresh materials, (b) sundried materials and (c) salted materials (about 30% salt contained).

The fresh materials have been collected by fry net (1.5 m in diameter at the mouth ring) hauled with a rope on board in 30 minutes just after sunset.

The materials thus collected, after being rid of sea water as much as possible, were immersed in equal weight of acetone on board.

2.3.1.2 Extraction of lipids from the materials

The above mentioned three materials of different conditions (a, b and c) had their lipids extracted by different procedures A, B and C.

Procedure A: The acetone solution was first removed from the materials immersed in acetone through filtering by means of the Buchner funnel, and the residues were treated with an equal weight of acetone, then filtered. The same treatment with acetone was repeated three times, and the final residues, dried at 80°C for one hour, were extracted by ether for 24 hours in a Soxhlet apparatus.

The acetone was distilled off the acetone solution which removed the residues, and the resulting solution, removed of acetone, was shaked with two volumes of ether in a separatory funnel. This procedure was repeated five times. All the ether solutions thus obtained were united, then the lipids were obtained from them.

Procedure B: The sun-dried materias re-dried at 80°C for one hour were extracted by ether using a Soxhlet apparatus for 40 hours, and the lipids were obtained.

Procedure C: The salted materials were lightly washed with water for desalting, and immersed in acetone (500 ml acetone to 1 kg of material), then treated

Table 18. Lipid contents of plankton

Sample No.	Plankton	Origin of sample		Procedure of obtaining lipid	Lipid content on wet weight (%)
		Locality	Date		
1.	*Calanus plumchrus*	42°30′N, 150°08′E	July '56	B	25.39*
2.	〃 〃	51°30′N, 176°01′E	June '57	A	3.19
3.	〃 〃	48°30′N, 166°12′E	June '58	A	5.85
4.	〃 〃	42°20′N, 158°00′E	May '58	A	2.58
5.	〃 〃	46°30′N, 153°33′E	June '58	A	6.20
6.	〃 〃	Fukushima (Hokkaido)	Apr. '58	A	—
7.	〃 〃	43°43′N, 150°30′E	May '58	A	3.58
8.	*Calanus cristatus*	51°08′N, 169°28′E	June '58	A	6.72
9.	〃 〃	46°30′N, 153°33′E	June '58	A	4.62
10.	*Calanus finmarchicus*	54°50′N, 155°08′E	July '60	A	2.11
11.	*Metridia longa*	52°20′N, 151°00′E	July '58	A	4.07
12.	*Parathemisto japonica*	Fukushima (Hokkaido)	Apr. '58	A	1.55
13.	〃 〃	52°30′N, 152°36′E	July '58	A	2.12
14.	〃 〃	51°00′N, 153°22′E	July '58	A	1.56
15.	〃 〃	44°-51°N, 149°-155°E	Aug. '58	A	2.56
16.	〃 〃	51°23′N, 155°15′E	Aug. '58	A	1.85
17.	〃 〃	51°23′N, 155°15′E	Aug. '58	A	1.47
18.	〃 〃	52°10′N, 154°25′E	Aug. '58	A	1.62
19.	〃 〃	50°35′N, 154°45′E	Aug. '58	A	1.41
20.	〃 〃	52°20′N, 151°00′E	July '58	A	0.98
21.	*Acetes japonicus*	Tomo (Hiroshima)	Nov. '63	A	1.25
22.	*Neomysis nakazawai*	Yakumo (Hokkaido)	June '55	B	6.5*
23.	〃 〃	〃	June '62	A	1.35
24.	*Neomysis intermedia*	Kasumigaura (Ibaragi)	'56	B	3.9*
25.	*Neomysis spinosa*	Hachinohe (Aomori)	May '62	A	1.96
26.	*Neomysis awatschensis*	Urayasu (Chiba)	May '62	A	1.34
27.	*Acanthomysis sp.*	Azuma (Nagasaki)	Aug. '65	C	0.9
28.	*Proneomysis sp.*	Suminoe (Saga)	June '65	C	1.5
29.	*Euphausia pacifica*	Onagawa (Miyagi)	Apr. '58	A	—
30.	〃 〃	44°20′N, 149°40′E	Aug. '58	A	1.32
31.	〃 〃	Senzaki (Yamaguchi)	Apr. '63	A	1.49
32.	〃 〃	Kesen-numa (Miyagi)	Apr. '63	A	1.02
33.	*Thysanoessa longipes*	43°01′N, 147°30′E	May '58	A	1.04

* Lipid content on dry weight

Table 19. Characteristics

Sample No.	Plankton	Acetone-soluble fraction (%)	Color and state
1.	*Calanus plumchrus*	98.3	Bloodred, liquid
2.	〃 〃	99.1	〃 〃
3.	〃 〃	97.8	Reddish brown, liquid
4.	〃 〃	82.4	Yellowish brown, liquid
5.	〃 〃	98.6	Reddish brown, liquid
6.	〃 〃	94.8	Yellowish brown, liquid
7.	〃 〃	91.8	Reddish brown, liquid
8.	*Calanus cristatus*	97.2	〃 〃
9.	〃 〃	98.5	〃 〃
10.	*Calanus finmarchicus*	97.1	Dark reddish brown, liquid
11.	*Metridia longa*	98.9	Dark yellowish brown, liquid
12.	*Parathemisto japonica*	98.3	Deep yellowish brown, viscous liquid
13.	〃 〃	95.0	〃 〃
14.	〃 〃	88.3	〃 liquid
15.	〃 〃	95.7	Yellowish brown, viscous liquid
16.	〃 〃	94.5	〃 〃
17.	〃 〃	96.3	Light reddish brown, liquid
18.	〃 〃	97.7	Yellowish brown, viscous liquid
19.	〃 〃	92.5	〃 〃
20.	〃 〃	98.9	〃 〃
21.	*Acetes japonicus*	96.2	Blackish brown, semi-solid
22.	*Neomysis nakazawai**	—	Reddish brown, 〃
23.	〃 〃	97.9	Blackish brown, 〃
24.	*Neomysis inermedia**	—	〃 〃
25.	*Neomysis spinosa*	96.9	〃 〃
26.	*Neomysis awatschensis*	95.8	〃 〃
27.	*Acanthomysis sp.*	97.5	Dark reddish brown, 〃
28.	*Proneomysis sp.*	95.2	〃 〃
29.	*Euphausia pacifica*	97.9	Reddish brown, 〃
30.	〃 〃	94.4	〃 liquid
31.	〃 〃	94.6	Deep reddish brown, semi-solid
32.	〃 〃	95.6	Reddish brown, 〃
33.	*Thysanoessa logipes*	92.0	Blackish green yellow, liquid

* Data about total lipid ** Sterol content in unsaponifiable matter, 67.3%

as in Procedure A.

Catching locality and date, and lipid contents are shown in Table 18. The lipid contents of plankton were 1–6% on wet weight.

2.3.1.3 Characteristics of acetone-soluble lipids

The lipids obtained above were treated with tenfold acetone, once the acetone-insoluble matters were removed, the acetone-sollble lipids were obtained.

Characteristics of acetone-soluble lipids are shown in Table 19. About the lipids of *Neomysis nakazawai*, the content of vitamin A was determined. The lipid in isopropanol was examined by the spectrophotometric method, and the value $E^{1\%}_{1cm}$ at 328 mμ was found to be 3.098.

of plankton lipids

d_4^{15}	n_D^{20}	S.V.	I.V.	A.V.	Unsap. matter (%)	Fatty acid		
						N.V.	I.V.	Bromides insoluble in ether (%)
0.8891	1.4670	119.3	156.9	19.9	50.58	201.5	179.8	63.38
0.8929	1.4670	124.1	150.9	14.9	50.36	200.3	189.2	66.77
0.8824	1.4698	115.4	135.8	14.5	48.06	214.6	151.7	54 51
—	1.4779	131.8	150.5	21.2	42.91	202.3	182.1	63.31
0.8891	1.4694	119.6	160.1	12.3	46.99	200.1	199.5	62.37
0.8896	1.4733	122.7	155.0	12.0	45.72	198.3	186.1	57.52
—	1.4732	117.5	166.0	9.0	49.41	199.1	211.7	75.02
0.8885	1.4863	103.9	169.3	10.6	47.89	200.4	214.1	79.48
0.8955	1.4725	116.6	167.4	15.3	45.63	199.1	208.7	74.94
d_4^{25}0.9016	1.4773	133.2	174.9	38.6	37.8	197.3	205.2	74.5
0.9024	1.4725	147.8	161.0	28.2	27.61	201.4	197.8	62.14
0.9305	1.4784	141.2	194.6	35.4	26.73	192.2	222.7	82.26
—	1.4774	144.9	183.5	42.2	29.06	199.8	205.6	73.31
0.9484	1.4829	150.7	187.8	43.7	21.89	195.4	209.7	74.84
—	1.4818	162.1	197.0	48.7	17.17	186.1	201.3	—
—	1.4805	167.2	198.3	37.1	14.22	191.2	211.0	74.48
—	1.4795	163.9	189.0	26.9	14.30	192.2	201.1	67.01
—	1.4775	150.3	180.6	40.4	23.94	190.9	201.0	68.22
—	1.4787	152.6	187.8	45.1	22.46	192.8	200.8	68.89
—	1.4815	156.7	212.0	59.9	20.96	186.5	225.8	86.15
d_{20}^{25}0.9414	—	161.7	196.7	140.1	16.84	194.9	211.7	—
—	1.4845	163.6	181.3	133.2	10.62	190.0	207.9	73.7
0.9468	—	186.9	200.4	90.4	11.12	193.1	192.0	79.92
0.9561	1.4821	150.0	109.8	76.7	17.89	197.6	121.5	26.75
—	1.4866	179.5	202.6	76.0	10.1**	195.2	208.1	74.95
0.9407	1.4838	175.2	195.7	116.8	11.3***	—	196.6	69.55
0.9521	1.4884	148.9	194.0	133.9	24.93	190.6	213.3	—
0.9492	1.4894	163.7	177.1	147.6	19.74	187.9	200.8	—
0.9410	1.4863	169.6	243.4	145.0	24.16	190.8	260.7	102.09
—	1.4773	153.1	191.4	60.7	28.05	195.8	210.7	—
0.9328	—	180.9	236.8	154.4	10.42	190.8	250.1	99.73
0.9398	—	176.4	210.4	144.5	13.29	196.4	231.7	98.56
—	1.4802	140.6	174.3	63.8	33.32	192.4	189.3	—

*** Sterol content in unsaponifiable matter, 73.8%

2.3.2 Fatty acids of acetone-soluble lipids

2.3.2.1 *Neomysis nakazawai* (1)

The properties of mixed fatty acid are shown in Table 19 (Sample No. 22). The mixed fatty acids were separated into solid and liquid acids by the lead salt alcohol method, and a highly unsaturated acid was obtained by the lithium salt acetone method. The results are shown in Table 20. The methyl esters prepared from the fatty acids (S.V. 181.4, I.V. 198.3, A.V. 0.66) were fractionated using Widmer spiral with the results shown in Table 21. The saturated esters in each fraction were estimated by the permanganate oxidation method, and the saponification equivalent of the saturated esters was determined. The results are shown

Table 20. Solid acid, liquid acid and highly unsaturated acid (*Neomysis nakazawai*)

	Yield % to total fatty acid	Neut. V.	I.V.	mp	n_D^{20}
Solid acid	23.4	207.0	40.5	50-52	
Liquid acid	76.9	185.7	254.5		1.4837
Highly unsaturated acid	38.5		316.3		1.4939

Table 21. Fractionation of the methyl esters (*Neomysis nakazawai*)

Fraction No.	Distillation temp. °C	Pressure (mmHg)	n_D^{20}	S.V.	S.E.	I.V.	Yield	
							(g)	(%)
1	-135	1	1.4394	230.1	243.8	22.6	2.3	3.7
2	135-145	〃	1.4428	218.0	257.3	40.5	2.9	4.6
3	145-150	〃	1.4453	209.4	268.0	47.2	9.6	15.2
4	147-152	0.5	1.4456	206.5	271.7	56.1	3.7	5.9
5	152-157	〃	1.4558	191.4	293.1	116.5	5.1	8.1
6	157-165	〃	1.4686	184.1	304.7	211.7	9.4	14.9
7	165-174	〃	1.4811	176.5	317.8	295.6	12.0	19.0
8	174-175	〃	1.4858	172.0	326.3	318.8	4.5	7.2
9	175-185	〃	1.4939	165.8	338.5	340.5	4.3	6.8
10	185-202	〃	1.4964	165.6	338.8	292.3	2.6	4.1
	Residue			163.2*		180.9**	6.6 (diff.)	10.5
	Residne	(Extract with petroleum ether)		166.1*		203.6**		

* Neut. V. of acid ** I.V. of acid

Table 22. Saturated esters in each fraction (*Neomysis nakazawai*)

Fraction No.	Saturated methyl esters in each fraction (%)	Saturated methyl esters to total methyl esters (%)	S.V.	S.E.	Carbon number of fatty acid
1	79.6	2.9	229.9	244.0	14.1
2	65.3	3.0	219.0	256.2	15.0
3	60.1	9.1	210.3	266.8	15.8
4	55.3	3.3	207.2	270.8	16.1
5	21.5	1.7	194.1	289.1	17.4
6	8.9	1.3	189.6	296.0	17.9
7	3.2	0.7	188.1	298.0	18.0
8	2.0	0.1	—	—	
9	2.1	0.1	—	—	
10	3.3	0.1	—	—	

in Table 22. From the observed data, the saponification equivalent and the iodine value of the unsaturated esters together with the carbon number and the unsaturation of the unsaturated acids in each fraction were deduced by calculation. The results are shown in Table 23. The composition of the saturated and unsaturated esters in each fraction was calculated by assuming each of these ester

Table 23. Unsaturated esters in each fraction (*Neomysis nakazawai*)

Fraction No.	Unsaturated esters in each fraction (%)	Unsaturated esters to total methyl esters (%)	S.E.	I.V.	Unsaturation (−H)	Carbon number of fatty acid
1	20.4	0.8	243.1	110.8	2.12	14.2
2	34.7	1.6	256.1	116.7	2.35	15.4
3	39.9	6.1	267.0	118.3	2.48	16.3
4	44.7	2.6	272.7	125.5	2.69	16.3
5	78.5	6.4	294.1	148.4	3.44	18.0
6	91.1	13.6	305.6	232.4	5.59	18.9
7	96.3	18.3	318.6	307.4	7.70	20.0
8	98.0	7.1	326.3	325.3	8.36	20.6
9	97.0	6.7	338.5	347.8	9.27	21.5
10	96.7	4.0	338.8	302.3	8.07	21.5

Table 24. Fatty acid composition (*Neomysis nakazawai*)

Saturated fatty acid	23.0%	Unsaturated fatty acid	77.0%
C_{14}	5.4%	C_{14} (−2.1 H)	1.3%
C_{16}	13.9%	C_{16} (−2.5 H)	8.9%
C_{18} and $C_{>18}$	3.7%	C_{18} (−3.4 H)	15.8%
		C_{20} (−7.7 H)	33.5%
		C_{22} (−>9.3 H)	17.5%

Table 25. Fractionation of the methyl esters (*Neomysis intermedia*)

Fraction No.	Distillation temp. °C	Pressue (mmHg)	n_D^{20}	S.V.	S.E.	I.V.	Yield (g)	Yield (%)
1	-155	3	1.4395	230.2	243.7	20.7	1.80	2.7
2	155-165	〃	1.4402	220.6	254.4	23.4	2.25	3.3
3	165-166	〃	1.4421	212.4	264.2	35.1	3.45	5.1
4	166-167	〃	1.4439	204.9	273.8	40.0	7.05	10.4
5	167-172	〃	1.4462	201.3	278.8	46.5	11.50	17.0
6	160-161	2.5	1.4482	199.7	281.0	59.2	6.90	10.2
7	161-169	〃	1.4486	197.8	283.7	60.7	4.05	6.0
8	169-179	〃	1.4526	187.6	299.1	88.2	4.15	6.2
9	179-184	〃	1.4556	184.8	303.6	119.6	8.05	12.0
10	184-199	〃	1.4688	176.4	318.0	178.7	3.10	4.6
11	199-214	〃	1.4740	168.4	333.1	210.4	3.45	5.1
12	214-231	〃	1.4787	163.4	343.4	201.2	1.65	2.4
	Residue		1.4829	156.2*		144.8**	10 60 (diff.)	15.0
	Residue	(Extract with petroleum ether)		157.1*		147.0**		

* Neut V. of acid ** I.V. of acid

fractions containing two adjacent homologous esters of even acid. The unsaturated esters in the residue were regarded wholly as those of C_{22} acids. The average unsaturation for the unsaturated esters of C_{14}, C_{16}, C_{18}, C_{20} and C_{22} acids were obtained by plotting the carbon number against the unsaturation for the unsaturat-

Table 26. Saturated esters in each fraction (*Neomysis intermedia*)

Fraction No.	Saturated esters in each fraction (%)	S.V.	S.E.	Carbon number of fatty acid
1	81.7	230.4	243.6	14.1
2	79.1	220.8	254.6	14.8
3	69.2	213.2	263.2	15.5
4	63.8	206.1	272.3	16.1
5	62.4	201.8	278.0	16.5
6	55.5	200.8	279.5	16.6
7	54.8	193.9	280.9	16.7
8	33.6	188.7	297.3	17.8
9	28.0	186.9	300.2	18.1
10	17.8	184.3	304.5	18.4
11	13.1	171.9	326.5	20.0
12	15.0	—	—	—

Table 27. Unsaturated esters in each fraction (*Neomysis intermedia*)

Fraction No.	Unsaturated esters in each fraction (%)	S.E.	I.V.	Unsaturation (−H)	Carbon number of fatty acid
1	18.3	244.5	113.2	2.18	14.3
2	20.9	255.5	112.0	2.25	15.1
3	30.8	266.4	114.0	2.39	15.9
4	36.2	276.5	111.0	2.41	16.6
5	37.6	280.1	123.7	2.73	16.9
6	44.5	282.8	133.0	2.96	17.1
7	45.2	287.0	134.3	3.04	17.4
8	66.6	299.9	132.4	3.13	18.3
9	72.0	304.9	166.1	3.99	18.7
10	82.2	320.9	217.4	5.49	20.0
11	86.9	334.2	242.1	6.37	21.0
12	85.0	343.4	236.7	6.41	21.7

Table 28. Fatty acid composition (*Neomysis intermedia*)

Saturated fatty acid	42.0%	Unsaturated fatty acid	58.0%
C_{14}	4.5%	C_{14} (−2.1 H)	1.0%
C_{16}	24.5%	C_{16} (−2.4 H)	11.0%
C_{18}	11.5%	C_{18} (−>3.0 H)	18.0%
C_{20}	1.5%	C_{20} (−>5.5 H) C_{22} (−>6.6 H)	28.0%

ed acids in each fraction. Thus, by the calculation of weight composition of individual esters in total esters, approximate values of fatty acid composition (per cent weight) were obtained with the results shown in Table 24.

2.3.2.2 *Neomysis intermedia*

The properties of mixed fatty acids are shown in Table 19 (Sample No. 24). The methyl esters prepared from the fatty acids, having S.V. 188.3, I.V. 113.9, A.V. 0.03. were fractionated with the results shown in Table 25. The values of

Table 29. Fractionation of the methyl esters (*Calanus plumchrus*)

Fraction No.	Distillation temp. °C	Pressure (mmHg)	n_D^{20}	S.V.	S.E.	I.V.	Yield (g)	Yield (%)
1	-135	3	1.4356	229.0	245.0	12.5	0.61	3.5
2	135-142	〃	1.4360	225.5	248.9	9.8	2.48	14.3
3	142-154	〃	1.4438	211.9	264.8	51.3	3.42	19.7
4	-166	2	1.4608	194.7	288.1	188.4	2.95	17.0
5	166-176	〃	1.4749	182.7	307.1	282.7	2.00	11.5
6	176-185	〃	1.4800	172.4	325.5	292.6	1.54	8.9
7	-190	1.5	1.4810	166.2	337.7	295.7	0.78	4.5
8	190-200	〃	1.4830	162.6	345.2	281.5	1.72	9.9
	Residue			161.5*		185.2**	1.87	10.7

* Neut. V. of acid ** I.V. of acid

Table 30. Saturated esters in each fraction (*Calanus plumchrus*)

Fraction No.	Saturated esters in each fraction (%)	Saturated esters to total methyl esters (%)	S.V.	S.E.	Carbon number of fatty acid
1	88.6	3.1	229.3	244.7	14.2
2	90.9	13.0	228.0	246.1	14.3
3	69.5	13.7	216.2	259.5	15.2
4	29.8	5.1	204.9	273.9	16.2
5	5.4	0.6	186.6	300.6	18.2
6	2.9	0.3	—	—	
7	4.5	0.2	—	—	
8	5.3	0.5	—	—	

Table 31. Unsaturated esters in each fraction (*Calanus plumchrus*)

Eraction No.	Unsaturated esters in each fraction (%)	Unsaturated esters to total methyl esters (%)	S.E.	I.V.	Unsaturation (−H)	Carbon number of fatty acid
1	11.4	0.4	247.6	109.5	2.14	14.5
2	9.1	1.3	276.3	107.7	2.35	16.6
3	30.5	6.0	277.3	168.2	3.67	16.7
4	70.2	11.9	294.2	268.2	6.22	18.1
5	94.3	10.9	307.5	299.8	7.26	19.2
6	97.1	8.6	325.5	301.3	7.73	20.5
7	95.5	4.3	337.7	309.6	8.24	21.4
8	94.7	9.4	345.2	297.3	8.09	21.9

saturated and unsaturated esters obtained by the process as before are shown in Table 26 and Table 27. The fatty acid composition (per cent weight) is shown in Table 28.

2.3.2.3 *Calanus plumchrus*

The properties of mixed fatty acids are shown in Table 19 (Sample No. 1-7). The methyl esters prepared from the fatty acids, having S.V. 191.6, I.V. 178.2,

Table 32. Fatty acid composition (*Calanus plumchrus*)

Saturated fatty acid	36.4%	Unsaturated fatty acid	63.6%
C_{14}	19.3%	C_{14} (−2.1 H)	0.3%
C_{16}	15.0%	C_{16} (−2.7 H)	4.9%
C_{18} and $C_{>18}$	2.1%	C_{18} (−5.9 H)	18.2%
		C_{20} (−7.5 H)	15.3%
		C_{22} and $C_{>22}$ (> −8.2 H)	24.9%

Table 33. Fractionation of the methyl esters (*Calanus finmarchicus*)

Fraction No.	Distillation temp. °C	Pressure (mmHg)	n_D^{20}	S.V.	S.E.	I.V.	Yield (g)	Yield (%)
1	-110	0.5	1.4400	228.4	245.7	22.2	1.53	6.7
2	111-115	〃	1.4415	223.9	250.6	31.3	1.26	5.5
3	115-120	〃	1.4456	216.0	259.8	57.6	1.36	5.9
4	120-125	〃	1.4491	209.6	267.7	79.7	1.83	8.0
5	125-130	〃	1.4510	203.2	276.1	91.3	1.99	8.7
6	130-135	〃	1.4606	196.8	285.1	160.9	1.21	5.3
7	135-140	〃	1.4676	187.4	299.4	313.3	1.57	6.8
8	140-145	〃	1.4710	183.6	305.6	234.2	2.03	8.9
9	145-150	〃	1.4791	175.2	320.3	279.5	1.55	6.8
10	150-155	〃	1.4822	173.4	323.5	295.4	1.22	5.4
11	155-160	〃	1.4850	170.6	328.9	307.0	1.65	7.2
12	160-165	〃	1.4883	166.3	337.5	298.7	1.03	4.5
13	165-	〃	1.4887	161.6	347.2	288.5	1.71	7.5
	Residue					179.3	3.55	12.8

Table 34. Saturated esters in each fraction (*Calanus finmarchicus*)

Fraction No.	Saturated esters in each fraction (%)	Saturated esters to total methyl esters (%)	S.V.	S.E.	Carbon number of fatty acid
1	79.44	5.31	232.0	241.9	14.0
2	75.09	4.13	229.2	244.8	14.2
3	56.66	3.37	222.9	251.7	14.7
4	42.73	3.41	214.4	261.7	15.4
5	42.67	3.71	207.2	270.8	16.0
6	25.04	1.32	206.0	272.4	16.2
7	14.51	1.00	187.8	298.9	18.0
8	12.47	1.11	—		
9	5.74	0.39	—		
10	3.57	0.19	—		
11	5.69	0.41	—		
12	7.27	0.32	—		
13	6.21	0.46	—		

Table 35. Unsaturated esters in each fraction (*Calanus finmarchicus*)

Fraction No.	Unsaturated esters in each fraction (%)	Unsaturated estes to total methyl esters (%)	S.E.	I.V.	Unsaturation (−H)	Carbon number of fatty acid
1	20.56	1.37	260.6	107.9	2.22	15.5
2	24.91	1.37	268.0	125.8	2.67	16.1
3	43.37	2.57	270.3	132.9	2.83	16.2
4	57.27	4.58	272.2	139.1	2.98	16.4
5	57.33	4.98	280.1	159.3	3.52	17.0
6	74.96	3.97	290.7	214.6	4.91	17.8
7	85.49	5.86	299.5	249.5	5.89	18.5
8	87.53	7.76	305.6*	267.6	6.44	19.0
9	94.26	6.38	320.3*	296.6	7.48	20.1
10	96.43	5.23	323.5*	306.4	7.81	20.4
11	94.31	6.80	328.9*	325.5	8.44	20.8
12	92.73	4.18	337.5*	322.1	8.57	21.4
13	93.79	7.01	347.2*	307.6	8.42	22.1
	Residue					22.2

* S.E. of mixed fatty acid methyl esters were applied.

Table 36. Fatty acid composition (*Calanus finmarchicus*)

Saturated fatty acid	25.2%	Unsaturated fatty acid	74.8%
C_{14}	12.3%	C_{14} (−2.0 H)	0.3%
C_{16}	8.9%	C_{16} (−2.5 H)	11.2%
C_{18}	4.0%	C_{18} (−5.2 H)	15.6%
		C_{20} (−7.4 H)	20.9%
		C_{22} (−8.7 H)	25.2%
		C_{24} (−9.0 H)	1.6%

Table 37. Fractionation of the methyl esters (*Parathemisto japonica*)

Fraction No.	Distillation temp. °C	Pressure (mmHg)	n_D^{20}	S.V.	S.E.	I.V.	Yield	
							(g)	(%)
1*	-120	1	1.4427					
1a	-115	〃	1.4388	224.8	249.7	29.8	2.3	4.3
1b			1.4493	201.0	279.1	88.1	2.4	4.5
2	120-130	〃	1.4388	221.9	252.9	28.3	3.4	6.3
3	130-140	〃	1.4446	205.4	273.2	52.9	5.7	10.6
4	140-147	〃	1.4526	197.6	283.9	106.3	5.4	10.1
5	-120	0.5	1.4686	180.2	311.4	216.3	4.1	7.6
6	120-135	〃	1.4739	175.1	320.4	248.8	10.6	19.8
7	135-150	〃	1.4762	172.9	324.6	255.1	4.1	7.6
8	150-165	〃	1.4780	170.6	328.9	270.5	5.5	10.3
9	165-175	〃	1.4792	167.6	334.9	282.6	2.6	4.9
10	175-185	〃	1.4869	158.7	353.6	310.6	2.9	5.4
11	185-190	〃	1.4918	158.6	353.7	305.9	1.0	1.9
	Residue			165.1**			3.6	6.7

* Fraction 1 was refractionated into Fraction 1a and 1b.

** N.V. of acid

Table 38. Saturated esters in each fraction (*Parathemisto japonica*)

Fraction No.	Saturated esters in each fraction (%)	Saturated esters to total methyl esters (%)	S.V.	S.E.	Carbon number of fatty acid
1a	77.2	3.3	226.1	248.2	14.4
2	76.6	4.8	222.6	252.0	14.7
3	58.1	6.2	211.2	265.6	15.7
1b	50.0	2.3	210.5	266.6	15.8
4	38.5	3.9	202.5	277.0	16.5
5	8.2	0.6	194.4	288.6	17.2
6	8.6	1.7	—		
7	8.9	0.7	—		
8	6.3	0.6	—		
9	6.7	0.3	—		
10	8.7	0.5	—		
11	3.3	0.1	—		

Table 39. Unsaturated esters in each fraction (*Parathemisto japonica*)

Fraction No.	Unsaturated esters in each fraction (%)	Unsaturated estes to total methyl esters (%)	S.E.	I.V.	Unsaturation (−H)	Carbon number of fatty acid
1a	22.8	1.0	254.8	130.5	2.62	15.0
2	23.4	1.5	256.0	120.9	2.44	15.1
3	41.9	4.4	284.8	126.3	2.83	17.2
1b	50.0	2.2	291.6	176.3	4.05	17.7
4	61.5	6.2	288.3	172.8	3.92	17.5
5	91.8	7.0	313.4	235.6	5.81	19.5
6	91.4	18.1	320.4	272.2	6.87	20.0
7	91.1	6.9	324.6	280.0	7.16	20.4
8	93.7	9.7	328.9	288.7	7.48	20.7
9	93.3	4.6	333.5	302.8	7.96	21.1
10	91.3	4.9	351.6	340.2	9.42	22.4
11	96.7	1.8	353.7	316.3	8.82	22.6

Table 40. Fatty acid composition (*Parathemisto japonica*)

Saturated fatty acid	25.0%	Unsaturated fatty acid	75.0%
C_{14}	7.0%	C_{14} (−2.4 H)	1.0%
C_{16}	12.5%	C_{16} (−2.5 H)	5.0%
C_{18} and $C_{>18}$	5.5%	C_{18} (−4.4 H)	11.0%
		C_{20} (−6.9 H)	37.0%
		C_{22} and $C_{>22}$ (−9.0 H)	21.0%

A.V. 3.6, were fractionated with the results shown in Table 29. The values of saturated and unsaturated esters are shown in Table 30 and Table 31.

The fatty acid composition is shown in Table 32.

2.3.2.4 *Calanus finmarchicus*

The properties of mixed fatty acids are shown in Table 19 (Sample No. 10).

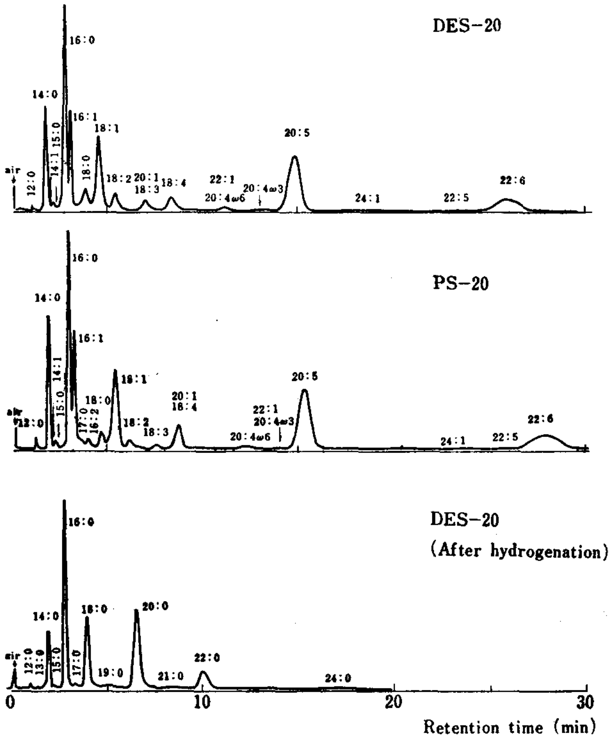

Fig. 6. Gas-liquid chromatograms of methyl esters of *Neomysis spinosa* lipid fatty acids
Operating conditions:

Instrument ……	Yanagimoto GCG-2		
Column ……….	1) Diethylene glycol succinate polyester on Celite 545 (60-80 mesh), 1/5wt/wt, 2m×5mm i.d., (DES-20) 2) Propylene glycol succinate polyester on Celite 545 (60-80 mesh), 1/5wt/wt, 2m×1.5 mm i.d., (PS-20)		
Detector ……….	TCD		
Sample ……….	Fatty acid methyl esters		Hydrogenated esters
Column ……….	DES-20	PS-20	DES-20
Carrier gas, He ….	60 ml/min	70 ml/min	60 ml/min
Temperature ……	225°C	230°C	220°C
Bridge current ….	170 mA	200 mA	170 mA
Sens. …………	2mV	2mV	2mV
Chart speed ……	10 ml/min	10ml/min	10 ml/min
Sample size ……	2μl-6μl		

The liquid stationary phases were prepared on referring to the reprot of Ito and Fukuzumi[68], and others.[69],[70]

The methyl esters prepared from the fatty acids were fractionated with the results shown in Table 33. The values of saturated and unsaturated esters are shown in Table 34 and Table 35. The fatty acid composition is shown in Table 36.

2.3.2.5 *Parathemisto japonica*

The properties of mixed fatty acids are shown in Table 19 (Sample No. 12–20).

The methyl esters prepared from the fatty acids were fractionated with the results shown in Table 37. The values of saturated and unsaturated esters are shown in Table 38 and 39. The fatty acid composition is shown in Table 40.

2.3.2.6 *Neomysis spinosa*

The properties of mixed fatty acids are shown in Table 19 (sample No. 25).

The methyl esters prepared from the fatty acids by the sulphric acid-methanol methods (S.V. 189.1, I.V. 207.3) were analyzed on gas-liquid chromatography. The gas-liquid chromatograms obtained on two kinds of column and by hydrogenated methyl ester are shown in Fig. 6. The peaks were identified by the comparison with esters of standard acids, myristic, palmitic, stearic, arachidic, palmitoleic, oleic, linoleic, and linolenic acids, and from the straight-line relationship of semilogarithmic plot of retention times versus the number of carbon atoms. At the same time the reports by Ito and Fukuzumi63),68),71) were referred to. The peak areas were measured by the half value width method. From the generalized results of fatty acid methyl esters and hydrogenated esters, approximate values of fatty acid composition (per cent weight) were obtained. The results are shown in Table 41.

Table 41. Fatty acid composition (*Neomysis spinosa*)

Saturated acid wt. %		Monoenoic acid wt. %		Polyenoic acid wt. %	
10:0	trace	14:1	0.6	16:2	1.6
12:0	0.5	16:1	10.3	18:2	1.8
13:0	trace	18:1	11.5	18:3	1.0
14:0	6.9	20:1	1.6	18:4	2.8
15:0	0.8	22:1	1.6	20:4ω6	1.0
16:0	19.9	24:1	trace	20:4ω3	trace
17:0	0.9			20:5	24.0
18:0	3.3			22:5	trace
19:0	0.4			22:6	9.0
21:0	0.5				
Total	33.2	Total	25.6	Total	41.2

2.3.2.7 *Neomysis awatschensis*

The properties of mixed fatty acids are shown in Table 19 (Sample No. 26).

The methyl esters prepared from the fatty acids by the sulphric acid-methanol method, having S.V. 191.6, I.V. 200.8, were analyzed on gas-liquid chromatography under the same conditions as in the analysis of fatty acid methyl esters of *Neomysis spinosa*. The gas-liquid chromatograms obtained are shown in Fig. 7.

The fatty acid composition is shown in Table 42.

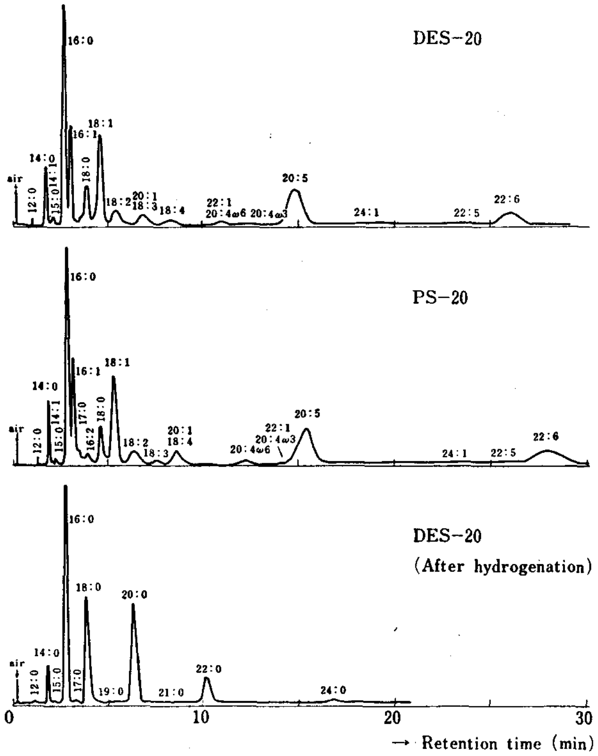

Fig. 7. Gas-liquid chromatograms of methyl esters of *Neomysis awatschensis* lipid fatty acids
Operating conditions are the same as given in Fig. 6.

Table 42. Fatty acid composition (*Neomysis awatschensis*)

Saturated acid wt. %		Monoenoic acid wt. %		Polyenoic acid wt. %	
12:0	0.2	14:1	0.5	16:2	1.3
14:0	3.8	16:1	10.8	18:2	3.4
15:0	0.8	18:1	13.2	18:3	1.5
16:0	21.2	20:1	1.3	18:4	2.3
17:0	1.9	22:1	1.7	20:4ω6	0.9
18:0	5.4	24:1	trace	20:4ω3	trace
19:0	0.7			20:5	17.4
21:0	0.4			22:5	trace
				22:6	11.3
Total	34.4	Total	27.5	Total	38.1

2.3.2.8 4:6 mixture of *Acanthomysis pseudomacropsis and Euphausia sp.*

The materials were collected at the adjacent sea of Miyako, Iwate Prefecture in 1962, and immersed in an equal weight of acetone. The lipids were obtained from the materials immersed in acetone by Procedure A. The lipid content of the

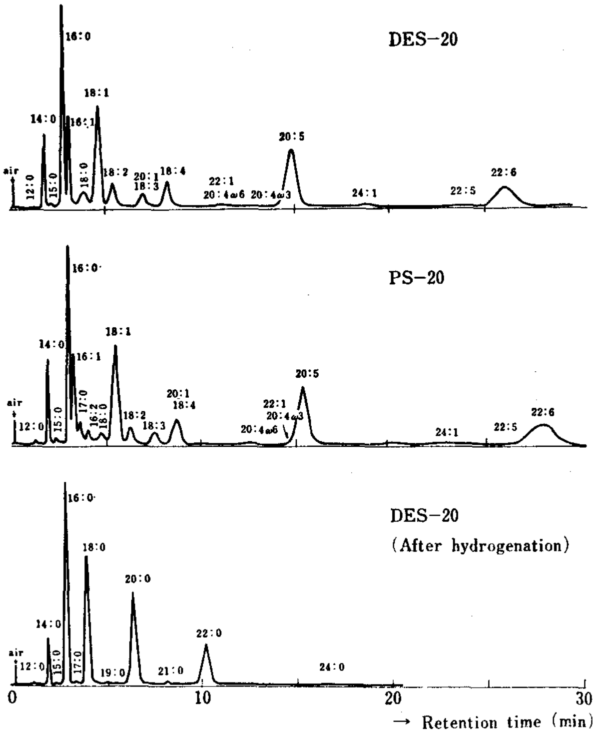

Fig. 8. Gas-liquid chromatograms of methyl esters of *Acanthomysis pseudomacropsis* and *Euphausia pacifica* (4:6 mixture) lipid fatty acids
Operating conditions are the same as given in Fig. 6.

Table 43. Fatty acid composition (*Acanthomysis pseudomacropsis*: *Euphausia sp.*=4:6)

Saturated acid wt. %		Monoenoic acid wt. %		Polyenoic acid wt. %	
14:0	4.4	14:1	trace	16:2	2.0
15:0	0.6	16:1	7.8	18:2	2.2
16:0	17.8	18:1	14.7	18:3	1.9
17:0	2.8	20:1	2.0	18:4	3.1
18:0	1.8	22:1	1.3	20:4ω6	0.9
19:0	1.0	24:1	trace	20:4ω3	trace
21:0	1.0			20:5	22.1
				22:5	trace
				22:6	12.6
Total	29.4	Total	25.8	Total	44.8

material was 1.51% on wet weight. The acetone-soluble lipid (98.9% of the lipid) was obtained. The acetone-soluble lipid was a blackish semi-liquid at ordinary temperature: n_D^{20} 1.4891, d_4^{15} 0.9429, A.V. 111.4, S.V. 188.8, I.V. 224.4, Unsap. matter 10.4%, sterol content in unsap. matter 75.7%. The fatty acids had I.V.

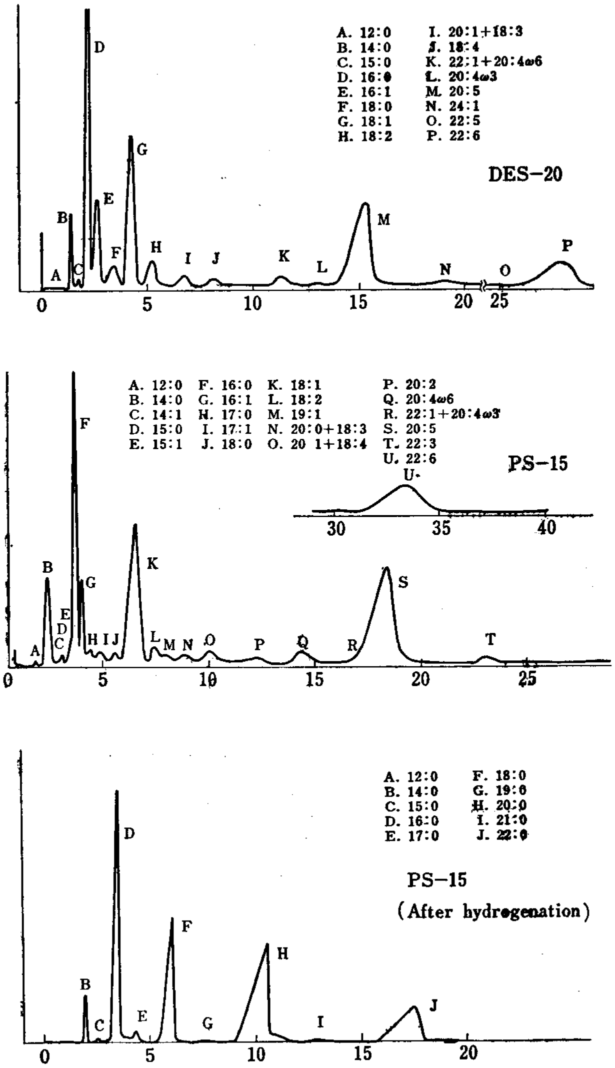

Fig. 9. Gas-liquid chromatograms of methyl esters of *Euphausia pacifica* (Senzaki) lipid fatty acids

Operating conditions:

Instrument	Yanagiomoto GCG-2
Column	1) Diethylene glycol succinate polyester on Celite 545 (60–80 mesh), 1/5wt/wt, 2m×5mm i.d., (DES-20) 2) Propylene glycol succinate polyester on Celite 545 (60–80 mesh), 3/20wt/wt, 2m×5mm i.d., (PS-15)
Detector	TCD

Sample	Fatty acid methyl ester		Hydrogenated ester
Column	DES-20	PS-15	PS-15
Carrier gas, He	60ml/min	60ml/min	50ml/min

Temperature	220°C	220°C	220°C
Bridge current	170 mA	170 mA	170 mA
Sens.	2mV	2mV	2mV
Chart speed..	10 ml/min	10 ml/min	10 ml/min
Sample size	3μl	3μl	2μl

Table 44. Fatty acid composition (*Euphausia pacifica*) (*per cent wt*)

Locality of catching	Senzaki	Kesen-numa
Saturated acid		
12:0	trace	0.1
14:0	2.7	4.4
15:0	0.4	0.6
16:0	20.1	18.3
17:0	1.5	1.4
18:0	1.9	2.0
19:0	trace	1.5
Total	26.6	28.3
Monoenoic acid		
14:1	trace	0.5
15:1	trace	—
16:1	5.8	7.0
17:1	2.1	2.3
18:1	16.4	13.6
19:1	trace	trace
20:1	0.8	0.7
22:1	trace	trace
24:1	trace	trace
Total	25.1	24.1
Polyenoic acid		
16:2	—	3.0
18:2	2.2	1.2
18:3	1.5	2.5
18:4	1.1	4.6
20:2	trace	trace
20:4ω6	2.9	1.8
20:4ω3	trace	0.5
20:5	25.9	20.5
21:5	trace	1.1
22:5	trace	trace
22:6	14.7	12.8
Total	48.3	48.0

248.9. Bromination of the fatty acids yielded 90.13% of ether-insoluble bromide.

The methyl esters prepared from the fatty acids by the sulphuric acid-methanol method (S.V. 183.3, I.V. 225.7) were analyzed on gas-liquid chromatography under the same conditions as in the analysis of fatty acid methyl esters of *Neomysis spinosa*. The gas-liquid chromatograms obtained are shown in Fig. 8. The fatty acid composition is shown in Table 43.

2.3.2.9 *Euphausia pacifica*

The materials were collected at the adjacent sea of Senzaki, Yamaguchi Prefecture and the adjacent sea of Kesen-numa, Miyagi Prefecture. The properites of fatty acids are shown in Table 19 (Sample No. 31–32). The methyl esters prepared from the fatty acids by the hydrochloric acid-methanol method were analyzed on gas-liquid chromatography. The gas-liquid chromatograms obtained are shown in Fig. 9. The fatty acid composition is shown in Table 44.

2.3.2.10 *Neomysis nakazawai* (2)

Neomysis nakazawai (1) reported in (2.3.2.1) was a sun-dried material. *Neomysis nakazawai* (2) is a fresh material. The properties of fatty acids are shown in Table 19 (Sample No. 23). The methyl esters prepared from the fatty acids by the hydrochloric acid-methanol method were analyzed on gas-liquid chromatography under the same conditions as in *Euphausia pacifica.* The fatty acid composition obtained is shown in Table 45.

2.3.2.11 *Acetes japonicus*

The properties of the fatty acids are shown in Table 19 (Sample No. 21).

Table 45. Fatty acid composition (*Neomysis nakazawai*)

Saturated acid wt. %		Monoenoic acid wt. %		Polyenoic acid wt. %	
12:0	0.3	14:1	0.8	18:2	1.5
13:0	trace	15:1	0.4	18:3	0.6
14:0	4.6	16:1	10.5	18:4	2.6
15:0	1.2	17:1	1.5	20:2	trace
16:0	21.2	18:1	15.7	20:4ω6	0.7
17:0	1.6	19:1	trace	20:4ω3	trace
18:0	3.0	20:1	2.2	20:5	21.1
19:0	trace	22:1	trace	21:5	trace
		24:1	trace	22:5	trace
				22:6	10.5
Total	31.9	Total	31.1	Total	37.0

Table 46. Fatty acid composition (*Acetes japonicus*)

Saturated acid wt. %		Monoenoic acid wt. %		Polyenoic acid wt. %	
11:0	0.2	14:1	0.1	18:2	2.9
12:0	0.2	15:1	trace	18:3	0.5
13:0	trace	16:1	11.1	18:4	1.6
14:0	3.0	17:1	2.4	19:2 ?	0.3
15:0	0.8	18:1	12.2	20:2	0.3
16:0	18.2	19:1	0.5	20:4ω6	3.5
17:0	1.0	20:1	1.9	20:4ω3	trace
18:0	4.1	22:1	trace	20:5	20.0
20:0	1.4			22:6	13.8
22:0	trace				
Total	28.9	Total	28.2	Total	42.9

The methyl esters prepared from the fatty acids by the hydrochloric acid-methanol method were analyzed on gas-liquid chromatography under the same conditions as in *Euphausia pacifica* excluded column temp: 210°C, carrier gas: He 45 ml/min (PS-15). The fatty acid composition obtained is shown in Table 46.

2.3.2.12. *Daphnia carinata*

The materials were prepared at Freshwater Fisheries Research Laboratory, Fisheries Agency, Tokyo, in 1964 as dried form.

The lipids were obtained from the dried material by Procedure B.

Table 47. Fatty acid ocmposition (*Daphnia carinata*)

Saturated acid wt. %		Monoenoic acid wt. %		Polyenoic acid wt. %	
12:0	0.2	12:1	0.4	16:2	1.6
13:0	0.4	13:1	0.7	18:2	7.4
14:0	2.1	14:1	5.0	18:3	13.9
15:0	2.9	15:1	1.4	18:4	2.2
16:0	7.6	16:1	15.5	20:4ω6	1.9
17:0	2.4	17:1	2.4	20:4ω3	1.2
18:0	3.8	18:1	21.1	20:5	3.8
20:0	1.4	19:1	0.7		
		20:1	trace		
		22:1	trace		
Total	20.8	Total	47.2	Total	32.0

The lipid content of the material was 5.5% on dry weight. Acetone-soluble lipids (89.2% of the lipid) were obtained. The properties of the acetone-soluble lipid are as follow: n_D^{20} 1.4811. S.V. 169.9, I.V. 151.6, Unsap. M. 14.77%.

The methyl esters prepared from the fatty acids by the BF_3-methanol method were analyzed on gas-liquid chromatography under the same conditions as in *Acetes japonicus*. The fatty acid composition obtained is shown in Table 47.

2.3.2.13 *Acanthomysis sp.*

The properties of the mixed fatty acids are shown in Table 19 (Sample No. 27).

The methyl esters prepared from the fatty acids by the hydrochloric acid-methanol method were analyzed on gas-liquid chromatography.

Operating conditions:

Instrument.... Yanagimoto Gas Chromatograph GCG-5DH
Column ...... (1) 20% Diethylene glycol adipate polyester (DEA) on Celite 545 (60–80 mesh), 200°C, 3 mm i.d. × 1.5 m.
(2) 15% Polypropylene glycol succinate ester (PS) on Celite 545 (60–80 mesh), 205°C, 3 mm i.d. × 1.5 m.
Carrier gas.... He 15 ml/min (DEA), 25 ml/min (PS)
Detector .... FID, H_2 18 ml/min, Air 600 ml/min, Attenu. 1/8, Sens. 10^9

The fatty acid composition obtained is shown in Table 48.

Table 48. **Fatty acid composition (*Acanthomysis sp.*)**

Saturated acid wt. %		Monoenoic acid wt. %		Polyenoic acid wt. %	
14:0	2.9	14:1	0.4	16:2	0.6
15:0	0.9	15:1	0.4	16:3	0.3
16:0	19.0	16:1	10.4	18:2	1.5
17:0	0.9	17:1	1.4	18:3	0.5
18:0	5.4	18:1	8.1	18:4	1.0
19:0	1.0	19:1	0.8	20:2	0.4
20:0	1.3	20:1	1.1	20:4ω6	3.9
21:0	0.4	22:1	trace	20:4ω3	0.6
22:0	1.0	24:1	0.7	20:5	18.3
				21:5	0.2
				22:4	0.7
				22:5	0.9
				22:6	12.7
Total	32.8	Total	23.3	Total	41.6

Unidentified component 2.2%

Table 49. **Fatty acid composition (*Proneomysis sp.*)**

Saturated acid wt. %		Monoenoic acid wt. %		Polyenoic acid wt. %	
12:0	0.9	12:1	0.3	16:2	1.6
13:0	0.4	13:1	trace	18:2	2.5
14:0	2.4	14:1	0.8	18:3	3.8
15:0	0.9	15:1	0.3	18:4	2.2
16:0	20.5	16:1	8.4	20:2	0.4
17:0	trace	17:1	0.8	20:3	2.6
18:0	6.8	18:1	11.9	20:4ω6	0.4
19:0	trace	19:1	trace	20:4ω3	0.7
20:0	0.7	20:1	1.3	20:5	14.7
21:0	trace	22:1	0.8	22:2	trace
22:0	trace	24:1	trace	22:3	trace
23:0	trace			22:4	0.7
				22:5	trace
				22:6	13.1
Total	32.6	Total	24.6	Total	42.7

2.3.2.14 *Proneomysis sp.*

The properties of mixed fatty acids are shown in Table 19 (Sample No. 28). The methyl esters prepared from fatty acids by the BF_3-methanol method were analyzed on gas-liquid chromatography under the same conditions as in *Acanthomysis sp.*. The fatty acid composition obtained is shown in Table 49.

2.3.3 Unsaponifiable matters of the lipids of plankton

Very little is known as to the unsaponifiable matters of the lipids of plankton. About seven species the contents of unsaponifiable matters in the lipids were reported. Those are as follows:

Calanus finmarchicus ....25%.[19] 32%.[7] 28.2%[25]

Calanus cristatus	43.1%[26]
Euphausia superba	4.3%,[27] 5.5%,[31] 5.53%[33]
Euphausia pacifica	16.8%[27]
Cyclops strenuus	20%[7]
Daphnia galeata	22.3%[7]
Diaptomus gracilis	17.5%[7]

As to the unsaponifiable components, the presence of large amounts of alcohols (cetyl alcohol, C_{20} unsaturated alcohol) and small amounts of sterol with *Calanus finmarchicus*[19],[25] and C_{14-16} saturated and C_{18-20} unsaturated alcohols with *Euphausia superba*[33] was reported. The author's results on the contents of unsaponifiable matters in the lipids of plankton are summarized as follows:

Calanus plumchrus	42.9–50.6%	*Calanus cristatus*	45.6–47.9%
Calanus finmarchicus	37.8%	*Metridia longa*	27.6%
Parathemisto japonica	14.2–29.1%	*Acetes japonicus*	16.8%
Neomysis nakazawai	10.6–11.1%	*Neomysis intermedia*	17.9%
Neomysis spinosa	10.1%	*Neomysis awatschensis*	11.3%
Acanthomysis sp.	24.9%	*Proneomysis sp.*	19.7%
Euphausia pacifica	10.4–28.1%	*Thysanoessa longipes*	33.3%
Daphnia carinata	14.8%		

As it was reported, the contents of unsaponifiable matters in the lipids of plankton are much larger than those in many marine fishes, but the detailed analysis of unsaponifiable components was not yet done. The author examined the unsaponifiable matters of the lipids with seven species of plankton, *Parathemisto japonica, Calanus plumchrus, Neomysis nakazawai, Neomysis intermedia, Euphausia pacifica, Acetes japonicus* and *Acanthomysis sp.*.

2.3.3.1 *Parathemisto japonica*

2.3.3.1.1 Material

The unsaponifiable matter obtained from the samples No. 12–20 (Table 18 and Table 19) was a deep yellowish brown liquid at ordinary temperature: n_D^{20} 1.4779, I.V. 108.9*, sterol content 19.38% (digitonide method).

2.3.3.1.2 Column chromatography

Unsaponifiable matter (20.5477 g) dissolved in 400 ml of petroleum ether (bp 40–60°C) were fractionated by adsorption column chromatography.

Adsorbent: alumina (Brockmann), 200 g (tenfold of sample weight), activity 3–4 (activity test: Brockmann's method[72])

Column: 3.8 cm i.d. × 16.5 cm

One fraction: 40 ml

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* The iodine values recorded in this paper were determined by the Wijs method for the lipids and fatty acid components, and by the pyridine sulfate dibromide method for unsaponifiable components.

Table 50. Chromatography of unsaponifiable matter (*Parathemisto japonica*)

Solvent	Fraction No.	n_D^{20}	Color and state	Yiled		L.B.*
				(g)	(%)	
Petroleum ether	6-12		Milky white, liquid	0.1748		
	13-26	1.4626	Yellow, 〃	5.9854		
	27-39	1.4603	Light yellow, 〃	3.8832		—
	40-45	1.4588	〃 〃	0.3004		—
	46-50		Yellow, 〃	0.1419		—
			Total	10.4857	51.0	
Ether	51-53		Reddish brown, liquid	2.5547		+
	54		Orange, solid	0.9875		+
	55-58		Yellowish brown, 〃	2.2491		+
			(54-58)	(3.2366)		
	59-64		Reddish brown, liquid containing small amount of solid	0.6938		
	65-67		Dark yellow, liquid	0.0635		
	68-69		Reddish yellow, 〃	0.0352		
			(59-69)	(0.7925)		
			Total	6.5838	31.5	
2% Ethanol-Ether	70-74		Dark yellow, liquid	0.0097		
96% Ethanol	75-79		Deep reddish brown, 〃	0.3202		
	80-82		〃 〃	0.0977		
	83-88		Reddish brown, 〃	0.0249		
			Total	0.4428		
			(70-88)	(0.4525)		
2% HCl-Ethanol	89-93		Brown, liquid	0.0308		
8% HCl-Ethanol	94-101		Reddish brown, 〃	1.4707		
	102-107		Light reddish brown, 〃	1.1709		
			Total	1.6416		
			(89-107)	(1.6724)		
(Total	19.0944g,	Residue	1.4533g)			

* Liebermann-Burchard reaction

Flow rate: 1 fraction/ 1 hr

The sample dissolved in petroleum ether was loaded on the column, and eluted with 2,000 ml of petroleum ether (bp 40–60°C), 760 ml of ether, 200 ml of 2% ethanol in ether, 560 ml of 96% ethanol, 200 ml of 2% HCl in ethanol, and 560 ml of 8% HCl in ethanol. The operation was carried out in a current of N_2. The yields and properties of the fractions by each solvent are shown in Tabe 50.

2.3.3.1.3 Analyses of each fraction

(A) Eluate with petroleum ether,

yield 10.4857 g (51.0% of unsaponifiable matter)

The fraction had n_D^{20} 1.4612, I.V. 92.4 and oxine-vanadate test[73] +. An acetylated product was prepared from the fraction. After the acetylation of the

Table 51. Fractional distillation of acetate of eluate with petroleum ether (*Parathemisto japonica*)

Fraction No.	Distillation temp. °C	Pressure (mmHg)	Yield		S.V.	I.V.
			(g)	(%)		
1	140–145	0.5	0.47	19.4	187.7	44.5
2	145–150	〃	0.82	33.8	176.6	72.7
3	150–155	〃	0.54	22.3	161.4	79.5
4	Residue		0.37	24.5		70.1
	Loss		0.22			

fraction, a separated matter did not yield from the acetylated product, which had n_D^{20} 1.4538, S.V. 176.2, I.V. 80.4 and Liebermann-Burchard reaction –. It was considered that this fraction was mainly composed of straight chain alcohols. The acetylated product was fractionated as the results show in Table 51.

Fraction-1: S.V. 187.7, I.V. 44.5

S.V. of Fraction-1 lies between those of cetyl acetate (S.V. 198) and oleyl acetate (S.V. 180). The free alcohol obtained by the saponification of Fraction-1 was recrystallized from petroleum ether, yielding a crystal of mp 45.4–46.7°C. This value is close to mp of hexadecanol (49.3°C).

Fraction-2: S.V. 176.6, I.V. 72.7

The hydrogenated product prepared from the unsaponifiable portion obtained by the saponification of Fraction-2 was recrystallized from 95% ethanol, yielding a crystal of mp 65.0–65.8°C, which showed no depression of melting point when mixed with eicosanol (mp 65.5°C). This crystal was idetnified with eicosanol. The crystal obtained from the mother liquor of crystal identified with eicosanol showed mp 58.61–61.2°C. This crystal was yet impure, the melting point of which is close to that of octadecanol (mp 58°C).

Fraction-3: S.V. 161.3, I.V. 79.5

The hydrogenated product prepared from the unsaponifiable portion obtained by the saponification of Fraction-3 was recrystallized from 95% ethanol, yielding a crystal of mp 68.3–69.4°C, which is close to the melting point of docosanol (mp 70.6°C). It is considered that Fraction-3 before hydrogenation contained lower unsaturated C_{22} alcohol.

(B) Eluate with ethyl ether

This fraction was divided into the following three portions by color and properties.

B_1) Fraction No. 51–53, yield 2.5547 g

Fraction B_1 was found to contain 34.1% of sterol by the digitonide method. The residual portion removed sterol by the digitonide method was a reddish brown liquid: n_D^{20} 1.4725, I.V. 102.5. The acetylated product prepared from the portion had S.V. 223.0, I.V. 81.9. The portion was recrystallized from petroleum ether, yielding a crystal of mp 48.5–49.4°C. This crystal was identified with exadecanol by the mixed melting point test. The acetylated product prepared from the sterol portion was recrystallized from methanol, yielding a crude steryl acetate of mp 104.0–106.8°C.

B_2) Fraction No. 54–58, yield 3.2366 g

Fraction B_2 was a yellowish orange solid: Liebermann-Burchard reaction +, sterol content 94.7% (digitonide method), I.V. 104.2. The fraction was recrystallized from 90% ethanol and acetone, yielding a crystal of mp 133.5–135.6°C. Its ultraviolet absorption spectrum is shown in Fig. 10. The spectrum shows the characteristic absorption of provitamin D, whose content in the crude steryl acetate is found to be 0.22%*. Three crystallizations of crude steryl acetate from methanol gave a fraction of mp 137.2–139.2°C, $[\alpha]_D^{16}=-40.52$ (chloroform), I.V. 88.0. A change of color as the time proceeded by the Liebermann-Burchard reaction on this fraction is shown in Fig. 11. It was considered that the sterol components of this fraction consist cheifly of sterol of Δ^5 series. The fraction was recrystallized many times from methanol, yielding a crystal of mp 146.5–147.0°C. The crystal was identified with cholesterol by the mixed melting point test.

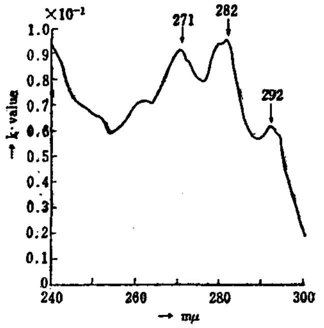

Fig. 10. Ultraviolet absorption of the crystal obtained from eluate with ethyl ether on alumina chromatography of unsaponifiable matter (*Parathemisto japonica*)

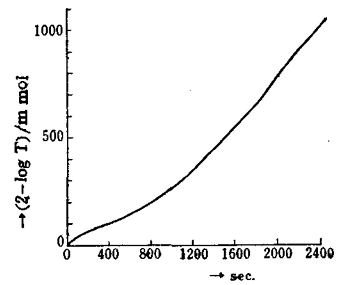

Fig. 11. Change of color as the time proceeded by Liebermann-Burchard reaction on the crystal (mp 137.2–139.2°C) obtained from eluate with ethyl ether on alumina chromatography of unsaponifiable matter (*Parathemisto japonica*)

B_3) Fraction No. 59–69, yield 0.7925 g

Fraction B_3 had n_D^{20} 1.4861, sterol content 25.0% (digitonide method). The residual part removed of sterol by the digitonide method was a light yellowish brown semi-solid. The acetylated product prepared from this part had S.V. 274.4

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* Provitamin D(%) $=12.2\times[k_{282}-(8k_{277}+5k_{290})/13]$74) k: extinction coefficient (g/l)

and I.V. 41.9. The unsaponifiable portion obtained by the saponification of the acetylated product was recrystallized repeatedly, yielding a crystal of mp 60.5–61.5°C, which showed no depression of melting point when mixed with chimyl alcohol. This crystal was identified with chimyl alcohol.

(C) Eluate with 2% ethanol in ethyl ether and eluate with ethanol,
yield 0.4525 g

This fraction was found to contain 12.1% of sterol by the digitonide method. The residual part removed of sterol by the digitonide method was a light reddish brown semi-solid. The acetate of this part had S.V. 281.3, I.V. 83.9, which was considered to contain unsaturated glyceryl ether (acetyl V. and I.V. of hexadecenyglyceryl ether $C_{19}H_{38}O_3$ are 281.7 and 80.8 respectively). The recrystallization of the compound freed from the acetate gave a crystal of mp 54.9–56.6°C.

(D) Eluate with HCl-ethanol,
yield 1.6724 g

This fraction was a reddish brown liquid, having n_D^{20} 1.4779, sterol content 0.91% (digitonide method). The residual part removed of sterol by the digitonide method was a reddish brown liquid having n_D^{20} 1.4817. The acetate of this part, having S.V. 235.1 and I.V. 162.9, resembles the acetate of docosatrienylglyceryl ether $C_{25}H_{46}O_3$ (acetyl V. 234.6, I.V. 159.2).

2.3.3.1.4 Summary of the results

(1) The unsaponifiable matter content in the lipids was 26.2%.

(2) The unsaponifiable matter was a yellowish brown liquid, having n_D^{20} 1.4779, I.V. 108.9.

(3) The unsaponifiable matter contained ca. 50% of alcohol, ca. 20% of sterol and some amount of glyceryl ether.

Alcohols consisted of C_{16}, C_{18} and C_{20} saturated alcohols and C_{18}, C_{20} and C_{22} unsaturated alcohols. Sterols consisted chiefly of cholesterol. Glyceryl ethers consisted chiefly of chimyl alcohol.

2.3.3.2 *Calanus plumchrus*

2.3.3.2.1 Material

An unsaponifiable matter was obtained from sample No. 1–7 in Table 18 and Table 19 and its content in the acetone-soluble lipid was 47.7% (average). The unsaponifiable matter was a reddish brown liquid of n_D^{20} 1.4620, I.V. 98.7, sterol content 2.0% (digitonide method).

2.3.3.2.2 Column chromatography

The unsaponifiable matter (48.84 g) dissolved in 2,100 ml of n-hexane was fractionated by column chromatography.

Adsorbent: silicic acid (Mallinckrodt, 100 mesh), 1,464 g (thirty times weight of the unsaponifiable matter). Used after drying for five hours at 100°C and allowing to stand for three days in the desiccator with silica gel.

Column: 6.3 cm i.d. × 91.5 cm
One fraction: 50 ml
Flow rate: 1 fraction/ 40 minutes

The unsaponifiable matter sample dissolved in n-hexane and loaded on the column was eluted in turn with 2,200 ml of n-hexane, 1,975 ml of 2% benzene in n-

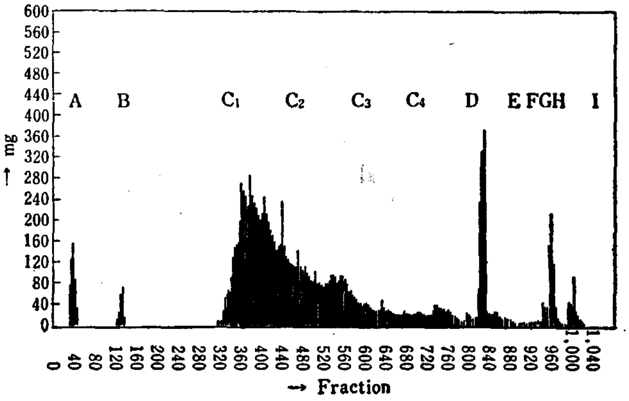

Fig. 12. Chromatogram of unsaponfiiable matter (*Calanus plumchrus*)
A: n-Hexane, B: 20% Benzene-n-Hexane, C: 5% Ether-n-Hexane, D: 8% Ether-n-Hexane, E: Ether, F: 2% Ethanol-Ether, G: 8% Ethanol-Ether, H: Ethanol, I: 8% HCl-Ethanol

Table 52. Characteristics of unsaponifiable matter (*Calanus plumchrus*)

Solvent	Fraction No.	Yield	
		(g)	(%)
n-Hexane	31- 44	0.960	2.0
2% Benzen-n-Hexanee 10% Benzene-n-Hexane	45- 121	0	
20% Benzene-n-Hexane	122- 135	0.437	0.9
30% Benzene-n-Hexane 2% Ether-n-Hexane	136- 313	0	
5% Ether-n-Hexane (1)	314- 330	0.135	0.3
(2)	331- 484	25.110	51.4
(3)	485- 609	9.353	19.1
(4)	610- 718	3.001	6.1
8% Ether-n-Hexane	719- 820	2.509	5.1
Ether (1)	821- 825	0.783	1.6
(2)	826- 831	1.526	3.1
(3)	832- 850	0.576	1.2
(4)	851- 899	0.655	1.3
2% Ethanol-Ether	900- 939	0.197	0.4
8% Ethanol-Ether	940- 960	0.501	1.0
Ethanol	961- 991	1.275	2.6
8% HCl-Ethanol	992-1025	0.957	2.0
	Total	47.977	98.1
	Residue	0.863	1.8

hexane, 1,000 ml of 10% benzene in n-hexane, 1,900 ml of 20% benzen in n-hexane, 1,900 ml of 30% benzene in n-hexane, 2,300 ml of 2% ethyl ether in n-hexane, 27,900 ml of 5% ethyl ether in n-hexane, 6,000 ml of 8% ethyl ether in n-hexane, 6,000 ml of ethyl ether, 2,500 ml of 2% ethanol in ethyl ether, 2,300 ml of 8% ethanol in ethyl ether, 2.500 ml of ethanol and 3,135 ml of 8% HCl in ethanol. The operation was carried out in a current of N_2. Yields of fractions separated with each solvent are shown in Table 52 and the chromatogram in Fig. 12.

2.3.3.2.3 Analyses of each fraction

(A) Eluate with n-hexane,
yield 0.960 g (2.0% of unsaponifiable matter)

This fraction was a colorless and somewhat viscous liquid, having n_D^{20} 1.4390, I.V. 3.3, not dissolved in methanol, and did not solidify after leaving at -15°C for a day. This fraction dissolved in hexane was purified by rechromatography with thirty times weight of silicic acid. The purified fraction was identified with pristane on gas-liquid chromatography by comparing with standard pristane. The retention time of sample was 0.55 min. in accordance with that of standard pristane under the operating conditions shown below.

Operating conditions of gas-liquid chromatography:
Instrument....Yanagimoto Gas Chromatograph GCG-2
Column....Ethyleneglycol succinate polyester on diasolid M, 1/10, w/w. 5 mm i.d. × 2 m, 210°C
Detector....TCD, bridge current 170 mA, recorder sens. 2 mV
Carrier gas....He, 60 ml/min
Pressure of column inlet....1.2 kg/cm²
Chart speed....10 mm/min
Sample size....1 μl

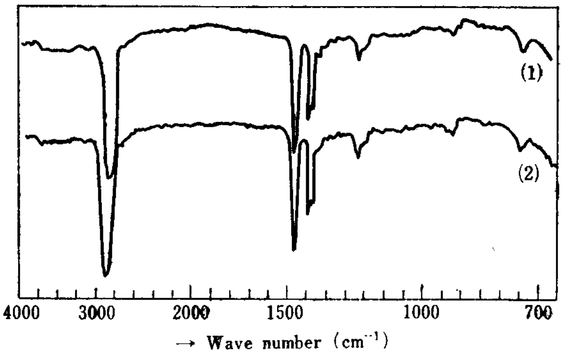

Fig. 13. Infrared spectra of pristane
1: Pristane standard
2: Colorless liquid obtained from eluate with n-hexane on chromatography of unsaponifiable matter

Moreover, infrared spectrum of this fraction was measured with the results shown in Fig. 13. This fraction showed bands by the isopropyl splitting at 1380 cm^{-1} and 1365 cm^{-1}, and by the skeletal vibrations of the isopropyl groups at 1170 cm^{-1} with a shoulder at 1145 cm^{-1} the same as standard pristane, and was confirmed as pristane.*

(B) Eluate with 5% ethyl ether in n-hexane and 8% ethyl ether in n-hexane, yield 40.106 g (82.0% of unsaponifiable matter)

The properties of each fraction of this eluate are shown in Table 53. Each fraction is considered as fatty alcohol. The acetylated products of the eluate with 5% ethyl ether in n-hexane (2), (3) and (4) were put together, freed by saponifica-

Table 53. Characteristics of eluates with 5% and 8% ether-n-hexane (*Calanus plumchrus*)

Fraction	State	OH-group**	L.B.***	Acetate	
				S.V.	I.V.
Eluate with 5% ether-n-hexane (1)	Liquid	+	—	200.3	62.9
(2)	Solid	+	—	165.6	70.4
(3)	Solid	+	—	178.4	54.4
(4)	Liquid	+	—	197.5	67.5
Eluate with 8% ether-n-hexane	Liquid	+	—	202.7	98.3

** Oxine-vanadate test　　*** Liebermann-Burchard reaction

Table 54. Fractional distillation of eluates with 5% ether-n-hexane (2), (3) and (4) (*Calanus plumchrus*)

Fraction No.	Distillation temp. °C	Pressure (mmHg)	Yield (g)	n_D^{20}	S.V.	I.V.
1	-150	2	0.89	1.4444	196.7	21.8
2	150-155	〃	1.70	1.4432	203.0	24.7
3	155-160	〃	1.56	1.4440	202.1	27.8
4	160-165	〃	1.37	1.4470	190.4	31.4
5	165-170	〃	8.16	1.4525	172.4	72.1
6	170-175	〃	3.54	1.4532	171.3	73.8
7	175-180	〃	5.93	1.4535	171.1	74.4
8	180-185	〃	3.01	1.4540	167.1	71.9
9	185-190	〃	5.73	1.4548	162.5	69.7
10	-160	1	1.23	1.4550	158.2	69.6
11	〃	<1	1.84	1.4554	158.8	70.0
12	〃	<1	0.60	1.4575	157.9	76.5
13	Residue		0.87	1.4703	148.5	85.0
	Loss		0.87			

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* Pristane can by distinguished by the splitting of the 1380 cm^{-1} band of deformation frequencies into two components, one at 1383 cm^{-1} and the other at 1368 cm^{-1} ("isopropyl splitting"), and by the band at 1170 cm^{-1} with a shoulder at about 1150 cm^{-1}, due to skeletal vibrations of the isopropyl groups.27).

Table 55. Redistillation of fractions 5, 6 and 7 (*Calanus plumchrus*)

Fraction No.	Distillation temp. °C	Pressure (mmHg)	Yield (g)	n_D^{20}	S.V.	I.V.
14	-168	2	0.96	1.4508	185.0	64.3
15	168-171	〃	3.74	1.4524	171.7	—
16	171-172	〃	1.39	1.4531	171.3	—
17	172-176	〃	5.79	1.4532	170.4	—
18	176-	〃	1.30	1.4533	167.7	—
19	Residue		1.46	1.4542	158.7	—
	Loss		0.08			

Table 56. Redistillation of fraction 9 (*Calanus plumchrus*)

Eraction No.	Distillation temp. °C	Pressure (mmHg)	Yield (g)	n_D^{20}	S.V.
20	-176	1.5	1.84	1.4545	163.5
21	176-181	〃	1.83	1.4548	161.6
22	181-	〃	1.06	1.4551	161.5

tion followed by re-acetylation. The re-acetylated product, having n_D^{20} 1.4525, S.V. 171.9, I.V. 65.9, 37.30 g of which were fractionally distilled by Widmer spiral with the results shown in Table 54. Fractions 5, 6 and 7 were put together, 14.72 g of which were redistilled fractionally with the results shown in Table 55. Fraction 9 (4.77 g) was also re-fractionated with the results shown in Table 56. Among the above fractions, the fractions closely resembling the saponification value were put together. Hereupon, fractions 1, 2 and 3 were put together into fraction a (3.2 g). Fractions 15, 16 and 17 were put together into fraction b (9.0 g). Fractions 8 and 18 were put together into fraction c (3.6 g). Fractions 20, 21 and 22 were put together into fraction d (4.5 g). Fractions 10, 11, 12 and 19 were put together into fraction e (3.7 g). These fractions (a, b, c, d, e) were saponified to yield free alcohols respectively, to which tenfold 90% acetone were added, leaving -5°C for a day. The crystals coming out of 90% acetone were separated from the mother liquor by filtration, and the separated crystals were examined by recrystallization about the saturated alcohol components contained. The acetone was distilled out from the filtrate, then the residues dissolved in tenfold ethyl ether were brominated at -20°C. The ethyl ether-insoluble bromide yielded only with fraction e in a very small amount was removed by filtration, the ethyl ether was distilled out of the ethyl ether solution of bromide. Residual bromides now dissolved in tenfold petroleum ether were allowed to stand at -5°C for a day, then the petroleum ether-insoluble bromide was crystallized out for fractions c, d and e. They were separated from the mother liquor by filtration, from which petroleum ether-soluble bromide were obtained. De-bromination of petroleum ether-insoluble and soluble bromides gave free alcohols. In this, (I) alcohols coming out of 90% acetone solutions by leaving -5°C for a day, (II) alcohols freed from petroleum ether-insoluble bromides by de-bromination, and (III) alcohols freed from petroleum ether-soluble bromides by de-bromination, were further examined.

(I) Alcohols coming out of 90% acetone solutions by leaving at -5°C for a day

a-I: The crystal of mp 49.5–49.7°C was obtained by recrystallization, which was identified with hexadecanol by the mixed melting point test. The infrared spectrum of this crystal showed eight bands in progression series between 1350 cm^{-1} and 1180 cm^{-1}, consequently this crystal was confirmed as hexadecanol.[75)]

b-I: The crystal of mp 61–64°C was obtained by recrystallization, which was considered as the mixture of octadecanol and eicosanol.

c-I and de-I (unity of d-I and e-I): A small amount of obtained crystals were washed with petroleum ether lightly. The infrared spectrum of the crystal showed ten bands in progression series between 1350 cm^{-1} and 1180 cm^{-1}, consequently this crystal probably contains eicosanol. (see Fig. 14 and Fig. 15)

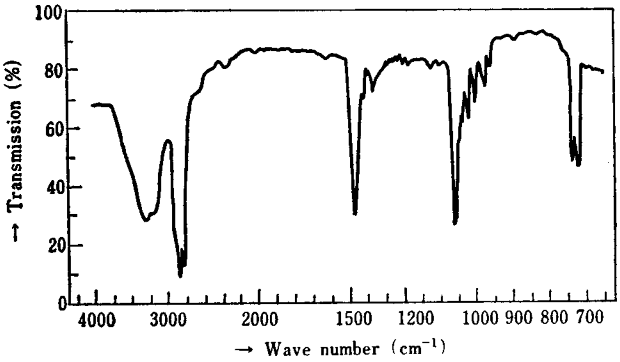


Fig. 14. Infrared spectrum of n-octadecanol in KBr

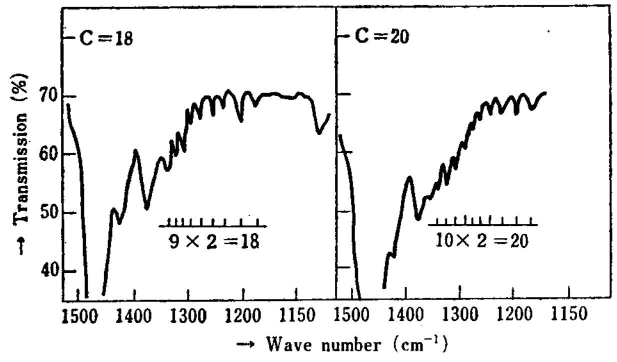


Fig. 15. Infrared spectra of normal aliphatic alcohols in KBr

Table 57. Characteristics of acetate of (II) and (III) (*Calanus plumchrus*)

Fraction	II		III	
	S.V.	I.V.	S.V.	I.V.
a	Not yielded		196.6	65.5
b	Not yielded		178.6	73.0
c	173.8	72.3	180.9	72.2
d	166.5	69.8	167.6	69.0
e	164.4	70.3	166.2	68.0

Table 58. Fractional distillation of b-III acetate (*Calanus plumchrus*)

Fraction No.	Distillation temp. °C	Pressure (mmHg)	Yield (g)	n_D^{20}	S.V.
b-III-1	-145	1	0.710	1.4538	176.1
b-III-2	145-148	〃	1.624	1.4545	175.4
b-III-3	148	〃	0.686	1.4558	175.2
b-III-R	Residue		0.881	1.4570	170.4
	Loss		0.005		

(II) Alcohols freed from petroleum ether-insoluble bromides by de-bromination and,

(III) Alcohols freed from petroleum ether-soluble bromides by de-bromination

The acetylated products prepared from (II) and (III) had S.V. and I.V. as shown in Table 57. There is little difference between (II) and (III) as to their saponification value and iodine value and the presence of the alcohols containing two or more double bonds are presumed to be in a small amount.

a-III: A crystal of mp 50.5–52.2°C was obtained from the hydrogenated product of free alcohols. Consequently the alcohols before hydrogenation were considered to contain hexadecenol and octadecenol.

b-III: The acetylated products (3.906 g) of the alcohol were fractionally redistilled with the results shown in Table 58. The hydrogenated product prepared from free alcohols obtained by saponification of b-III-2 gave a crystal of mp 64.9–65.3°C by recrystallization. This crystal was identified with eicosanol by the mixed melting point test. The infrared spectrum of the crystal showed ten bands in progression series between 1350 cm^{-1} and 1180 cm.$^{-1}$ Consequently the alcohol before hydrogenation was confirmed as eicosenol.

e-III: The hydrogenated product prepared from free alcohols gave a crystal of mp 69.5–70.3°C by recrystallization. This fraction was considered to contain docosenol.

(C) Eluate with ethyl ether (1), (2), (3) and (4),
yield 3.540 g (7.2% of unsaponifiable matter)

The results of Liebermann-Burchard reaction and sterol content (digitonide

Table 59. Characteristics of eluate with ether (*Calanus plumchrus*)

Fraction	Liebermann-Burchard reaction	Sterol content (%)
1	++	9.9
2	+++	35.6
3	++	9.3
4	+	7.4

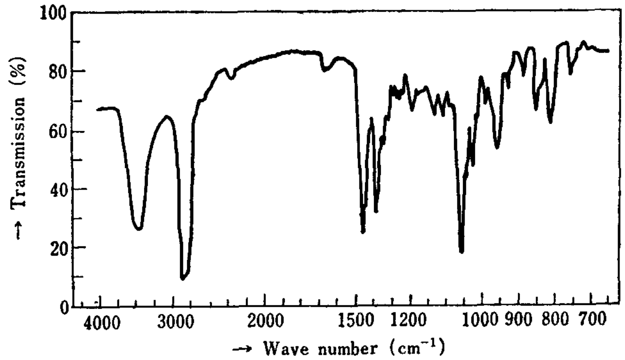


Fig. 16. Infrared spectrum of sterol in KBr

Table 60. Characteristics of the residue sterol-removed from eluate with ether (*Calanus plumchrus*)

Fraction	n_D^{25}	Acetate	
		S.V.	I.V.
1	1.4733	235.2	141.2
2	1.4802	241.1	150.1
3	1.4850	329.7	69.4
4	1.4880	321.4	49.4

method) of this fraction are shown in Table 59. Free sterol recovered from the digitonide of each fraction, was recrystallized from methanol, yielding a crystal of mp 146.5–147°C. This crystal was identified with cholesterol by the mixed melting point test. The infrared spectrum of the crystal was measured with the results shown in Fig. 16. The crystal showed a pair of bands at 850–790 cm^{-1} due to δ C-H of $R_1R_2C{=}CHR_3$ the same as the bands of standard cholesterol, and was confirmed as cholesterol. The residues removed of sterol from each fraction of eluate with ethyl ether had the properties shown in Table 60. The presence of glyceryl ether in fractions 1 and 2 was considered.

(D) Eluate with 2% ethanol in ethyl ether, 8% ethanol in ethyl ether, ethanol and 8% HCl in ethanol,

Table 61. Characteristics of eluates with ethanol-ether, ethanol and HCl-ethanol (*Calanus plumchrus*)

Fraction	n_D^{25}	Acetate	
		S.V.	I.V.
Eluate with ethanol-ether	1.5092	374.0	72.5
Eluate with ethanol	1.5154	452.9	66.3
Eluate with HCl-ethanol	1.4748	236.1	72.2

yield 2.930 g (6.0% of unsaponifiable matter)

The properites of eluate with ethanol in ethyl ether, ethanol and HCl in ethanol are shown in Table 61. This fraction probably contained unknown polar substances.

2.3.3.2.4 Summary of the results

(1) The unsaponifiable matter content in the lipids was 47.7% (average).

(2) The unsaponifiable matter was a reddish brown liquid having n_D^{25} 1.4620, I.V. 98.7.

(3) The unsaponifiable matter contained ca. 80% of alcohol, ca. 2% of pristane and ca. 2% of sterol. The alcohols consisted of saturated alcohols (hexadecanol predominantly, octadecanol and eicosanol) and lower unsaturated alcohols (hexadecenol, octadecenol, eicosenol and docosenol). Highly unsaturated alcohols were contained in a small amount or not at all. Sterol consisted chiefly of cholesterol. A small amount of glyceryl ether was present.

2.3.3.3 *Neomysis nakazawai*

2.3.3.3.1 Material

Sun-dried products of the plankton caught at Funka Bay, Hokkaido, in summer 1961, were obtained by the courtesty of Yakumo Fisherman's Union, Hokkaido. The sun-dried products (14,900 g), after drying at 80°C for one hour, were extracted with ether, giving 1,046 g of ether extract (7.0% of sundried product). The ether extract was then treated with tenfold acetone, once the acetone-insoluble matter was removed, an acetone-soluble lipid (96.1% of ether extract) was obtained which had d_{25}^{25} 0.9617, n_D^{25} 1.4870, S.V. 170.3, I.V. 174.5, A.V. 136.6, unsaponifiable matter 11.91%. The acetone-soluble lipid (920 g) was saponified, and the unsaponifialbe matter (109.5 g) was obtained in the usual way. The unsaponifialbe matter was a yellowish solid at ordinary temperature: I.V. 95.3, sterol content (digitonide method) 61.2%.

2.3.3.3.2 Removal of sterol from unsaponifiable matter

After the unsaponifiable matter (106.8 g) dissolved in thirtyfold methanol was allowed to stand at room temperature for a day, the crystal came out from the solution which was removed by filtration. This procedure was repeated more than two times, then the original unsaponifiable matter was divided into two portions, one of them was a portion of light yellow crude sterol (54.8 g, 51.3%) and the other one was a portion of a yellowish brown liquid from which crude sterol was removed (52.0 g, 48.7%).

2.3.3.3.3 Column chromatography of the liquid portion of unsaponifiable matter

The liquid portion of unsaponifiable matter (52.0 g) dissolved in 2,000 ml of n-hexane was analyzed by column chromatography.

Adsorbent: Mixture of silicic acid (Mallinckrodt, 100 mesh) and celite 545 (mixing ratio 2:1), 1,300 g (25 times weight of unsaponifiable matter). Used after drying at 100°C for five hours and allowing to stand for three days in the desiccator with silica gel.

Column: 6.8 cm i.d. × 108 cm

One fraction: 200 ml

Flow rate: 1 fraction/ 50 min.

The liquid part of unsaponifialbe matter dissolved in n-hexane and loaded on the column was eluted in turn with 6,000 ml of n-hexane, 6,000 ml of 10% benzene in n-hexane, 5,000 ml of 20% benzene in n-hexane, 5,000 ml of 30% benzene in n-hexane, 4,000 ml of 2% ethyl ether in n-hexane, 30,000 ml of 5% ethyl ether in n-hexane, 10,000 ml of ethyl ether, 5,500 ml of 2% ethanol in ethyl ether, 11,000 ml of 8% ethanol in ethyl ether, 7,000 ml of ethanol, and 6,000 ml of 8% HCl in ethanol.

The operation was carried out in a current of N_2. The yields of fractions

Table 62. Chromatography of liquid portion of unsaponifiable matter (*Neomysis nakazawai*)

Solvent	Fraction No.	n_D^{25}	Color and state	Yield		L.B.*
				(g)	(%)	
(A) n-Hexane	10- 29	1.4629	Colorless, liquid	0.761	1.46	
(B) 10% Benzene-n-Hexane	30- 56		Yellow, solid	0.330	0.63	
(C) 20% Benzene-n-Hexane	57- 80	1.5023	Light yellowish brown, liquid	0.068	0.13	
(D) 30% Benzen-n-Hexane	81- 96	1.4990	〃 semi-solid	0.099	0.19	
(E) 2% Ether-n-Hexane	97-108	1.5000	〃 liquid	0.248	0.48	
(F) 5% Ether-n-Hexane (1)	126	1.5063	〃 liquid	0.286	0.55	
(2)	127-139		〃 solid	5.351	10.29	−
(3)	140-173	1.4693	〃 liquid	8.185	15.74	−
(4)	174-191	1.4961	Yellowish brown, liquid	0.987	1.90	+
(5)	192-256		〃 solid	8.710	16.75	++
(6)	257-285	1.5140	Yellow, viscous liquid	0.572	1.10	+
(G) Ether (1)	286-293		Blackish brown, solid	15.379	29.58	−
(2)	294-303	1.5073	〃 viscous liquid	2.262	4.35	−
(3)	304-334		〃 solid	1.590	3.06	−
(H) 2% Ethanol-Ether	335-353		〃 〃	0.391	0.75	
(I) 8% Ethanol-Ether	354-395		〃 〃	1.798	3.46	
(J) Ethanol	396-426		〃 〃	2.634	5.07	
(K) 8% HCl-Ethanol	427-452		〃 〃	1.481	2.85	

* Liebermann-Burchard reaction

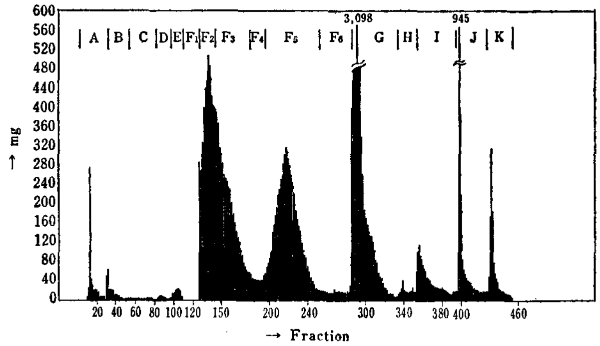


Fig. 17. Chromatogram of liquid portion of unspaonifiable matter (*Neomysis nakazawaki*) A: n-Hexane, B: 10% Benzene-n-Hexane, C: 20% Benzene-n-Hexane, D: 30% Benzene-n-Hexane, E: 2% Ether-n-Hexane, F: 5% Ether-n-Hexane, G: Ether, H: 2% Ethanol-Ether, I: 8% Ethanol-Ether, J: Ethanol, K: 8% HCl-Ethanol

separated with each solvent appear in Table 62, and the chromatogram is shown in Fig. 17.

2.3.3.3.4 Analyses of each fraction

Portion of crude sterol

The crystal obtained by treating the original unsaponifiable matter with methanol was recrystallized repeatedly from methanol, yielding a crystal of mp 145.5–146.6°C (mp of acetate: 112.5–113.5°C). The infrared spectrum of the crystal showed a pair of bands at 850–790 cm^{-1} due to δC-H of $R_1R_2C{=}CHR_3$ the same as those of standard cholesterol. This crystal was identified with cholesterol.

Eluate with 5% ethyl ether in n-hexane (2) and (3)

(designating F–2 and F–3 respectively)

F–2 Yield 5.551 g (10.3% of liquid part of unsap. matter)

F–3 Yield 8.185 g (15.7% of liquid part of unsap. matter)

F–2 and F–3 were both Liebermann-Burchard reactions—, oxine-vanadate test +, and yielded no separated product from the acetylated product of these fractions.

The acetylated products of F–2 and F–3 had S.V. 172.0, 191.7, and I.V. 59.5, 126.9 respectively. Both fractions were considered to be composed chiefly of alcohols.

Eluate with 5% ethyl ether in n-hexane (4), (5) and (6)

(designating F–4, F–5 and F–6 respectively)

F–4 Yield 0.987 g (1.9% of liquid part of unsap. matter)

F–5 Yield 8.710 g (16.8% of liquid part of unsap, matter)

F–6 Yield 0.572 g (1.1% of liqiud part of unsap. matter)

F–4, F–5 and F–6 were all Liebermann-Burchard reactions +, and the sterol contents (digitonide method) of each fraction were 2.8%, 90.7% and 27.2% respectively.

The free sterol recovered from digitonide of F–5, was recrystallized from methanol, yielding a crystal of mp 145.5–146.0°C. This crystal was identified with cholesterol by the mixed melting point test and infrared spectrum.

Eluate with ethyl ether (1), (2) and (3)

(designating G–1, G–2 and G–3 respectively)

G–1 Yield 15.379g (29.6% of liquid part of unsap. matter)

G–2 Yield 2.262g (4.4% of liquid part of unsap. matter)

G–3 Yield 1.590g (3.1% of liquid part of unsap. matter)

G–1, G–2 and G–3 were all Liebermann-Burchard reactions –. The acetylated product prepared from G–1 was saponified, and an unsaponifiable portion was recovered, which was recrystallized from petroleum ether, yiedling a crystal of mp 60.5–61.5°C. This crystal was identified with chimyl alcohol (mp 60.5–61.5°C) by the mixed melting point test.

2.3.3.3.5 Summary of the results

(1) The unsaponifiable matter content in the lipids was 11.9%.

(2) The unsaponifiable matter was a yellowish brown solid,having I.V. 95.3.

(3) The unsaponifiable matter contained ca. 1.3% of alcohol, ca. 60% of sterol (composed chiefly of cholesterol), and probably 15% of glyceryl ether.

2.3.3.4 *Acanthomysis sp.*

2.3.3.4.1 Material

The acetone-soluble lipids obtained from the plankton caught at Ariake Sea, Nagasaki Prefecture (Sample No. 28 in Table 18 and Table 19) were used for the present study.

The acetone-soluble lipids (140.7 g) were saponified, and the unsaponifiable matter (35.0g, 24.9% of acetone-soluble lipid) was obtained in the usual way.

The unsaponifiable matter was a reddish brown solid: I.V. 88.3, sterol content (digitonide method) 67.6%.

2.3.3.4.2 Removal of sterol from unsaponifiable matter

After the unsaponifiable matter (32.038g) was dissolved in 500 ml of methanol, it was allowed to stand at room temperature for a day, then the crystal came out from the solution which was removed by filtration. This procedure was repeated once more (methanol 100 ml), then the original unsaponifiable matter was divided into two portions, one was a portion of crude sterol (19.177g, 59.9%) and the other one was a portion of a deep reddish viscous liquid (12.699g, 39.6%).

2.3.3.4.3 Alkali column chromatography of the liquid portion of unsaponifiable matter

The liquid portion of unsaponifiable matter contained 4.2% of free fatty acid. An alkali column chromatography using silicic acid impregnated with KOH

Table 63. Alkali column chromatography of liquid portion of unsaponifiable matter (*Acanthomysis sp.*)

Adsorbent:	Silica gel (Mallinckrodt, 100 mesh) 381 g (30 times the sample weight) impregnated with 38 g KOH/760 ml iso-propanol
Column :	66.5 cm × 6.2 cm i.d.
Flow rate:	16 ml/min

Fraction	Solvent	ml	Yield		Ratio to total unsap. matter (%)
			(g)	(%)	
Purified unsap. matter	Ether	6750	9.936	78.2	31.0
Acidic substances	2% Formic acid-ether Ether	2500 3500	1.257	9.9	3.9
Adsorbed substances			1.507	11.9	4.7

was carried out with the liquid portion of unsaponifiable matter to remove the free fatty acid according to the method of McCarthy and Duthie,[76] and that portion removed free from the fatty acid was obtained through eluate with ether.

The operating conditions of chromatography and the results of chromatography are shown in Table 63.

2.3.3.4.4 Column chromatography of the purified liquid portion of unsaponifiable matter

The purified liquid portion of unsaponifiable matter thus obtained (8 g),

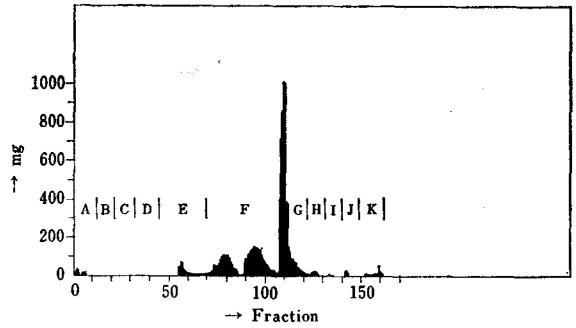


Fig. 18. Chromatogram of liquid portion of unsaponifiable matter (*Acanthomysis sp.*)
A: n-Hexane, B: 5% Benzene-n-Hexane, C: 10% Benzene-n-Hexane, D: 30% Benzene-n-Hexane, E: 5% Ether-n-Hexane, F: 10% Ether-n-Hexane, G: Ether, H: 2% Ethanol-n-Hexane, I: 8% Ethanol-Ether, J: Ethanol, K: Methanol

Operating conditions:

Adsrobent . . Silicic acid: Celite 545 (mixing ratio 2:1), fortyfold sample weight. Used after decantation with n-hexane, benzene, ether, ethanol and methanol, and allowing the mxiture to air-dry, acitivated at 100°C for five hours.

Column . . . . 66.5cm × 6.2 cm i.d.

1 fraction . . 150 ml

Flow rate . . 1 fraction/20–23 min

Table 64. Chromatography of liquid portion of unsaponifiable matter (*Acanthomysis sp.*)

Solvent	Fraction No.	Color and state	Yield (mg)	Yield (%)	Ratio to total unsap. matter (%)
n-Hexane	1~12	White, semi-solid	102	1.3	0.4
5% Benzene-n-Hexane	13~22	Light yellow, liquid	38	0.5	0.2
10% Benzene-n-Hexane	23~32	〃 〃	14	0.2	0.1
30% Benzene-n-Hexane	33~45	〃 sem-solid	18	0.2	0.1
5% Ether-n-Hexane (1)	46~55	〃 liquid	7	0.1	—
(2)	56~69	Yellowish brown, liquid	310	3.9	1.3
10% Ether-n-Hexane (1)	70~71	〃 semi-solid	37	0.5	0.2
(2)	72~77	〃 〃	316	4.0	1.3
(3)	78~87	〃 liquid	776	9.7	3.3
(4)	88	Light yellow, sem-solid	22	0.3	0.1
(5)	89~107	〃 solid	1,777	22.2	7.5
Ether (1)	108~109	Dark red, liquid	1,109	13.9	4.7
(2)	110~115	Red, semi-solid	2,626	32.8	11.1
(3)	116~122	〃 liquid	281	3.5	1.2
2% Ethanol-Ether	123~131	Dark red, liquid	105	1.3	0.4
8% Ethanol-Ether	132~140	Brown, liquid	43	0.5	0.2
Ethanol	141~149	〃 〃	69	0.9	0.3
Methanol	150~162	Reddish brown, semi-solid	181	2.3	0.8
	Residue		169	2.1	0.7
	Total		8,000	100.2	33.9

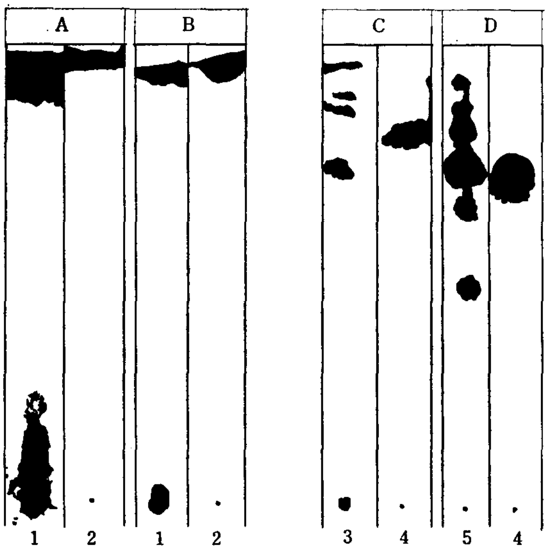


Fig. 19. Thin-layer chromatograms of fractions of liquid portion of unsaponifiable matter (1) (*Acanthomysis sp.*)
Solvent: plate A, petroleum ether (40–60°C); plate B, benzene; plate C, petroleum ether-ether, 9:1 (V/V); plate D, benzene.
Samples: 1, 1–12 fraction; 2, squalane; 3, 56–69 fraction; 4, undecylmethyketone; 5, 56–69 fraction (re-chromatography)

Table 65. TLC conditions of main fractions (*Acanthomysis sp.*)

Adsorbent : Wako gel B-5
Activation : 110°C, 45 min
Distance : 12 cm
Thickness : 250 μ
Solvent : Petroleum ether (bp 40–60°C), Benzene,
Petroleum ether-Ether (9:1, 7:3 and 4:6)

Observed blackish spots obtained by charring at 180°C after spraying with $K_2Cr_2O_7$-H_2SO_4.

Fraction	Standard	Solvent
Eluate with n-hexane	Squalane	Petroleum ether, Benzene
Eluate with 5% ether-n-hexane (2)	Undecylmethylketone	Petroleum ether-Ether (9:1)
Eluate with 5% ether-n-hexane (2) (rechromatography)*	Undecylmethylketone	Benzene
Eluate with 10% ether-n-hexane (2)	Stearyl alcohol	Petroleum ether-Ether (9:1), Benzene
Eluate with 10% ether-n-hexane (3)	Stearyl alcohol	Petroleum ether-Ether (9:1), Benzene
Eluate with 10% ether-n-hexane (5)	Cholesterol	Petroleum ether-Ether (9:1), Benzene
Eluate with ether (1)	Chimyl alcohol	Petroleum ether-Ether (9:1) and (7:3)
〃 (2)	Chimyl alcohol	Petroleum ether-Ether (9:1), and (7:3)
〃 (3)	Chimyl alcohol	Petroleum ether-Ether (7:3)
Eluate with ether (2) (rechromatography)**	Chimyl alcohol	Petroleum ether-Ether (4:6)

* Rechromatographic conditions of eluate with 5% ether-n-hexane (2)
Adsorbent: silica gel (30 times the sample weight)
Column: 30 cm × 1.5 cm i.d.
Eluate with 5% ether-n-hexane was collected after eluting with n-hexane followed by 30% benzene-n-hexane.

** Rechromatographic conditions of eluate with ether (2)
Adsorbent: activated alumina (50 times the sample weight)
Column: 30 cm × 1.5 i.d.
Eluate with 10% methanol-methylene chloride was collected after eluting with methylene chloride.

once dissolved in 60 ml of n-hexane, was analyzed by column chromatography.

The operating conditions of chromatography and chromatogram obtained are shown in Fig. 18, and the yields and states of each fraction are shown in Table 64.

The main fractions among each fraction were eluate with n-hexane (fraction No. 1-12), eluate with 5% ether in n-hexane (2) (fraction No. 56–69), eluate with 10% ether in n-hexane (2), (3) and (5) (fraction No. 72–77, 78–87, and 89–107), eluate with ether (1), (2) and (3) (fraction No. 108–109, 110–115 and 116–122), eluate with 2% ethanol in ether (fraction No. 123–131) and eluate with methanol (fraction No. 150–162).

2.3.3.4.5 Thin-layer chromatography of the main fractions

A thin-layer chromatographic analysis of the main fractions was carried out to identify the main component of the fractions. The operating conditions of the analysis are shown in Table 65, and the chromatograms obtained are shown in

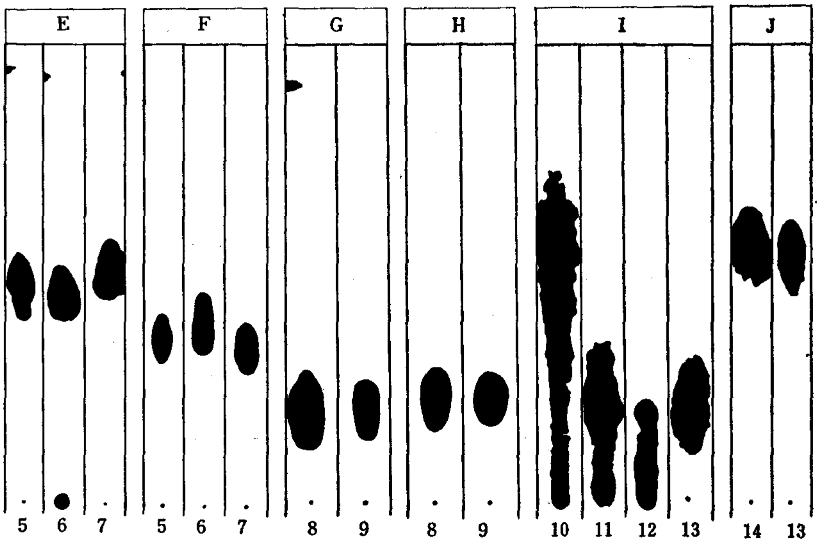


Fig. 20. Thin-layer chromatograms of fractions of liquid portion of unsaponifiable matter (2) (*Acanthomysis sp.*)
Solvent: plate E, petroleum ether-ether, 9:1 (V/V); plate F, benzene;
plate G, petroleum ether-ether, 9:1 (V/V): plate H, benzene;
plate I, petroleum ether-ether, 7:3 (v/v); plate J, petroleum ether-ether, 4:6 (v/v)
Samples: 5, 72–77 fraction; 6, 78–87 fraction; 7, stearyl alcohol; 8, 89–107 fraction; 9, cholesterol; 10, 108–109 fraction; 11, 110–115 fraction; 12, 116–122 fraction; 13, chimyl alcohol; 14, 110–115 fraction (re-chromatography)

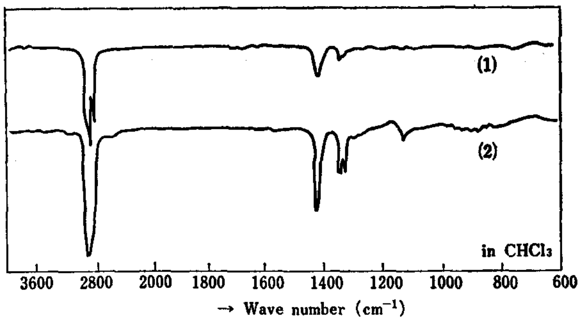


Fig. 21. Infrared spectra of pristane (*Acanthomysis sp.*)
1: Fraction 1–12 (re-chromatography) 2: Pristane standard

Fig. 19 and Fig. 20. As for the results the same value of Rf were obtained between eluate with n-hexane (fraction No. 1–12) and squalane, main component of eluate with 5% ether in n-hexane (2) (fraction No. 56—69) (rechromatography) and undecylmethylketone, eluate with 10% ether in n-hexane (2) and (3) (fraction No. 72–77 and No. 78–87) and stearyl alcohol, eluate with 10% ether in n-hexane (5) (fraction No. 89–107) and cholesterol, and eluate with ether (2) (fraction No.

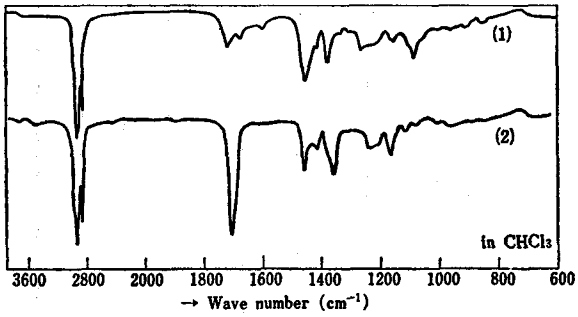


Fig. 22. Infrared spectra of undecylmethylketone (fraction 56–69) (*Acanthomysis sp.*)
1: Fraction 56–69 (rechromatography) 2: Undecylmethylketone standard

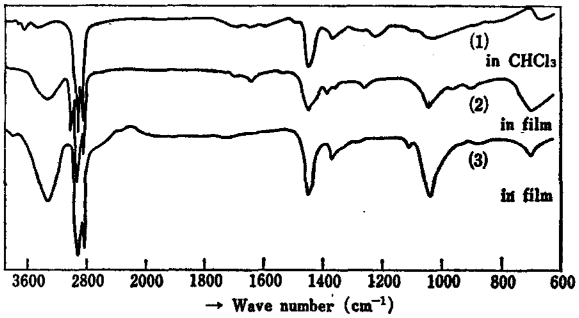


Fig. 23. Infrared spectra of lauryl alcohol (fraction 72–77) (*Acanthomysis sp.*)
1: Fraction 72–77 2: Fraction 78–87 3: Lauryl alcohol standard

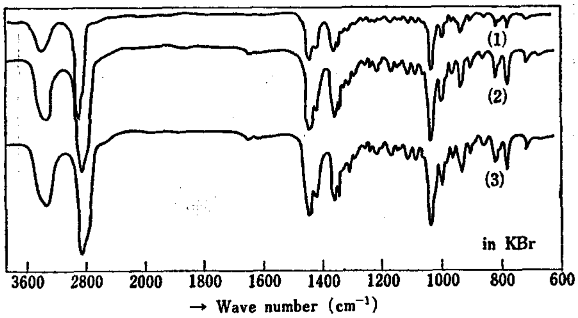


Fig. 24. Infrared spectra of cholesterol (fraction 89–107) (*Acanthomysis sp.*)
1: Fraction 89–107 2: Recrystallized product of crude sterol portion 3: Cholesterol standard

110–115) (rechromatography) and chimyl alcohol.

2.3.3.4.6 Infrared spectra of the main fractions

The infrared spectra of the main fractions are shown in Fig. 21 to Fig. 26.

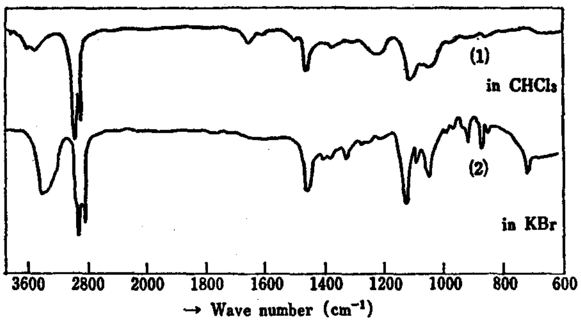


Fig. 25. Infrared spectra of chimyl alcohol (fraction 110–115) (*Acanthimysis sp.*)
1: Fraction 110–115 2: Chimyl alcohol standard

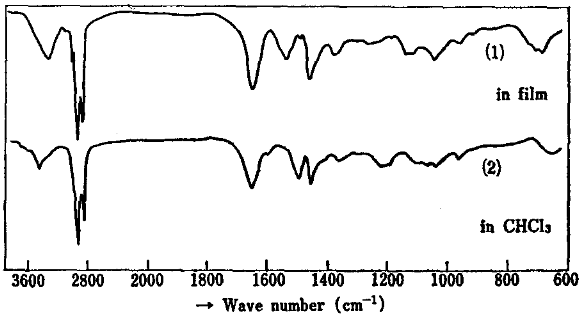


Fig. 26. Infrared spectra of fraction 116–122 (*Acanthomysis sp.*)
1: Fraction 108–109 2: Fraction 116–122

(1) 1–12 rechromatography fraction (eluate with n-hexane purified by rechromatography. Chromatographic conditions: Silicic acid; thirtyfold sample wt., activated at 100°C for 5 hrs, column; 30 cm × 1.5 cm i.d., n-hexane)

The same as in pristane, bands due to νasCH_2 of -CH_2- at 2925 cm^{-1}, νsCH of -CH_3 at 2870 cm^{-1}, δasCH_3 of -CH_3 at 1460 cm^{-1}, and δsCH_3 of -CH_3 at 1380 cm^{-1} were observed. Bands due to νOH and νC=O were not observed.

(2) 56–69 rechromatography fraction (eluate with 5% ether in n-hexane (2) purified by rechromatography and furher by thin-layer chromatography)

The same as in undecylmethylketone, band due to νC=O of -CO- at 1710 cm^{-1} (solvent: chloroform) was observed. Band due to νCH of $-C\begin{smallmatrix}\diagup O\\ \diagdown H\end{smallmatrix}$ at 2860–2700 cm^{-1} was not observed.

(3) 72–77 fraction (eluate with 10% ether in n-hexane (2)) and 78–87 fraction (eluate with 10% ehter in n-hexane (3))

The same as in lauryl alcohol, bands due to monomeric, dimeric or polymeric νOH was observed. Other bands resemble that of lauryl alcohol.

(4) 89–107 fraction (eluate with 10% ether in n-hexane (5)) and purified product of the porition of crude sterol (by bromination and debromination followed by repeated recrystallization from acetone and methanol)

The same as in cholesterol, a pair of bands due to δC-H of $R_1R_2C{=}CHR_3$ at 850–790 cm^{-1} were observed. Other bands agree with cholesterol.

(5) 110–115 fraction (eluate with ether (2))

The same as in chimyl alcohol, bands due to νOH, $\nu asCH_2$ at 2925 cm^{-1}, νsCH_2 at 2850 cm^{-1}, $\delta asCH_3$ at 1460 cm^{-1} and antisymmetric stretching vibration of C-O-C at 1150-1070 cm^{-1} were observed.

(6) 108–109 fraction (eluate with ether (1)) and 116–122 fraction (eluate with ether (3))

Table 66. Main components in main fractions (*Acanthyomysis sp.*)

Fraction	Component
Eluate with n-hexane (fraction 1–12)	Higher hydrocarbon
Eluate with 5% ether-n-hexane (2) (fraction 56–69)	Ketone
Eluate with 10% ether-n-hexane (2) (fraction 72–77)	Higher alcohol
Eluate with 10% ether-n-hexane (3) (fraction 78–87)	Higher alcohol
Eluate with 10% ether-n-hexane (5) (fraction 89–107)	Sterol
Eluate with ether (1) (fraction 108–109)*	Unidentified
〃 (2) (fraction 110–115)	Glyceryl ether
〃 (3) (fraction 116–122)*	Unidentified

* Infrared spectra of eluate with ether (1) and (3) resemble that of chimyl alcohol. In addition to this, band due to νC=C was observed. Eluate with ether (3) may be unsaturated glycerylether.

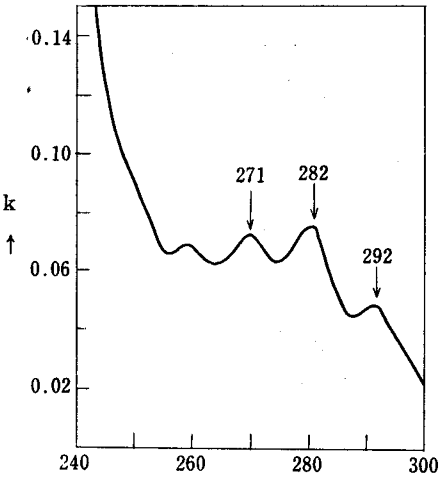


Fig. 27. Ultraviolet absorption of crude sterol portion (*Acanthomysis sp.*)

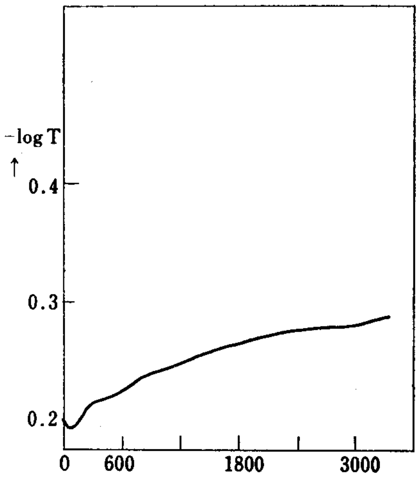


Fig. 28. Change of color as the time proceeded by Liebermann-Burchard reaction on crude sterol portion (*Acanthomysis sp.*)

Band due to $\nu C{=}C$ near 1650 cm^{-1} was observed. Other bands resemble that of chimyl alcohol.

2.3.3.4.7 Confirmation of main components in each fraction

In addition to the thin-layer chromatography and infrared spectroscopy, the formation of a red crystal by 2,4-dinitrophenylhydrazine reaction in eluate with 5% ether in n-hexane (2) (fraction No. 56–69) was observed, and in eluate with 10% ether in n-hexane (5) (fraction No. 89–107) the Liebermann-Burchard reaction was positive.

The main components of each main fraction were generalized from the results of thin-layer chromatography, infrared spectra and others as shown in Table 66.

2.3.3.4.8 Examination of the portion of crude sterol

The crystal coming out of the methanol solution of this portion leaving at room temperature was positive to the Liebermann-Burchard reaction and its ultraviolet absorption, as shown in Fig. 27, showed the presence of sterol of $\Delta^{5,7}$ series, but the content was very low (=0.20%). Moreover, a change of color by the Liebermann-Burchard reaction as the time proceeded as shown in Fig. 28, suggested that the sterol was composed chiefly of cholesterol. The purification of this crystal by bromination and debromination was followed by a repeated crystallization from acetone and methanol yielding a crystal of mp 147.0–148.0°C, mp of acetate 116.0–116.8°C. This crystal was identified with cholesterol by the mixed melting test and

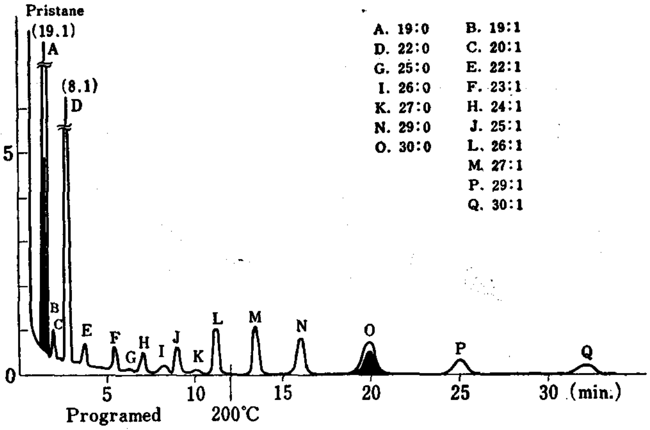


Fig. 29. Gas-liquid chromatogram of Fraction 1–12 (rechromatography) (*Acanthomysis sp.*)
Operating conditions:

Instrument	Yanagimoto GCG–5DH
Column..............	Diethylene glycol succinate polyester on Neosorb (80–100 mesh), 1/10wt/wt, 1.5m×3mm i.d., 200°C
Detector	FID, H_2 15 ml/min, Air 700 ml/min, Attenu. 1/8, Sens. 10^9
Carrier gas	He 0.8 kg/cm^2
Chart speed..........	10 mm/min
Sample size	0.6μl

Table 67. Composition of hydrocarbons of eluate with n-hexane (fraction 1–12) (re-chromatography) (*Acanthomysis sp.*)

Saturated hydrocarbon			Mono-unsaturated hydrocarbon		
Peak	Component	wt. %	Peak	Component	wt. %
A	19:0	29.8	B	19:1	1.2
D	22:0	15.2	C	20:1	trace
G	25:0	trace	E	22:1	1.5
I	26:0	15.4	F	23:1	1.7
K	27:0	trace	H	24:1	2.4
N	29:0	5.6	J	25:1	2.8
O	30:0	5.9	L	26:1	4.4
			M	27:1	5.3
			P	29:1	4.6
			Q	30:1	4.3
	Total	71.9		Total	28.2

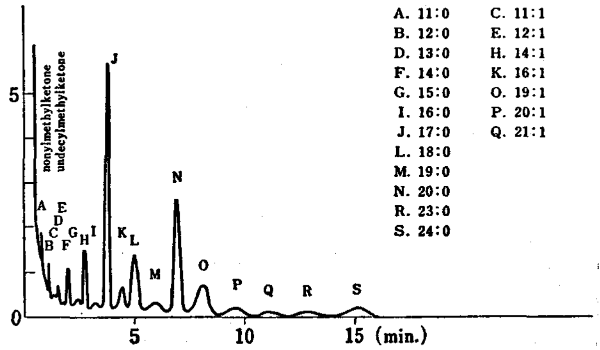


Fig. 30. Gas-liquid chromatogram of Fraction 56–69 (rechromatography, purified by TLC) (*Acanthomysis sp.*)

Operating conditions:

Instrument ....Yanagimoto GCG–5DH
Column ....Propylene glycol succinate polyester on Neosorb (60–80 mesh), 3/20wt/wt, 1.5m×3mm i.d., 200°C
Detector ....FID, H_2 15 ml/min, Air 600 ml/min, Attenu. 1/32, Sens. 10^9
Carrier gas ....He 0.7 kg/cm^2
Chart speed..........10 mm/min
Sample size..........1 μl

infrared spectrum.

2.3.3.4.9 Gas-liquid chromatography of main fractions

The gas-liquid chromatography was carried out on Yanagimoto Gas Chromatograph GCG-5DH (FID).

(A) Eluate with n-hexane (fraction No. 1–12)

This fraction, as described above, purified by rechromatography, was analyzed by gas-liquid chromatography. Pristane and squalane were used as internal

Table 68. Composition of ketones of eluate with 5% ether-n-hexane (2) (fraction 56–69) (re-chromatography) (*Acanthomysis sp.*)

Saturated ketone			Mono-unsaturated ketone		
Peak	Component	wt. %	Peak	Component	wt. %
A	11:0	4.3	C	11:1	trace
B	12:0	2.1	E	12:1	trace
D	13:0	1.6	H	14:1	6.8
F	14:0	2.6	K	16:1	3.9
G	15:0	1.5	O	19:1	10.4
I	16:0	1.3	P	20:1	4.6
J	17:0	35.4	Q	21:1	3.5
L	18:0	11.0			
M	19:0	3.4			
N	20:0	2.6			
R	23:0	1.5			
S	24:0	3.5			
	Total	70.8		Total	29.2

standards. The gas-liquid chromatogram is shown in Fig. 29. The composition of each component calculated from the peak areas is shown in Table 67.

(B) Eluate with 5% ether in n-hexane (2) (fraction No. 56–69)

This fraction, as described above, purified by rechromatography followed by thin-layer chromatography, was analyzed by gas-liquid chromatography. Nonylmethylketone and undecylmethylketone were used as internal standards. The gas-liquid chromatogram is shown in Fig. 30, and the composition of components calculated from the peak areas is shown in Table 68.

(C) Eluate with 10% ether in n-hexane (2) (fraction No. 72–77) and eluate with 10% ether in n-hexane (3) (fraction No. 78–87)

The acetates prepared from this fraction by heating and refluxing with acetic anhydride, purified by thin-layer chromatography, were analyzed by gas-liquid chromatography. The acetates of myristyl, palmityl, stearyl and oleyl alcohols were used as internal standards. Moreover, alcohol standards were also prepared by the reduction of identified methyl esters of mixed fatty acids obtained from the marine lipids with $LiAlH_4$.[77] The gas-liquid chromatograms obtained are shown in Fig. 31 and 32, and the compositions of each component are shown in Table 69 and Table 70. The composition of each component obtained by the generalization of both fractions is shown in Table 71.

(D) Eluate with ether (2) (fraction No. 110–115)

This fraction purified by rechromatography, as described above, was esterified by heating with acetic anhydride in boiling water for 12 hours using pyridine as assitant and the acetate thus obtained was analyzed by gas-liquid chromatography.

The acetates of chimyl and bathyl alcohols were used as internal standards.

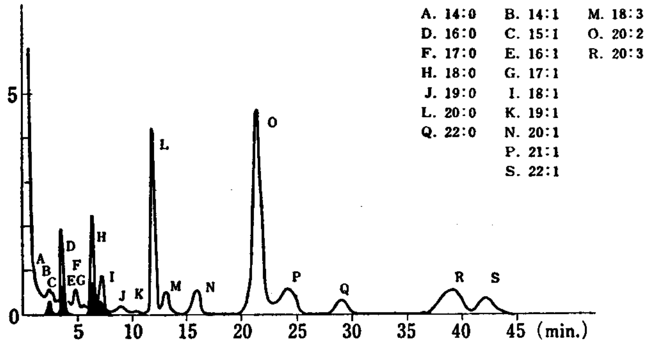


Fig. 31. Gas-liquid chromatogram of Fraction 72–77 (*Acanthomysis sp.*)

Operating conditions:

Instrument	Yanagimoto GCG–5DH
Column	Diethylene glycol succinate polyester on Neosorb (80–100 mesh), 1/10wt/wt, 1.5m×3mm i.d., 200°C
Detector	FID, H_2 15 ml/min, Air 600 ml/min, Attenu. 1/8, Sens. 10^9
Carrier gas	He 1.0 kg/cm^2
Chart speed	10 mm/min
Sample size	0.6 μl

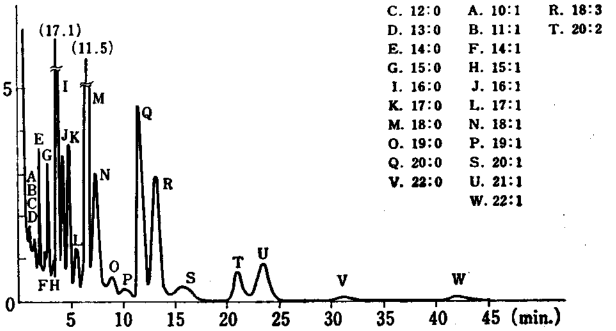


Fig. 32. Gas-liquid chromatogram of Fraction 78–87 (*Acanthomysis sp.*)
Operating conditions are the same as given in Fig. 31, sample size (0.8 μl) excluded.

The gas-liquid chromatogram obtained is shown in Fig. 33, and the composition of each component calculated from the peak areas is shown in Table 72.

2.3.3.4.10 Summary of the results

(1) The unsaponifiable matter content in the acetone-soluble lipid was 24.9%.

(2) The unsaponifiable matter contained ca. 74.0% of sterol, ca. 4.6% of alcohol, 11.1% of glycerylether, 1.3% of ketone and 0.4% of hydrocarbon.

(3) The sterol was composed chiefly of cholesterol. The main components of alcohol were 16:0, 18:0, 20:0 and 20:2. Saturated alcohol 45.8%, monoenoic

Table 69. Composition of alcohols of eluate with 10% ether-n-hexane (2) (fraction 72–77) (*Acanthomysis sp.*)

Saturated alcohol			Monoenoic alcohol			Polyenoic alcohol		
Peak	Component	wt. %	Peak	Component	wt. %	Peak	Component	wt. %
A	14:0	1.1	B	14:1	2.2	M	18:3	2.9
D	16:0	3.8	C	15:1	0.9	O	20:2	35.2
F	17:0	1.1	E	16:1	1.3	R	20:3	8.0
H	18:0	5.4	G	17:1	1.0			
J	19:0	0.8	I	18:1	2.2			
L	20:0	15.8	K	19:1	0.7			
Q	22:0	3.1	N	20:1	3.2			
			P	21:1	6.1			
			S	22:1	5.7			

Table 70. Composition of alcohols of eluate with 10% ether-n-hexane (3) (fraction 78–87) (*Acanthomysis sp.*)

Saturated alcohol			Monoenoic alcohol			Polyenoic alcohol		
Peak	Component	wt. %	Peak	Component	wt. %	Peak	Component	wt. %
C	12:0	0.5	A	10:1	0.5	R	18:3	7.9
D	13:0	0.2	B	11:1	0.1	T	20:2	3.2
E	14:0	2.9	F	14:1	2.9			
G	15:0	3.1	H	15:1	1.7			
I	16:0	16.0	J	16:1	4.7			
K	17:0	3.8	L	17:1	2.2			
M	18:0	17.1	N	18:1	6.4			
O	19:0	1.7	P	19:1	1.6			
Q	20:0	11.6	S	20:1	0.9			
V	22:0	3.9	U	21:1	5.9			
			W	22:1	1.4			

Table 71. Composition of alcohols of eluate with 10% ether-n-hexane (2) and (3) (fraction 72–87) (*Acanthomysis sp.*)

Saturated alcohol		Monoenoic alcohol		Polyenoic alcohol	
Component	wt. %	Component	wt. %	Component	wt. %
12:0	0.2	10:1	0.2	18:3	5.4
13:0	0.1	11:1	trace	20:2	19.2
14:0	2.0	14:1	2.5	20:3	4.0
15:0	1.5	15:1	1.3		
16:0	9.9	16:1	3.0		
17:0	2.4	17:1	1.6		
18:0	11.3	18:1	4.3		
19:0	1.2	19:1	1.1		
20:0	13.7	20:1	2.0		
22:0	3.5	21:1	6.2		
		22:1	3.5		
Total	45.8	Total	25.7	Total	28.6

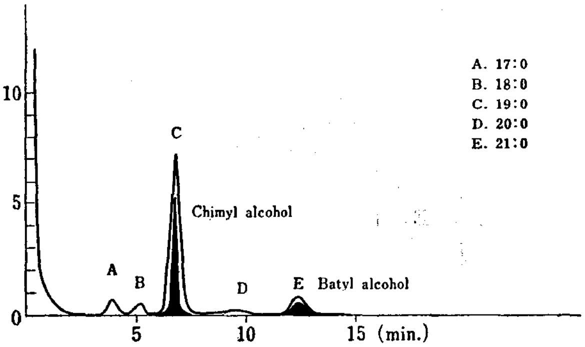


Fig. 33. Gas-liquid chromatogram of Fraction 110–115 (*Acanthomysis sp.*)
Operating conditions:
Instruments..........Yanagimoto GCG–5DH
Column..............SE–30 on Chromosorb W (60–80 mesh), 1/20wt/wt, 75 cm × 3mm i.d., 240°C
Detector ....FID, H_2 15 ml/min, Air 600 ml/min, Attenu. 1/4, Sens. 10^9
Carrier gas ....He 0.5 kg/cm^2
Chart speed..........10 mm/min
Sample size ....0.4 μl

Table 72. Composition of glycerylethers of eluate with ether (2) (fraction 110–115) (re-column chromatography) (*Acanthomysis sp.*)

Peak	Component (Saturated)	wt. %
A	17:0	5.4
B	18:0	4.8
C	19:0	77.2 (chimyl alcohol)
D	20:0	trace
E	21:0	12.7 (bathyl alcohol)

alcohol 25.7% and polyenoic alcohol 28.6%. Glyceryl-ether was composed chiefly of chimyl alcohol. Ketone was composed chiefly of methylketone. Hydrocarbon was composed of pristane 29.8%, squalane 5.9% and many other homologues.

2.3.3.5 *Neomysis intermedia*

2.3.3.5.1 Material

The salted material (ca. 30% of salt contained) of the plankton caught at Lake Kasumiga-ura, Ibaragi Prefecture in 1963, was used for the present study. From the salted material (30,900g) the lipids (377g, 1.22% of material) were obtained by Procedure C, as described before. The lipids were treated with tenfold acetone, then acetone-soluble lipids (95% of the lipid) were obtained. The acetone-soluble lipids (306 g) were saponified and the unsaponifiable matter (50.3 g, 16.4% of the acetone-soluble lipid) was obtained in the usual way. The unsaponifiable matter thus obtained was purified by the re-saponification procedure. The purified unsaponifiable matter was a reddish brown solid at the ordinary temperature, having I.V. 86.5, sterol content (digitonide method) 66.7%.

2.3.3.5.2. Removal of sterol from unsaponifiable matter.

The unsaponifiable matter was divided into two portions by the process described above: a portion of crude sterol (28.20g, 61.7%) and a portion of blackish brown viscous liquid removed of crude sterol (17.53g, 38.3%).

2.3.3.5.3 Column chromatography of liquid portion of unsaponifiable matter

A liquid portion of unsaponifiable matter (17.53g) dissolved in 700 ml of n-hexane was analyzed by column chromatography.

Adsorbent: Mixture of silicic acid (Mallinckrodt, 100 mesh) and celite 545 (mixing ratio 2:1), thirtyfold sample weight. Used after drying at 100°C for five hours and allowing to stand in the desiccator with silica gel for three days.

Column: 42.5 cm × 6.2 cm i.d.

One fraction: 120 ml.

Table 73. Chromatography of liquid portion of unsaponifiable matter (*Neomysis intermedia*)

Solvent		Fraction No.	Yield		%*	L.B.**
			(g)	(%)		
n-Hexane	(1)	8- 12	0.37	2.1	0.8	
	(2)	13- 26	0.08	0.5	0.2	
5% Benzene-n-Hexane		27- 37	0.17	1.0	0.4	
10% Benzene-n-Hexane		38- 53	0.21	1.2	0.5	
20% Benzene-n-Hexane		54- 80	0.14	0.8	0.3	
30% Benzene-n-Hexane		81-100	0.23	1.3	0.5	
2% Ether-n-Hexane		101-114	—	—	—	
5% Ether-n-Hexane	(1)	115-124	0.34	2.0	0.8	
	(2)	125-132	0.24	1.4	0.5	
	(3)	133-163	1.79	10.2	3.9	
	(4)	164-198	1.05	6.0	2.3	
10% Ether-n-Hexane	(1)	199-201	0.09	0.5	0.2	−
	(2)	202	0.06	0.3	0.1	+
	(3)	203-240	3.23	18.4	7.0	⧺
	(4)	241-260	0.31	1.8	0.7	+
Ether	(1)	261-264	2.36	13.5	5.2	−
	(2)	265-266	2.15	12.2	4.7	
	(3)	267-269	0.94	5.3	2.0	
	(4)	270-286	1.29	7.3	2.8	
	(5)	287-292	0.35	2.0	0.8	
	(6)	293-305	0.20	1.1	0.4	
2% Ethanol-Ether		306-326	0.44	2.5	1.0	
8% Ethanol-Ether		327-345	0.45	2.6	1.0	
Ethanol		346-357	0.34	1.9	0.7	
8% HCl-Ethanol		358-365	0.39	2.3	0.9	
		Residue	0.31	1.8	0.7	
		Total	17.53	100.0	38.3	

* Ratio to total unsaponifiable matter

** Liebermann-Burchard reaction

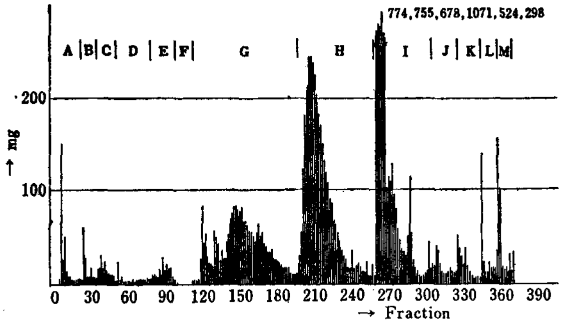


Fig. 34. Chromatogram of liquid portion of unsaponifiable matter (*Neomysis intermedia*) A: n-Hexane, B: 5% Benzene-n-Hexane, C: 10% Benzene-n-Hexane, D: 20% Benzene-n-Hexane, E: 30% Benzene-n-Hexane, F: 2% Ether-n-Hexane, G: 5% Ether-n-Hexane, H: 10% Ether-n-Hexane, I: Ether, J: 2% Ethanol-Ether, K: 8% Ethanol-Ether, L: Ethanol, M: 8% HCl-Ethanol

Flow rate: 1 fraction/ 30 min.

The liquid portion of unsaponifiable matter dissolved in n-hexane was loaded on the column and eluted with 1,500 ml of n-hexane, 2,000 ml of 5% benzene in n-hexane, 2,000 ml of 10% benzene in n-hexane, 3,000 ml of 20% benzene in n-hexane, 3,000 ml of 30% benzene in n-hexane, 3,000 ml of 2% ether in n-hexane, 10,000 ml of 5% ether in n-hexane, 8,000 ml of 10% ether in n-hexane, 6,000 ml of ether, 3,000 ml of 2% ethanol in ether, 3,000 ml of 8% ethanol in ether, 1,500 ml of ethanol and 3,000 ml of 8% HCl in ethanol.

The yields of each fraction and the chromatogram are shown in Table 73 and Fig. 34.

2.3.3.5.4 Analyses of each fraction

(A) Portion of crude sterol

The crude sterol was purified by the process described above, yielding a crystal of mp 147–148°C, which was identified with cholesterol by the mixed melting point test and infrared spectrum. An attempt to isolate any sterol other than cholesterol by silicic acid column chromatography and thin-layer chromatography was not successful.

(B) Eluate with n-hexane (1)

A colorless and semi-transparent solid, purified by silicic acid column chromatography, having I.V. 9.3 and not dissolve in methanol, was considered to be hydrocarbon. The sample was analyzed by gas-liquid chromatography, and the composition of each component was determined, as shown in Table 74.

(C) Eluate with 5% ether in n-hexane (3) and (4)

Fraction (3) had S.V. 165.2, I.V. 65.3 and fraction (4) had S.V. 219.8, I.V. 134.3. These fractions were considered to be alcohols in reference to the results of chromatograms about the unsaponifiable matter of the plankton lipid obtained

Table 74. Composition of hydrocarbons of eluate with n-hexane (*Neomysis intermedia*)

Saturated hydrocabron			Unsaturated hydrocarbon		
Peak	Component	wt. %	Peak	Component	wt. %
A	15:0	1.0	G	20:1	2.8
B	16:0	0.6	I	21:1	9.0
C	17:0	1.9	K	22:1	7.7
D	18:0	3.1	M	23:1	6.8
E	19:0 (Pristane)	9.3	P	25:1	3.8
F	20:0	6.7	S	27:1	2.9
H	21:0	5.2			
J	22:0	6.5			
L	23:0	6.0			
N	24:0	5.9			
O	25:0	5.4			
Q	26:0	4.1			
R	27:0	4.1			
T	28:0	2.6			
U	29:0	1.6			
V	30:0 (Squalane)	1.3			
W	32:0	1.7			
	Total	67.0		Total	33.0

Table 75. Composition of alcohols of eluate with 5% ether-n-hexane (3) and (4) (*Neomysis intermedia*)

Saturated alcohol		Monoenoic alcohol		Polyenoic alcohol	
Component	wt. %	Component	wt. %	Component	wt. %
11:0	1.9	13:1	1.2	20:4ω6(20:3)	1.4
12:0	3.2	14:1	2.1	20:4ω3	0.4
13:0	1.3	15:1	1.3	20:5	1.6
14:0	1.6	16:1	3.8	21:5?	4.0
15:0	1.7	17:1	0.8	22:5	trace
16:0	6.1	18:1	17.6	22:6	trace
17:0	5.2	19:1	0.6	24:3?	2.0
18:0	3.8	20:1	1.4		
19:0(18:2)	1.1	21:1	0.6		
20:0	13.2	22:1	0.6		
21:0(22:2)	4.0	24:1	1.9		
22:0	14.2				
Total	57.3	Total	31.9	Total	9.4

Unidentified component 1.4%

up to the present. The acetates of these fractions were analyzed by gas-liquid chromatography, and the composition of each component was determined. The results are shown in Table 75.

(D) Eluate with 10% ether in n-hexane (2), (3) and (4)

Every fraction was solid and was positive to the Liebermann-Burchard reaction, and from the isolation of fraction (3) a crystal of mp 147–148°C was identified

Table 76. Characteristics of eluate with ether (*Neomysis intermedia*)

Fraction	Color and state	Content in unsaponifiable matter (%)	Acetate	
			S.V.	I.V.
(1)	Reddish brown, solid	5.2	196.5	63.6
(2)	Deep reddish brown, solid	4.7	275.3	26.6
(3)	Reddish brown, solid	2.0	272.2	57.6
(4)	Reddish brown. solid	2.8	254.3	67.7
(5)	Deep reddish brown, semi-solid	0.8	—	—
(6)	Orange, solid	0.4	—	—

with cholesterol.

(E) Eluate with ether

The properties of each fraction of eluate with ether are shown in Table 76. Among each fraction, fraction (1), fraction (2) and fraction (3) were recrystallized from methanol, yielding crystals of mp 94–95°C, 64–65°C and 61–62°C. The crystals from fraction (2) and fraction (3) agreed with chimyl alcohol in their infrared spectra.

2.3.3.5.5 Summary of the results

(1) The unsaponifiable matter content in the acetone-soluble lipid was 16.4%.

(2) In the unsaponifiable matter were contained 0.8% of hydrocarbon, 6% of alcohol, 67% of sterol and 7% of glyceryl ether.

(3) The hydrocarbon composed of C_{15-32} saturated hydrocarbon and C_{20-27} unsaturated hydrocarbon (pristane homologues). Alcohol contained 60% of saturated alcohol. Sterol was composed mainly of cholesterol, and glyceryl ether was composed mainly of chimyl alcohol.

2.3.3.6 *Euphausia pacifica*

2.3.3.6.1 Material

The acetone-soluble lipid obtained from the plankton caught at adjacent seas of Senzaki, Yamaguchi Prefecture and Kesen-numa, Miyagi Prefecture in 1963 (Sample No. 31–32 in Table 18 and Table 19) was saponified and some unsaponifiable matter (11.3% of acetone-soluble lipid) was obtained. The unsaponifiable matter thus prepared was purified by the re-saponification procedure. The unsaponifiable matter was a reddish brown solid at ordinary temperture, having I.V. 97.8, sterol content (digitonide method) 72.5%.

2.3.3.6.2 Removal of sterol from unsaponifiable matter

The unsaponifiable matter (37.893g) was divided into two portions by the process described above: a portion of crude sterol (24.373g, 64.4%) and a portion of blackish brown viscous liquid (13.520g, 35.6%).

2.3.3.6.3 Column chromatography of liquid portion of unsaponifiable matter

The liquid portion of unsaponifiable matter (13.520g) dissolved in 550 ml of n-hexane was analyzed by column chromatography.

Adsorbent: Mixture of silicic acid (Mallinckrodt, 100 mesh) and celitie 545 (mixing ratio 2:1), 541 g (fortyfold sample weight). Used after drying at 100°C for five hours and allowing to stand in the desiccator with silica gel for three days.

Column: 41.5 cm × 6.2 cm i.d.

One fraction: 150 ml.

Flow rate: 1 fraction/55–60 min.

The liquid portion of unsaponifiable matter dissolved in n-hexane was loaded on the column and eluted with 2,000 ml of n-hexane, 2,000 ml of 5% benzene in n-hexane, 2,000 ml of 10% benzene in n-hexane, 2,000 ml of 30% benzene in n-hexane, 8,000 ml of 5% ether in n-hexane, 5,000 ml of 10% ether in n-hexane, 5,000 ml of ether, 3,000 ml of 2% ethanol in ether, 2,000 ml of 8% ethanol in ether, 2,000 ml of ethanol and 2,000 ml of methanol. The yields of each fraction and the chromatogram are shown in Table 77 and Fig. 35.

Table 77. Chromatography of liquid portion of unsaponifiable matter (*Euphausia pacifica*)

Solvent	Fraction No.	Yield		%*
		(g)	(%)	
n-Hexane (1)	6- 8	0.55	4.1	1.5
(2)	9- 19	0.27	2.0	0.7
5% Benzene-n-Hexane	20- 33	0.23	1.7	0.6
10% Benzene-n-Hexane	34- 45	0.05	0.4	0.1
30% Benzene-n-Hexane	46- 59	0.07	0.5	0.2
5% Ether-n-Hexane (1)	68- 72	0.25	1.8	0.6
(2)	73- 75	0.19	1.4	0.5
(3)	76-112	3.18	23.4	8.3
10% Ether-n-Hexane (1)	113-116	0.21	1.5	0.5
(2)	117-124	1.54	11.4	4.1
(3)	125-128	0.50	3.7	1.3
(4)	129-145	0.31	2.3	0.8
Ether (1)	146	0.23	1.7	0.6
(2)	147-151	2.34	17.3	6.2
(3)	152-153	0.18	1.4	0.5
(4)	154-155	0.09	0.7	0.3
(5)	156-175	0.36	2.7	1.0
2% Ethanol-Ether	176-190	0.17	1.2	0.4
8% Ethanol-Ether (1)	191-201	0.24	1.8	0.6
(2)	202-205	0.03	0.2	0.1
Ethanol (1)	206-210	0.27	2.0	0.7
(2)	211-218	0.07	0.5	0.2
Methanol (1)	219-224	0.12	0.9	0.3
(2)	225-231	0.06	0.5	0.2
H_2O	232-233	0.04	0.3	0.1
	Total	11.55	85.4	30.4
	Residue	1.97	14.6	5.2

* Ratio to total unsaponifiable matter

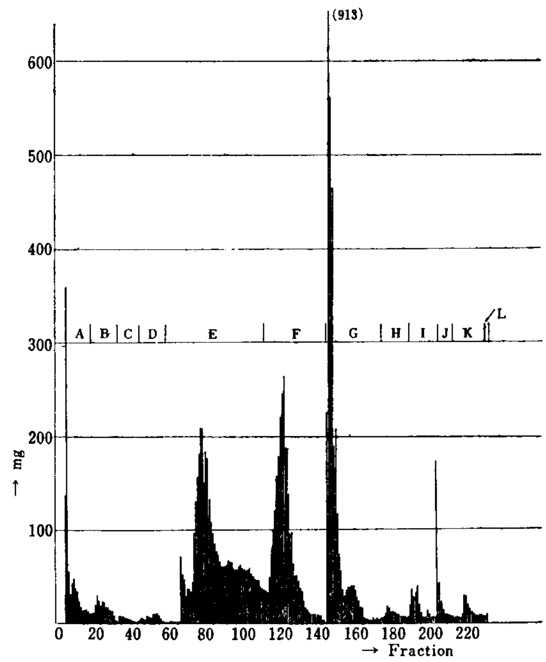


Fig. 35. Chromatogram of liquid portion of unsaponifiable matter (*Euphausia pacifica*) A: n-Hexane, B: 5% Benzene-n-Hexane, C: 10% Benzene-n-Hexane, D: 30% Benzene-n-Hexane, E: 5% Ether-n-Hexane, F: 10% Ether-n-Hexane, G: Ether, H: 2% Ethanol-Ether, I: 8% Ethanol-Ether, J: Ethanol, K: Methanol, L: water

2.3.3.6.4 Analyses of each fraction

(A) Portion of crude sterol

The ultraviolet absorption spectrum of the first crystal obtained by the crystallization of the crude sterol portion from methanol is shown in Fig. 36, and sterol of $\Delta^{5,7}$ series content was 0.28%. The crude sterol was purified by the bromination and the debromination followed by many times of recrystallization, yielding a crystal of mp 147–148°C, which was identified with cholesterol by the mixed melting point test and infrared spectrum.

(B) Eluate with n-hexane (1)

A colorless and transparent liquid, having n_D^{20} 1.4714, I.V. 4.5.

Infrared spectrum of this fraction, as shown in Fig. 37, showed the presence of hydrocarbon with isopropyl groups the same as in Fig. 13. This fraction was analyzed by gas-liquid chromatography, and the composition of each component was determined, as shown in Table 78.

(C) Eluate with 5% ether in n-hexane (3)

This fraction was a light yellowish brown liquid: n_D^{20} 1.4790, I.V. 194.9, Liebermann-Burchard reaction –. Infrared spectrum of this fraction showed a

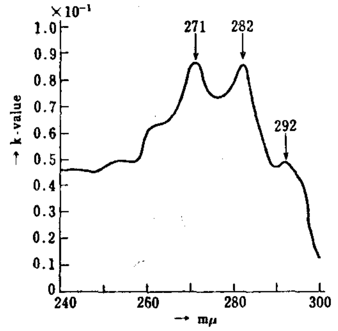


Fig. 36. Ultraviolet absorption of first crystal of crude sterol (*Euphausia pacifica*)

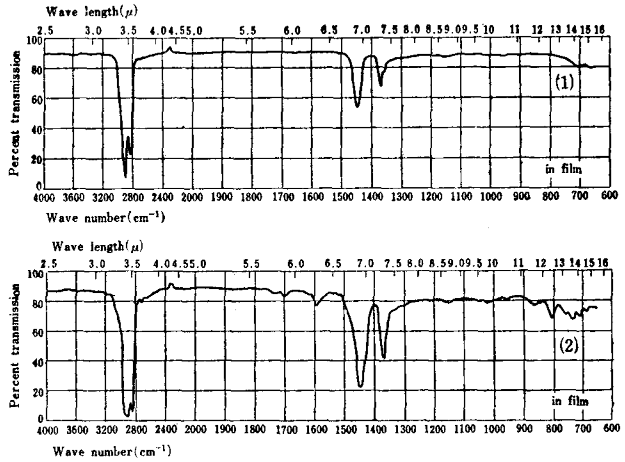


Fig. 37. Infrared spectra of eluate with n-hexane (1) and (2) (*Euphausia pacifica*)
1: Eluate with n-hexane (1) 2: Eluate with n-hexane (2)

band of O-H near 3330 cm^{-1}. The acetate of this fraction was analyzed by gas-liquid chromatography, and the composition of alcohols was determined. The results are shown in Table 79.

(D) Eluate with 10% ether in n-hexane (1), (2), (3) and (4)

Every fraction was solid, and was positive to the Liebermann-Burchard reaction, and from the isolation of fraction (2) a crystal of mp 147–148°C was identified with cholesterol.

(E) Eluate with ether (2)

This fraction was a deep red viscous solid, having I.V. 95.5. Infrared spectrum of this fraction showed bands of O-H at 3600 cm^{-1} and 3460 cm^{-1}.

This fraction was recrystallized repeatedly by ethanol and n-hexane, yielding a crystal of mp 60.0–61.5°C. The infrared spectra of the same are shown in

Table 78. Composition of hydrocarbons of eluate with n-hexane (1) (*Euphausia pacifica*)

Saturated hydrocarbon			Unsaturated hydrocarbon		
Peak	Component	wt. %	Peak	Component	wt. %
C	18:0	0.9	A	15:1	0.2
D	19:0 (Pristane)	2.5	F	20:1	4.1
E	20:0	5.6	H	21:1	9.8
G	21:0	8.5		Total	14.1
I	22:0	12.2			
J	23:0	12.1	B	?	0.2
K	24:0	13.4			
L	25:0	8.5			
M	26:0	6.4			
N	27:0	5.6			
O	28:0	5.0			
P	29:0	5.0			
	Total	85.7			

Table 79. Composition of alcohols eluate with 5% ether-n-hexane (3) (*Euphausia pacifica*)

Saturated alcohol		Monoenoic alcohol		Polyenoic alcohol	
Component	wt. %	Component	wt. %	Component	wt. %
12:0	2.8	14:1	0.6	16:2	2.0
13:0	2.0	15:1	0.5	18:2	1.6
14:0	1.3	18:1	52.5	18:3+20:0	0.5
15:0	1.2	19:1	0.6	20:4ω6	1.8
16:0	3.0	20:1+18:4	2.2	20:5	0.8
		22:1+20:4ω3	11.2	22:6	5.2
		24:1	6.7		

Unidentified component 3.5%

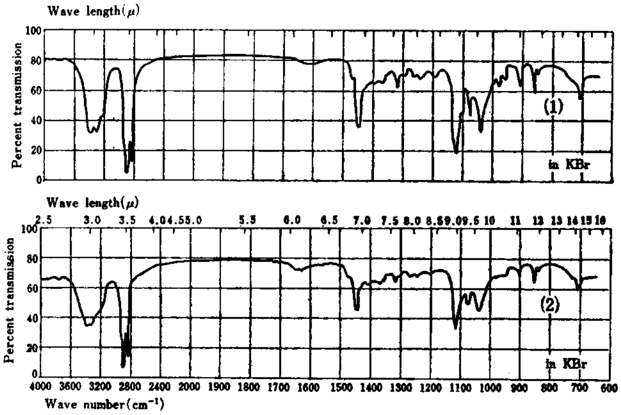


Fig. 38. Infrared spectra of the crystal (mp 60.0–61.5°C) obtained from eluate with ether (2) (*Euphausia pacifica*)

1: Crystal (mp 60.0–61.5°C) from eluate with ether (2)
2: Chimyl alcohol standard

Fig. 38.

The crystal was identified with chimyl alcohol by the mixed melting point test, infrared spectrum and Rf values of thin-layer chromatography.

2.3.3.6.5 Summary of the results

(1) The unsaponifiable content in the acetone-soluble lipid was 11.3%.

(2) The main components of unsaponifiable matter were ca. 1.5% of hydrocarbon (mixture of pristane homologues), ca. 8% of alcohol (18:1, 50%), 72.5% of sterol (mainly cholesterol) and ca. 6% of glyceryl ether (mainly chimyl alcohol).

2.3.3.7 *Acetes japonicus*

2.3.3.7.1 Material

The acetone-soluble lipid obtained from the plankton caught at adjacent sea of Tomo, Yamaguchi Prefecture, in 1963 (Sample No. 21 in Talbe 18 and Table 19), was saponified and an unsaponifiable matter (16.8% of acetone-soluble lipid) was obtained. The unsaponifiable matter was a blackish brown viscous liquid, having I.V. 102.7, sterol content (digitonide method) 52.8%.

2.3.3.7.2 Removal of sterol from unsaponifiable matter

The unsaponifiable matter (37.12 g) was treated with methanol and divided into two portions by the process described above: a portion of crude sterol (17.11 g, 46.1%) and a portion of blackish brown viscous liquid (20.1 g, 53.9%).

2.3.3.7.3 Column chromatography of liquid portion of unsaponifiable matter

The liquid portion of unsaponifiable matter (20.01 g) dissolved in 100 ml of n-hexane was analyzed by column chromatography.

Adsorbent: Mixture of silicic acid (Mallinckrodt, 100 mesh) and celite 545 (above 80 mesh) (mixing ratio 2:1), 800 g (fortyfold sample weight). Used after heating at 110°C for five hours and allowing to stand in the desiccator with silica gel for one day.

Column: 68.5 cm × 6.2 cm i.d.

One fraction: 150 ml

Flow rate: 1 fraction/55–60 min.

The liquid portion of unsaponifiable matter disslved in n-hexane was loaded on the column and eluted with 2,500 ml of n-hexane, 2,000 ml of 5% benzen in n-hexane, 3,000 ml of 10% benzene in n-hexane, 3,000 ml of 30% benzene in n-hexane, 8,000 ml of 5% ether in n-hexane, 6,000 ml of 10% ether in n-hexane, 7,000 ml of ether, 5,000 ml of 8% ethanol in ether, 2,500 ml of ethanol and 3,000 ml of methanol. The yields and states of each fraction are shown in Table 80, and the chromatogram obtained is shown in Fig. 39.

The thin-layer chromatographic analysis was carried out for the fractions marked with asterisks in Table 80 to examine the main components of each fraction.

The results are shown in Fig. 40. The results of TLC analysis showed that

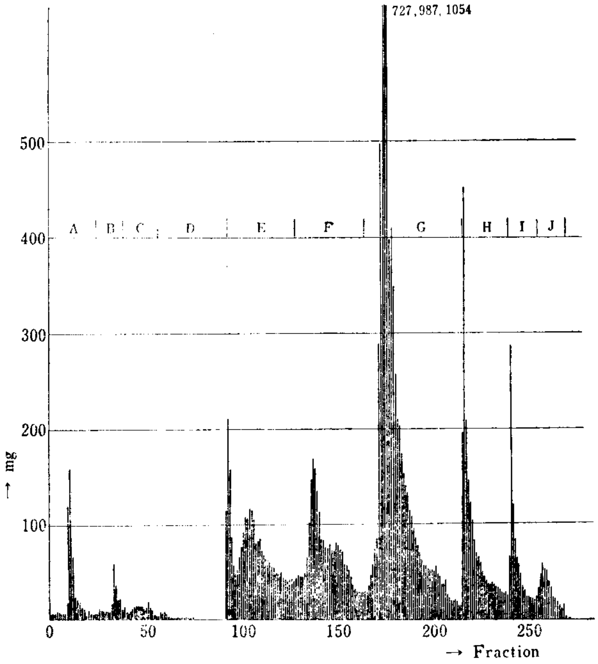


Fig. 39. Chromatogram of liquid portion of unsaponifiable matter (*Acetes japonicus*)
A: n-Hexane, B: 5% Benzene-n-Hexane, C: 10% Benzene-n-Hexane, D: 30% Benzene-n-Hexane, E: 5% Ether-n-Hexane, F: 10% Ether-n-Hexane, G: Ether, H: 8% Ethanol-Ether, I: Ethanol, J: Methanol

each fraction contained the following components as their main components:

Eluate with n-hexane, 5% benzene in n-hexane, and 10% benzene in n-hexane ....hydrocarbon
Eluate with 5% ether in n-hexane....unknown compound
Eluate with 10% ether in n-hexane....alcohol
Eluate with ether (1) and (2)........sterol
Eluate with ether (3) ....sterol and glyceryl ether
Eluate with ether (4) and (5)........glyceryl ether

2.3.3.7.4 Analyses of each fraction

(A) Portion of crude sterol

The ultraviolet absorption of the crystal coming out of methanol at first showed the presence of sterol of $\Delta^{5,7}$ series, the content of which is 0.26%. This crystal was recrystallized repeatedly from methanol followed by the bromination and debromination, and many times of re-crystallization, yielding a crystal of mp 147–148°C which was identified with cholesterol.

(B) Eluate with n-hexane (2)

A colorless and transparent somewhat viscous liquid, not dissolved in metha-

Table 80. Chromatography of liquid portion of unsaponifiable matter (*Acetes japonicus*)

Solvent	Fraction No.	Yield (mg)	Yield (%)	(%)*	Color and state
n-Hexane (1)	8- 10	33	0.2	0.1	Milky white, solid
(2)**	11- 13	345	1.7	0.9	Colorless, transparent liquid
(3)	14- 25	89	0.6	0.3	〃 〃
5% Benzene-n-Hexane (1)	26- 32	70	0.4	0.2	〃 〃
(2)**	33- 39	173	0.9	0.5	Light yellow, liquid
10% Benzene-n-Hexane (1)	40- 46	78	0.4	0.2	Light brown, 〃
(2)**	47- 57	127	0.6	0.3	〃 〃
30% Benzene-n-Hexane	58- 92	76	0.4	0.2	〃 liquid containing small amount of solid
5% Ether-n-Hexane (1)	93- 98	678	3.4	1.8	Reddish brown, liquid
(2)	99-116	1,554	7.7	4.2	Brown, solid
(3)	117-128	557	2.8	1.5	〃 liquid
10% Ether-n-Hexane (1)**	129-135	351	1.8	1.0	Light brown, liquid containing small amount of solid
(2)**	136-146	1,247	6.2	3.3	Reddish brown, liquid
(3)**	147-164	1,022	5.1	2.8	〃 〃
Ether (1)**	165-172	651	3.3	1.8	Light brown, solid
(2)	173-174	1,215	6.1	3.3	Reddish brown, 〃
(3)**	175-179	2,355	16.7	9.0	Deep reddish brown, semi-solid
(4)**	180-195	2,670	13.3	7.2	〃 solid
(5)**	196-216	797	4.0	2.2	〃 〃
8% Ethanol-Ether	217-240	2,031	10.1	5.4	Blackish brown, solid
Ethanol	241-255	943	4.7	2.5	〃 〃
Methanol	256-270	472	2.4	1.3	〃 〃
	Residue	1,476	7.3	3.9	
	Total	20,010	100.1	53.9	

* Ratio to total unsaponifiable matter

** Fractions were examined about their components by TLC.

nol, having n_D^{23} 1.4657. The infrared spectrum of this fraction showed that this fraction is the hydrocarbon with isopropyl groups the same as in *Calanus plumchrus* (Fig. 13). The gas-liquid chromatographic analysis of this fraction was carried out, and the composition of hydrocarbons was determined, as shown in Table 81. All the hydrocarbons present were pristane homologues.

(C) Eluate with 5% benzene in n-hexane (2) and eluate with 10% benzene in n-hexane (2)

The former was a colorless transparent liquid, having n_D^{23} 1.5270, I.V. 71.9, and the latter was a light brownish transparent liquid, having n_D^{23} 1.5673, I.V. 141.5. The infrared spectra of both fractions showed the presence of absorptions due to νCH of -CH_3 at 2960 cm^{-1}, νasCH_2 of -CH_2- at 2925 cm^{-1} and 2850 cm^{-1}, δasCH_3 of- CH_3 at 1460 cm^{-1}, δsCH_3 of -CH_3 at 1380 cm^{-1}, polymethylenic chain at 725 cm^{-1} and νC=C near 1600 cm^{-1}, but the absence of absorption was due to νOH and νC=O. Accordingly, these fractions were considered to be higher hydrocarbon with one or more double bonds.

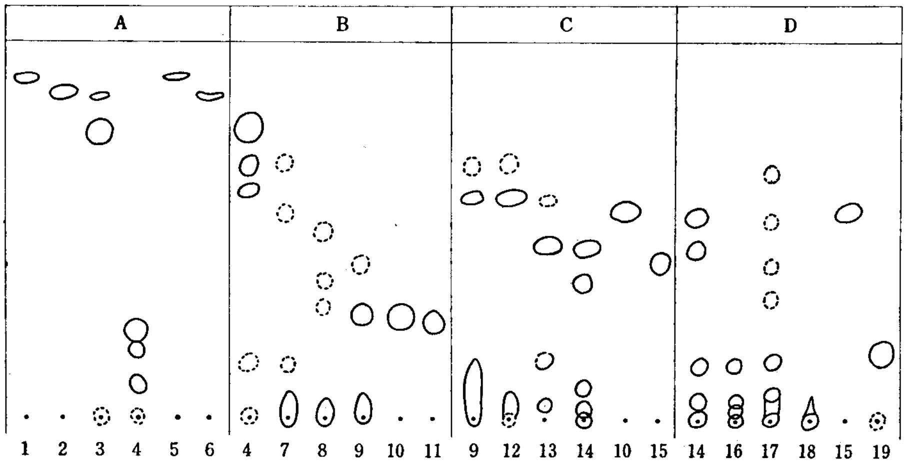


Fig. 40. Thin-layer chromatograms of each fraction (*Acetes japonicus*)

Solvents: plate A, petroleum ether (bp 40–60°C); plate B, petroleum ether-ether, 9:1 (v/v); plate C, petroleum ether-ether, 7:3 (v/v); plate D, petroleum ether-ether, 6:4 (v/v).

Samples: 1, Fraction n-Hexane (2); 2, Fraction 5% Benzene-n-Hexane (2); 3, Fraction 10% Benzene-n-Hexane (2); 4, Fraction 5% Ether-n-Hexane (1); 5, Pristane; 6, Squalane; 7, Fraction 5% Ether-n-Hexane (2); 8, Fraction 5% Ether-n-Hexane (3); 9, Fraction 10% Ether-n-Hexane (2); 10, Lauryl alcohol; 11, Octyl alcohol; 12, Fraction 10% Ether-n-Hexane (3); 13, Fraction Ether (1); 14, Fraction Ether (3); 15, Cholesterol; 16, Fraction Ether (4); 17, Fraction Ether (5); 18, Fraction 8% Ethanol-Ether; 19, Chimyl alcohol.

Blackish spots obtained by charring at 180°C after spraying with $K_2Cr_2O_7$-H_2SO_4 were observed.

Table 81. Composition of hydrocarbons of eluate with n-hexane (2) (*Acetes japonicus*)

Saturated hydrocarbon			Unsaturated hydrocarbon		
Peak	Component	wt. %	Peak	Component	wt. %
C	17:0	2.7	A	13:1	0.1
E	18:0	4.4	D	17:1	3.7
F	19:0 (Pristane)	8.3	H	20:1	17.0
G	20:0	8.8	J	21:1	12.3
I	21:0	9.4			
K	22:0	8.3		Total	33.1
L	23:0	8.0			
M	24:0	6.9			
N	25:0	3.6	B	?	0.7
O	26:0	2.0			
P	27:0	1.2			
Q	28:0	1.2			
R	29:0	1.3			
S	30:0	0.1			
	Total	66.2			

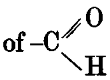
(D) Eluate with 5% ether in n-hexane (1), (2) and (3)

On the thin-layer chromatograms of these fractions, in fraction (1) several spots were observed, and in fractions (2) and (3) larger amounts of components stayed at the starting point.

The infrared spectra of these fractions were chracterized by the band due to νOH at 3540 cm^{-1} or 3380 cm^{-1} and νC=0 of $>$C=0 near 1700 cm.$^{-1}$ Consequently these fractions were more or less thought to be fatty acids or carbonyl compounds.

From the results of a further analysis by thin-layer chromatography, the component which stayed at the starting point was confirmed as fatty acid, thus fractions (2) and (3) were considered to be almost fatty acids.

Fraction (1) was purified by saponification followed by procedure of recovery of unsaponifiable matter and by thin-layer chromatography (TLC conditions were the same as before) the spot isolated from fatty acid was obtained.

The infrared spectrum of the substance obtained from this spot, as shown in Fig. 41, showed the band due to νC=0 near 1710 cm.$^{-1}$ This substance produced a reddish precipitation with 2,4-dinitrophenylhydrazine. Moreover, on the thin-layer chromatography using methylethylketone, methylisobuthylketone, methylisoamylketone, methylnonylketone, methylundecylketone, benzophenone, propionaldehyde, octaldehyde, and dodecylaldehyde as standards, this substance had the same Rf value as that of methylnonylketone, methylundecylketone, benzophenone and dodecylaldehyde, but the bands due to benzene nucleus at 1600 cm^{-1}, 1580 cm^{-1} and 1450 cm^{-1} which was found in the infrared spectrum of benzophenone, and the band due to νCH of  $-\mathrm{C}\begin{smallmatrix}\diagup\!\!\!\diagup\,\mathrm{O}\\ \diagdown\,\mathrm{H}\end{smallmatrix}$ at 2857 cm^{-1}–2703 cm^{-1} found in the infrared spectrum of dodecylaldehyde were not observed in the infrared spectrum of this substance, which resemble those of methylnonylketone and methylundecylketone.

In addition to this, in the spectrum of this substance the band due to νC=C at 1610 cm^{-1} was found. Furthermore this substance showed red color by halochromy reaction. From these results this substance was idetified with unsaturated ketone.

(E) Eluate with 10% ether in n-hexane (2) and (3)

Fraction (2) had I.V. 298.5, S.V. of acetate 241.9, and fraction (3) had I.V.

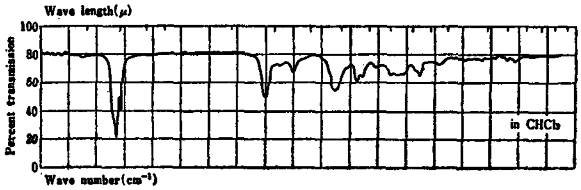


Fig. 41. Infrared spectrum of spot from eluate with 5% ether-n-hexane (1) (*Acetes japonicus*)

271.5, S.V. of acetate 219.9. The infrared spectra of these fractions showed the band due to νOH. From the results of thin-layer chromatography and infrared spectra, in reference to the results of chromatography on the unsaponifiable matter of the plankton lipids, these fractions were considered to be those which had alcohol as a main component.

(F) Eluate with ether (1), (2) and (3)

Fraction (1) was a light brown solid, fraction (2) a red brown solid and fraction (3) was a brown viscous liquid. These fractions were all positive to the Liebermann-Burchard reaction. The crystal obtained fromthese fractions by repeated recrystallization was identified with cholesterol.

(G) Eluate with ether (4)

This fraction was a dark reddish brown solid and had I.V. 90.0. It was recrystallized from ethanol and n-hexane, yielding a crystal of mp 60–61°C which was identified with chimyl alcohol.

2.3.3.7.5 Summary of results

(1) The unsaponifiable matter content in the acetone-soluble lipid was 16.8%.

(2) The main components in unsaponifiable matter were ca. 1.7% of hydrocarbon (pristane and its homologues), ca. 6% of alcohol, 52% of sterol (mainly cholesterol) and 13–14% of glyceryl ether. Small amounts of ketone were also observed.

3. Discussion and conclusion

3.1 The lipids of fishes

Fourteen marine teleosts ("SABA" (mackerel), "KICHIJI" (spiny-cheek), "HATAHATA" (a kind of sandfish), "MA-AJI" (horse mackerel), "IBODAI" (butterfish), "MAGAREI" (flounder), "BABA-GAREI" (slime flounder), "ISHI-GAREI" (stone flounder), "NISHIN" (herring), "BENIMASU" (sockeye salmon), "CHIKA" (surf smelt), "ANKOU" (goosefish), "MADARA" (cod), "SUKETOU-DARA" (Alaskan pollack)), four fresh-water teleosts ("WAKASAGI" (pond smelt), "AYU" (sweet smelt), "SHISHAMO" (longfin smelt), "FUNA" (crucian carp)), one elasmobranch ("YOSHIKIRI-ZAME" (great blue shark)), two cephalopods ("SURUME-IKA" (squid), "SODE-IKA" (squid)) and one shellfish ("EZO-AWABI" (abalone)) were examined about their lipids.

(1) Saponification values lay between 170–200 in a large majority of the lipids. Iodine values were lower in the lipids of herring (flesh), sockeye salmon (flesh), spiny-cheek (flesh), and longfin smelt (flesh), while the values were higher in the lipidsof slime flounder (flesh), cod (liver), "SURUME-IKA" (liver), "SODE-IKA" (liver) and great blue shark (liver). The unsaponifiable matter content was higher in slime flounder (viscera) and pond smelt (whole body), 10.30% and 7.06% respectively. In other members this value was given within 1–5%.

(2) The flesh lipid had a somewhat higher iodine value than the viscera lipid. The difference between the fatty acid composition of flesh lipid and of liver lipid was not very significant, but the former was somewhat richer in the contents of 20:5 and 22:6 than the latter. In cod and Alaskan pollack the lipids of testis and ovary contained smaller amounts of monoenoic acid and larger amounts of 20:5 and 22:6 than the lipids of liver. The above observations are in accordance with the indication of Nicolsky[78] that more unsaturated lipids are preserved temporarily in the body for every day's consumption, on the other hand more saturated lipids are stored as a depot lipid for reserve supply, which may be consumed for instance for the passing of the winter or the coming migration.

(3) The mean values of the main fatty acid compositions of the lipids of thirteen marine teleosts (sockeye salmon was excluded because it was anadromous) and four fresh-water teleosts are given in Table 82. Comparing the marine fishes with the fresh-water fiishes, there is not so large a difference between the two in saturated fatty acids. Of the monoenoic acids the total amounts and the amounts of 18:1 are the same between the two, but the former is rich in 20:1 and 22:1, on the other hand the latter is rich in 16:1. In the polyenoic acid 20:5 and 22:6 are the predominant constitutents of the marine fish lipids, but in the fresh-water fish lipids, the smaller amounts of 20:5 and 22:6 and the larger amounts of 18:2 and 18:3 than the marine fish lipids were found. In the marine fish lipids monoenoic acid is the largest component among saturated, monoenoic and polyenoic acids. The fresh-water fish lipids contained larger amounts of saturated and smaller amounts of monoenoic and polyenoic acids than the marine fish lipids, but the monoeonoic acid was yet the main component of the fatty acids the same as in the marine fish lipids. The distributions of fatty acids by carbon numbers in unsaturated fatty acids of the marine and fresh-water fish lipids are given in Table

Table 82. Average compositions of main fatty acids of lipids of marine fishes and fresh-water fishes (per cent wt)

Saturated acid			Monoenoic acid			Polyenoic acid		
Fatty acid	Marine fish	Fresh-water fish	Fatty acid	Marine fish	Fresh-water fish	Fatty acid	Marine fish	Fresh-water fish
14:0	5.0	6.4	14:1	0.6	1.4	18:2	1.3	3.0
16:0	16.1	17.0	16:1	9.4	15.5	18:3	0.7	4.2
18:0	3.0	3.2	18:1	21.0	19.6	18:4	1.6	1.5
			20:1	7.0	1.3	20:4	2.0	3.1
			22:1	5.5	1.9	20:5	9.8	5.8
						22:5	1.9	1.5
						22:6	8.5	3.9
Total	27.2	31.4	Total	46.4	43.4	Total	26.2	23.9

Table 83. The distributions of carbon numbers of unsaturated fatty acids of marine and fresh-water fish lipids (per cent wt)

	Carbon number				
	14	16	18	20	22
Marine fish	0.6	9.7	25.0	17.2	15.8
Fresh-water fish	1.4	16.6	28.3	9.9	7.3

83. The data are the same as those obtained by Lovern[5)], shown in Table 3, with a difference of degree about the predominance of C_{20} and C_{22} unsaturated fatty acids in marine fishes and C_{16} and C_{18} unsaturated fatty acids in fresh-water fishes.

(4) The lipid of herring flesh had larger amounts of 20:1 and 22:1 and smaller amounts of 22:6 than the average values of the lipids of marine fishes. The flesh lipid of sockeye salmon also had larger amounts of 20:1 and 22:1 and smaller amounts of 20:5 and 22:6 than the average. The lipid of "SODE-IKA" (liver) had relalively large amounts of saturated acids, 20:5 and 22:6. The lipid of the great blue shark liver contained larger amounts of saturated acids and smaller amounts of monoenoic acids. The lipid of the abalone liver contained large amounts of 14:0 and 16:0, and smaller amounts of 20:5 and 22:6. The lipids above mentioned showed respectively the peculiarities of the fatty composition.

(5) The muscle lipid of "KICHIJI", spiny-cheek (*Sebastolobus macrochir*), a marine fish, contained larger amounts of 18:1 (34.1% of the total acids compared with the average values of marine fishes, 21%) and also larger amounts of 16:1 than the average value (12.2% compared with the average value of marine fishes, 9.4%), simultaneously much smaller amounts of 20:5 and 22:6 (6.1% and 3.3% compared with the average value of marine fishes, 9.8% and 8.5% respectively) which were accompanied by somewhat smaller amounts of 16:0 (13.0% compared with the average value of marine fishes, 16.1%).

Lewis[38)] examined the temperature and pressure effects on the fatty acids of some marine ectotherms, and reported that the fishes and plankton from deep water off the Southern California coast had a fatty acid distribution which was unique for the high levels of oleic acid. Furthermore, Lewis[79)] studied on the fatty acid compositions of 20 species of marine animals, primarily fishes and crustaceans which came from various depths down to 4.400 m. He found that the medium-chain saturated and the long-chain polyunsaturated acids decreased with increasing depth, while oleic acid increased. This peculiarity in the fatty acid composition of marine animals from deep sea was thought to be originated from the large amounts of wax esters presented in these animals. Nevenzel, Rodegker and Mead[80)] and Nevenzel, Rodegker, Mead and Gordon[81)] studied on the lipids of deep sea fishes, such as castor oil fish, *Ruvettus pretiosus* and living coelacanth, *Latimeria chalumnae.* They found large amounts of wax esters in muscle lipids and glycerides as main components in liver lipids, and also found the presence of large amounts of 18:1 in the fatty acid composition of both classes of lipids. Mori, Saito, Nakanishi and Hashimoto[82], and Mori, Saito and Nakanishi[83] examined the lipids of castor oil fishes, *Lepidocybium flavobrunneum* and *Ruvettus pretiosus,* and an African fish, "KURO-ŌMATŌDAI", *Allocyttus verrucosus.* They found that the muscle lipids of these fishes contained wax esters as main components which were accompanied by small amounts of glycerides, and that the wax esters and the glycerides are similar in the fatty acid composition which are composed of 18:1, 20:1 and 22:1 as main components, among these 18:1 is of the largest amounts.

The muscle lipids of "KICHIJI" had unsaponifiable matter 1.17%, contained almost no wax esters and mainly composed of glycerides, but contained much larger amounts of 18:1 and smaller amounts of 20:5, 22:6 and 16:0 than the average value of the fatty acids of the marine fish lipids. These characteristics of fatty acid composition of "KICHIJI" lipids are common to the lipids of the deep sea fishes mentioned above, and were considered to be derived from that "KICHIJI" lives in mesopelagic* environment. ("KICHIJI" were caught at depth of 300–400 m).

(6) The marine and fresh-water fishes have respectively a peculiar pattern of fatty acid compositions containing large amounts of highly unsaturated fatty acids in the former. It is very interesting to elucidate the physiological functions of highly unsaturated fatty acids *in vivo.* The author compared the life environment in sea water with one in fresh-water about the difficulty or the easiness of oxygen respiration in regard to the organism. The dissolved oxygen decreases with the increasing of concentration of chlorine ion in water, but pH being ca. 8.2 in sea water to below 7 in fresh-water, CO_2 expirated by the organism is more absorptive in sea water than in fresh-water. Consequently the respiratory condition is rather better in sea water than in fresh-water. Moreover, sea water is more advantageous for the organism than fresh-water in the conditions of envionmental temperature, growth of larva, and others[85]. Neverthless it is the problem why the fresh-water fishes contain more saturated lipid with smaller amounts of 20:5 and 22:6, on the other hand, the marine fishes contain large amounts of highly unsaturated fatty acids which may be more easily converted into energy by decomposition. In the sea, the life environment compared with fresh-water, one of the factors which demand consumption of much energy for the

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* The oceanic environment has been divided by Hedgpeth[84] as follows: epipelagic designates the zone from the surface to 150 m, mesopelagic that from 150 to 1000 m, and bathypelagic that from 1000 to 4000 m. Benthic (bottom living) designations are as follows: sublittoral to depths of 300 m, mesobenthic to 1000 m, bathybentic to 4000 m, and abyssobentic at greater depths.

organism is the width of the sea. Marine fishes are likely to migrate in the sea over far wider areas and to far longer diatances than fresh-water fishes. As a source of energy in the fish during these migrations the highly unsaturated fatty acids are possible to be useful for the continuous consumption of energy. However, another factor which demands more energy consumption for the organism in the sea as life environment is the depth of the sea. Increasing every 10 m of depth of water, one atm of hydraulic pressure adds to the water as life environment. Whether the fishes are of the deep sea species or not, many species of fishes go in or out of their environment of considerably increasing pressure for the mgiration in a daily or in an annual cycle. Under such environment as the condition of increasing pressure the fishes may be subjected to some accelerations in metabolism, and for which the highly unsaturated fatty acids preserved in large amounts are capable to be consumed. It was appointed in the foregoing paragraph that the mesopelagic fish, "KICHIJI", contained very large amounts of 18:1 accompanied with considerably smaller amounts of 16:0, 20:5, and 22:6 compared with the average composition of marine fishes. Moreover, as described already, Lewis[79] reported that marine animals contain increasing amounts of 18:1 and decreasing amounts of 20:5 and 22:6 with increasing depths of the habitats. The results of the author and those of Lewis may support that assumption on the consumption of highly unsaturated fatty acids under the environment of increasing pressure. For clarifying the facts, of course, it is necessary to study on the lipid metabolism of fishes under the condition of increasing pressure.

3.2. The lipids of plankton

The lipids of fifteen species of plankton (*Calanus plumchrus, Calanus cristatus, Calanus finmarchicus, Metridia longa, Parathemisto japonica, Acetes japonicus, Neomysis nakazawai, Neomysis intermedia, Neomysis spinosa, Neomysis awatschensis, Euphausia pacifica, Thysanoessa longipes, Acanthomysis sp., Proneomysis sp., Daphnia carinata*) were exmained, the results obtained were discussed and concluded.

(1) The lipid contents of the plankton were relatively high on the whole. Maximum values were obtained from calanoida. They amounted to 3–6% on wet weight in calanoida and 1–2.5% in the others. Nakai[21] reported the percentage of dry weight to wet weight in zooplankton as 12–24% (average 17%). On the basis of these figures the percentage of lipid on wet weight was converted to the percentage of lipid on dry weight. Consequently the values of 3–6% on wet weight in Calanoida correspond to the values of 17–35% on dry weight. the values of 1–2.5% on wet weight in the others correspond to 6–12% on dry weight.

(2) The lipid had a reddish brown or yellowish brown color. The acetone-soluble fractions occupied the greater parts of the lipids. Specific gravity was

especially lower in Calanoida than in the others. The lipids of plankton had generally a low saponification value and the content of unsaponifiable matter was higher than that of the usual fats and oils. Especially in *Calanus*, saponification values were very low, such as 104–133, and its unsaponifiable matter content was considerably higher, amounting to 38–50%. Even in other families unsaponifiable matter contents were relatively higher and amounted to 10–30%. Iodine values were not always definite in the same species of plankton, usually lower in *Calanus*, brackish plankton (*Neomysis intermedia*) and fresh-water plankton (*Daphnia carinata*), and especially higher in *Parathemisto* (belongs to Amphipoda) and *Euphausia*. Neutralization values of the mixed fatty acids were generally high, especially higher in *Calanus*. This discriminated the *Calanus* from others including its high content of unsaponifiable matter. Moreover in *Euphausia* iodine values of mixed fatty acids were much higher, and its values amounted to 210–260. As a whole, there were some variations on the values of lipids even in the same species of plankton, and yet distinct differences were found between their genus, especially the lipids of *Calanus* were peculiar.

(3) The average value of composition of main fatty acid components of eight species of marine plankton (*Neomysis nakazawai*, *Neomysis spinosa*, *Neomysis awatschensis*, *Euphausia pacifica*, 4:6 mixture of *Acanthomysis pseudomacropsis* and *Euphausia pacifica*, *Acetes japonicus*, *Acanthomysis sp.* and *Proneomysis sp.*) and of one species of fresh-water plankton (*Daphnia carinata*) are shown in Table 84. In comparing marine plankton with freshwater plankton, as to saturated acids, 16:0 was contained in the former in much larger amounts. As to the moneonoic acid, the latter contained larger amounts of 16:1 and 18:1, but 20:1 and 22:1 were the components of very small amounts in both kinds of plankton. As to the polyenoic acid, as against the former containing 20:5 and 22:6 in very large amounts, the latter was relatively rich in 18:2 ans 18:3, containing 20:5 in small amounts and

Table 84. Average compositions of main fatty acids of the lipids of marine and fresh-water plankton (per cent wt)

Saturated acid			Monoenoic acid			Polyenoic acid		
Fatty acid	Marine ~~fish~~	Fresh-water ~~fish~~	Fatty acid	Marine ~~fish~~	Fresh-water ~~fish~~	Fatty acid	Marine ~~fish~~	Fersh-water ~~fish~~
14:0	3.9	2.1	14:1	0.4	5.0	16:2	1.1	1.6
16:0	19.6	7.6	16:1	9.1	15.5	18:2	2.1	7.4
18:0	3.7	3.8	18:1	13.0	21.1	18:3	1.5	13.9
			20:1	1.4	trace	18:4	2.4	2.2
			22:1	0.5	trace	20:4	2.0	3.1
						20:5	20.5	3.8
						22:5	trace	—
						22:6	13.2	—
Total	30.9	20.8	Total	26.1	47.2	Total	42.7	32.0

Table 85. The distributions of carbon numbers of unsaturated fatty acids of marine and fresh-water plankton lipids (per cent wt)

	Carbon number				
	14	16	18	20	22
Marine plankton	0.5	9.6	17.6	25.0	15.9
Fresh-water plankton	5.0	17.1	44.6	6.9	trace

lacking 22:6. Furthermore marine plankton contained 30.9% of saturated acid, 26.1% of monoenoic acid and 42.7% of polyenoic acid, and was meagerer in monoenoic acid and richer in polyenoic acid than freshwater plankton. In addition to this, in the plankton of *Calanus* amounts of 14:0 were considerably great beyond that of 16:0.

The distributions of fatty acids by carbon numbers in unsaturated fatty acids of the marine and fresh water plankton lipids are given in Table 85. The data are characteristic about the predominance of C_{20} and C_{22} unsaturated fatty acids in marine species and C_{14}, C_{16} and C_{18} unsaturated fatty acids in fresh water species. This tendency about the plankton lipids was the same as about the fish lipids.

(4) The fatty acid composition of the lipid of marine plankton was different from that of fish lipid in regard to the content of monoenoic and polyenoic acids.

In the case of marine fish lipid the monoenoic acid is the principal ingredient,[63), 86)] while in marine plankton lipid monoenoic acid content was considerably lower and polyenoic acid was the main component. As already described, DeWitt[39)] reported that the iodine value of cod liver oil at White Sea and Iceland varied regularly in an annual cycle over a range of 20–30 units, and the maximum value occurred in early winter, an abrupt fall took in March, when the cod spawned, and the unsaturation slowly increased again during the summer period of intensive feeding, (The cod also feed voraciously, mainly on pelagic crustacea, during the summer. In winter, the rate of feeding slackens, the diet consisting largely of small fish.) and that these changes in iodine value correspond to complementary changes in the total polyunsaturated fatty acids and the total manoenoic acids, the saturated fatty acids of the oil remaining virtually constant throughout the cycle. The facts reported by DeWitt coincide with the observation that marine fish lipid contained monoenoic acid as the main component, on the other hand marine plankton lipid was rich in polyunsaturated acid. As already stated, Kayama, Tsuchiya and Mead[40)], in the model experiment as to the aquatic food chain (phytoplankton—zooplankton—fish), did not find highly unsaturated acid in phytoplankton lipid, but observed the formation of arachidonic and eicosapentaenoic acids in zooplankton and docosatetraenoic, docosapentaenoic and docosahexaenoic acid in fish. Kelly, Reiser and Hood[37)] reported that crustaceans convert the lower unsaturated acid to the higher unsaturated acid in a higher degree than the terrestrial animals and the teleosts do.

In any way, it is considered that the zooplankton plays an important role as the carrier of highly unsaturated fatty acids to contribute to the formation and transmission of them.

(5) In the aquatic food chain fish is a plankton feeder. When fishes feed on zooplankton, highly polyenoic fatty acids (C_{20} and C_{22}) of the plankton lipids in considerable amounts must convert to monoenoic acids in the fish body.

As to the saturation of fatty acids in the fish body Lovern previously reported with tunny87), eel9) and herring88), but here the convertion process did not clarify. Ritterberg and Schoenheimer89) demonstrated that saturation and desaturation of the fatty acids in animals are a physiologically reversible process. However polyenoic acids in marine animals belong to $\omega 3$ and $\omega 6$ type, on the other hand the monoenoic fatty acids of them are mainly of $\omega 11$ type with C_{20}, C_{22} and C_{24}, so the assumption that the saturation of polyenoic acid to monoenoic acid goes on according to the reversed process of formation of polyenoic acid in fish90)'91)'92) is not applicable. Polyenoic acid may once decompose to acetate, which may be synthesized to monoenoic acid in fish body93).

(6) Unsaponifiable matter of the lipids of plankton

The properties and components of unsaponifiable matter of the lipids of plankton are summarized as follows:

1) *Calanus plumchrus*
   Unsap. matter content in the lipid 45–50%
   liquid, n_D^{25}, 1.4620, I.V. 99
   Components of unsap. matter
      pristane 2%
      alcohol (mainly saturated and monoenoic) 80%
      sterol 2%
      chimyl alcohol
2) *Parathemisto japonica*
   Unsap. matter content in the lipid 20–25%
   liquid, n_D^{20} 1.4779, I.V. 109
   Components of unsap. matter
      alcohol (saturated and lower unsaturated) 50%
      sterol 20%
      chimyl alcohol
3) *Neomysis nakazawai*
   Unsap. matter content in the lipid 10–15%
   solid, I.V. 95
   Components of unsap. matter
      hydrocarbon 0.7%

alcohol	13%
sterol	60%
glyceryl ether	15%

4) *Neomysis intermedia*

Unsap. matter content in the lipid 16–18%

solid, I.V. 87

Components of unsap. matter

hydrocarbon (pristane and its homologues)	0.8%
alcohol (saturated 60%, monoenoic 30%)	6%
sterol	67%
glyceryl ether	7%

5) *Acetes japonicus*

Unsap. matter content in the lipid 17%

viscous liquid, I.V. 103

Components of unsap. matter

hydrocarbon (pristane and its homologues)	1.7%
ketone	a small amount
alcohol	6%
sterol	53%
glyceryl ether	13–14%

6) *Euphausia pacifica*

Unsap. matter content in the lipid 11%

solid, L.V. 98

Components of unsap. matter

hydrocarbon (pristane and its homologues)	1.5%
alcohol (18:1 50%)	8%
sterol	73%
glyceryl ether	6%

7) *Acanthomysis sp.*

Unsap. matter content in the lipid 25%

solid, I.V. 88

Components of unsap. matter

hydrocarbon (pristane and its homologues)	0.4%
ketone	1.3%
alcohol (saturated 46%, monoenoic 26%)	5%
sterol	74%
glyceryl ether	11%

With regard to unsaponifiable matter, in the plankton lipids which contain large amounts of unsaponifiable matter, the unsaponifiable matter consisted of large amounts of alcohols as seen in *Calanus* lipid. On the other hand, in the plankton lipids which contain a small amounts of unsaponifiable matter, the unsaponifiable matter contained relatively large amounts of sterols as in *Neomysis* lipid.

As to hydrocarbon almost only pristane was found in *Calanus* lipid, and in the others pristane accompanied with its homologues were found.

In a part of plankton a small amount of ketone was observed, which belongs relatively to higher members so that it is considered to be an ordinary components, and not to be the decomposed products derived from the autoxidation of the lipid.

As to the presence of ketone in the lipids, Park, Keeney, Katz and Schwartz[94] reported that the triglyceride containing one β-ketonic acid and two fatty acids was found in butter fat, and that is a precursor of methyl ketone.

The main components of alcohols were saturated and monoenoic alcohols, not involving highly unsaturated alcohol excluded Euphausia lipid.

The sterol consisted mainly of cholesterol, and glyceryl ether containing chimyl alcohol as main component.

(7) *Calanus* was the genus containing large amounts of unsaponifiable matter which amounted to 45–50% (one sample excluded) in the lipid. Furthermore the unsaponifiable matter contained 80% of alcohol and the specific gravity (d_4^{15}) of the lipid was 0.88–0.89 which is smaller than that of other genus of plankton and many other fishes.

What functions does such a lipid carry out in the body of the plankton ?, or what functions ought to be acquired by the plankton containing such a lipid ?

As the author already discussed[95], it seems that these functions are related to the plankton moving vigorously and especially vertically with depth, namely may be for buoyancy in such plankton, and moreover for physiological functions of alcohol or wax.

The sperm whale and the sharks living in deep sea have lipids rich in fatty alcohols and hydrocarbons respectively.

It seems that the formation and presence of such non-glyceride constituents as fatty alcohols, hydrocarbons[97] and glyceryl ethers[98] are related to the ecological or physiological functions common to the marine organisms living in deep sea.

As mentioned above, Nevenzel et al.[80],[81] and Mori et al.[82],[83] reported on the lipid of deep-sea fishes. They found that wax esters constituted the greater part of the lipids from muscle, and Nevenzel et al. suggested that the wax esters may serve to achieve netural buoyancy. These findings are in accordance with the author's information on the lipid rich in fatty alcohols.

Subsequently, it seems that there is a close connection between wax and the diving of the whale.[96] Ishikawa[99] exmained the solubility and absorption rate of oxygen and other respiratory gases in the oily substances and water in his studies on the diving of whales. He found that the solubility of oxygen in oils, particularly in the whale wax, is much higher than that in water, and that the absorption rate is especially greater for the wax than for the whale oil and other glycerides. Consequently it is considered that the wax ester plays an important role in the respiration of the sperm whale as an oxygen depot. Moreover, the wax ester absorbs also N_2 considerably, but the reason why the whale can not get the caison disease seems to be rather attributed to the fact that its "mono breath dive" does not reserve N_2 so much as to get caison disease. These characterictics of the wax ester must be regarded as important to provide one of its physiological functions in deep sea.

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