選択イオン質量分析法によるカツオ普通筋ジアシルグリセロリン脂質の分子種分析

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著者	大島, 敏明 和田, 俊 小泉, 千秋
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Application of Selected-Ion Monitoring Gas Chromatography/Mass Spectrometry to the Analysis of Molecular Species of 1,2-Diacylglycerophospholipids of Bonito White Muscle

Toshiaki Ohshima,* Shun Wada,* and Chiaki Koizumi*

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The molecular species of 1, 2-diacylglycerophospholipids of bonito *Euthynnus pelamis* (Linnaeus) white muscle were determined by selected-ion monitoring gas chromatography/mass spectrometry using electron impact ionization. The 1, 2-diacylglycerol *tert*-butyldimethylsilyl ether derivatives obtained from the glycerophospholipids were separated on a polar open-tubular column gas liquid chromatography based on the numbers of both total acyl carbons and double bonds. Peak assignment was carried out by locating the characteristic fragment ions, two types of $[RCO+74]^+$ ions due to fatty acyl residues on sn-1 and sn-2 positions of 1, 2-diacylglycerol and $[M-57]^+$ ions indicating the corresponding molecular weight. Consequently, the highly unsaturated 1, 2-diacylglycerophospholipids such as 20: 5n-3–20: 5n-3, 20: 5n-3–22: 6n-3 and 22: 6n-3–22: 6n-3 species were identified in the bonito white muscle.

With recent progress in high temperature fused silica open-tubular column, molecular species of naturally occurring triacylglycerols has become possible to be separated based on the combination of the number of total acyl carbons (TC) and double bonds (DB) by using high temperature methylphenylsilicone phase.[1] More polar SP 2330 phase has been applied successfully to the separation of *tert*-butyldimethylsilyl ether derivatives of glycerophospholipids into their molecular species.[2,3] In these studies, peak assignment was carried out on the basis of cochromatography with reference standards, relative retention times, rechromatography of compounds recovered from argentation thin layer chromatography and/or gas chromatography/mass spectrometry (GC/MS) on apolar columns.[1] In general, these techniques are quite time consuming and therefore troublesome for unsaturated lipids which are susceptible to oxidation. GC/MS equipped with either electron impact (EI) or chemical ionization (CI) modes and an apolar column has been usually used for identification of glycerides.[4,5] In the case of polar open-tubular columns, however, mass spectrometry is disturbed markedly by the higher level of back ground caused by bleeding from stationary phase, especially at higher column temperature. This appears to be one of the reasons why application of polar open-tubular columns in combination with MS has not been described so far.[1] In order to identify the peak components separated on a polar open-tubular column, we recently applied selected-ion monitoring GC/MS technique to the analyses of vegetable oil triacylglycerols.[6]

In the previous papers, the glycerophospholipids having two polyunsaturated fatty acyl residues, such as 20: 5–20: 5, 20: 5–22: 6 and 22: 6–22: 6, were estimated to be present in bonito white muscle.[7,8] In the present study, therefore, the molecular species of glycerophospholipids were investigated by using selected-ion monitoring mass spectrometry coupled with the gas chromatography equipped with a polar open-tubular column.

Materials and Methods

Samples

Twelve bonito *Enthynnus pelamis* (Linnaeus) samples, weighing 1.6 kg on the average, were supplied from Katsuura, Chiba Prefecture on 3rd May in 1987. Three bovine heart muscle specimens were purchased from a local retailer.

Extraction and Fractionation of Lipids

Total lipids of bonito white muscle and bovine

* Department of Food Science and Technology, Tokyo University of Fisheries, Konan, Minato, Tokyo 108, Japan (大島敏明，和田　俊，小泉千秋：東京水産大学食品生産学科).

heart muscle were extracted with chloroform/methanol according to the Bligh and Dyer procedure.[9] The phospholipid fraction (PL) was separated from other lipid classes by column chromatography on Bio-Beads S-X2 using benzene as eluant.[10]

Lipid Derivatization

An aliquot of a chloroform solution of the PL was hydrolyzed by phospholipase C (from *Bacillus sereus*, Boehringer Mannheim GmbH-Yamanouchi, Tokyo) to diradylglycerol,[11] followed by Silica Gel G preparative thin-layer chromatography (TLC, E. Merck, FRG) using a mixture of petroleum ether/diethyl ether/acetic acid (70: 30: 1, v/v/v) as the developing solvent. The diradylglycerol recovered from the TLC plates was converted to the corresponding *tert*-butyldimethylsilyl (*tert*-BDMS) ether derivatives by heating with *tert*-butyldimethylchlorosilane / imidazole reagent (Applied Science Laboratories Inc., State College, PA, USA) at 80°C for 1 h.[12] The corresponding 1,2-diacylglycerol *tert*-BDMS derivatives were separated from other derivatives by one dimensional Silica Gel G TLC using petroleum ether/diethyl ether/acetic acid (90: 10: 1, v/v/v) as the first developing solvent and toluene as the second one.[13]

The fatty acid methyl ester (FAME) of the phospholipids was obtained by saponification with 1 N KOH-ethanol at 85°C for 1 h, followed by interesterification with 14% BF_3-methanol at 95°C for 20 min.

The 1,2-diacylglycerol *tert*-BDMS ether derivative was hydrogenated in hexane over a catalytic amount of PtO_2 at 0.2 kg/cm^2 of hydrogen pressure for 2 h.

Gas Liquid Chromatography of FAME

Analytical gas liquid chromatography (GLC) of the fatty acid methyl ester (FAME) was carrried out on a Shimadzu GC12A instrument equipped with a flame ionization detector (FID) and a SUPELCOWAX-10 fused silica open-tubular column (0.25 mm id×30 m, Supelco Japan, Tokyo). Helium was used as the carrier gas at 1.8 kg/cm^2 (split ratio was about 1: 50) and the oven temperature was held at 195°C.

GLC and SIM-GC/MS of 1,2-Diacylglycerol tert-BDMS Ether Derivatives

The gas-liquid chromatography of the 1,2-diacylglycerol *tert*-BDMS ether derivatives was carried out on a Shimadzu GC12A instrument equipped with a Shimadzu movable solvent-less and on-column injector and a MP65HT (65% methylphenylsilicone) fused silica open-tubular column (0.25 mm id×25 m, Quadrex, New Haven, CT, USA). The injection port was held isothermally at 370°C. Depending on the type of sample, the column oven temperature was variable between 320 and 360°C. Helium was used as the carreir gas at a constant inlet pressure of 2 kg/cm^2. Usually 1–2 μl of 0.02% hexane solution of the sample were injected.

A selected-ion monitoring (SIM) GC/MS of the 1,2-diacylglycerol *tert*-BDMS ether derivatives was performed on a Shimadzu QP1000

Table 1. Fatty acid composition of bonito white muscle glycerophospholipids (Mean±S.D., n=4) (area %)

Fatty acid*1		Fatty acid	
14: 0	0.43±0.04	18: 2n-6	0.94±0.42
A15: 0	1.00±0.01	19: 0	0.45±0.01
15: 0	0.28±0.03	18: 3n-3	0.17±0.01
16: 0	19.89±1.00	19: 3n-6	0.22±0.01
16: 1n-9	0.11±0.10	20: 1n-11	0.51±0.27
16: 1n-7	0.99±0.05	20: 2n-6	0.28±0.02
I17: 0	0.27±0.01	20: 4n-6	3.02±0.06
A17: 0	0.36±0.02	20: 4n-3	0.17±0.00
16: 2n-4	0.42±0.03	20: 5n-3	4.82±0.10
17: 0	0.96±0.04	22: 4n-6	0.15±0.04
17: 1n-8	0.31±0.03	22: 5n-3	0.87±0.03
17: 2n-8	0.22±0.10	22: 6n-3	31.88±0.11
18: 0	15.04±0.29	24: 1n-9	0.80±0.11
18: 1n-9	9.70±0.22		
18: 1n-7	2.77±0.05	Other	2.97

*1 I, iso; A, anteiso.

quadrupole mass spectrometer equipped with an electron impact (EI) source to which an outlet of a MP65HT open-tubular column (0.25 mm id × 15 m) was connected directly. The conditions were 70 eV electron beam energy, 3 kV accelerating energy and a source temperature of 310°C.

Results and Discussion

GLC and SIM-GC/MS of 1, 2-*Diacylglycerol tert-BDMS Ether Derivatives*

In the case of triacylglycerol, the peak assignment was accomplished by locating the fragment ions having the same retention times on the SIM profile, that is, three kinds of $[RCO]^+$ ions due to the fattty acyl residues on the glycerol moiety and three kinds of $[M\text{-}OCOR]^+$ ions indicating the corresponding molecular weight.[14] The 1, 2-diacylglycerol *tert*-BDMS ether derivatives yield in general $[M\text{-}57]^+$ ions indicating the molecular weight and $[RCO]^+$, $[RCO+74]^+$ and $[M\text{-}OCOR]^+$ ions due to the fatty acyl residues.[12] Our preliminary examination showed that there was no notable difference between $[RCO]^+$ and $[RCO+74]^+$ in ion intesity (data not shown here). There-

Table 2. Calculated m/z of fragment ions from different molecular species of 1, 2-diacylglycerol *tert*-BDMS ether derivatives

TC: DB*	Acyl–Acyl	m/z of fragment ion		
		$[RCO+74]^+$		$[M\text{-}57]^+$
32: 0	16: 0–16: 0	313	313	625
	15: 0–17: 0	299	327	625
32: 1	16: 0–16: 1	313	311	623
33: 1	16: 0–17: 1	313	325	637
34: 0	16: 0–18: 0	313	341	653
34: 1	16: 0–18: 1	313	339	651
34: 2	16: 0–18: 2	313	337	649
36: 1	18: 0–18: 1	341	339	679
	16: 0–20: 1	313	367	679
36: 2	18: 1–18: 1	339	339	677
	16: 0–20: 2	313	365	677
	18: 0–18: 2	341	337	677
36: 3	18: 1–18: 2	339	337	675
36: 4	18: 2–18: 2	337	337	673
36: 5	16: 0–20: 5	313	359	671
38: 4	18: 0–20: 4	341	361	701
38: 5	18: 0–20: 5	341	359	699
38: 6	16: 0–22: 6	313	385	697
40: 7	18: 1–22: 6	313	385	723
42: 11	20: 5–22: 6	359	385	743
44: 12	22: 6–22: 6	385	385	769

* TC, the number of total acyl carbons; DB, the number of double bonds.

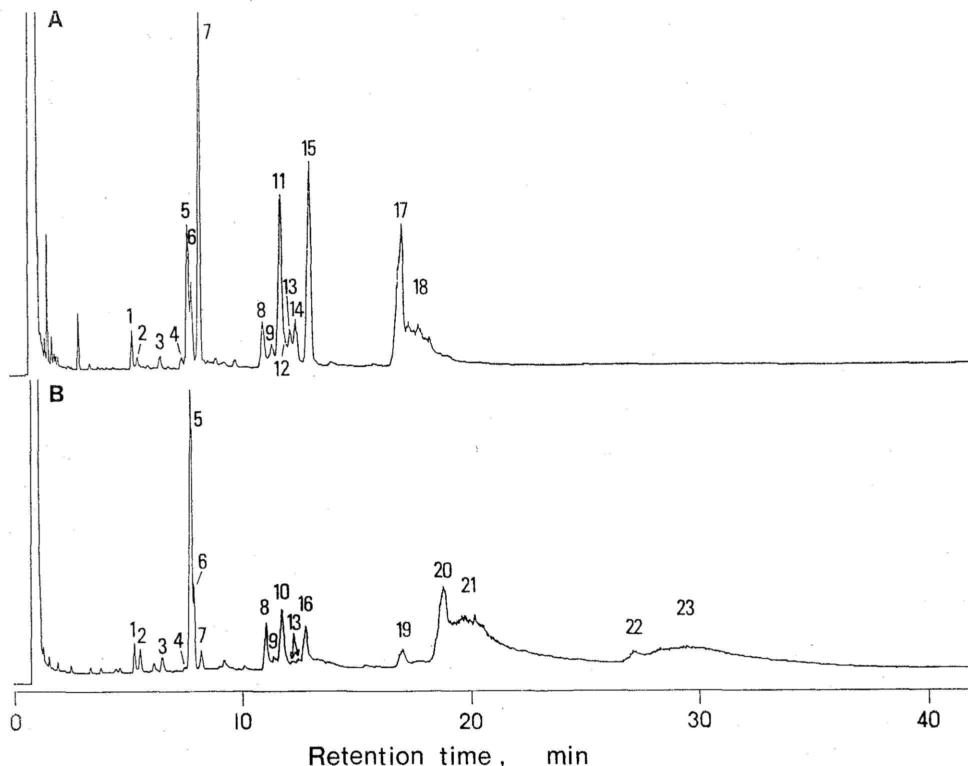

Fig. 1. Open-tubular gas liquid chromatograms of 1, 2-diacylglycerol *tert*-BDMS ether derivatives of bovine heart (A) and bonito white (B) muscles. 65% methylphenylsilicone, 0.25 mm id × 25 m, 290°C; He, 1.75 kg/cm².

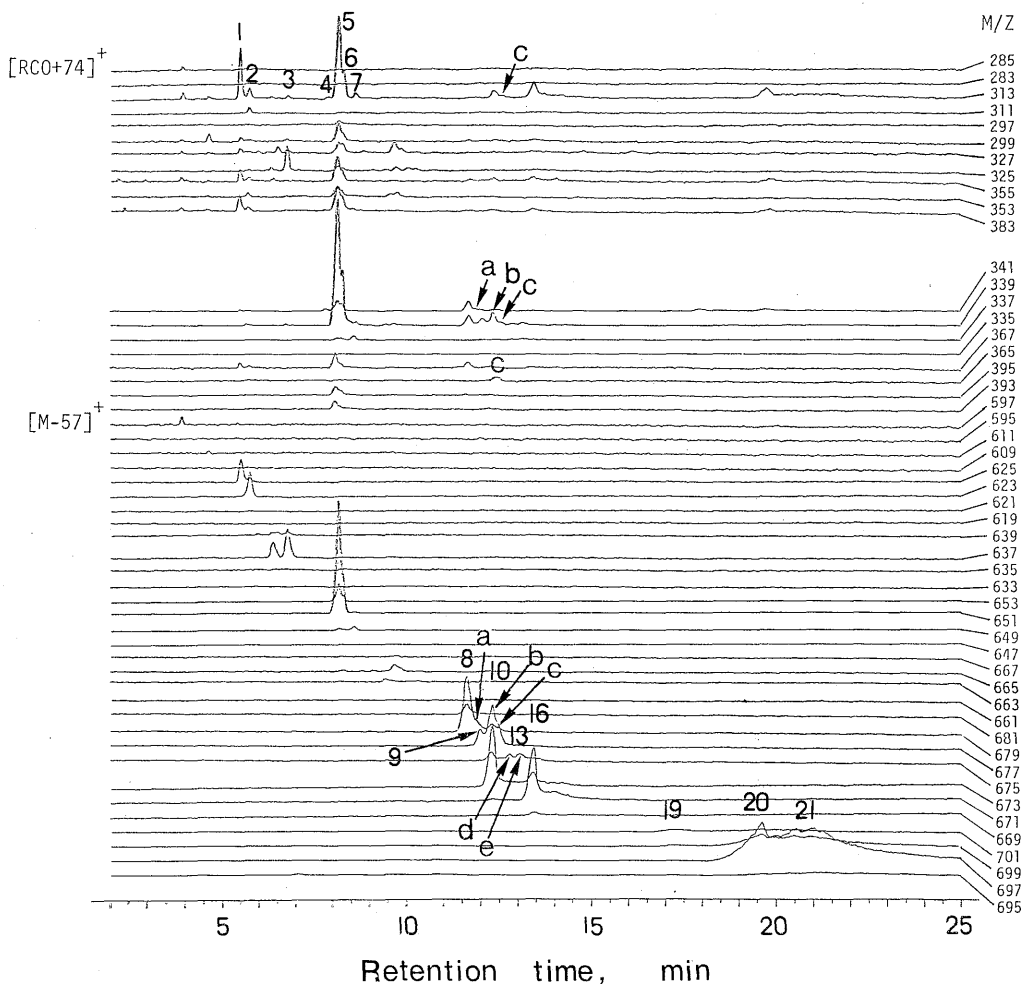

Fig. 2. Selected-ion monitoring of 1, 2-diacylglycerol *tert*-BDMS ether derivative of bonito white muscle. 65% methylphenylsilicone, 0.25 mm id × 15 m, 240°C; IS, 3 kV, 70 eV and 310°C.

fore, in this study we determined 1, 2-diacylglycerol *tret*-BDMS ether derivatives by locating the fragment ions corresponding to two types of $[RCO+74]^+$ and $[M-57]^+$ at the same retention time on the SIM profile. In order to simplify this peak assignment of the fragment ions, m/z of $[RCO+74]^+$ and $[M-57]^+$ yielded from bonito white muscle phospholoipids were previously estimated from their fatty acid compositions (Table 1) and summarized in Table 2.

Typical FID gas chromatograms of the 1, 2-diacylglycerol *tert*-BDMS ether derivatives from bovine heart muscle (A) and bonito white muscle (B) are shown in Fig. 1. As shown in Fig. 1-A, the bovine heart muscle glycerophospholipids gave more than 19 peaks (the prominent peaks were numbered in the figure) within 19 min. Peak No. 18 was eluted as a broad and unresolved peak. The bonito white muscle contained the compounds having longer retention times as shown in Fig. 1-B; peaks No. 20, 21, 22 and 23 appeared after 20 min under the similar analytical conditions. The shape of these delayed peaks were broad and the separations between peaks were somewhat incomplete.

Figure 2 shows the profile of the selected fragment ions yielded from the 1, 2-diacylglycerol *tert*-BDMS ether derivatives of bonito white muscle phospholipids. Since the liquid phase of the open-tubular column used in the GC/MS was the same as that used in the FID/GLC, although the column length was half of the latter, the elution order of the compounds should be unchanged from that in FID/GLC. Therefore, the numbers

of peak components appeared in the SIM profile (Fig. 2) corresponds to those in the FID gas chromatogram (Fig. 1-B).

When monitoring was carried out at m/z 313 and m/z 625 as the selected fragment ions, the ion peaks appeared at 5.5 min. From the results of previous calculation (Table 2), it was clear that m/z 313 ion is due to the $[RCO+74]^+$ yielded from palmitic acid residue and that m/z 625 ion is ascribed to the $[M-57]^+$ corresponding to the 1, 2-diacylglycerol having the number of total acyl carbons (TC) 32 and no double bond (DB). From the characteristic combination of the fragment ions, the particular molecular species in peak No. 1 is certainly 16: 0–16: 0. As a minor component of peak No. 1, 15: 0–17: 0 species was also identified, because the ion peaks appeared at the same retention time when monitoring was carried out at m/z 299 and 327 as the selected fragment ions (see Table 2 for the combination).

In a similar manner, the components of peaks No. 2, 3, 4 and 7 were readily identified as 16: 0–16: 1n-7, 16: 0–17: 1n-7, 16: 0–18: 0 and 16: 0–18: 2n-6, respectively. However, the peak assignment of the others was somewhat complicated as mentioned below.

Peak No. 5 was unresolved from peak No. 6 as shown in the SIM profile as well as in the FID gas chromatogram (Fig. 1-B). The intense ion peaks on m/z 339 suggested that not only peak No. 5 but also peak No. 6 has 18: 1 fatty acyl residue(s), since 18: 1 fatty acids of the bonito white muscle phospholipids consisted of two positional isomers, 18: 1n-9 and 18: 1n-7 (see Table 2); the ion peaks on m/z 339 of peak No. 5 and peak No. 6 arised from 18: 1n-9 and 18: 1n-7, respectively. Consequently, peaks No. 5 and 6 were identified as positional isomers; 16: 0–18: 1n-9 and 16: 0–18: 1n-7, respectively, as expected from the elution order of fatty acid methyl esters on this column.[15] Although the fragment ion peaks due to 15: 0, 17: 0, 19: 0, 21: 0 and 22: 1 were also detected at the same retention time, no fragment ion peak corresponding to $[M-57]^+$, expected from any combinations between above five fatty acyl residues, appeared at the same retention time. These unexpected fragment ions arised probably from the above certain molecular species of glycerophospholipids, because their concentrations in the PL seemed so high.

As shown in the FID gas chromatogram (Fig. 1-B), peak No. 8 was not separated completely from peak No. 9. This suggests that an unresolved small peak possibly exists between these two peaks. Indeed, the fragment ion peak appearing on m/z 679 profile due to the molecular species having TC 36 and DB 1 (see Table 1), showed a shoulder in the tailling portion of peak No. 8

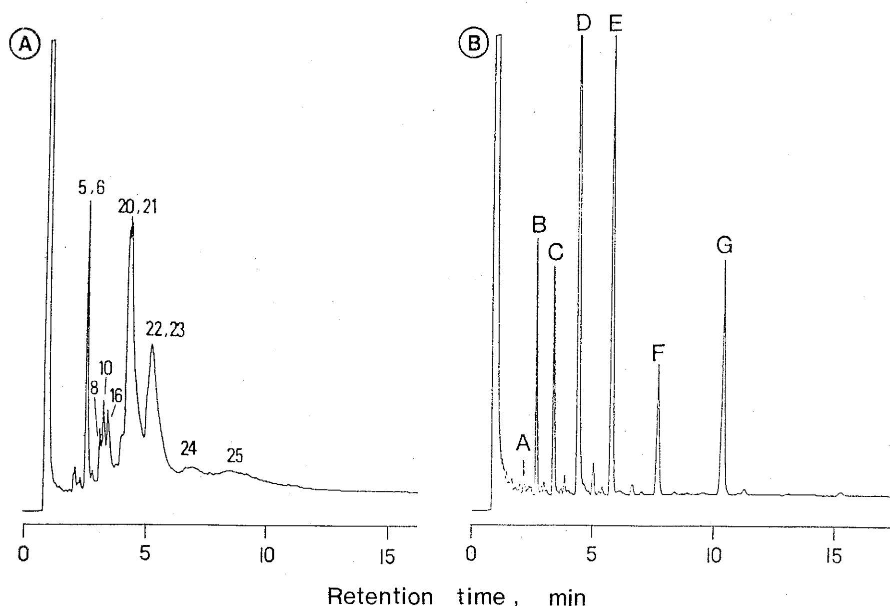

Fig. 3. Open-tubular gas liquid chromatograms of 1, 2-diacylglycerol *tert*-BDMS ether derivatives of bonito white muscle before (A) and after (B) hydrogenation at 320 and 350°C, respectively. Peak Nos. are in coincidence with those in Fig. 1.

(marked "a" in Fig. 2) immediately before peak No. 9. Although the component at the tailing portion on peak No. 8 on m/z 339 was coeluted with that of peak No. 9, there was apparent peak tailing on m/z 341 ion profile (marked also "a" in the figure). In consideration of the fatty acid composition shown in Table 1, peak No. 8 and its tailing portion "a" were identified as 18:0–18:1n-9 and 18:0–18 1n-7 species, respectively. The fragment ion peaks on m/z 313 and 367 also eluted at the same retention time. From Table 2, therefore, the molecular species 16:0–20:1n-11 was also estimated as a minor part of peak No. 8.

Peak No. 9 was readily identified as 18:1–18:1 by means of the fragment ion peak on m/z 339 and 677 (see Table 2). The positional isomers of the molecular species were characterized as follows.

Peak No. 10 consisted of at least two components; the prominent peak "b" and the shoulder "c". The component of peak "b" was certainly identified as 18:1–18:1 species based on its m/z 339 and m/z 677 ions. The other component, shoulder "c", included two molecular species; one was identified as 16:0–20:2n-6 species from the ion peaks at m/z 313, 365 and 677, and the other as 18:1–18:1 species from the ion peaks at m/z 339 and 677. Consequently, three molecular species of 18:1–18:1, the components of peak No. 9, the major portion "b" and a part of shoulder "c" of peak No. 10, were separated from

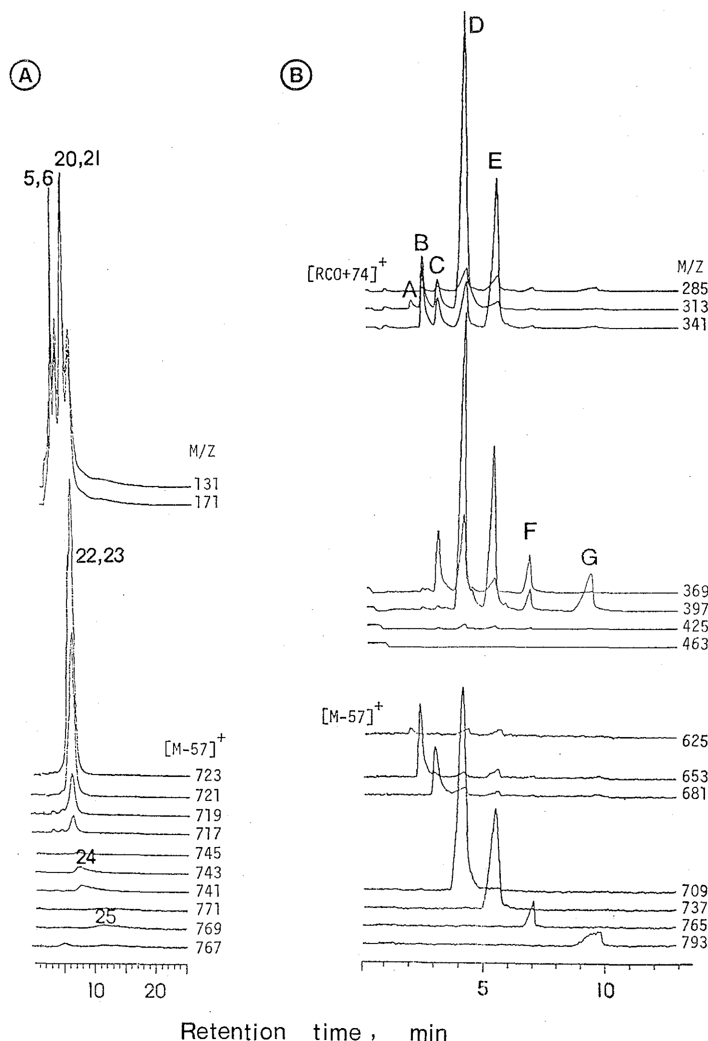

Fig. 4. Selected-ion monitoring of 1,2-diacylglycerol *tert*-BDMS ether derivatives of bonito white muscle glycerophopholipids before (A) and after (B) hydrogenation. 65% methylphenylsilicone, 0.25 mm id × 15 m; Is, 3 kV, 70 eV and 310°C.

one another on the basis of positional isomers of fatty acyl residues. Although close specification of these positional isomers of the molecular species of 18:1–18:1 seems impossible under these experimental conditions, the molecular species of peak No. 9, "b" and "c" are tentatively 18:1n-9–18:1n-7 (and/or 18:1n-7–18:1n-9) and 18:1n-7–18:1n-7, respectively, as expected from the elution order of fatty acid methyl esters on this column.[15] There was also an unexpected intense ion peak on m/z 673, which seemed due to the molecular species of TC: DB=36:4. However, no intense fragment ions corresponding to the molecular weight (730) estimated from m/z 673 ions were detected on any profiles of $[RCO+74]^+$ examined here. Consequently, the ion peak at m/z 673 was considered to be resulted from the molecular species identified above.

Peak No. 13 consisted of two peaks, "d" and "e", when monitoring was carried out at m/z 339 due to 18:1 residue and m/z 675 due to TC: DB=36:3. The peak components "d" and "e" were identified as 18:1n-9–18:2n-6 and 18:1n-7–18:2n-6, respectively, since they showed the fragment ion peaks on m/z 339, 337 and 675.

From a combination of the ion peaks on m/z 313 and 671 of peak No. 16, the molecular species of 16:0–20:5n-3 was tentatively estimated; although 20:5n-3 was contained in the original phospholipids (Table 1), m/z 359 ion which must be arised from 20:5 fatty acyl residue was not detected under these experimental conditions. In general, $[RCO+74]^+$ as well as $[RCO]^+$ due to fatty acyl residue on the glycerol skelton decrease in ion intensity when the fatty acyl chains contain double bonds.[16] Indeed, we confirmed these phenomena in the analyses of certain vegetable oil triacylglycerols such as soybean, palm, cottonseed, and safflower oils.[6] The fragment ion due to 20:5 fatty acyl residue was therefore probably very weak in ion intensty in the case of 16:0–20:5n-3 species. Peaks No. 20 and 21 were separated partly from each other in the analytical GLC as shown in Fig. 1. However, the components of both peaks yielded the intense fragment ions of m/z 697 as shown in Fig. 2. The ion of m/z 313 also detected at the same retention time. The ions of m/z 313 and 697 correspond to $[RCO+74]^+$ of 16:0 residue and $[M-57]^+$ of TC: DB of 38:6, respectively as seen in Table 2. Therefore, both of peaks No. 20 and 21 were estimated as 16:0–22:6n-3 species. The above conclusion implies that a single molecular species, 16:0–22:6n-3, was separated into two broadened peaks in GLC. Geeraert[17] pointed out that one of the defects of this type of column is that polyunsaturated triacylglycerols might polymerize on methylphenylsilicone phase. Peaks No. 20 and 21, which are indeed polyunsaturated glycerol derivatives, are probably polymerized under the condition of analysis.

Phosphatidylcholine and phosphatidylethanolamine, the major phospholipid classes of bonito white muscle, prominently consist of the molecular species having TC 40, 42 and 44 as reported previously.[7,8] Under the experimental conditions of Figs. 1 and 2, however, molecular species having TC over 38 were not detected. This suggests that there are probably some molecular species eluted after 25 min. Therefore, the samples before and after hydrogenation were analyzed again at the elevated oven temperature of 350°C and 320°C, respectively. Fig. 3 shows the typical gas chromatograms before (A) and after (B) hydrogenation of the 1,2-diacylglycerol *tert*-BDMS ether derivatives of bonito white muscle phospholipids. The hydrogenated sample was separated into seven well resolved peaks (marked A to G in Fig. 3-B). As shown in the SIM-profile in Fig. 4, peak E yielded the fragment ions of m/z 341 due to 18:0 fatty acyl residue, m/z 397 due to 22:0 fatty acyl residue and m/z 737 due to $[M-57]^+$ of TC: DB=40:0. From these results, peak E was certainly identified as 18:0–22:0 species. In a similar manner, peaks F and G were assigned to 20:0–22:0 and 22:0–22:0, respectively. The above observations strongly suggest that this sample contains also the molecular species of TC 40, 42 and 44, as expected to some extent from the previous results.[7,8] The original sample before hydrogenation (Fig. 3-A) shows additional three peaks (numbered 22 and 23, 24 and 25) after the unresolved peaks No. 20 and 21. The components of unresolved peaks No. 22 and 23 yielded the intense fragment ion peaks on m/z 723 (due to TC: DB=40:7) as shown in Fig. 4-A. Addition to this, the hydrogenated molecular species of TC 40 (peak E in Fig. 4-B) contained 18:0 and 22:0 as fatty acyl residues. Therefore, TC 40 species seemed to contain 18:1n-9–22:6n-3.

The weak fragment ion peaks of peak No. 24 appeared on m/z 743 (due to TC: DB=42:11) as shown in Fig. 4-A. Therefore, peak No. 24 seems to be 20:5n-3–22:6n-3 species. The fragment ion peak on m/z 741 (due to TC: DB=42:

Table 3. Compositions of the molecular species of 1, 2-diacylglycerophospholipids of bonito white and bovine heart muscles

TC: DB*1	Peak	Acyl –Acyl	Bovine*3,4	Bonito*4
32: 0	1	16: 0 –16: 0	1.21	{0.74
		15: 0 –17: 0	—	
32: 1	2	16: 0 –16: 1n-7	0.61	0.76
33: 1	3	16: 0 –17: 1n-8	0.48	0.47
34: 0	4	16: 0 –18: 0	0.54	0.14
34: 1	5	16: 0 –18: 1n-9	6.92	11.28
	6	16: 0 –18: 1n-7	3.65	1.92
34: 2	7	16: 0 –18: 2n-6	15.18	0.53
36: 1	8	18: 0 –18: 1n-9	2.76	{2.37
	8a	18: 0 –18: 1n-7	—	
36: 2	9	18: 1n-9–18: 1n-9	1.66	0.69
	10b	18: 1n-9–18: 1n-7	—	{4.10
	10c	{16: 0 –20: 2n-6	—	
		18: 1n-7–18: 1n-7	—	
	11	18: 0 –18: 2n-6	12.84	—
36: 3	12	16: 0 –20: 3n-3	0.45	—
	13d	18: 1n-9–18: 2n-6	2.44	0.48
	13e	18: 1n-7–18: 2n-6	3.23	—
36: 4	15	18: 2n-6–18: 2n-6	15.08	—
36: 5	16	16: 0 –20: 5n-3	—	3.36
38: 4	17	18: 0 –20: 4n-6	15.52	—
	18*2	18: 0 –20: 2n-6	17.44	—
38: 5	19	18: 0 –20: 5n-3	—	1.39
38: 6	20	16: 0 –22: 6n-3	—	13.92
	21*2		—	22.64
40: 7	22	18: 1n-9–22: 6n-3	—	1.96
	23*2		—	18.49
42: 11	24	20: 5n-3–22: 6n-3	—	5.81
42: 12	25	22: 6n-3–22: 6n-3	—	8.93

*1 TC, the number of total acyl carbons; DB, the number of dobule bonds.
*2 shoulder separation with peak broadened.
*3 unpublished data.
*4 area% on the gas chromatogram.

12) was also detected at the same retention time as peak No. 24, however as shown in Fig. 4-B there was no ion peak which could explain a reasonable combination of two fatty acyl residues with TC 42 and DB 12. Unsaturated 1, 2-diacylglycerol *tert*-BDMS ether serivatives usually yield the weak frgment ions having the range of mass number of $[(M\text{-}57)\pm 2]^+$ as well as $[M\text{-}57]^+$. Therefore, m/z 741 of the peak No. 24 is probably $[(M\text{-}57)\text{-}2]^+$.

The TC 44 species having only 22: 0 as the fatty acyl residue in the hydrogenated sample was identified as 22: 6n-3–22: 6n-3, because peak No. 25 of the original sample before hydrogenation yielded m/z 796 (due to 22: 6 fatty acyl residue).

The molecular species of bonito white muscle thus identified are summarized in Table 4, as well as those of bovine heart muscle glycerophospholipids analyzed as a comparative sample in our separated study. The most highly unsaturated fatty acyl residue of the 1, 2-diacyl glycerophospholipids of bovine heart muscle was 20: 4n-6. Indeed, most of mammalian glycerophospholipids have been well known to contain polyunsaturated fatty acyl residue (PUFA) on its sn-2 position of glycerol moiety.[18-20] Contrary to this, bonito glycerophospholipids included novel

molecular species in which both sn-1 and sn-2 positions were occupied by PUFA such as 20:5n-3 and 22:6n-3. The highly unsaturated glycerophospholipids characterized here were estimated to occur at a relatively higher level in many marine fish muscles such as cod,[21-23] sardine,* jack mackerel,* flying fish,* red barracuda,* fluounder,* and also other several species.[24]

In general, better separation based on the degree of unsaturation between highly unsaturated molecular species of glycerol derivatives are achived by polar columns.[1] Myher and Kuksis[2,3] accomplished better separations of each of 1,2-diacyl and 1-*O*-alk-1′-enyl-2-acyl *tert*-BDMS ether derivatives of rat liver phospholipids by using SP 2330 open-tubular column which is more polar than 65% methylphenylsilicone gum. Under these conditions, the derivatives of highly unsaturated molecular species such as 16:0–22:6 of 1,2-diacylglycerophospholipid and 18:0″–22:6 of plasmalogen seemed not to polymerize under analytical GLC. However, the maximum temperature of SP 2330 column (around 250°C) is too low to elute the highly unsaturated diacylglycerol derivatives, especialy in fish muscle phospholipids, even though hydrogen as the carrier gas and a shorter column were equipped. Although GC/MS offers much valuable information concerning the molecular species of glycerides, no application of polar open-tubular column in combined with mass specitrometer has yet been achieved[1] exceptfor certain vegetable oil triacylglycerols.[6] From this point of view, development of the capillary column with both higher polarity and higher maximum temperature is necessary for better separation and accurate quantification of highly unsaturated marine lipids.

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