ARTICLE

Molecular Species Composition of the Major Diacyl Glycerophospholipids from Muscle, Liver, Retina and Brain of Cod (*Gadus morhua*)

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The molecular species composition of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) from white muscle, liver, retina and brain of cod (*Gadus morhua*) were determined by high-performance liquid chromatography of the respective 1,2-diacylglycerol 3,5-dinitrobenzoyl derivatives. A minimum of 69 diacyl species was identified. In muscle and liver saturated fatty acid/polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid/PUFA molecular species were predominant, particularly 16:0/20:5 and 16:0/22:6 in PC, 16:0/22:6 and 18:1/22:6 in PE and 18:0/22:6 and 18:1/22:6 in PS. Didocosahexaenoyl species were major components of PC, PE and PS from retina, comprising 29.3, 71.8 and 59.7% of the respective totals. Didocosahexaenoyl species were also abundant in PE and PS from brain, accounting for 13.8 and 24.0% of the totals, respectively. DiPUFA species were important in muscle, totalling 21.2% in PC and 38.3% in PE. PC from all tissues had the largest amounts of species containing only saturated or monounsaturated fatty acids, accounting for 59.8% of PC from brain, including 12.8% of 18:1/24:1 plus 24:1/18:1.
Lipids 26, 565-573 (1991).

The main structural glycerophospholipids from vertebrate neural tissue contain large amounts of docosahexaenoic acid (DHA; 22:6n-3). Polyunsaturated fatty acids (PUFA) are very tenaciously retained by neural tissue (1), and two or three generations of dietary deprivation are required to reduce significantly the n-3 PUFA content of brain in rat (2) and brain and retina of rhesus monkeys (3). Depletion of n-3 PUFA in brain and retina results in reduced visual and cognitive abilities in rhesus monkeys (4). However, the precise role of 22:6n-3 in neural tissue is unknown.

Early attempts to separate phospholipids from bovine rod outer segments (ROS) according to the degree of unsaturation by adsorption thin-layer chromatography (TLC) (5), and later by argentation TLC (6), showed the presence of fractions containing more than six double bonds per molecule. Positional analyses of the fatty acids in PC and PE from frog ROS showed 22:6n-3 to be on both the *sn*-1 and *sn*-2 positions of the glyceride and it was deduced that diPUFA species were present (7). The development of methods for analyzing the molecular species composition of glycerophospholipids by high-performance liquid chromatography (HPLC) directly (8) or by preparing derivatives (9-12), or by gas-liquid chromatography (GLC) of volatile derivatives (13) has revealed the fully complexity of the lipid components of membrane structures, and these techniques have now been applied to many systems. The presence of diPUFA molecular species in frog retina was confirmed by direct analysis (14), while analyses of glycerophospholipids from the brain and retina of trout showed large amounts of di22:6n-3 species, especially in phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) from retina and in PE and PS from brain (15). Small amounts of diPUFA species also have been found in non-neural tissues including rat erythrocyte (11), rat testes (16), fish muscle (9) and cod roe (17). Marine fish are particularly rich in n-3 PUFA (18), and this paper presents molecular species analyses of PC, PE and PS of four tissues of cod (*Gadus morhua*). The results from two neural tissues, brain and retina, are compared with those from liver and muscle.

MATERIALS AND METHODS

Cod (*Gadus morhua*) (300-600 g weight) were obtained from the Marine Station (Millport on the Firth of Clyde, Scotland), maintained in a seawater aquarium at 10-14°C on a diet of chopped squid and used within two weeks of capture. Fish were killed by decapitation, and the eyes, liver and fillet of muscle removed and frozen at −70°C until required. Brains were removed and used immediately.

Phospholipase C from *Bacillus cereus* was obtained from Boehringer Corporation (London) Ltd. (Lewes, East Sussex, England). Butylated hydroxytoluene (BHT) was from Sigma Chemical Co. (Poole, Dorset, England). 3,5-Dinitrobenzoylchloride was from Aldrich Chemical Co. (Gillingham, Dorset, England) and was recrystallized from carbon tetrachloride before use. Standard lipids were obtained from Sigma and from Nu-Chek Prep (Elysian, MN), as detailed in (15). TLC and high-performance thin-layer chromatography (HPTLC) plates were coated with silica gel 60 (Merck, Darmstadt, Germany). Analar grade glacial acetic acid, carbon tetrachloride, propan-2-ol and pyridine were purchased from BDH Ltd. (Poole, Dorset, England). All other solvents of HPLC grade were from Rathburn Chemicals (Walkerburn, Peeblesshire, Scotland).

Ultrasphere ODS and Ultrasphere octyl HPLC columns (25 × 0.46 cm, 5 micron particle size) were obtained from Altex/Beckman (Beckman Instruments U.K. Ltd., High Wycombe, Bucks, England).

Extraction and purification of lipids. All solvents, apart from those used for TLC and HPLC, contained 0.01% (w/v) BHT. Samples were stored at −20°C under nitrogen between preparative procedures.

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Abbreviations: BHT, butylated hydroxytoluene; GLC, gas-liquid chromatography; HPLC, high-performance liquid chromatography; HPTLC, high-performance thin-layer chromatography; MUFA, monounsaturated fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; ROS, rod outer segment; SFA, saturated fatty acid; TLC, thin-layer chromatography; VLCPUFA, very long chain PUFA. Molecular species are abbreviated as follows: *e.g.*, 16:0/22:6 PC is

moved and the remaining tissue (8.3 g) homogenized in 10 volumes of chloroform/methanol (2:1, v/v) using a Polytron tissue disrupter. The retinas from 40 frozen cod eyes (5.7 g tissue) and 20 chopped frozen livers (89 g) were each homogenized in 10 volumes of chloroform/methanol (2:1, v/v), and finely shipped frozen muscle fillets (211 g) from 20 fish were homogenized in 5 volumes of solvent. After homogenization total lipid was extracted by the method of Folch *et al.* (19).

Neutral lipid was first removed from the brain and liver extracts by preparative TLC in hexane/diethyl ether/acetic acid (70:30:1, v/v/v). Phospholipids were eluted from the origin with chloroform/methanol/water (5:5:1, v/v/v), dried by rotary evaporation under vacuum at 35°C and finally under a stream of nitrogen. Phospholipid classes were separated by TLC in chloroform/methanol/water/triethylamine (30:35:6:35, by vol) (20) and the lipids detected under UV light after spraying with 0.1% (w/v) 2',7'-dichlorofluorescein in methanol containing 0.01% (w/v) BHT. Phospholipids were eluted from the silica gel with three 40-mL washes of chloroform/methanol/water (5:5:1, v/v/v) and dried as before. 2',7'-Dichlorofluorescein was removed from lipids by extraction with a solution of 2% (w/v) $KHCO_3$. PC and PS were further purified by TLC in chloroform/acetone/methanol/acetic acid/water (10:4:2:2:1, by vol) (21), and detected and extracted as before. All phospholipids were finally checked for purity by HPTLC in methyl acetate/propan-2-ol/chloroform/methanol/0.25% (w/v) KCl (25:25:25:10:9, by vol) (22). Lipids were detected by spraying with 3% (w/v) copper acetate in 8% (v/v) phosphoric acid and charring at 160°C for 15 min.

Fatty acid methyl esters were prepared by esterification in 2 mL of 1% (v/v) concentrated sulfuric acid in methanol at 50°C for 16 hr under nitrogen. The fatty acid composition of the phospholipids was determined by GLC of fatty acid methyl esters in a Packard 436 chromatograph fitted with a CP Wax 52CB fused silica capillary column (50 m × 0.32 mm i.d.) (Chrompack U.K. Ltd., London, England) (23), and with a CP Sil5CB fused silica capillary column (50 × 0.34 mm i.d.) (Chrompack) (24), using hydrogen as carrier gas.

Preparation of 3,5-dinitrobenzoyl derivatives. One-mg portions of phospholipid were hydrolyzed with phospholipase C using a two phase system of 1 mL diethyl ether and 1 mL 0.1M sodium borate buffer, pH 7.5, at room temperature under nitrogen for 5 hr (25). At the end of the incubations 1,2-diacylglycerols were extracted and purified by TLC in hexane/diethyl ether/acetic acid (50:50:1, v/v/v) (17). 1-Alkenyl-2-acylglycerols and 1-alkyl-2-acylglycerols were separated at this stage. Amounts of phospholipid remaining after phospholipase C digestion were less than 2% of the starting material. Diacylglycerols were derivatised in dry pyridine with 3,5-dinitrobenzoyl chloride at 60°C for 45 min under nitrogen, extracted and washed as described by Takamura *et al.* (12). The purity of the product was checked by HPTLC in hexane/diethyl ether/acetic acid (70:30:1, v/v/v).

Separation of molecular species. The 1,2-diacyl-3-dinitrobenzoyl-*sn*-glycerols were separatd by HPLC at 19–21°C on reverse phase columns using a Pye Unicam 4010 pump (Pye Unicam Ltd., Cambridge, England) and three isocratic solvent systems. An ODS column was used with methanol/propan-2-ol (95:5, v/v), flow rate 1.0 mL/min; and acetonitrile/propan-2-ol (80:20, v/v), flow rate 1.0 mL/min (12); a C8 column was used with methanol/water/acetonitrile (93:5:2, v/v/v), flow rate 1.2 mL/min (17). Peaks were detected at 254 nm with a Pye Unicam 4020 detector and quantified using a Shimadzu C-R6A recording integrator (Anachem, Luton, England).

Peaks were identified from plots of $\log_{10}$ (relative retention time, RRT) *vs.* the effective carbon number on the *sn*-1 position of the glyceride as described by Patton *et al.* (8) and Takamura *et al.* (12) using 16:0/22:6 as a reference peak. Didocosahexaenoylglycerol (Nu-Chek Prep) was also available for direct comparison of retention times. The main n-3 PUFA-containing molecular species were identified directly by GLC of the fatty acid methyl esters (17), while 18:1/20:1 plus 20:1/18:1 and 18:1/24:1 plus 24:1/18:1 had previously been synthesized (15). Each sample was chromatographed three times in each of the solvent systems and the standard deviations calculated. Where final peak areas were calculated by subtraction, the standard deviations of the contributing peaks were added to give the final error. Results are given to one decimal place for clarity. The $\log_{10}$ (RRT × 10) of all the molecular species in each solvent system are given in the appendix.

RESULTS

In muscle SFA/PUFA species were the most abundant class, comprising over 50% of PC and PS with almost equal amounts of 16:0/20:5 and 16:0/22:6 in PC and mainly 16:0/22:6 in PS (Table 1). The most abundant MUFA/PUFA species in each class and a major component of PE and PS was 18:1/22:6. DiPUFA species were also abundant in PC (21.2%) and PE (38.3%), predominantly 22:6/20:5 plus 20:5/22:6 in PC and 22:6/22:6 in PE. Another nine diPUFA species were found. Only small amounts of species containing only saturated and monounsaturated fatty acids were present of which 16:0/18:1 was the most abundant comprising 6.4% of PC.

The molecular species composition of PC, PE and PS from liver was broadly similar to that of muscle (Table 2). SFA/PUFA were the most abundant species in PC and PS, and MUFA/PUFA species in PE. In all three glycerophospholipids 16:0/22:6 was a major species comprising a quarter of the PC, and 18:1/22:6 was abundant in PE and PS. In PS, 18:0/22:6 was a major species but was much less abundant than in PS from muscle; 18:1/22:6 and 18:0/22:6 were equally abundant in PS from liver. In PS from liver 20:1/22:6 was also a major species (Table 2). In addition, liver contained up to nine diPUFA species totaling 13.1% in PE with 22:6-containing species dominant. Species containing only saturated and monounsaturated fatty acids were again of minor importance with 16:0/18:1 comprising 6.4% of PC.

Di22:6 species were most abundant in PC, PE and PS from retina accounting for 29.3, 71.8 and 59.7%, respectively (Table 3). Other diPUFA species were of minor importance. In PC, 16:0/22:6, 16:0/18:1 and 18:0/22:6 were the only other species present at greater than 5.1%. Two unknown species totaling 9.2% were also present in PC and were probably very long chain PUFA-containing species (26). In PE, 16:0/22:6, 18:1/22:6 and 18:0/22:6 at 5–6% were the only other species present at more than 2%, while in PS 18:0/22:6 was again abundant (Table 3). Species com-

TABLE 1

Molecular Species Compositions of Phosphatidylcholine, Phosphatidylethanolamine and Phosphatidylserine from Cod Muscle[a]

Species	PC(mol%)	PE(mol%)	PS(mol%)
diPUFA			
20:5/20:5; (18:4/22:6)	2.4 ± 0.2	1.4 ± 0.0	—[b]
20:5/22:6	8.3 ± 0.0	9.5 ± 0.3	1.1 ± 0.1
22:6/22:6	6.4 ± 0.5	19.8 ± 1.1	5.0 ± 0.6
20:5/22:5; 20:4/22:6	0.9 ± 0.1	3.2 ± 0.1	tr[c]
22:5/22:6	1.8 ± 0.0	3.6 ± 0.1	1.8 ± 0.0
22:5/22:5; 22:4/22:6	1.1 ± 0.1	0.8 ± 0.0	2.0 ± 0.8
Total	21.2	38.3	10.2
SFA/PUFA			
14:0/22:6	1.6 ± 0.0	0.7 ± 0.0	—
16:0/20:5	18.2 ± 0.8	4.4 ± 0.1	0.9 ± 0.0
16:0/22:6	21.7 ± 0.2	13.0 ± 0.1	5.2 ± 0.1
16:0/20:4	4.7 ± 0.1	1.9 ± 0.0	1.0 ± 0.0
16:0/22:5	1.5 ± 0.1	1.1 ± 0.1	—
16:0/18:2	1.3 ± 0.0	0.5 ± 0.0	—
18:0/20:5	1.2 ± 0.1	2.5 ± 0.0	5.0 ± 0.1
18:0/22:6	0.8 ± 0.1	6.7 ± 0.1	35.7 ± 2.8
18:0/20:4	0.6 ± 0.0	0.7 ± 0.2	2.2 ± 0.0
18:0/22:5	tr	0.8 ± 0.0	4.9 ± 0.1
Total	54.3	33.5	57.5
MUFA/PUFA			
18:1/20:5	3.5 ± 0.1	5.4 ± 0.1	0.8 ± 0.0
18:1/22:6	4.0 ± 0.1	13.4 ± 0.3	10.9 ± 1.0
18:1/20:4	1.6 ± 0.2	1.8 ± 0.7	2.8 ± 0.1
18:1/22:5	1.6 ± 0.2	1.9 ± 0.4	3.4 ± 0.1
18:1/22:4	—	1.1 ± 0.0	1.5 ± 0.2
20:1/22:6	0.2 ± 0.1	1.9 ± 0.2	3.9 ± 0.1
Total	14.1	26.9	25.3
SFA/MUFA			
16:0/18:1	6.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.1
18:0/18:1; (16:0/20:1)	0.5 ± 0.1	0.1 ± 0.1	1.7 ± 0.1
Total	7.8	0.7	3.3
MUFA/MUFA			
16:1/18:1	1.1 ± 0.1	—	—
16:1/18:1; (16:1/20:1)	0.6 ± 0.2	0.5 ± 0.0	2.2 ± 0.0
Total	2.3	0.6	3.1
Very long chain			
Total	0.4	—	0.7

[a]Species in italics have defined *sn*-1 and *sn*-2 fatty acid distributions, otherwise it was assumed that the most saturated fatty acid was on the *sn*-1 position. Only one fatty acid combination is given for those species with two equally likely positional isomers. Bracketed species are minor components based on fatty acid composition data. The errors are given as ± 1 standard deviation rounded to the nearest decimal place (see text). The following molecular species were also detected at <0.9% in at least one of the glycerophospholipids: 18:4/20:5, 22:4/20:5, *14:0/20:5*, *14:0/22:5*, *16:0/18:4*, *16:0/22:4*, *18:0/18:2*, *18:0/22:4*, *16:1/20:5*, *16:1/22:6*, *16:1/22:5*, *20:1/20:5*, *20:1/20:4*, *20:1/22:5*, *20:1/22:4*, 16:0/14:0, 14:0/16:1, 18:0/20:1, 18:1/18:1, 18:1/20:1, *24:1/20:5*, *24:1/22:6*, 18:1/24:1.

[b]—, Not detected.

[c]tr, <0.1%.

prising only SFA and MUFA totalled just 2.1% and 5.9%, respectively, in PE and PS from retina.

PC from cod brain showed the most complex composition of any of the glycerophospholipids examined; a minimum of 54 species were found, including seven unknowns which totalled 6.6% (Table 4). At least 10 species containing 24:0, 24:1 and 26:1 fatty acids totalling 16.9% were found, the most important of which was 24:1/18:1 plus 18:1/24:1 (12.8%). These species containing very long chain fatty acids were found only as trace components of PE and as less than 1% of PS. PC from brain also contained 42.9% of species containing SFA or MUFA of which 16:0/18:1 (16.9%) and 18:0/18:1 (9.5%) were the most important. The only other major species present was

TABLE 2

Molecular Species Compositions of Phosphatidylcholine, Phosphatidylethanolamine and Phosphatidylserine from Cod Liver[a]

Species	PC(mol%)	PE(mol%)	PS(mol%)
diPUFA			
20:5/22:6	2.2 ± 0.1	3.7 ± 0.1	1.2 ± 0.1
22:6/22:6	1.9 ± 0.2	4.0 ± 0.5	1.8 ± 0.1
20:4/22:6; 20:5/22:5	2.0 ± 0.0	2.1 ± 0.1	0.1 ± 0.0
22:5/22:6; 22:4/22:6	—[b]	1.5 ± 0.1	0.6 ± 0.1
Total	6.6	13.5	4.0
SFA/PUFA			
14:0/22:6	2.0 ± 0.0	0.6 ± 0.0	0.4 ± 0.0
14:0/22:5	1.0 ± 0.1	0.4 ± 0.0	—
16:0/20:5	15.8 ± 1.0	7.5 ± 0.4	0.9 ± 0.1
16:0/22:6	26.2 ± 0.3	13.6 ± 0.6	13.5 ± 0.9
16:0/20:4	3.8 ± 0.0	0.1 ± 0.0	1.5 ± 0.1
16:0/22:5	2.1 ± 0.0	3.0 ± 0.0	1.8 ± 0.1
16:0/18:2	1.2 ± 0.0	1.4 ± 0.1	0.4 ± 0.1
18:0/20:5	1.5 ± 0.0	4.7 ± 0.3	1.0 ± 0.1
18:0/22:6	1.5 ± 0.3	4.6 ± 0.1	21.7 ± 0.1
18:0/20:4	1.0 ± 0.1	1.5 ± 0.1	2.1 ± 0.1
18:0/22:5	tr[b]	0.3 ± 0.1	2.0 ± 0.1
18:0/22:4	0.1 ± 0.0	0.4 ± 0.0	1.3 ± 0.5
Total	57.7	39.3	47.2
MUFA/PUFA			
16:1/20:5	1.8 ± 0.0	0.6 ± 0.1	tr
16:1/22:6	3.3 ± 0.0	0.7 ± 0.2	—
16:1/20:4	1.5 ± 0.1	0.6 ± 0.0	—
16:1/22:5	1.8 ± 0.0	0.1 ± 0.0	—
18:1/20:5	5.5 ± 0.1	8.7 ± 1.8	0.9 ± 0.0
18:1/22:6	5.9 ± 0.1	24.5 ± 0.1	22.0 ± 1.6
18:1/20:4	2.5 ± 0.1	0.7 ± 0.1	1.9 ± 0.1
18:1/22:5	0.7 ± 0.1	4.8 ± 0.1	3.8 ± 0.1
20:1/20:5	0.9 ± 0.1	0.9 ± 0.1	1.8 ± 0.1
20:1/22:6	0.9 ± 0.0	2.7 ± 0.2	10.8 ± 0.6
20:1/20:4	tr	0.5 ± 0.1	1.3 ± 0.1
Total	25.1	45.5	44.4
SFA/SFA			
Total	0.3	0.0	0.4
SFA/MUFA			
16:0/18:1	6.4 ± 0.1	0.2 ± 0.0	—
Total	7.4	0.7	1.4
MUFA/MUFA			
18:1/18:1	0.6 ± 0.1	0.3 ± 0.0	2.5 ± 0.4
18:1/18:1; (18:1/20:1)	1.3 ± 0.0	0.2 ± 0.0	—
Total	2.3	0.8	2.7
Very long chain			
Total	—	0.2	0.3

[a]Species in italics have defined *sn*-1 and *sn*-2 fatty acid distributions, otherwise it was assumed that the most saturated fatty acid was on the *sn*-1 position. Only one fatty acid combination is given for those species with two equally likely positional isomers. Bracketed species are minor components based on fatty acid composition data. The errors are given as ± 1 standard deviation rounded to the nearest decimal place (see text). The following molecular species were also detected at <0.9% in at least one of the glycerophospholipids: 20:5/20:5, 22:5/22:6, 22:4/20:5, *14:0/20:5*, *14:0/22:6*, *16:0/18:4*, *16:0/22:4*, *18:0/18:2*, *18:1/18:2*, *18:1/22:4*, *20:1/22:5*, *20:1/22:4*, 14:0/16:0, *16:0/16:0*, 14:0/16:1, 18:0/18:1 + (16:0/20:1), *16:1/16:1*, 18:1/20:1, *20:1/20:1*, *24:1/22:6*, 18:1/24:1.

[b]—, Not detected.

[c]tr, <0.1%.

TABLE 3

Molecular Species Compositions of Phosphatidylcholine, Phosphatidylethanolamine and Phosphatidylserine from Cod Retina[a]

Species	PC(mol%)	PE(mol%)	PS(mol%)
diPUFA			
20:5/22:6	0.7 ± 0.1	1.7 ± 0.1	0.4 ± 0.0
22:6/22:6	29.3 ± 0.9	71.8 ± 1.7	59.7 ± 1.4
22:5/22:6	0.2 ± 0.0	1.0 ± 0.1	0.4 ± 0.0
Total	30.2	74.6	60.5
SFA/PUFA			
16:0/20:5	2.6 ± 0.5	0.7 ± 0.1	0.1 ± 0.0
16:0/22:6	23.2 ± 0.1	5.9 ± 0.2	1.7 ± 0.1
16:0/20:4	1.7 ± 0.3	0.6 ± 0.0	0.4 ± 0.0
18:0/20:5	0.5 ± 0.1	0.8 ± 0.0	1.9 ± 0.5
18:0/22:6	9.1 ± 1.1	5.0 ± 0.1	16.2 ± 0.1
18:0/20:4	0.5 ± 0.0	1.0 ± 0.0	1.6 ± 0.5
Total	36.5	14.4	22.2
MUFA/PUFA			
18:1/20:5	0.6 ± 0.1	1.2 ± 0.2	0.4 ± 0.1
18:1/22:6	1.7 ± 0.5	5.9 ± 0.0	5.9 ± 0.4
20:1/20:4	0.2 ± 0.0	—[b]	1.7 ± 0.7
Total	4.1	9.0	11.3
SFA/SFA			
Total	0.7	tr[c]	0.5
SFA/MUFA			
16:0/18:1	13.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
18:0/18:1; (16:0/20:1)	1.9 ± 0.1	0.5 ± 0.0	1.6 ± 0.2
Total	15.3	0.8	2.3
MUFA/MUFA			
18:1/18:1; (16:1/20:1)	1.3 ± 0.1	0.6 ± 0.0	2.5 ± 0.1
Total	2.3	1.3	3.1
Unknowns			
U1	5.1 ± 0.2	—	—
U2	4.1 ± 0.1	—	—

[a]Species in italics have defined *sn*-1 and *sn*-2 fatty acid distributions, otherwise it was assumed that the most saturated fatty acid was on the *sn*-1 position. Only one fatty acid combination is given for those species with two equally likely positional isomers. Bracketed species are minor components based on fatty acid composition data. The errors are given as ± 1 standard deviation rounded to the nearest decimal place (see text). The following molecular species were also detected as <0.9% in at least one of the glycerophospholipids: *20:5/20:5*, 22:5/22:6, *22:5/22:5*, *16:0/22:6*, *16:0/22:5*, *18:0/22:5*, *18:1/20:4*, *18:1/22:5*, *20:1/22:6*, *20:1/22:5*, 14:0/16:0, 16:0/18:0, 18:0/16:1, 16:1/18:1, 18:1/20:1, *20:1/20:1*, 18:1/24:1.

[b]—, Not detected.

[c]tr, <0.1%.

16:0/22:6. PE and PS from brain were broadly similar in composition to those from muscle, liver and retina. D[22:6 was abundant in both classes, especially PS (24.0%), while 16:0/22:6, 18:1/22:6 and 18:0/22:6 were the other major species in PE, and 18:0/22:6 and 18:1/22:6 in PS.

DISCUSSION

The method used here is capable of resolving most of the molecular species present in glycerophospholipids from fish tissues. However, species containing fatty acids which are present in very small amounts (<0.5%) were not always detected, e.g., 16:4n-3 and 18:3n-3. Isomers of unsaturated fatty acids are not separated, *e.g.*, n-7 and n-9 MUFA or n-3 and n-6 PUFA. Analysis by GLC showed that 22:5 was the n-3 isomer, 22:4 the n-6 isomer and 20:4 predominantly the n-6 isomer; 20:4n-3 was a very minor component in most samples. The chromatographic systems could not separate diPUFA species containing different fatty acids but the same total carbon number and double bonds. Very long chain fatty acid-containing species were also difficult

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