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Furan Fatty Acids in the Lipids of Kokanee,  
*Oncorhynchus nerka* f. *adonis*

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Abstract

Furan fatty acids of the testis and liver lipids of kokanee, *Oncorhynchus nerka* f. *adonis* were analyzed by thin layer chromatography, gas liquid chromatography and spectroscopic analyses. The furan fatty acid contents of the testis triglycerides and liver lipids were 11.0% and 0.3% of the total fatty acids, respectively. Of the individual furan fatty acids, 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic ( $F_8$ ) acid was the most predominant, and its contents were 64.7% of the total furan fatty acids in the testis triglycerides and 71.5% in the liver lipids.

Furthermore, 14,17-epoxy-15,16-dimethyleicosa-14,16-dienoic ( $F_8'$ ) acid, an isomer of  $F_8$  acid, was found as a minor component (0.2% of the total furan fatty acids) in the testis triglycerides.

Introduction

Furan fatty acids<sup>1)</sup> (F acids:  $F_1$  to  $F_8$ ) with methyl or dimethyl substituents on the furan ring have been found in the lipids of various fishes.<sup>2-5)</sup> An F acid was first isolated from *Exocarpus cupressiformis* seed oil, but this F acid had no methyl substituent on the furan ring.<sup>6)</sup>

In fish lipids, F acids exist mainly in the triglycerides of testis and the cholesterol esters of liver. Glass et al.<sup>4)</sup> have reported that the F acids in the livers of some fish decrease at spawning season; on the contrary, they increase in the testes. Gunstone et al.<sup>5)</sup> have revealed that F acids were concentrated significantly in the cholesterol esters of the starved cod liver.

In this study, we have investigated on the identification and composition of F acids in the lipids of the testis and liver of kokanee, *Oncorhynchus nerka* f. *adonis*, to get knowledge on the distribution and biochemistry of the F acid in fish.

Materials and Methods

Kokanees, *Oncorhynchus nerka* f. *adonis* were caught by gill net from Lake Shikotsu-ko, Hokkaido in October, 1979. The testes and livers from five males with average body weight of 169 g were pooled.

The lipids of each tissue were extracted by the method of Bligh and Dyer.<sup>7)</sup> Triglycerides (TG) from the testis lipids were separated by preparative thin layer chromatography (TLC) on silica gel plates (Wakogel B-10 0.5 mm) using n-hexane-diethyl ether-acetic acid (85:15:1 by vol.) as development solvent. The

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fatty acid methyl esters from testis TG and liver lipids were prepared by refluxing the TG with methanolic sodium methoxide and/or by refluxing the fatty acids with 7%  $\text{BF}_3$ -methanol.

Preparative  $\text{AgNO}_3$ -TLC of the fatty acid methyl esters was carried out on silica gel plates (Wakogel B-10 0.5 mm) impregnated with 10%  $\text{AgNO}_3$ , using n-hexane-benzene (1:1 v/v) as development solvent. The separated bands were visualized by spraying with 0.1% ethanolic solution of 2',7'-dichlorofluorescein under UV light. The silica gel layer above the front of the monoenoic ester band was scraped off and the fatty acid methyl esters were extracted with petroleum ether (40–60°C). The recovered fatty acid methyl esters were then fractionated by urea adduct method into urea-complexing and non-urea-complexing fatty acid (NUCF) methyl esters.

Gas liquid chromatography (GLC) was carried out on a Yanagimoto G80 gas chromatograph equipped with a flame ionization detector, using glass columns (1.5 m  $\times$  3 mm i.d.) packed with 5% DEGS on Chromosorb W AW DMCS (100–120 mesh) at 190°C, 15% BDS on Chromosorb W AW DMCS (80–100 mesh) at 215°C and 5% Silar 10C on Gas Chrom Q (100–120 mesh) at 190°C.

Ultraviolet (UV) spectra were measured with a Hitachi 124 spectrophotometer using n-hexane as solvent. Infrared (IR) spectra were taken with a Nippon Bunko DS-301 spectrophotometer using chloroform as solvent.

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) was carried out using a Hitachi M-60 mass spectrometer equipped with a glass column (1 m  $\times$  3 mm i.d.) packed with Diasolid XF (80–100 mesh). The ionizing voltage was 20 eV and the column temperature was held at 180°C.

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

## Results and Discussion

Fig. 1 shows a gas chromatogram of the fatty acid methyl esters from the testis TG which constituted 6.2% of the total lipids. The fatty acids contained unknown compounds (No.1–6) with common fatty acids in fish lipids. These unusual components were concentrated in the non-urea-complexing fraction with multiple-branched fatty acids, according to  $\text{AgNO}_3$ -TLC and urea fractionation, and comprised about 11% of the total fatty acids (Fig. 2). Furthermore, these esters migrated with  $R_f$  0.52, below saturated esters and above monoenoic esters, on the plate of silica gel (Wakogel B-5 0.25 mm) impregnated with 10%  $\text{AgNO}_3$  using n-hexane-benzene (7:3 v/v) as development solvent. GLC analysis also showed that these components were not changed by hydrogenation.<sup>2)</sup>

The UV spectrum of NUCF methyl esters showed a single absorption at 224 nm which indicated the presence of a furan ring in the molecule.<sup>1)</sup> The IR spectrum showed the absorptions at 1596 and 1645  $\text{cm}^{-1}$  by the C=C stretching vibration in the furan ring. The NMR spectrum of NUCF methyl esters showed a single peak at 1.8 ppm ( $\delta$ ) which indicated the presence of methyl group on the furan ring.<sup>1)</sup> The signals at 2.3 ppm by the methylene proton next to the ester group and at 2.5

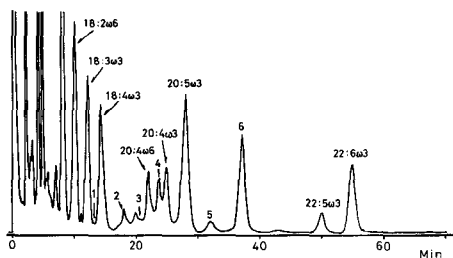


Fig. 1. Gas liquid chromatogram (BDS column) of fatty acid methyl esters from testis triglycerides.

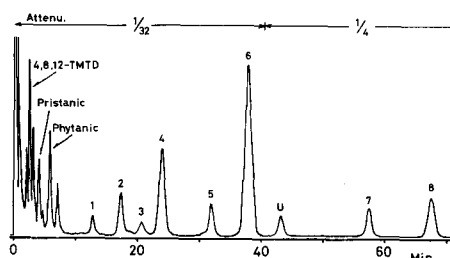


Fig. 2. Gas liquid chromatogram (BDS column) of non-urea-complexing fatty acid methyl esters from testis triglycerides.

ppm by the methylene proton next to the furan ring were also observed.

From these results, it was concluded that the NUCF methyl esters contained fatty acids with furan ring in the molecule.

The GLC-MS spectrum of a compound (No. 6), being the most predominant of the unknowns, is shown in Fig. 3 A. The mass fragmentation pattern with characteristic ions at  $m/e=364$  ( $M^+$ ), 333 ( $M-31$ ), 307 ( $M$ -alkyl), 179 ( $M$ -alkyl ester) and 123 (furan fragment) corresponds to the methyl ester of 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid ( $F_6$  ester) identified by Glass et al.<sup>1)</sup> Similarly, other unknown compounds were identified as methyl esters of furan fatty acids from  $F_1$  to  $F_8$  acid by GLC-MS analyses and by logarithmic plotting procedures of relative retention time (RRT: 18:1=100) vs. carbon number (Fig. 4).

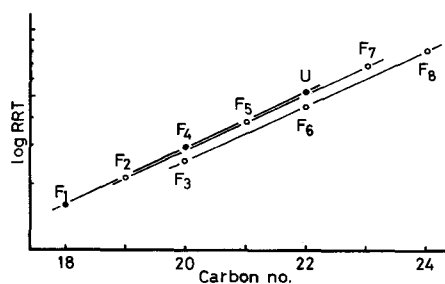
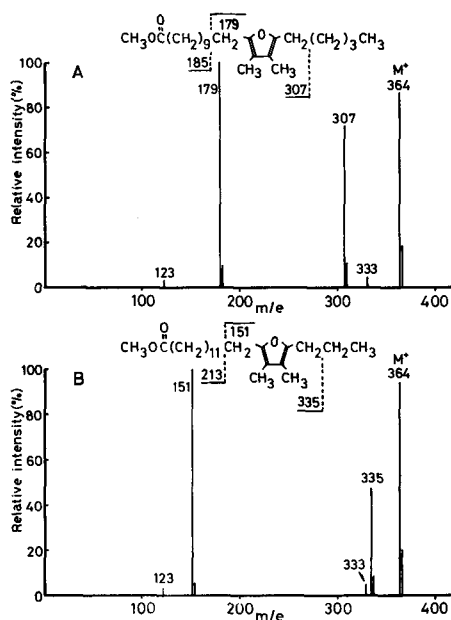


Fig. 4. Plot of log relative retention time (18:1=100) of furan fatty acid methyl esters against carbon number.  
●:  $\omega 4$  family, ○:  $\omega 6$  family

Fig. 3. GLC-MS spectra of furan fatty acid methyl esters having ECLs 23.54 (No. 6) (A) and 24.01 (U)(B) on BDS phase.

The equivalent chain length (ECL) values of F acids on three liquid phases used for GLC analysis are shown in Table 1. The ECL values on 5% DEGS column nearly with those reported by Scrimgeour.<sup>3)</sup>

Table 1. Equivalent chain length values of furan fatty acid methyl esters on three liquid phases.

Furan fatty acid*1	Liquid phases		
	DEGS	BDS	Silar 10C
F <sub>1</sub>	20.68	19.96	21.23
F <sub>2</sub>	21.68	20.96	22.16
F <sub>3</sub>	22.17	21.52	22.70
F <sub>4</sub>	22.63	21.99	23.21
F <sub>5</sub>	23.54	22.98	24.08
F <sub>6</sub>	24.12	23.54	24.67
F <sub>6</sub> ,*2	24.57	24.01	25.16
F <sub>7</sub>	25.45	24.98	25.99
F <sub>8</sub>	26.01	25.50	26.59

\*1 Named as in reference 1).

\*2 14,17-Epoxy-15,16-dimethyleicosa-14,16-dienoic acid.

A small peak (U) having the ECL 24.01 on BDS phase was detected after elution of F<sub>6</sub> ester as shown in Fig. 2. The log RRT of this peak was plotted on the line of  $\omega$ 4 family having dimethyl substituents on the furan ring. As shown in Fig. 3B, the molecular ion (M<sup>+</sup>) of U occurred at m/e=364. Furthermore, the mass spectrum showed peaks at m/e=333 (M-31), 335 (M-alkyl), 151 (M-alkyl ester) and 123 (furan fragment). From such results, this component was identified

Table 2. Lipid contents and furan fatty acid compositions of kokanee testis and liver.

Lipid content (%)	Testis		Liver	
	2.8		3.4	
Furan fatty acid	A*1	B*1	A	C*1
F <sub>1</sub>	1.9	0.21	1.9	0.01
F <sub>2</sub>	4.9	0.54	2.1	0.01
F <sub>3</sub>	2.6	0.29	2.9	0.01
F <sub>4</sub>	16.6	1.82	8.2	0.03
F <sub>5</sub>	6.6	0.72	4.9	0.02
F <sub>6</sub>	64.7	7.09	71.5	0.24
F <sub>6</sub> ,*2	0.2	0.02	-	-
F <sub>7</sub>	0.3	0.04	1.4	Tr*3
F <sub>8</sub>	2.2	0.24	7.1	0.02
Total	100.0	10.97	100.0	0.34

\*1 A: % of furan fatty acid mixture, B: % of furan fatty acids of total fatty acids in triglycerides, C: % of furan fatty acids of total fatty acids in total lipids.

\*2 14,17-Epoxy-15,16-dimethyleicosa-14,16-dienoic acid.

\*3 Trace (less than 0.005%).

as the methyl ester of 14,17-epoxy-15,16-dimethyleicosa-14,16-dienoic ( $F_{6'}$ ) acid which was an isomer of  $F_6$  acid.<sup>5)</sup>

The F acid composition in the lipids of kokanee testis and liver are shown in Table 2. The F acid contents in the testis TG and liver lipids were respectively 10.97% and 0.34% of the total fatty acids. These values were slightly low as compared with the results on several freshwater fish lipids reported by Glass et al.<sup>4)</sup> Of the individual F acids,  $F_6$  acid was the most predominant, and comprised 64.7% of the total F acids in the testis TG and 71.5% in the liver lipids.  $F_{6'}$  acid was present as a minor component (0.2% of the total F acids) in the testis TG, but was not detected in the liver lipids.

The presence of  $F_{6'}$  acid, one of  $\omega 4$  family, in the testis TG of kokanee is noteworthy in connection with the interrelations<sup>4)</sup> among the F acids. It is suggested that this acid exists usually in the testis lipids of fish as well as  $F_7$  and  $F_8$  acids which are the members of  $\omega 6$  family.

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