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Studies on antarctic whale oils by gas-liquid chromatography using a hydrogen flame ionization detector.

IV. Analysis of the branched-chain fatty acids from blue whale bone oil

By Yoshihiko Sano

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IV. Analysis of the Branched-Chain Fatty Acids from Blue Whale Y Bone Oil

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Studies on the Antarctic Whale Oils by Gas-Liquid Chromatography Using a Hydrogen Flame Ionization Detector. IV Analysis of the Branched-Chain Fatty Acids from Blue Whale Bone Oil

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The author reports in the present paper the separation and thntative identification of non-urea-complex-forming multi-branched-chain fatty acids as well as urea-complex-forming branched-chain ones of the iso, anteiso and of other series.

Blue whale bone oil was deacidified with a 20%-potassium hydroxide solution in n-hexane, followed by purification through a silicic acid column. The methyl esters, obtained by alkali-catalyzed methanolysis of the refined oil, were separated by silicic acid column chromatography and urea-complex fractionation. Each fraction was then analyzed by gas-liquid chromatography(GLC) on a polyester(PEGA) column before and after hydrogenation.

In order to separate the urea-complex-forming branched-chain fatty acids, some urea-complex-forming fractions separated aspmanfieñed above were converted into isopropyl esters by a BF_3-isopropyl alcohol reagent. The purified isopropyl esters and their hydrogenated products were separated by urea-complex fractionation. These fractions were analyzed by thin-layer, GLC and

infrared spectrometry.

Isopropyl esters of the branched-chain fatty acid concentrated in the non-urea-complex-forming fractions were saponified to the free acids.

The infrared absorption spectrum of thus fractionated fatty acids suggested the existence of an isopropyl group as a doublet at near 1380_{cm}-1, and consequently the presence of branched-chain fatty acids was corroborated. The branched-chain fatty acids having "fractional carbon numbers (FCN)"[5] of 0.2, 0.4, 0.6 and 0.8 on the PEGA column were found in the isopropyl ester of non-urea-complex-forming fraction.

The last two were tentatively identified to be iso and anteiso acids, respectively, according to the published data.

In addition, the component of ECL value 17.5 in the multi-branched-chain fatty acid fraction was presumed as phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) after comparison with dihydro-phytol acetate synthesized from phytol.

1. Introduction

The author has reported on his earlier analysis of the blubber oils of sei whale and fin whale, and pointed out that the oils were composed of numerous fatty acids including such trace ingredients as branched-chain fatty acids and polyunsaturated odd-numbered fatty acids and so forth.[1) 2)] It has also been reported in the previous paper of this series[2)] that multi-branched chain fatty acids were found to be present in the blubber oil of fin whale. To study the presence of these fatty acids in the oils of other whale species, the author deals with the Blue whale bone oil in the present paper and at the same time, examines mono-branched chain fatty acids.

Methyl esters were first prepared by methanolysis, followed by purification through the silicic acid column, as a result of which a coloured substance and polyunsaturated fatty acid methyl were removed. Fractionation was conducted mainly by urea-complex fractionation method. In order to achieve concentration of mono-branched chain fatty acids, isopropyl esters were prepared first and then fractionation by urea-complex fractionation in isopropyl alcohol followed, at which time concentration of branched-chain acids occurred in non-urea-complex-forming fractions.

The analysis of each fraction by gas-liquid chromatography using a Hydrogen Flame Ionization detector revealed the presence of a variety of mono-branched chain fatty acids.

And moreover by the infrared absorption spectra of fractions, the branched structure was corrobrated. On the other hand, from non-urea-complex-forming methyl ester fractions, multi-branched chain fatty acids were detected as in the case of fin whale oil. Of these, the component with ECL value 17.5 was assumed to have the same branch structure as phytanic acid.

2. Experiments

2-1. Sample Oil

Blue whale bone oil, the composition of its major fatty acids had been reported earlier[4] and 28.5 g in weight was deacidified and purified by the procedure similar to that described in the previous paper[2] Methanolysis was conducted on the refined oil and following which the n-hexane solution was purified through a glass filter precoated with 5g of silicic acid,as a result of which most of coloured substances as well as free fatty acids were removed. Thus 25.7 g of crude methyl esters was obtained.

2-2. Thin-Layer Chromatography(TLC)

Thin-layer chromatography was carried out to analyze each fraction or reaction product in the similar manner to what was delineated in the previous paper[4] For identification of spot locations, however, the spray of 1%-iodine-methanol reagent was employed. This method may be applied for making distinction between saturated and unsaturated fatty acids because spots of saturated components would

disappear within an hour after colouring at the room temperature.

2-3. Gas-Liquid Chromatography (GLC)

The method and conditions of gas-liquid chromatography conducted for this experiment are similar to that stated in the previous paper.[4] And the column which had been in use for considerable length of time was found quite stable.(Fig.-1*). Isopropyl esters of fatty acids and fat alcohol acetate were analyzed by GLC under the same conditions.

2-4. The Infrared Absorption Spectra

Autographic infrared spectrometer, Model IR-S manufactured by Nihon bunko was used and liquid membrane process was employed.

3 The Results

3-1. Removal of Polyunsaturated Fatty Acid Methyl by the Silicic Acid Column Chromatography

To purify the crude methyl esters obtained by methanolysis, that is, to remove foreign matter such as coloured substance and polyunsaturated fatty acid methyl as well, the column (inner diameter 50 mm, 102 mm thick silicic acid layer) packed with 100 g of silicic acid (manufactured by Mallinkrodt; 100 mech) was used. In other words, 25.7 g of crude methyl esters were dissolved in 25 ml of n-hexane and was then passed

through the above mentioned column developed with n-hexane and 2%-diethyl ether in n-hexane. The partial chromatograms were formed at each 200 ml and each fraction was subjected to GLC analysis, by which separation of fraction A without polyunsaturated fatty acid methyl (1.1g; the first six fractions) and fraction B (9.3g; the subsequent four fractions) was achieved (Table-1.).

Table-1 Methanolysis of the sample oil and fractionation of the resulting methyl esters by silicic acid column chromatography.

Sample oil (28.5 g)
↓ Deacidified with a 20%-KOH solution in *n*-hexane
↓ Purified by silicic acid column chromatography
↓ Alkali-catalyzed methanolysis
↓ Purified through a glass filter precoated with silicic acid (5 g)
Crude methyl esters (25.7 g)
↓ Silicic acid (100 g)
↓ Column (50 mm i.d. × 102 mm)
↓ *n*-Hexane
↓ 1200 m*l*
A 1.1 g
↓ 800 m*l*
B 9.3 g
↓ 2%-diethyl ether in *n*-hexane
Others

3-2. Urea-Complex Fractionation and GLC Analysis of Fraction A.

As is shown in Table-2, fraction A was fractionated into four fractions through urea-complex fractionation. Gas-liquid chromatography was then conducted on each of these fractions.

In fraction A, 14:0, 15:0, 16:0br, 16:0, 16:1, 17:0br, 17:0, 18:0br, 18:0, 18:1, 19:0br, 19:0, 19:1, 20:0, 20:1, 21:0, 21:1, 22:0, 22:1, 23:0, 23:1, 24:0, and 24:1 were detected. In fraction A_2, 23:0 disappeared while 15:0br emerged. In fraction A_3, 17:0br (6.8%) and 18:0br(7.5%) were present in relatively large quantity, and such unknown ingredients as ECL values 15.4, 16.3_5, 18.5_5, and 18.7 existed besides 14:0, 15:0br, 15:0, 16:0br, 16:0, 17:0, 17:1, 18:0, 18:1, 19:1, 20:1, 22:1. In non-urea-complex-forming fraction A_4, the constituents with ECL value 17.5 was detected most and 18:1 ranked second in quantity and the unknown ingredients with ECL values 16.2_5 and 16.5 were detected as well in considerably large amount as shown in Figure-1. p142

Table-2 Urea-complex fractionation of fraction A.

Fraction A (1.03 g)

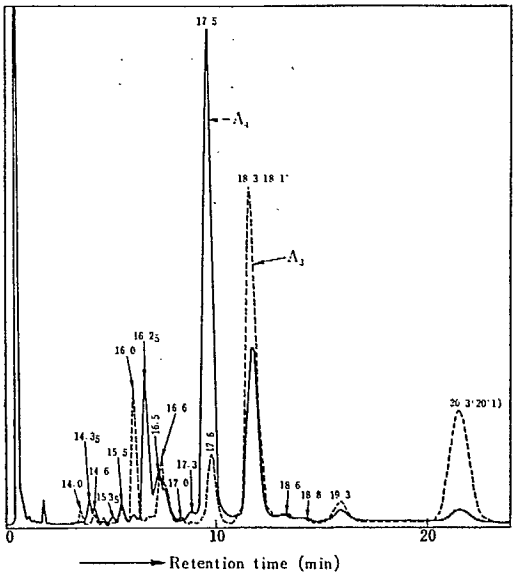
- Urea (1.0 g)
  Methanol (10 m*l*)
  ca. 15°C; 1 hr
  ⟶A_1 (242 mg)
- Urea(1.0 g)
  ca. 15°C; 30 min
  ⟶A_2 (513 mg)
- Recovered

251 mg

- Urea (250 mg)
  Methanol (2.5 m*l*)
  ca. 5°C; 3 hr
  ⟶A_3 (183 mg)
- Recovered

A_4 (58 mg)

The fractions A_1, A_2 and A_3 are urea-complex-forming, while fraction A_4 is non-urea-complex-forming.



The figures of each peak denote the ECL values.

GLC conditions :

- Apparatus ·····Shimadzu gas chromatograph model GC-1 B
- Column ·····4 mm i.d.×1.5 m, U-shaped stainless steel tubing; 10%-PEGA/Diabase B, 80-100 mesh; temp. 215°C
- Detector ·····Shimadzu hydrogen flame ionization detector HFD-1; H_2 40 m*l*/min; air 0.8 *l*/min; Sens. 100; Range 1.6 V
- Carrier gas ·····He 50-60 m*l*/min
- Chart speed·····5 mm/min

Fig.-1 Partial gas-liquid chromatograms of methyl ester fractions A_3 and A_4.

3-3. Urea-Complex Fractionation and GLC Analysis of Fraction B.

Table-3 shows the procedure of urea-complex fractionation of fraction B (9.00g) by which six fractions were fractionated. Again each fraction was analyzed by GLC and the results showed in fraction B_1, B_2, B_3 and B_4 similar components to those of fraction A_1 and A_2 were detected. Figure-2 shows gas-liquid chromatograms and ECL values of each constituent of methyl ester fraction B_5, in which the presence of 18:1 comprised the highest percentage of 70% and 16:1 (6.7%), 20:1(4.5%), 14:0(3.5%), 17:1(2.7%) and 19:1(2.2%) followed in that order. A small amount of branched-chain fatty acids were also recognized. Fraction B_6 is a non-urea-complex-forming fraction, in which the presence of multi-branched-chain fatty acids with ECL value 17.5 and many other unknown ingredients described in the previous report on the analysis of fin whale blubber oil[2] were corroborated.

Table-3 Urea-complex fractionation of fraction B.

Fraction B (9.00 g)

- Urea (9.0 g)
  Methanol (9 ml)
  15-18°C; 30 min
  ⟶B_1(1042 mg)
- Urea(8.0 g)
  ca. 15°C; 30 min
  ⟶B_2(2534 mg)
- Methanol saturated with urea(10 ml)
  10-15°C; 30 min
  ⟶B_3(957 mg)
- Standing for 2 hr in a refrigerator
  ⟶B_4(1271 mg)
- Concentrated to 1/10 vol
  ca. 15°C; 30 min
  ⟶B_5(2475 mg)
- Recovered

B_6(88 mg)

All fractions except B_6 are urea-complex-forming.

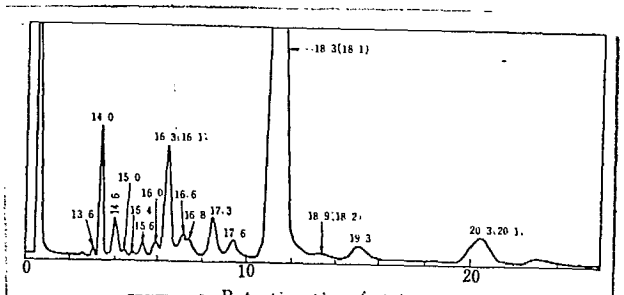


Fig.-2 Gas-liquid chromatogram of methyl ester fraction B_5.

3-4. Preparation of Isopropyl Esters of Fraction B5 and Its Fractionation and GLC Analysis

It is known that methyl esters of long chain fatty acids with *mono*branched-chain methyl are urea-complex-forming,[5] however, if converted into isopropyl esters, it is assumed that this substance would form non-urea-complex-forming fractions. In order to fractionate branched-chain fatty acids from straight-chain fatty acids, the above mentioned fraction B_5 was subjected to urea-complex fractionation, its methyl esters having been converted into isopropyl esters.

3-4-1. Isopropyl Estrification by BF_3 -Isopropyl Alcohol Reagent

Fatty acids(B_{5a}; 1.91g) was obtained through saponification of fraction B_5(2.22g) by the standard method. The fatty acids(1.46g) was dissolved in 2 ml of n-hexane, into which 25 ml of reagent of BF_3-isopropyl alcohol[Note 1)] was added. It was then filtered through a sheet of filter paper and was sealed. The treatment for 30 minutes at 50°C-55°C then followed and the extraction was conducted by n-hexane after adding 30 ml of water to the reaction liquid.

Footnote 1) Preparation of BF_3 - Isopropyl alcohol reagent: To 150 ml of absolute isopropyl alcohol cooled by ice water, 50 g of BF_3-ether solution (made by Morita Kagaku Kogyo) is added and after tightly sealed is stored in the refrigerator.

Light yellowish reaction products (B_5E_1) 1.44g in weight was then obtained by the standard method. The results of TLC analysis of B_5F_1 showed that formation of isopropyl esters constituted only about 50%.

B_5E_2(904 mg) was obtained by treating 898 mg of B_5E_1 for 30 minutes at approximately 60°C after adding 20 ml of BF_3-isopropyl alcohol reagent. B_5E_3(878 mg) was finally obtained from B_5E_2 obtained through the above mentioned treatment after repetition of the same process for several times as unreacting fatty acids were detected in it. However, it was learned that achievement of full estrification was difficult by this procedure.

3-4-2. Removal of Fatty acids in the Crude Isopropyl Esters p143

B_5E_3 (870mg) was dissolved in 2ml of n-hexane and was put through a column packed with 2.0g of silicic acid and was developed by 80 ml of n-hexane containing 2% ether. The coloured substance was removed. To this developing solution, was added a drop of 1%-phenol phthalein solution. While stirring it with a magnetic stirer, 20%-potassium hydroxide was dropped slowly until the solution became faintly coloured. It was immediately filtered through a glass filter packed with 5.0 g of silicic acid and the silicic acid layer was washed with n-hexane containing 2%-ether. After the removal of the solvent, 545 mg of refined isopropyl ester ($B5E_{3p}$) was obtained.

At the same time, the saponified matter in jelly form was gathered and decomposed by 2N-hydrochloric acid and then after n-hexane extraction, 208 mg of fatty acid fraction (B_5E_{3a}) was obtained. TLC analysis of these showed that B_5E_{3p} did not contain fatty acids while the major components of B_5E_{3a} were unsaturated fatty acids. Methyl esters(201mg) were obtained from saponifying esters of B_5E_{3a}(208mg) first and then by methyl estrification of it with BF_3-methanol reagent. The coloured sabstance was then removed from the methyl esters through the coloumn precoated with 1.0g of silicic acid. From the results of its TLC analysis, 191 mg of pure methyl esters of fatty acids was obtained. The GLC analysis brought about the results similar to those of fraction B5.

3-4-3. Urea-Complex Fractionation and GLC Analysis of Refined Isopropyl Esters (B_5E_{3p})

Urea-complex fractionation of B_5E_{3p}(580mg) was carried out in isopropyl alcohol and it was then fractionated into four fractions, namely, fractions P,Q,R, and S as shown in Table-4.

Fraction S, which is non-urea-complex-forming, was hydrogenated in n-hexane solution at room temperature using platinum black as catalyst, with resulting 128 mg of hydrogenated products(SH). The hydrogenated products(SH), 110mg in weight, was then dissolved in one ml of isopropyl alcohol and after addition of 120 mg of urea, was heated for resolution

then was left standing for 20 minutes in the water at 15-20°C. The urea-complex fractionation was conducted as shown in Table-4, which was repeated until four fractions were obtained.

Table-4 Urea-complex fractionation of the purified isopropyl esters (B_5E_{3p}) and the hydrogenated products (SH) of the non-urea-complex-forming fraction S

B_5E_{3p}(580 mg)
- Urea (0.6 g)
- *iso*-PrOH(6 m*l*)
- *ca.* 15°C; 1 hr
- → P(95mg)
- Urea (0.6 g)
- → Q(163 mg)
- Urea(0.6 g)
- → R(143 mg)
- Recovered

↓

S(139 mg)
- Hydrogenated

↓

SH(128 mg)

(110 mg)
- Urea (120 mg)
- *iso*-PrOH (1 m*l*)
- 15-20°C; 30 min
- → SH_1(20 mg)
- Urea (*ca.* 200 mg)
- 15-20°C; 30 min
- → SH_2(61 mg)
- Urea (*ca.* 200 mg)
- *ca.* 3°C; overnight
- → SH_3(12 mg)
- Recovered

↓

SH_4 (8 mg)

All fractions except S and SH_4 are urea-complex-forming.

The gas-liquid chromatogram of fraction B_5E_{3p} showed almost identical feature with that of fraction B_5 except for somewhat poor separation. The retention time of isopropyl esters and methyl esters of 18.1, one of its major conponents, showed a ratio of 1.17.

As a result of GLC analysis on fractions P, Q, R and S as shown in Figure-3, relatively numerous fatty acids presumably of multi-branched-chain were detected in fraction R. There were a large quantity of monoen ( ) acid with 16:1, 17:1,18:1, 19:1 and 20:1 etc. in the fraction.

As is clear from the gas-liquid chromatogram of hydrogenated products(SH) of fraction S, numerous other ingredients besides isopropyl esters of straight chain fatty acids were detected in this fraction. The first two fractions SH_1, and SH_2 fractionated from the fraction S mentioned above were made up largely of straight chain saturated fatty acids and the presence of branched- chain fatty acids was only minor. Thus the results coincided with the prediction that isopropyl esters of the branched-chain fatty acids would be non-urea-complex-forming.

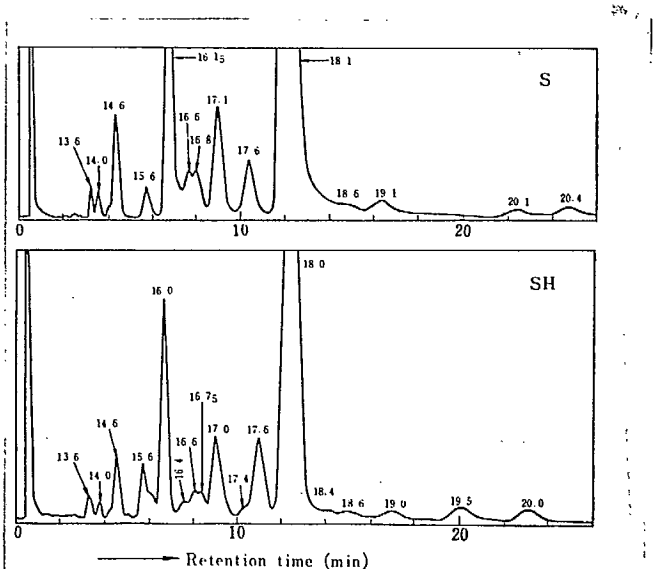
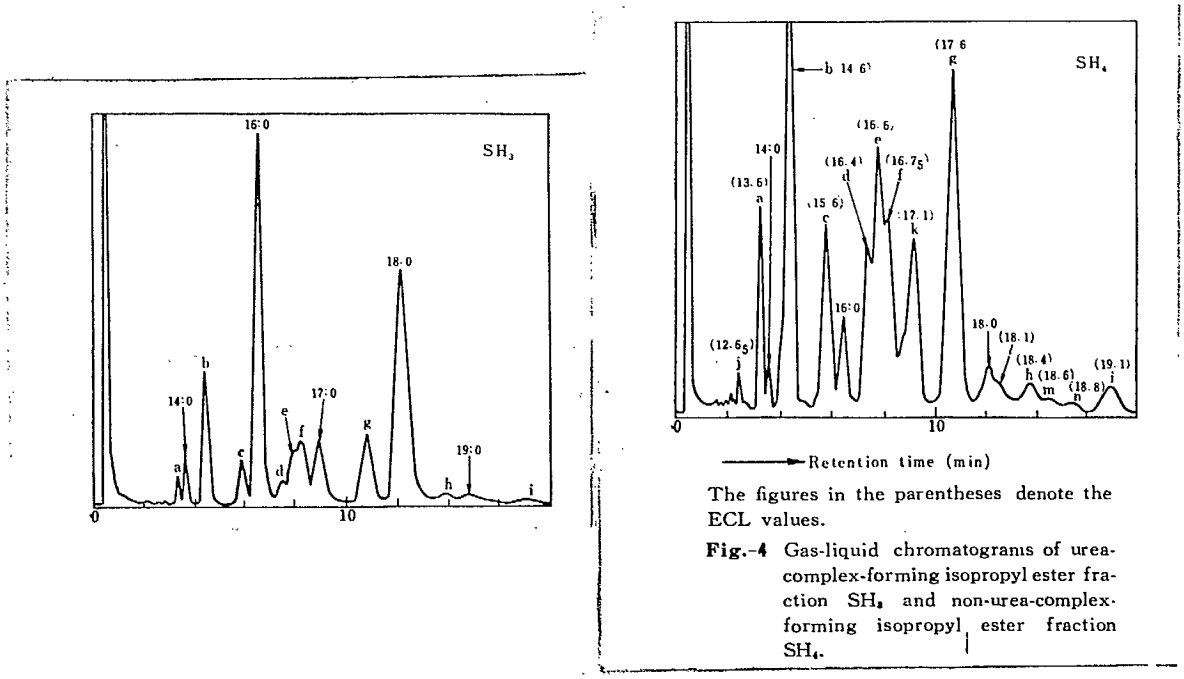


Fig.-3 Gas-liquid chromatograms of non-urea-complex-forming isopropyl ester fraction S and its hydrogenated products (SH).

Fraction SH_3 is isopropyl ester, obtained from the urea-complex which was formed by overnight standing of it in the refrigerator at about 3°C. Its gas-liquid chromatogram is shown in Figure-4. From this chromatogram, it was corroborated that isopropyl esters of the branched-chain fatty acids which practically were non-urea-complex-forming ingredients (a--i) at 15 -20°C became urea-complex-forming under the conditions of the overnight standing in the refrigerator. As shown in Figure-4, non-urea-complex-forming SH_4 contained smaller amount of 14:0, 16:0 and 18:0 etc. while a marked increase in ingredients(a-i) were detected as compared to those in fraction SH_3.

Emergence of ingredients(j--n) was noted as well. ECL values of each ingredients are given in the parentheses in Figure-4 below.

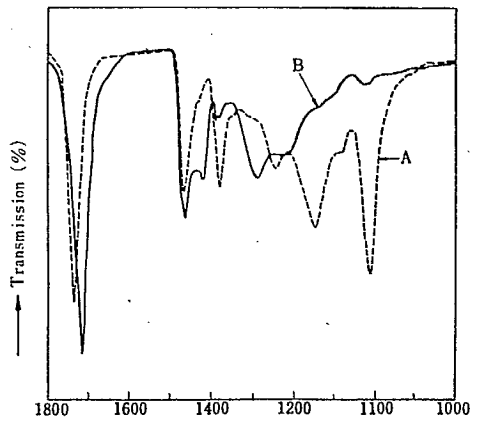


The figures in the parentheses denote the ECL values.

Fig.-4 Gas-liquid chromatograms of urea-complex-forming isopropyl ester fraction SH_3 and non-urea-complex-forming isopropyl ester fraction SH_4.

3-4-4. Corroboration of Branched-Chain Fatty Acids

Since it became clear that fractions SH_3 and SH_4 were composed of many fatty acdis other than the straight chain ones, these fractions were first mixed together to insure the adequate quantity for treatment and the mixture was saponified by the standard method with alkali thus 12 mg of fatty acids(SH_a) was obtained. As shown in Figure-5, the infrared absorption spectrum of these fatty acids(SH_a) revealed the absorption typical of fatty acids, and, moreover, the presence of the branched-chains was corroborated from a doublet found in the neighbourhood of 1380 cm^{-1}. On the other hand, the infrared spectrum of the mixed isopropyl esters showed a strong absorption at 1110cm^{-1}, and no doublet was recognized near 1380cm^{-1}.

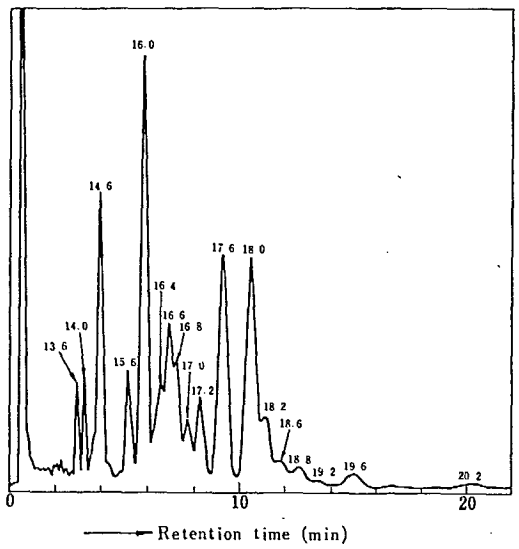


A : the mixed isopropyl esters (SH_3 and SH_4)
B : their acids (SH_a)

The infrared analysis were made on thin films between rock-salt disks by using a twin-beam spectrophotometer model IR-S (Japan Spectroscopic Co., Ltd.).

Fig.-5 Infrared absorption spectra of the mixed isopropyl esters (SH_3 and SH_4) and their fatty acids (SH_a).

Methyl esters, 5 mg in weight was obtained through methyl estrification of 5 mg of the fraction SH_a of the branched-chain fatty acids with BF_3-methanol reagent. Since its TLC analysis indicated a trace amount of unpurified matter, a small glass filter, the column packed with 300mg of silicic acid was used for refining it. GLC analysis of the refined methyl esters brought about gas-liquid chromatogram shown in Figure-6.



The figures of each peak denote the ECL values.

Fig.-6 Gas-liquid chromatogram of the concentrated fraction of branched-chain fatty acid methyl esters.

The branched-chain fatty acids with ECL values with 0.6 "fractional carbon numbers(FCN)" [6] such as 13.6(1.9%), 14.6(12.3%), 15.6(3.6%), 16.6(7.2%), 17.6(13.8%) and 19.6 p145 (0.5%) ranked the highest in quantity. Those with 0.4 FCNs such as 15.4, 16.4 and 18.4 and with 0.2 FCNs such as 16.2, 17.2(4.6%), 18.2(4.6%), 19.2(1.6%) and 20.2(0.7%) were also

found. The presence of those with 0.8 FCNs such as 16.8, (5.2%) and 18.8(0.6%) were also assumed.

3-5. Separation and Identification of Multi-Branched-Chain Fatty Acids

3-5-1. Urea-Complex-Fractionation and GLC Analysis of Hydrogenated Products of Fraction B_6

Figure-7 shows the gas-liquid chromatograms of non-urea-complex-forming fraction B_6 and its hydrogenated products (B_6H) previously referred to in the section 3-3 of this report.

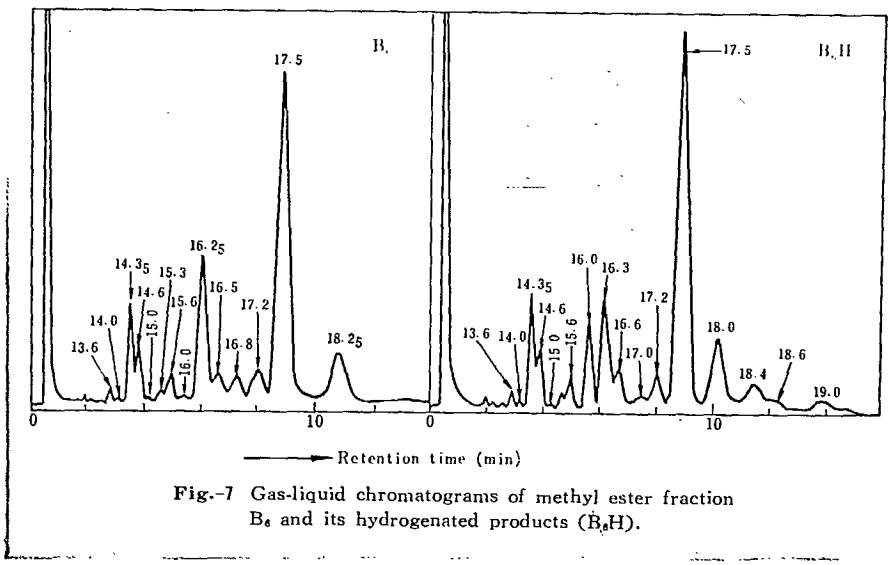


Fig.-7 Gas-liquid chromatograms of methyl ester fraction B_6 and its hydrogenated products (B_6H).

The major component of this fraction was one with ECL value 17.5, which showed no change in retention time after hydrogenation. This substance appears to be one identical to the multi-branched-chain fatty acids reported in the previous paper.[2] That those ingredients with ECL values 13.6, 14.3_5, 14.6, 15.6 and 17.2 and so forth were

saturated fatty acids and that those with ECL values 16.2_5, 16.8, 18.2_5 were unsaturated fatty acids were learned from the chromatograms. Relatively large amount of 16.3 component was detected.

The urea-complex fractionation of the hydrogenated products B_6H was conducted at -10°C and 33 mg of urea-complex forming fraction B_6H_1 and and 38 mg of non-urea-complex-forming fraction B_6H_2 were fractionated from it. Then GLC analysis of respective fractions were carried out. The gas-liquid chromatogram of the urea-complex-forming fraction B_6H_1 as shown in Figure-8 is similar to that shown in Figure-6. In this fraction, a relatively large quantity of the branched-chain fatty acids were detected. In non-urea-complex-forming fraction B_6H_2, the major component with ECL value of 17.5 comprized 53% while those with ECL values of 16.2_5, 14.3_5 were present with percentages respectively of 12% and 8%. Thus these components were presumably multi-branched-chain fatty acids.(Figure-9)

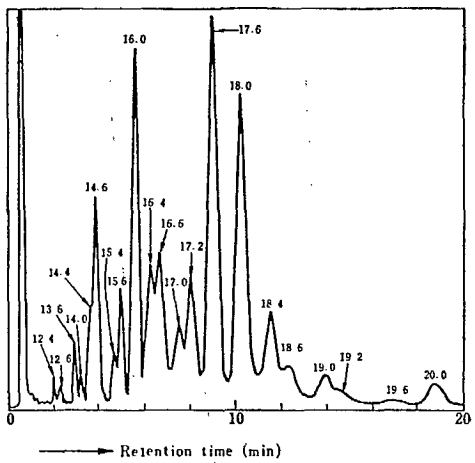


Fig.-8 Gas-liquid chromatogram of urea-complex-forming methyl ester fraction B_6H_1.

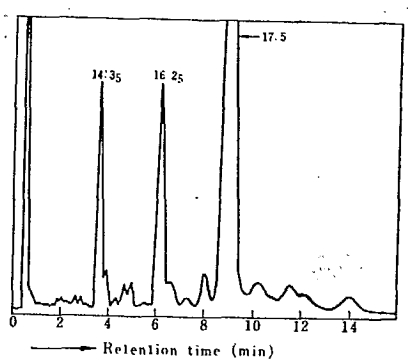


Fig.-9 Gas-liquid chromatogram of non-urea-complex-forming methyl ester fraction B_6H_2.

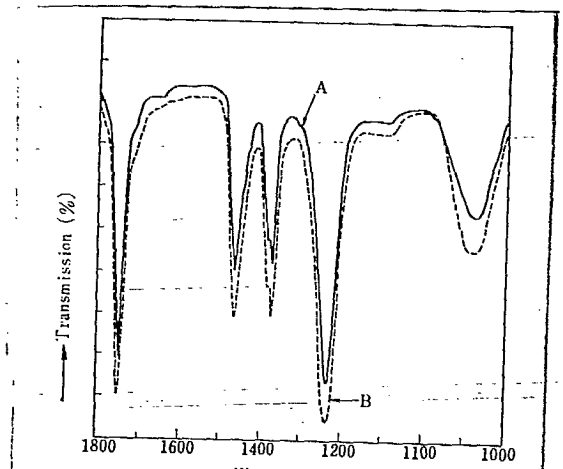
3-5-2. Reduction of Fraction B_6H_2 by Hydrogenated Alunimum Lithium

First,29 mg of fraction B_6H_2 was dissolved in 10 ml of absolute ethyl ether(dehydrated by metallic sodium) and while stirring with a magnetic stirer,about 2 ml of absolute ethyl ether containing about 20 mg of hydrogenated aluminum lithium(manufactured by Degussa Hanau Co.,) was dropped slowly. After stirring for 20 minutes and dropping of 15 ml of water and adding of 5ml of 2N-hydrochloric acid, the reaction solution turned transparant and the ether layer became separated. Then the solution was transferred to a separating funnel and was washed in the sodium chloride solution, rinsed in water and then dehydrated. After removal of solvent, 25 mg of B_6H_{2h} was obtained. As TLC analysis of p146
B_6H_{2h} detected no methyl ester, it was considered that the reduction worked out well.

3-5-3. Acetylation and GLC Analysis of Reduced Alcohol(B_6H_{2h}) derived from B_6H_2

In 10 ml of glacial acetic acid, 24 ml of alcohol (B_6H_{2h}) derived from B_6H_2 was dissolved and about 30 mg of sodium acetate was added. While stirring with a magnetic stirer, treatment for 2 hours at 90°C was carried out. After cooling down the reaction solution by running water and adding 30 ml of water and extraction by n-hexane, 25 mg of the acetylated product (B_6H_2AC) with a faint and unique odor was obtained. Its infrared absorption spectrum revealed those

absorptions typical of acetate. As is shown in Figure-10, no OH absorption was found in the neighbourhood of 3400cm^{-1}, whereas a doublet as an absorption of the branched-methyl group was observed near 1380cm^{-1}. As a result of the gas-liquid chromatography of acetylated products B_6H_2AC, as shown in Figure-11, a chromatogram was formed, although separation was somehwat inadequate compared to methyl esters illustrated in Figure-9.



A : the acetylated product (B_6H_2Ac)
B : dihydrophytol acetate

The analyses were made on thin films of the acetate.

Fig.-10 Infrared absorption spectra of the acetylated products (B_6H_2Ac) obtained from the alcohol (B_6H_{2h}) derived from B_6H_2 with $LiAlH_4$ and dihydrophytol acetate synthesized from phytol.

3-5-4. Synthesis of Dihydrophytol Acetate from Phytol and Its GLC Analysis

Phytol (manufactured by Tokyo Kasei Kogyo; Special grade reagent), 2.9 g in weight was dissolved in 50 ml of glacial acetic acid and after adding of 0.5g of sodium

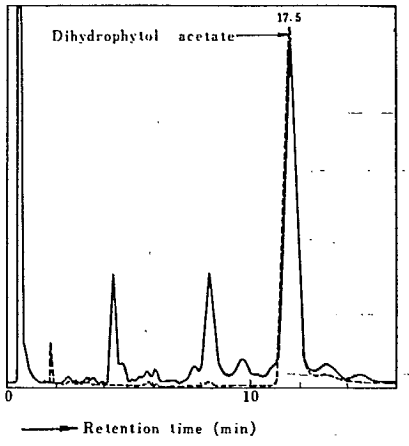


Fig.-11 Gas-liquid chromatograms of the acethylated products B_6H_2Ac and dihydrophytol acetate ($PAcH_2$).

Table-5 Branched-chain fatty acids occurring in the antarctic blue whale bone oil.

Series	Monomethyl branched-chain		Multibr-anched-chain	Unknown	
	Iso acids	Anteiso acids			
FCN	0.6	0.8		0.2	0.4
ECL values	12.6	16.8	14.3_5	17.2	12.4
	13.6	18.8	16.2_5	18.2	14.4
	14.6		17.5	19.2	15.4
	15.6			20.2	16.4
	16.6				17.4
	17.6				18.4
	18.6				
	19.6				

Abbreviations : FCN, "fractional carbon numbers"
ECL, "equivalent chain length"
The ECL values were determined a methyl ester on the PEGA column described in **Fig.-1** (cf., **Fig.-6**, **Fig.-8** and **Fig.-9**).

acetate, was stirred for 2.5 hours at approximately 90°C in the flow of nitrogen gas. The reaction solution was then extracted by n-hexane and thus 3.2 g of the crude acetylated product was obtained. As a result of further treatment by development and elution with n-hexane using a column packed with 5 g of silicic acid, coloured and unpurified substances were removed, thus 2.95 g of acetylated product with a very faint colour was obtained. Then according to the standard method, urea-complex fractionation was conducted on it, which fractionated 2.74 g of non-urea-complex-forming fraction. The acetylated produot was then dissolved in 20 ml of n-hexane and was hydrogenated for five hours at room temperature with 300 mg of platinum black as catalyst. This resulted in yield of 2.72 g of hydrogenated product(PAcH). The fractionation of this product resulted in obtainment of $PAcH_1$(857mg) and $PAcH_2$(1223 mg) as n-hexane developing fractions by a column(inner diameter 20mm) packed with 5 g of silicic acid. The infrared absorption spectra of these fractions indicated typical absorptions of acetate and a doublet absorption at 1380 cm^{-1}(Figure-10). TLC analysis showed only a trace amount of hydrogen carbide in $PAcH_1$, while no unpurified matter was detected in $PAcH_2$.

Figure-11 also shows gas-liquid chromatogram of$PAcH_2$ and there was complete agreement in retention time of dihydrophytol acetate and that of the component with ECL value of 17.5.

In Table-5 are compiled data on all branched-chain fatty acids in the antarctic blue whale bone oil mentioned above. The isolation of respective components appeared to be still difficult. It is also assumed that there may be some trace ingredients, presumably branched-chain fatty acids.

4. Observations

There have already been many reports published on the presence of the branched-chain fatty acids in both flora and fauna oils and fats. The authors also reported on the presence of the ingredients assumed to be one of iso-series and multi-branched chain fatty acids in whale oils.[1) 2)] In the present paper, the author attempted to describe the experiment conducted for corroboration of the presence of the branched-chain fatty acids in blue whale bone oil. As was already stated in the text of this report, urea-complex fractionation of isopropyl esters was carried out, first, by converting urea-complex-forming fractions containing methyl esters of branched-chain fatty acids into isopropyl ester. And by conducting urea-complex fractionation on them, the concentration of branched-chain fatty acids was achieved. From the infrared absorption spectra of these branched-chain fatty acids, a doublet was recognized near 1380cm^{-1}, thus corroborating the presence of branched-chain fatty acids. There were many ingredients detected in the concentrated fractions of the branched-chain fatty acids and these could be classified roughly into four groups of 0.2, 0.4, 0.6 and 0.8 according

to their "fractional carbon numbers(FCN)."[6] (Figure-6) Of these, FCN 0.6 ingredients were relatively predominant, which had been assumed to be one of iso series(terminal isopropyl). This assumption seems to hold on the basis of data published by E.C. Horning et al[7] and N. Nicolaides, T. Ray.[6] They reported that retention time of anteiso series (terminal second butyl) was longer than that of iso series. FCN values of 0.7[7], 0.75,[6] 0.8[7] were also reported by them. Since some deviations from these values would be expected under different conditions of GLC analysis, it is considered that the ingredients of whale oils with FCN value of 0.8 to be anteiso series.

It is generally known that in methyl esters of branched-chain fatty acids, the location of branched chain as well as the number of branches have some bearing on retention time and that with the increase of the number of branches, length of retention time shortens. Although the structures of the ingredients with ECL values of 14.3_5 and 16.2_5 that were detected in methyl ester fractions of multi-branched-chain fatty acids are not yet clarified, the author wishes to study further on this problem in the future.

The presence of multi-branched chain fatty acids in the blubber oil of fin whale was already reported by the author[2], however, they seem to exist also in bone oil of blue whale and sei whale as well as sperm whale.[8] A comparison of the acetylated alcohol obtained by reduction of the concentrated fraction of methyl of multi-branched-chain fatty acids and dihydrophytol acetate showed a complete agreement in

the retention time of these ingredients with ECL value of 17.5. They, therfore, are considered to have multi-branched chain structure of C20, the locations of branched-chains of these are considered identical to those of phytol. However, the detailed account on this point must be held until the forthcoming report of this series, since the question is still under study.

As was stated above, the presence of various branched-chain fatty acids as minor ingredients in blue whale bone oil besides straight chain fatty acids was corroborated. A fact worthy of particular attention and interesting as such is the presence of the components of multi-branched chain fatty acids in the blue whale bone oil which were considered to be similar to those discovered in butterfat of cow milk, namely, (3,7,11 and 15-tetramethylhexadecanoic acid).

In closing, the author wishes to extend his sincere thanks to the crew of the 17th Nisshin-maru fleet for their cooperation in preparation of sample oil, and his hearty appreciation is due Dr. Yozo ISHIKAWA, director of the Research Laboratory for his kind permission to publish the results of the present experiment. (Received for publication on November 10, 1965).

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