Stereospecific Analysis of Triacyl-*sn*-glycerols in Docosahexaenoic Acid-Rich Fish Oils

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ABSTRACT: This paper presents the positional distribution of fatty acids in docosahexaenoic acid (22:6n-3)-rich fish oil triacyl-*sn*-glycerols (TG). Stereospecific analysis of TG was carried out by a nonenzymatic method. The TG of bonito head oil, obtained after a winterization process, contained 22:6n-3 at concentrations of 28, 7, and 49 mole % in the *sn*-1, *sn*-2, and *sn*-3 positions, respectively. In the TG of oil before the winterization process, 22:6n-3 was concentrated in the *sn*-3 position, followed evenly by the *sn*-1 and *sn*-2 positions. Tuna orbital oil, obtained after winterization, showed the preferential association of 22:6n-3 to the *sn*-3 position, followed by the *sn*-1 position. This distribution pattern was similar to that observed for seal oil TG rather than sardine oil TG. The bonito head and tuna orbital oils are useful as fish oils with characteristics different from those of common fish oils, such as menhaden, sardine, and herring oils.

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KEY WORDS: Bonito, docosahexaenoic acid, fatty acid, fish oil, positional distribution, sardine, seal, stereospecific analysis, triacyl-*sn*-glycerols, tuna.

Docosahexaenoic acid (22:6n-3) is essential for the development and function of the retina and brain, and it has important physiological activities (1). Marine oils are an important source of longer-chain n-3 polyunsaturated fatty acids. Especially orbital fats of bonito and tuna are useful as an excellent source of 22:6n-3 (2). High content of this acid relative to other polyunsaturated fatty acids facilitates concentration of 22:6n-3. The orbital fats are dominated by triacyl-*sn*-glycerols (TG), and 22:6n-3 occurs in the from of TG.

There have been several reports on positional distribution of fatty acids in fish TG (3–6). Distributions of 22:6n-3 were detailed in these reports. The previous studies were focused on depot fat TG. Orbital fats of bonito and tuna seemed to play a role in the protection of their eyes, when the fish migrate in water at a high speed (7). Such an additional role leads us to expect a structure of orbital fat (TG) that is different from that of the usual depot fats TG. In mackerel, the positional distribution of fatty acids in TG was somewhat different between light and dark muscle (8).

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In the present study, stereospecific analyses of TG have been carried out for bonito head oil (essentially comprising orbital oil) and tuna orbital oil. When the results were compared with those in sardine and seal oils, specific distribution patterns of 22:6n-3 would indicate the potential for further beneficial use of the 22:6n-3-rich fish oils.

EXPERIMENTAL PROCEDURES

Materials. All of the oils (bonito head, tuna orbital, sardine, and seal oils) were industrial products and prepared in the Sagami Chemical Research Center (Sagamihara, Japan). Bonito head oils were obtained after and before a winterization process, and the tuna orbital oil was winterized oil. TG were isolated from the other lipids by preparative thin-layer chromatography (TLC) on Silica gel 60 G plates (0.5 mm thickness; Merck, Darmstadt, Germany) with *n*-hexane/diethyl ether (80:20, vol/vol) for development.

Gas–liquid chromatography (GLC). Fatty acid methyl esters were prepared by heating 1–2 mg of TG in a mixture of 1,2-dichloroethane (0.6 mL), methyl acetate (25 μL), and 1 M sodium methoxide–methanol solution (25 μL) at 50°C for 2 h. After adding acetic acid (6 μL) and removing the solvents, the products were taken up in *n*-hexane. GLC analysis of the methyl esters was performed on a Shimadzu GC-14A gas chromatograph (Shimadzu Co., Kyoto, Japan), equipped with a flame-ionization detector and a open-tubular column, Omegawax 320 (30 m × 0.32 mm i.d., 0.25 μm film thickness; Supelco Inc., Bellefonte, PA). The column temperature was 195°C, and injector and detector temperatures were 240°C. The carrier gas was hydrogen at a linear velocity of 18 cm/s. Peak area percentages were measured with a Shimadzu C-R6A integrator.

Stereospecific analysis of TG. The method for stereospecific analysis of fish TG (6,9,10) was used after modifications as follows. TG (10 mg), mixed with trinonadecanoylglycerol (0.5 mg), were partially hydrolyzed with ethyl magnesium bromide in dry diethyl ether (0.33 mL of 0.33 M solution) for 25 s before adding 0.1 mL acetic acid/diethyl ether (1:9, vol/vol) and 1 mL water. All products were immediately reacted with 3,5-dinitrophenyl isocyanate (50 mg) in dry toluene (1.0 mL), in the presence of dry pyridine (0.1 mL),

overnight at ambient temperature. Resulting di-3,5-dinitrophenylurethane derivatives of 1- and 2-monoacylglycerols (MG) were isolated by preparative TLC on Silica gel 60 G plates (0.5-mm thickness) with chloroform/acetone (96:4, vol/vol) for development. The 1-MG derivatives were resolved into *sn*-1 and *sn*-3-MG fractions by high-performance liquid chromatography with a Shimadzu LC-6A isocratic pump, a Hitachi L-4200 ultraviolet spectrophotometric detector (Hitachi Co., Tokyo, Japan), and a Shimadzu C-R6A integrator. Two columns of Sumichiral OA-4100 (25 cm × 4 mm i.d., 5 μm particles; Sumitomo Chemical Co., Osaka, Japan) in series were used with *n*-hexane/1,2-dichloroethane/ethanol (40:12:3, vol/vol/vol) as mobile phase at a flow rate of 0.5 mL/min at −10°C. Detection was 254 nm. Each of the MG derivatives was converted to fatty acid methyl esters and analyzed by GLC in a manner similar to that used for the analysis of fatty acids from TG. Assignments of each fatty acid to the three positions were obtained from the peak area ratio of each fatty acid to 19:0, and the fatty acid composition of each position was calculated on the basis of the assignments.

Pancreatic lipase hydrolysis of TG. A semimicro method developed by Luddy *et al.* (11) was used to form 2-MG from the TG.

Preparation of 1,2(2,3)-diacylglycerols (DG). Partial hydrolysis of TG with ethyl magnesium bromide was performed by the semimicro method developed by Christie and Moore (12,13) to form 1,2(2,3)-DG from the TG. The required 1,2(2,3)-DG were separated from other products by preparative TLC on boric acid-impregnated Silica gel 60 G plates (12,13).

RESULTS AND DISCUSSION

Positional distribution of fatty acids in TG. Tables 1 and 2 show the fatty acid compositions and positional distributions of fatty acids in the TG used in this study. Reproducibility of the stereospecific analysis was checked by quadruplicate analyses of bonito head oil (A) TG. Coefficient of variation, obtained by dividing standard deviation by mean value, was 2.7–12.3% for the major (>5 mole %) fatty acids in the fish oil TG, i.e., 16:0, 16:1n-7, 18:1n-9, 20:5n-3, and 22:6n-3. Other samples were subjected to duplicate analyses, and mean values of the results are shown in Tables 1 and 2.

The principal fatty acids at more than 3 mole % of the total fatty acids were 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 20:1n-11,13, 20:1n-9, 20:5n-3, 22:1n-11,13, 22:5n-3, and

TABLE 1
Positional Distribution of Fatty Acids in Triacyl-*sn*-glycerol of Bonito Head and Tuna Orbital Oils (mole %)

	Bonito head oil (A) (after winterization)				Bonito head oil (B) (before winterization)				Tuna orbital oil (after winterization)			
Fatty acid	Total	*sn*-1	*sn*-2	*sn*-3	Total	*sn*-1	*sn*-2	*sn*-3	Total	*sn*-1	*sn*-2	*sn*-3
14:0	3.2	2.5 ± 0.9[a]	7.4 ± 0.6	2.4 ± 1.3	4.7	3.5[b]	8.5	2.5	4.2	2.8[b]	9.0	2.2
15:0	0.7	0.6 ± 0.2	1.7 ± 0.1	0.4 ± 0.2	1.1	0.9	1.7	0.7	1.1	1.0	1.9	0.6
16:0	12.2	15.3 ± 1.3	21.0 ± 0.9	3.6 ± 0.3	20.7	31.0	22.4	10.4	22.1	24.5	31.5	13.5
16:1n-7	7.0	7.9 ± 0.3	11.6 ± 1.3	3.2 ± 0.2	7.1	8.5	8.9	4.5	6.3	4.7	9.9	5.1
16:2n-4	1.7	1.1 ± 0.4	3.9 ± 1.0	1.2 ± 0.5	1.7	1.0	2.8	1.5	1.9	0.6	3.4	2.0
16:3n-4	1.0	0.7 ± 0.3	1.4 ± 0.1	1.2 ± 0.5	0.9	0.9	1.1	0.8	0.9	1.1	1.1	0.5
17:0	0.5	0.6 ± 0.1	1.0 ± 0.1	0.2 ± 0.0	1.2	1.5	1.5	0.6	1.0	1.3	1.4	0.6
18:0	1.7	2.5 ± 0.3	1.7 ± 0.2	0.7 ± 0.1	4.1	0.2	0.9	0.1	4.5	7.1	2.8	3.4
18:1n-9	15.5	19.3 ± 0.8	13.5 ± 0.4	11.6 ± 0.6	13.2	16.8	10.1	14.7	15.2	15.4	12.5	16.8
18:1n-7	3.0	4.4 ± 0.4	2.6 ± 0.0	1.5 ± 0.1	2.6	3.9	2.2	2.0	2.6	2.9	2.5	2.5
18:2n-6	2.4	2.3 ± 0.1	3.8 ± 0.2	1.7 ± 0.1	1.5	1.4	1.7	1.4	2.2	1.7	3.3	2.0
18:3n-3	0.7	0.9 ± 0.1	0.9 ± 0.1	0.4 ± 0.1	0.6	0.7	0.7	0.6	0.6	0.6	0.3	0.7
18:4n-3	1.2	0.9 ± 0.1	2.5 ± 0.2	0.8 ± 0.1	1.0	0.7	1.7	0.8	0.9	0.5	1.7	0.7
20:0	0.1	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3
20:1n-11,13	0.4	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.4	0.3	0.4	0.4	0.5	0.4	0.3	0.6
20:1n-9	1.0	1.1 ± 0.1	0.9 ± 0.0	1.0 ± 0.1	0.9	0.9	0.7	1.5	1.0	0.9	0.5	1.4
20:1n-7	0.1	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.0	0.2
20:2n-6	0.2	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.2
20:3n-6	0.1	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.2	0.1
20:3n-3	0.2	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.1
20:4n-6	2.1	1.4 ± 0.2	3.3 ± 0.4	2.5 ± 0.1	1.9	1.0	2.3	2.8	1.6	1.6	2.3	1.1
20:4n-3	0.6	0.7 ± 0.0	0.7 ± 0.1	0.4 ± 0.1	0.5	0.6	0.5	0.4	0.4	0.4	0.4	0.4
20:5n-3	8.7	6.4 ± 0.8	10.7 ± 0.8	10.4 ± 0.5	7.1	5.0	8.2	8.1	5.8	4.0	6.4	7.1
21:5n-3	0.2	0.1 ± 0.1	0.1 ± 0.2	0.5 ± 0.1	0.2	0.1	0.1	0.3	0.0	0.0	0.0	0.1
22:1n-11,13	0.5	0.3 ± 0.1	0.7 ± 0.0	0.6 ± 0.0	0.4	0.3	0.4	0.7	0.5	0.4	0.2	0.9
22:1n-9	0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.2	0.2	0.1	0.1	0.3	0.1	0.1	0.0	0.2
22:5n-6	1.7	1.3 ± 0.1	0.0 ± 0.0	3.0 ± 0.2	1.5	0.6	0.7	3.3	1.4	1.0	0.7	2.1
22:5n-3	1.6	0.9 ± 0.3	2.3 ± 0.4	2.3 ± 0.7	1.3	0.7	2.2	1.1	1.1	1.0	1.1	1.1
22:6n-3	31.4	27.6 ± 2.3	6.9 ± 0.4	48.8 ± 1.5	24.0	18.3	19.6	37.6	22.9	24.7	5.5	33.2
24:1n-9	0.2	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	0.4	0.4	0.5	0.3	0.4	0.4	0.3	0.4

[a]Mean value ± standard deviation of quadruplicate analyses. [b]Mean value of duplicate analyses.

TABLE 2
Positional Distribution of Fatty Acids in Triacyl-*sn*-glycerol of Sardine and Seal Oils (mole %)

	Sardine oil				Seal oil			
Fatty acid	Total	*sn*-1	*sn*-2	*sn*-3	Total	*sn*-1	*sn*-2	*sn*-3
14:0	8.9	9.1[a]	11.5	5.9	6.3	3.3[a]	14.8	3.8
15:0	0.6	0.6	0.7	0.6	0.3	0.2	0.7	0.2
16:0	18.4	21.6	19.8	13.6	8.1	9.1	10.0	5.6
16:1n-7	7.9	7.2	9.6	6.9	22.8	17.5	33.6	19.4
16:2n-4	1.6	1.6	1.2	1.7	1.2	0.7	2.0	1.1
16:3n-4	0.8	0.6	1.3	0.5	0.6	0.1	1.8	0.4
17:0	0.4	1.6	0.4	0.6	0.1	0.2	0.1	0.1
18:0	2.5	2.9	2.6	1.8	0.9	0.8	1.0	0.8
18:1n-9	10.9	13.1	7.9	11.7	17.8	18.2	20.0	15.5
18:1n-7	3.3	4.9	2.0	3.0	5.6	6.9	4.3	5.2
18:1n-5	—	—	—	—	0.5	0.8	0.6	0.3
18:2n-6	1.2	1.1	2.0	1.1	1.5	1.3	3.0	0.7
18:3n-3	0.9	0.6	1.4	0.9	0.5	0.5	0.6	0.3
18:4n-3	2.8	1.9	2.2	2.3	1.2	1.4	0.4	1.5
18:4n-1	—	—	—	—	0.2	0.1	0.2	0.2
20:0	0.2	0.1	0.3	0.2	—	—	—	—
20:1n-11,13	4.4	4.2	2.8	6.1	1.2	1.8	0.5	1.3
20:1n-9	2.3	2.2	1.9	2.7	6.7	11.5	1.4	5.9
20:1n-7	0.2	0.2	0.3	0.2	0.5	0.9	0.1	0.3
20:2n-6	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1
20:3n-6	0.1	0.1	0.2	0.0	0.1	0.1	0.2	0.0
20:3n-3	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
20:4n-6	0.8	0.8	0.9	1.0	0.4	0.4	0.4	0.4
20:4n-3	0.7	0.1	0.7	0.7	0.4	0.6	0.8	0.3
20:5n-3	12.4	9.8	11.1	16.5	8.0	7.3	1.4	13.4
21:5n-3	—	—	—	—	0.4	0.0	0.0	0.8
22:1n-11,13	5.8	4.5	2.7	4.9	2.2	4.9	0.3	1.0
22:1n-9	0.8	1.2	0.1	0.7	0.4	0.8	0.2	0.3
22:5n-6	0.4	0.0	0.1	0.2	—	—	—	—
22:5n-3	1.4	1.0	1.7	1.4	4.5	3.0	0.6	8.7
22:6n-3	9.8	9.3	11.7	8.3	7.6	7.2	1.4	12.5
24:1n-9	0.6	0.4	0.6	0.8	—	—	—	—

[a]Mean value of duplicate analyses.

22:6n-3. The proportions of 22:6n-3 in total fatty acids were 31.4 and 24.0 mole % in the bonito head oil (A) and (B) TG, respectively, and 22.9 mole % in the tuna orbital oil TG. The contents of 22:6n-3 in TG of these oils were much higher than those in TG of sardine (9.8 mole %) and seal (7.6 mole %) oils, as expected from previous reports (2,7).

In the bonito oil (A) TG, the *sn*-1 position was high in 16:0, 18:1n-9, and 22:6n-3; the *sn*-2 position in 16:0, 16:1n-7, 18:1n-9, and 20:5n-3; and the *sn*-3 position in 18:1n-9, 20:5n-3, and 22:6n-3. The bonito oil (B) TG had 16:0, 18:1n-9, and 22:6n-3 as the major fatty acids in all three positions. Both of the *sn*-1 and *sn*-3 positions of the tuna orbital oil TG attracted 16:0, 18:1n-9, and 22:6n-3; and the *sn*-2 position, 16:0 and 18:1n-9. In the sardine oil TG, the *sn*-1 position mainly had 16:0 and 18:1n-9; the *sn*-2 position, 14:0, 16:0, 20:5n-3, and 22:6n-3; and the *sn*-3 position 16:0, 18:1n-9, and 20:5n-3. The seal oil TG had 16:1n-7, 18:1n-9, and 20:1n-9 as the primary fatty acids in the *sn*-1 position; 14:0, 16:0, 16:1n-7, and 18:1n-9 in the *sn*-2 position; and 16:1n-7, 18:1n-9, 20:5n-3, and 22:6n-3 in the *sn*-3 position.

In the bonito head oil (A) TG, 22:6n-3 was preferentially esterified in the *sn*-3 position, followed by the *sn*-1 position. The contents of this fatty acid were 27.6, 6.9, and 48.8 mole % in the *sn*-1, *sn*-2, and *sn*-3 positions, respectively. The tuna orbital oil TG showed the distribution pattern of 22:6n-3 similar to that in the bonito head oil (A) TG. The tuna orbital oil TG contained 22:6n-3 at concentrations of 24.7, 5.5, and 33.2 mole % in the *sn*-1, *sn*-2, and *sn*-3 positions, respectively. Both of the 22:6n-3-rich fish oils were winterized oils. The bonito head oil (B) obtained before the winterization process had 18.3, 19.6, and 37.6 mole % of 22:6n-3 in the *sn*-1, *sn*-2, and *sn*-3 positions of TG, respectively. This fatty acid was primarily located in the *sn*-3 position, and distributed almost evenly between the *sn*-1 and *sn*-2 positions. The sardine oil TG contained 22:6n-3 in the *sn*-2 position (11.7 mole %) at the concentration higher than that in the *sn*-1 (9.3 mole %) and *sn*-3 (8.3 mole %) positions. In the seal oil TG, 22:6n-3 was more concentrated in the *sn*-3 position (12.5 mole %), followed in sequence by the *sn*-1 (7.2 mole %) and *sn*-2 (1.4 mole %) positions.

Comparison with usual marine oil TG. Brockerhoff *et al.* (3) studied the positional distribution of fatty acids in TG isolated from fatty fish, such as herring and mackerel. The overall distribution among the positions showed a predominance of monounsaturated fatty acid of all chainlengths in the *sn*-1 position. The *sn*-2 position contained high proportions of polyunsaturated fatty acids, but was also high in 16:0. The *sn*-3 position was occupied to a great extent by longer-chain unsaturated fatty acids. The analysis of blubber fat from marine mammal was also reported (3,14). The TG showed a preferential occupation of the *sn*-1 and *sn*-3 positions by the polyunsaturated fatty acids 20:5n-3 and 22:6n-3, especially of the *sn*-3 position. The *sn*-2 position was esterified by high proportions of saturated and C_{16}–C_{18} monounsaturated fatty acids. The longer-chain monounsaturated fatty acids were relegated to the *sn*-1 position. Brockerhoff *et al.* (3) pointed out the general tendency of longer-chain polyunsaturated fatty acids to be preferentially esterified in the *sn*-2 position in fish TG and in the *sn*-3 position in marine mammal TG. Litchfield (4,5) reported that the positional distribution of 22:6n-3 can be predicted by the following proportionality equations: for fish TG,

$$y_1 = 0.28x;\ y_2 = 2.06x;\ \text{and}\ y_3 = 0.66x \quad [1]$$

and for marine mammal TG,

$$y_1 = 0.94x;\ y_2 = 0.22x;\ \text{and}\ y_3 = 1.84x \quad [2]$$

where x shows the mole % of 22:6n-3 in the total TG and $0 < x < 30$; and y_1, y_2, and y_3 show the mole % of 22:6n-3 in the *sn*-1, *sn*-2, and *sn*-3 positions, respectively. These relationships essentially indicate that about 10, 70, and 20% of 22:6n-3 were esterified in the *sn*-1, *sn*-2, and *sn*-3 positions of fish TG, and about 30, 10, and 60% of it were in the *sn*-1, *sn*-2, and *sn*-3 positions of marine mammal TG.

Proportional distributions of 22:6n-3 among the *sn*-1, *sn*-2, and *sn*-3 positions of the TG analyzed in this study are shown in Figure 1. The general tendency for fish TG did not hold for the 22:6n-3-rich fish oils, particularly the oils obtained after

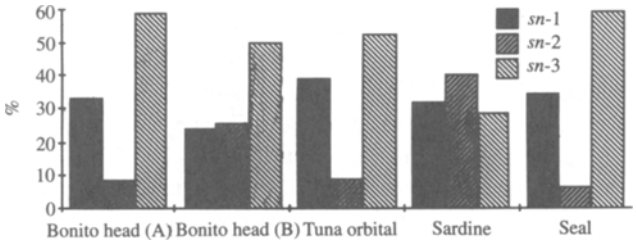

FIG. 1. Proportional distribution of 22:6n-3 among the *sn*-1, *sn*-2, and *sn*-3 positions of the fish oil triacyl-*sn*-glycerol (% of 22:6n-3 present).

winterization. Less than 10% of 22:6n-3 was esterified in the *sn*-2 position of the bonito head (A) oil and tuna orbital oil TG, whereas 30–40 and 50–60% of this acid were located in the *sn*-1 and *sn*-3 positions, respectively. Such a distribution pattern resembles that observed for marine mammal TG (seal oil TG) rather than fish TG (sardine oil TG). Assignment of 22:6n-3 in the *sn*-3 position higher than in the *sn*-2 position was also observed for the bonito head oil (B) TG, confirming that the general tendency cannot be applied to the 22:6n-3-rich fish oils.

Stereospecific and regiospecific analysis of TG. Sawada *et al.* (15) investigated the binding position of 22:6n-3 in major TG species of bonito and tuna orbital oils and the composition of TG species in them. The enzymatic hydrolysis of TG by lipase *Rhyzopus delemar* showed the predominance of 22:6n-3 in the *sn*-2 position. The opposite distribution pattern was obtained by the nonenzymatic method used in the present study.

Figure 2 compares the analytical data on distribution of 22:6n-3 obtained by the chemical and enzymatic methods. Pancreatic lipase hydrolysis has been used to determine the fatty acid composition of the *sn*-2 position of TG. When the enzyme is used on TG that contain a normal range of fatty acid components, little fatty acid specificity is evident. However, the enzymatic method is not suitable for TG that contain long-chain polyunsaturated fatty acids in the primary positions at high levels (12,16). Ester bonds of long-chain polyunsaturated fatty acids, such as 22:6n-3, to glycerol are hydrolyzed more slowly (12,16). In the *sn*-2 position of the bonito head oil (A) and tuna orbital oil TG, enzymatic hydrolysis gave values of 22:6n-3 much higher than those given by the nonenzymatic hydrolysis used in this study. The value obtained by the enzymatic hydrolysis of the bonito head oil (B) TG was also higher than that obtained by the nonenzymatic method. In the sardine and seal oil TG, similar values were obtained by both methods. Although the enzymatic analysis has not been performed in duplicate on each sample, these results indirectly support the view that 22:6n-3 was esterified in the primary (*sn*-1 and *sn*-3) positions at much higher concentrations in the bonito head oil (A and B) and tuna orbital oil TG.

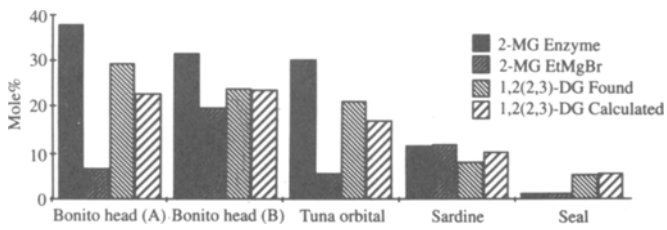

FIG. 2. Analytical data on the distribution of 22:6n-3 in the fish oil triacyl-*sn*-glycerol obtained by enzymatic and nonenzymatic hydrolysis procedures (mole %); MG, monoacylglycerol; DG, diacylglycerol.

For each TG sample, 1,2(2,3)-DG were prepared by the nonenzymatic hydrolysis. The 22:6n-3 contents in the 1,2(2,3)-DG prepared are shown in Figure 2, together with those calculated from the data presented in Tables 1 and 2. In the bonito head oil (A) and tuna orbital oil TG, the values found in this investigation were higher than the calculated values, whereas the sardine oil TG showed a calculated value that was higher than the found value. The *sn*-2 position of the 1,2(2,3)-DG was reported to be contaminated by up to 4% of fatty acids that had migrated from the *sn*-1 and *sn*-3 positions, although the fatty acid compositions of the primary positions appeared to be identical to those of the original TG (12,13). All positions of TG shown in Tables 1 and 2 were also contaminated with about 3% of fatty acids that had migrated from the other positions (9). When the fatty acid compositions of 1,2(2,3)-DG are calculated from the data shown in Tables 1 and 2, the contaminations are compensated. The preparation of 1,2(2,3)-DG also has not been performed in duplicate. However, the higher values of found data suggest that significant migration of 22:6n-3 had occurred to the *sn*-2 position from the primary positions, and confirm the view that 22:6n-3 was predominantly esterified in the *sn*-1 and *sn*-3 positions of the bonito oil (A) and tuna orbital oil TG.

The specific distribution pattern of 22:6n-3 in the fish TG. Leger *et al.* (17) showed that feeding a diet with saturated fatty acids in the *sn*-2 position did not change the accumulation of polyunsaturated fatty acids in the *sn*-2 position of rainbow trout depot TG. This result suggested that resynthesis of the TG might go through the *sn*-3 glycerophosphate pathway. On the other hand, accumulation of the polyunsaturated fatty acids in the *sn*-3 position was suggested as typical for TG that were isolated from mammals fed marine oils. Polyunsaturated fatty acids did not take preference over other fatty acids for esterification in the *sn*-2 position (18). Recent findings for rats that were fed fish oil were in accordance with this theory (19,20). The present study has revealed the positional distribution of fatty acids in TG of the 22:6n-3-rich bonito head and tuna orbital oils. The distribution pattern of 22:6n-3 resembled that in marine mammal oils rather than fish oils. This similarity suggests that the metabolism of the fish TG is in part controlled in a manner similar to that of mammal TG.

Bonito head and tuna orbital oils have been useful as 22:6n-3-rich fish oils. Enzymatic of chemical preparation of 22:6n-3 and 20:5n-3-rich TG is of recent interest (21–23). The special positional distribution of 22:6n-3 in these fish oil TG must confirm the potential for some beneficial uses in biological tests, dietary effects, medical areas, and preparation of TG with specific structures.

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