FATTY ACID COMPONENTS OF GANGES RIVER DOLPHIN OIL

HIDEO TSUYUKI* AND SHINGO ITOH*

ABSTRACT

The purpose of the present work is an examination of chemical components of Ganges river dolphin oil by TLC, CC, and GLC.

The analysis by TLC and CC showed the presence of triglycerides, phospholipids, sterol esters, diglycerides, hydrocarbons, monoglycerides, sterols and free fatty acids. A substantial part in all fractions was 80.6% of triglycerides fraction contained a small amount of free fatty acids.

The analysis by GLC showed the presence of fatty acids with chain lengths from 10 to 22 carbon atoms and with zero to six double bonds. The Ganges river dolphin oil was found to be rich in unsaturated fatty acids (72.73%), particularly octadecamonoenoic (32.30%) and hexadecamonoenoic (24.47%). On the other hand, saturated fatty acids were contained in small amounts (27.27%) in Ganges river dolphin oil.

INTRODUCTION

Although we have reported on the fatty acid composition of the oil of Finless porpoise (Tsuyuki and Itoh, 1969a) and Many-toothed pilot whale (Tsuyuki and Itoh, 1969b) which are cetaceans in salt-water, the literatures on the oil of fresh-water cetaceans are few.

Expedition of the Ganges river dolphin (*Platanista gangetica*) was done in East Pakistan on the various field of study from Oct. 1969 through March 1970 organized by Prof. M. Nishiwaki of the Ocean Research Institute, University of Tokyo. The materials were brought us through Prof. M. Nishiwaki.

This study is reported as a part of the examination of Platanistidae which has been continuing to date by organized Japanese scientists.

It was a pleasure for us to join in the study.

MATERIAL AND METHODS

Material

Ganges river dolphin used in the present investigation was caught at the Ganges River in East Pakistan, in January 1970 by Prof. Dr. Nishiwaki. Then sample oil was extracted by boiling the blubber of Ganges river dolphin with water. The chemical properties of sample oil are shown in Table 1.

* Department of Food Engineering, College of Agriculture & Veterinary Medicine, Nihon University.

Sci. Rep. Whales Res. Inst.,
No. 23, 1971. 141–147

TABLE 1. PROPERTIES OF GANGES RIVER DOLPHIN OIL

n_D^{20}	1.4635
Acid value	1.3
Iodine value (Wijs)	104.4
Saponification value	195.3
Unsaponifiable matter (%)	1.01

Thin-layer chromatography

TLC was carried out qualitatively according to the method of Doizaki and Zieve (1962). Commercially available silica gel (Wakogel B-5, Wako Pure Chemical Industries, Ltd. in Tokyo) was applied in 0.25 mm thickness on 5×20 cm glass plate. The plate was dried in air for 15 min after spreading, and activated for 1 hr at 110° just before use. The sample oil was applied on 1.5 cm place from the bottom of the plate with a capillary tube. The chromatograms were developed in the glass chamber saturated with petroleum ether-ethyl ether-glacial acetic acid, 90: 10: 1 (v/v/v). The developed plate was sprayed with 50% solution of sulfuric acid in water, and the separated spots were identified by comparison with the each standard mixture which was developed by the same procedure.

Silicic acid column chromatography

To separate Ganges river dolphin oil quantitatively, silicic acid column chromatography was carried out according to the modified method of Barron and Hanahan (1958). A glass column (height 70 cm, ID 20 mm) was packed with 60 g silicic acid (Wakogel C-200, Wako Pure Chemical Industries, Ltd. in Tokyo) prepared by activation at 120° for 1 hr and washing with hexane. 0.526 g of sample oil was placed on the column as 10% solution in hexane, and constituents in sample oil were eluted. Also the flow rate was adjusted to 1.5–1.8 ml/min, and then each fraction was respectively collected in amount of 20 ml. The solvent mixture was replenished from a reservoir filled consecutively with 120 ml of hexane, 180 ml of 15% benzen in hexane, 660 ml of 5% ether in hexane, 320 ml of 15% ether in hexane, 280 ml of 30% ether in hexane, 240 ml of 90% ether in hexane and 320 ml of 20% methanol in ether. Each fraction was identified by chromatographed known compounds on silicic acid column by the same procedure.

Preparation of methyl ester

Fatty acid methyl ester was prepared with boron trifluoride-methanol reagent according to Duron and Nowotny (1963). 100 mg of Ganges river dolphin oil was added 10 ml of boron trifluoride-methanol reagent (140 g BF_3 per liter of methanol) under nitrogen atmosphere, and the conical beaker was closed with the screw cap. Then, the beaker was heated in a boiling water bath for 45 min. After the reaction, the ester was extracted by adding 2 volumes of ethyl ether, so 1 volume of water and shaking. The ethyl ether solution was dehydrated with anhydrous sodium sulfate and then evaporated. Finally the methyl ester was obtained in amount of 92 mg.

Gas-liquid chromatography

GLC was analyzed with a Shimadzu Gas Chromatograph Model GC-1C using a flame ionization attachment. A 180 cm long, 3 mm ID, U shaped stainless steel column was packed with Shimalite W (60–80 mesh), coated with 10% diethylene glycol succinate (commercially available DEGS made by Shimadzu Seisakusho Ltd, in Kyoto). Column temperature was kept constantly at 200°, and temperature at injection port and detector block were respectively 275° and 240°. Flow rates for air, nitrogen as carrier and hydrogen were 600, 70 and 75 ml/min respectively.

Chromatographic peaks were identified either by comparison of retention times with those of standards or from a graph representing the relationship between log retention time and the number of carbon atoms by applying the methods of Ackman (1963), Nelson and Freeman (1960).

Each peak was quantitated by the method of Ettre and Kabot (1963). All fatty acids were calculated as weight percentages of the total known fatty acids present.

RESULTS AND DISCUSSION

Fig. 1 shows the fractionation of Ganges river dolphin oil by a TLC plate qualitatively. The obtained fractions were from above, as follows: hydrocarbons, sterol esters, triglycerides, free fatty acids, sterols, diglycerides, monoglycerides and phos-

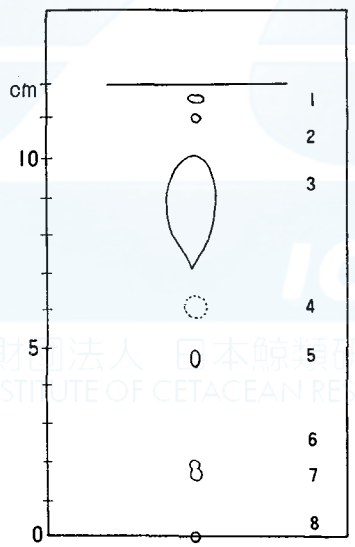

Fig. 1. Thin-layer chromatogram of Ganges river dolphin oil.

1. Hydrocarbons
2. Sterol esters
3. Triglycerides
4. Free fatty acids
5. Sterols
6. Diglycerides
7. Monoglycerides
8. Phospholipids

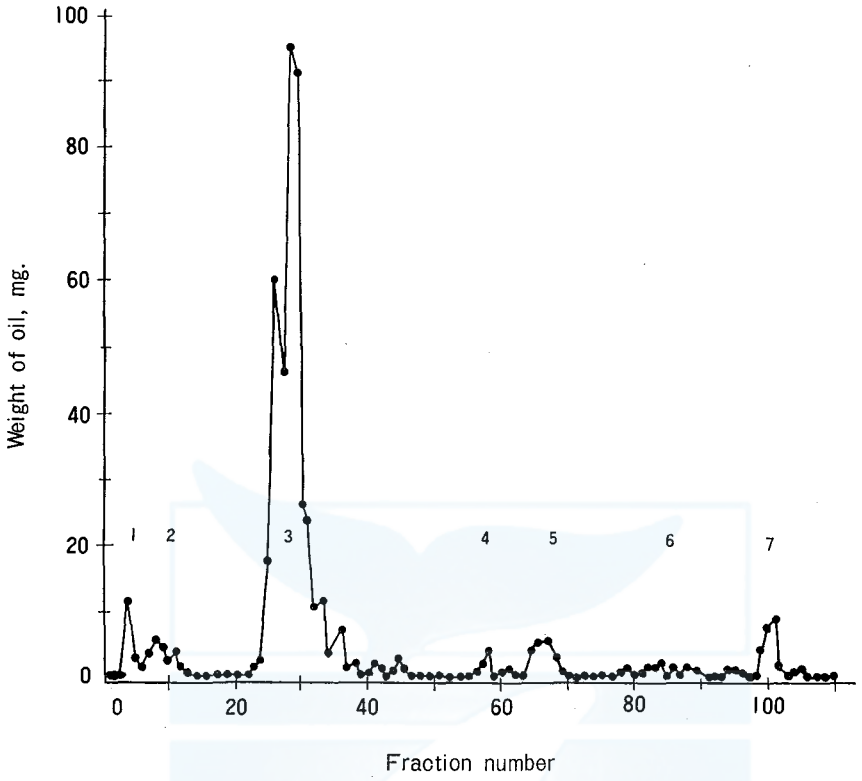

Fig. 2. Separation of Ganges river dolphin oil by silicic acid column.

No.	Fraction	Eluting solvent
1	Hydrocarbons	Hexane
2	Sterol esters	15% benzen in hexane
3	Triglicerides & free fatty acids	5% ether in hexane
4	Sterols	15% ether in hexane
5	Diglycerides	30% ether in hexane
6	Monoglycerides	90% ether in hexane
7	Phospholipids	20% methanol in ether

pholipids. It was seemed that triglycerides fraction was contained in sample oil at the largest quantity as compared to other fractions, because of the spot color was darker than others. The seven fractions except triglycerides seem to be contained respectively only trace amount as compared to triglycerides fraction.

Fig. 2 shows the fractionation of Ganges river dolphin oil by a silicic acid column quantitatively. The seven fractions were eluted as follows: hydrocarbons, sterol esters, triglycerides and free fatty acids, sterols, diglycerides, monoglycerides, and phospholipids. The largest proportion was 80.6% as triglycerides and free fatty acids fraction, and other proportions were as follows: 4.9% phospholipids, 4.1% sterol esters, 3.2% diglycerides, 2.8% hydrocarbons, 2.5% monoglycerides and 1.9% sterols.

TABLE 2. FATTY ACID COMPONENTS OF GANGES RIVER DOLPHIN OIL

No. of carbon atoms	No. of double bonds	Weight per cent	No. of carbon atoms	No. of double bond	Weight per cent
12	0	0.39	18	3	0.43
12	1	0.09	18	4	0.24
14	0	4.89	20	0	2.51
14	1	2.35	20	1	0.54
14	2	1.40	20	2	0.11
16	0	18.11	20	3	0.29
16	1	24.47	20	4	0.98
16	2	1.32	20	5	0.42
16	3	1.71	22	0	0.10
18	0	1.27	22	4	0.53
18	1	32.30	22	5	0.41
18	2	4.65	22	6	0.49

TABLE 3. A COMPARISON OF SATURATED AND UNSATURATED FATTY ACIDS OF GANGES RIVER DOLPHIN OIL

No. of carbon atoms	Weight per cent of total fatty acids		
	Saturated	Unsaturated	Total
12	0.39	0.09	0.48
14	4.89	3.75	8.64
16	18.11	27.50	45.61
18	1.27	37.62	38.89
20	2.51	2.34	4.85
22	0.10	1.43	1.53
Total	27.27	72.73	100.00

From the results of thin layer chromatogram and column chromatogram, it was found that Ganges river dolphin oil was approximately consisted of triglycerides.

Table 2 shows the fatty acid components of Ganges river dolphin oil by quantitative GLC analysis. There were 24 kinds of fatty acids, having from 12 to 22 carbon atoms. The majority were hexadecanoic, hexadecamonoenoic and octadecamonoenoic fatty acids, because of the total of the above three fatty acids held really 74.88% of the total fatty acids. The next prominents were tetradecanoic, tetradecamonoenoic and octadecadienoic fatty acids. On the other hand, the total proportion of other fatty acids was slightly 9.72%. In comparison with saturated and unsaturated fatty acids, the proportion of total saturated fatty acids was 27.27%, and those of total unsaturated fatty acids was 72.73%, as shown in Table 3. Moreover, to compare with the proportion of each carbon numbers, the most prominent was 45.61% of 16 carbon atoms fatty acids, and the next was 38.89% of 18 carbon atoms fatty acids. The rest were as follows: 8.64% 14 carbon atoms, 4.85% 20 carbon atoms, 1.53% 22 carbon atoms and 0.48% 12 carbon atoms.

Finally, the principal ingredient of Ganges river dolphin oil was triglycerides, and main fatty acid components of sample oil were hexadecanoic, hexadecamonoenoic and octadecamonoenoic.

SUMMARY

1. The fractionation of Ganges river dolphin oil was done by thin layer chromatography and silicic acid chromatography. The largest fraction was triglycerides and free fatty acids, and its proportion was 80.6%. The other proportions were as follows: phospholipids 4.9%, sterol esters 4.1%, diglycerides 3.2%, hydrocarbons 2.8%, monoglycerides 2.5%, and sterols 1.9%.

2. Fatty acid components of Ganges river dolphin oil were analyzed by gas-liquid chromatography.

The results obtained were as follows:

Total saturated fatty acids	27.27%;
dodecanoic	0.39%
tetradecanoic	4.89%
hexadecanoic	18.11%
octadecanoic	1.27%
eicosanoic	2.51%
docosanoic	0.10%
Total unsaturated fatty acids	72.73%;
dodecamonoenoic	0.09%
tetradecamonoenoic	2.35%
tetradecadienoic	1.40%
hexadecamonoenoic	24.47%
hexadecadienoic	1.32%
hexadecatrienoic	1.71%
octadecamonoenoic	32.30%
octadecadienoic	4.65%
octadecatrienoic	0.43%
octadecatetraenoic	0.24%
eicosamonoenoic	0.54%
eicosadienoic	0.11%
eicosatrienoic	0.29%
eicosatetraenoic	0.98%
eicosapentaenoic	0.42%
docosatetraenoic	0.53%
docosapentaenoic	0.41%
docosahexaenoic	0.49%

REFERENCES

ACKMAN, R. G., 1963. Structural correlation of unsaturated fatty acid esters by graphycal comparison of gas-liquid chromatographic retention times on a polyester substrate. *J. Am. Oil Chemists' Soc.*, 40: 558–564.

BARRON E. J. and D. J. HANAHAN, 1958. Silicic acid chromatography of the neutral lipides of rat liver, beef liver, and yeast. *J. Biol. Chem.*, 231: 493–503.

DOIZAKI, W. M. and L. ZIEVE, 1962. Rapid thin-layer chromatographic separation of phospholipids and

neutral lipids of serum. *J. Biol. Chem.*, 3: 138–140.

DURON, O. S. and A. NOWOTNY, 1963. Microdetermination of longchain carboxylic acids by transesterification with boron trifluoride. *Anal. Chem.*, 35: 370–372.

ETTRE, L. S. and F. J. KABOT, 1963. Relative response of fatty acid methyl esters on the flame ionization detector. *J. Chromatog.*, 11: 114–116.

NELSON, G. J. and N. K. FREEMAN, 1960. Phospholipide and phospholipide-fatty acid component of human serum lipoprotein fraction. *J. Biol. Chem.*, 235: 578–583.

TSUYUKI, H. and S. ITOH, 1969a. Fatty acid composition of finless porpoise oil. *Sci. Rep. Whales Res. Inst.*, 21: 131–135.

TSUYUKI, H. and S. ITOH, 1969b. Fatty acid composition of Many toothed pilot whale oil. *Sci. Rep. Whales Res. Inst.*, 21: 137–141.