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Occurrence of 5-Methyl-4-Tetradecenoic Acid in the Lipids of Kokanee, *Oncorhynchus nerka* f. *adonis*

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Abstract

GLC analysis of the fatty acids from the flesh lipids of kokanee, *Oncorhynchus nerka* f. *adonis* indicated the presence of an unusual fatty acid having ECL 14.93 on DEGS column. The unknown fatty acid was isolated by urea fractionation, $AgNO_3$-column chromatography and preparative GLC, and identified as 5-methyl-4-tetradecenoic acid by analyses using IR, NMR, GLC-MS and oxidative fission.

This acid constituted 0.2% of the total fatty acids and was also found as a minor component in the lipids of *Acanthodiaptomus pacificus*, a food of kokanee. This fact suggests that dietary plankton is an important source of the branched monoenoic acid in the kokanee lipids.

Introduction

The occurrence, distribution, biosynthesis, metabolism and GLC data of branched fatty acids have been reviewed by several authors[1)2)3)4)]. The monomethyl branched fatty acids having a double bond are contained in the marine animal lipids[5)6)7)8)9)10)]. However, there are few reports of such unusual acids in freshwater fish lipids. Ackman and Hooper[11)] concluded that a minor fatty acid having the ECL 16.29 on BDS column in the hydrogenated products of fatty acids obtained from freshwater fish lipids was 7-methlhexadecanoic acid formed from 7-methyl-6-hexadecenoic acid (7–M–6HDE). Previously, Ota and Yamada[12)] identified 7–M–6HDE in the flesh lipids of kokanee. Concurrently, an unknown fatty acid of ECL 14.9 on DEGS column was found in the kokanee flesh lipids and was presumed from the ECL to be a lower homologue of 7–M–6HDE.

The present paper describes the isolation and identification of this unknown fatty acid. Furthermore, the component fatty acids of the triglycerides of *Acanthodiaptomus pacificus*, a food of kokanee, were analyzed by $AgNO_3$-TLC and GLC in order to clarify the effect of dietary lipids.

Materials and Methods

Eight kokanees, *Oncorhynchus nerka* f. *adonis*, caught by gill net from Lake Shikotsu, Hokkaido in July were used in this experiment. They were all 4-year-old females having body weight 183 g and body length 22.7 cm on the average. The total lipids were extracted from the flesh with chloroform-methanol according to the method of Bligh and Dyer[13)]. The lipid content of the flesh was 7.0%. The

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iodine value was 158.0 and the content of unsaponifiable material of the flesh lipids was 1.3%. The flesh lipids were saponified with 1N KOH in ethanol. The fatty acids recovered by the usual method were esterified by refluxing for 1 hr with 1% H_2SO_4 in methanol. The methyl esters (4g) were then warmed with a saturated solution of urea in methanol (120 ml). After cooling the solution for 1 hr at 5°C in a refrigerator, the non-urea-complexing fatty acid (NUCF) methyl esters were extracted with petroleum ether (40–60°C). The NUCF methyl esters were subsequently separated according to the degree of unsaturation by column chromatography on silicic acid (Wakogel C-200) impregnated with 20% $AgNO_3$ using the solvent system of petroleum ether containing increasing amounts of diethyl ether[14]. The monoenoic acid ester fraction was eluted with 5% diethyl ether in petroleum ether. The separated methyl esters were further analyzed by $AgNO_3$-thin-layer chromatography (TLC) on silica gel (Wakogel B-5 0.25 mm) impregnated with 10% $AgNO_3$, using n-hexane-benzene (1:1 or 7:3 v/v) as development solvent. Preparative gas liquid chromatography (GLC) for the isolation of unknown component was carried out by using a Yanagimoto G80 gas chromatograph fitted with a 1.8 m×6 mm i.d. stainless steel column packed with 10% DEGS on 80–100 mesh chromosorb W. The column temperature was programmed from 150°C to 210°C at 2°C/min. Analytical GLC was carried out on 1.5m×3 mm i.d. glass columns packed with 5% DEGS on 100–120 mesh chromosorb W AW at 160°C and 5% Silar 10C on 100–120 mesh Gas Chrom Q at 165°C. Injection port temperature was maintained at 230°C.

Hydrogenation was carried out with a palladium carbon (Pd 5%) catalyst in n-hexane.

Infrared (IR) spectra were measured with a Nippon Bunko model DS-301 spectrometer using chloroform solution. Nuclear magnetic resonance (NMR) spectra were measured with a JNM-PMX 60 spectrometer at 60 MHz in deuterated chloroform. Gas liquid chromatography-mass spectrometry (GLC-MS) analyses were performed on a Hitachi RMU-6MG mass spectrometer equipped with 5% DEGS column (2 m×3 mm i.d.) at 180°C. The ionizing voltage was 20 eV. Oxidative fission was carried out on periodate-permanganate oxidation using the procedure of Downing and Greene[15]. The products were analyzed by GLC on 5% DEGS column at 120°C and identified by comparison with standard materials.

The fatty acids of the triglycerides of freshwater zooplankton, *Acanthodiaptomus pacificus* were esterified by using 14% BF_3 in methanol[16]. The methyl esters were separated according to the degree of unsaturation by preparative TLC on silica gel (Wakogel B-10 0.5 mm) impregnated with 10% $AgNO_3$ using 5% ethyl acetate in n-hexane as development solvent. Each fraction recovered was analyzed by GLC on 5% DEGS column at 170°C and/or 190°C. Methyl nonadecanoate was used as an internal standard for the determination of the fatty acid composition.

Results and Discussion

The fatty acids of kokanee flesh lipids contained 14:0, 16:0, 16:1, 18:1, 18:2 ω6, 18:3ω3, 18:4ω3, 20:5ω3 and 22:6ω3 as major fatty acid (more than 3% of the total fatty acids) as previously reported[12] and showed a typical type of

freshwater fish lipids with the ratio of total $\omega 3$ to $\omega 6$ acids 2.5 in the fatty acid composition.

The monoenoic acid fraction separated by $AgNO_3$-column chromatography contained two unusual components having ECLs (Equivalent chain length) 14.93 and 16.92 on 5% DEGS column with straight chain monoenoic acids. Of these acids, the component with ECL 16.92 was determined to be 7-M-6HDE by comparison of gas chromatographic behaviors with the results already described[12]. On the other hand, the component with ECL 14.93 on DEGS column was isolated with a purity of 82% by preparative GLC. After hydrogenation, this unknown fatty acid methyl ester showed the ECL 14.52 on DEGS column (Fig. 1).

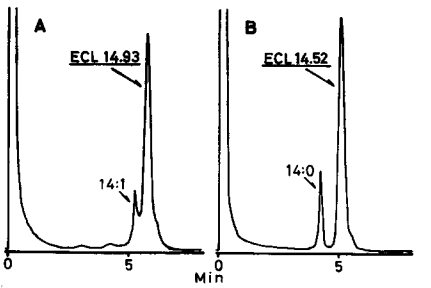

Fig. 1. Gas liquid chromatograms of unknown fatty acid methyl ester isolated by preparative GLC.
A: Before hydrogenation
B: After hydrogeneation

The IR spectrum of the methyl ester showed absorptions at 1670 cm^{-1} due to C=C stretching vibration, and at 840 cm^{-1} due to C-H out of plane deformation of a trisubstituted ethylenic double bond.

The NMR spectrum of this ester showed the signal at 5.36 ppm (δ) indicating that the methyl branch was located on the double bond[9].

The GLC-MS spectra of the unknown fatty acid methyl ester before and after hydrogenation are shown in Fig. 2. The original ester had a molecular ion at m/e 254 and there were major fragment ion peaks at m/e 74, 87, 110 (M–113–31), 127 and 167 (M–87). The hydrogenated methyl ester had a molecular ion at m/e 256. In addition, there were fragment ion peaks at m/e 101 and 129 corresponding to cleavage at the branched methyl group.

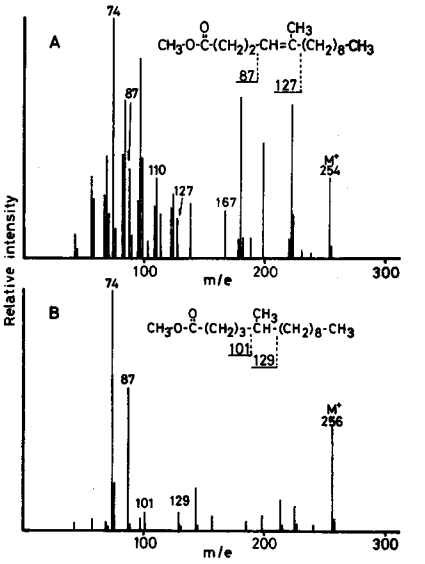

Fig. 2. GLC-MS spectra of unknown fatty acid methyl ester isolated by preparative GLC.
A: Before hydrogenation
B: After hydrogenation

The oxidative fission products of the unknown methyl ester were analyzed by GLC on 5% DEGS column at 110°C and identified as 2-undecanone and dimethyl succinate by comparison of retention times with standard.

From these results, it is apparent that the unknown fatty acid methyl ester with ECL 14.93 on DEGS column was 5-methyl-4-tetradecenoate (5-M-4TDE). The

Table 1. *Fatty acid composition of triglycerides of A. pacificus and kokanee flesh lipids (as wt. %).*

Fatty acid	*A. pacificus*	Kokanee[12]	Fatty acid	*A. pacificus*	Kokanee
Saturated acids			Polyunsaturated acids		
12:0	0.2	Tr*	16:2ω7	0.1	0.2
13:0	0.1	Tr	16:2ω4	0.8	0.9
Iso 14:0	0.1	0.1	17:2ω5	—	0.1
14:0	13.7	8.5	18:2ω6	4.9	7.4
Iso 15:0	0.5	0.5	19:2ω5	—	0.2
Anteiso 15:0	0.3	0.5	20:2ω9	Tr	Tr
15:0	0.4	0.9	20:2ω6	0.2	0.2
Iso 16:0	0.2	0.2	16:3ω4	0.5	0.2
16:0	13.8	14.0	18:3ω6	2.2	1.0
Iso 17:0	0.8	0.8	18:3ω3	3.3	4.7
Anteiso 17:0	0.3	0.5	20:3ω6	0.2	0.4
17:0	0.2	0.4	20:3ω3	0.8	—
Iso 18:0	—	0.2	16:4ω3	0.1	0.1
18:0	1.2	2.3	16:4ω1	0.4	0.1
20:0	Tr	0.2	18:4ω3	4.0	5.5
22:0	Tr	Tr	20:4ω6	2.6	2.4
Monounsaturated acids			20:4ω3	0.2	1.8
			22:4ω6	0.1	0.5
14:1	Tr	0.3	20:5ω3	5.8	4.3
5-M-4TDE**	0.2	0.2	21:5ω2	0.1	Tr
15:1	Tr	0.1	22:5ω6	1.2	0.4
16:1	15.2	11.8	22:5ω3	0.2	1.2
7-M-6HDE***	0.2	0.2	22:6ω3	9.1	6.5
17:1	0.2	0.3			
18:1	14.7	19.2	Unknowns	0.7	0.1
19:1	Tr	0.2			
20:1	0.2	0.4			
22:1	Tr	Tr			

* Trace (less than 0.05%)
** 5-Methyl-4-tetradecenoic acid
*** 7-Methyl-6-hexadecenoic acid

kokanee flesh lipids contained this acid in the content of 0.2% of the total fatty acids.

Pascal and Ackman[9] revealed the occurrence of monomethyl branched monoenoic acids (7-M-7HDE 0.37–1.37%, 7-M-6HDE 0.23–0.68%, 5-M-4TDE 0.10–0.39%) in sperm whale oils and stated that these acids might be distributed widely in aquatic animal lipids. In our experiment on the fatty acids of freshwater fish lipids[12], it was found that these unusual fatty acids were present as minor components in the lipids of fish living in lake, whereas not detected clearly in the lipids of fish living in streams. Hence, the presence of these acids in aquatic animals may be closely related to the dietary lipids.

In order to clarify the relation between fish lipids and dietary lipids, the component fatty acids of triglycerides from *A. pacificus*, which was a part of the diet of kokanee, were investigated. As shown in Table 1, the fatty acid composition tended to have 14:0, 16:1, 20:5ω3 and 22:6ω3 more, and 18:1 and 18:2ω6 less

than those of kokanee flesh lipids. Furthermore, the monomethyl branched monoenoic acids were also contained as minor components in the plankton lipids.

From these results, it was suggested that these unusual fatty acids were biochemically inert materials for fish, because of the trace amounts of the fish lipids as stated by Pascal and Ackman[9], and were concentrated into fish organ from the dietary lipids.

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