Similarities in the Lipid Class Profiles of Oils from Atlantic and Pacific Dogfish Livers[1]

S-J. Kang[2], M.C.A. Timmins, and R.G. Ackman*

Canadian Institute of Fisheries Technology, DalTech, Dalhousie University, Halifax, Nova Scotia, Canada B3J 2X4

ABSTRACT: Liver oils from Atlantic and Pacific dogfish (*Squalus acanthias*) have been compared for lipid classes, fatty acids of the total oil and of important lipid classes, and details of the alkyl chains in the 1-*O*-alkyl-2,3-diacylglycerol ethers (DAGE). In general there were few striking differences, confirming the view of biologists that these sharks are one species. The Pacific dogfish liver oil had a higher content (41.2%) of DAGE than the oil from Atlantic dogfish (18.2%). Both oils had all common and expected fatty acids in the proportions usual for marine oils, but they differed in the eicosenoic chains of the glycerol ethers (GE). The Pacific oil was unusual in having low but similar proportions of two alkyl chain isomers, 20:1n-11 and 20:1n-9. The Atlantic oil was very high in the 20:1n-11 isomer, which is usually lower than 20:1n-9 in the fatty acids of most regional marine oils. Unexpectedly, the DAGE of both oils had further unusual 20:1 isomer proportion in the GE chain, with 20:1n-7 > 20:1n-9. Minor oddities in the fatty acids may reflect different basic food sources.

JAOCS 75, 1667–1672 (1998).

KEY WORDS: 1-*O*-Alkyl-2,3-diacylglycerol ethers, dogfish liver oil, fatty acids, glycerol ethers, shark liver oil.

Oil from the liver of the Pacific dogfish was at one time a major source of natural vitamins A and D. No similar industry existed on the Atlantic coast of Canada, and the original research on the commercial utilization of the oils from this species (1) antedated modern chromatographic technology. Subsequently the biochemistry of the 1-*O*-alkyl-2,3-diacylglycerol ethers (DAGE) was the subject of much research (2,3), and more recently a concentrate of omega-3 polyunsaturated fatty acids was prepared from the total fatty acids of a Pacific dogfish liver oil (4). Biologists have more or less concluded that there is only one species of dogfish, *Squalus acanthias,* in the Atlantic and Pacific Oceans (5,6). In addition to our wish to confirm this through chemotaxonomy of the liver oils, it was desirable to compare Atlantic and Pacific dogfish liver oils for details of their fatty acids and DAGE, since dogfish is potentially a large fishery resource for industrial oil for eastern Canada and the United States (7).

MATERIALS AND METHODS

Oil from the livers of Atlantic dogfish caught off southwest Nova Scotia in 1991 and Pacific dogfish caught off British Columbia in 1997 were recovered by comminuting equal parts of several freshly thawed livers in a Cuisinart DLC-X food processor. The slurry was heated at 44–45°C for approximately 1 h, resulting in separation of an oil layer from a protein-rich aqueous layer. The oil was filtered and dried over Na_2SO_4 with recoveries of 55 and 53%, respectively.

Lipid class composition and isolation. Lipid class compositions were determined by thin-layer chromatography–flame-ionization detection (TLC–FID) (8,9) using silica gel Chromarods-SIII for separation. A Mark III Iatroscan (Iatron Laboratories, Inc., Tokyo; Canadian distributor, Scientific Products & Equipment, Concord, Ontario) was used for quantitation. After spotting, rods were placed in a constant humidity tank over a saturated sodium chloride solution for 10 min, and then transferred immediately to the developing tank. Different solvent systems were used to enhance separation of different lipid classes. Initially, each sample on the Chromarod-SIII was developed in hexane/diethyl ether/formic acid (97:3:1, vol/vol/vol) for 55 min, to separate nonpolar lipids. The triacylglycerol (TAG), DAGE, and polar lipids were well resolved from most other lipid classes. However, free fatty acids (FFA) overlapped with DAGE. In a second solvent system, FFA, sterol, diacylglycerol (DAG), and monoacylglycerol (MAG) were resolved using a double development technique. The rods were first developed in hexane/chloroform/isopropanol/formic acid (85:14.5:0.75:0.1, by vol) for 40 min and partially scanned from the top to the lowest point behind the DAG peak. In this solvent, FFA could be separated easily from DAGE and TAG, as the DAGE moved ahead of the FFA, leaving a satisfactory gap between DAGE and FFA. However, in this solvent system TAG and DAGE were found to overlap. The final development was performed in chloroform/methanol/water (65:35:4, vol/vol/vol) for 50 min to separate the polar lipids from unknown components. Solutions of standards such as cholesteryl ester, FFA, TAG, cholesterol, DAG, MAG, diacylglycerylether (1-*O*-hexadecyl-2,3-dipalmitoyl-*rac*-glycerol), phos-

[1]Presented in part at the 84th Annual Meeting of the American Oil Chemists' Society, Atlanta, GA, May 8–12, 1994.

[2]Present address: Department of Aquaculture, College of Fisheries, Gyeongsang National University, Tongyeong 650-160, Korea.

*To whom correspondence should be addressed at the Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, P.O. Box 1000, Halifax, Nova Scotia, Canada B3J 2X4.
E-mail: odorjr@tuns.ca

phatidylcholine, phosphatidylethanolamine, and lysophosphatidylcholine were used to identify the lipid classes.

Separate fractions of DAGE, TAG, and FFA were obtained by preparative TLC by streaking on "Prekotes" (Adsorbosil-5 silica gel TLC plates, 20 × 20 cm; Applied Science Laboratories, College Park, PA). The solvent development system was hexane/diethyl ether/acetic acid (85:15:1, vol/vol/vol). Bands were visualized by spraying with a 0.01% solution of 2′,7′-dichlorofluorescein in ethanol and viewing under ultraviolet light. Each band was scraped off the TLC plate and extracted with a mixture of chloroform/methanol (50:50, vol/vol) according to the procedure of Fine and Sprecher (10). The isolated DAGE were saponified with KOH/C_2H_5OH according to AOCS official method Ca 6b-53 (11). The unsaponifiable matter containing 1-*O*-alkyl glyceryl ether (GE) was recovered with freshly distilled diethyl ether, and the DAGE fatty acids after acidification with HCl.

Preparation of derivatives of glycerol ethers. Two derivatives of the deacylated GE were used in this experiment. The GE fraction from the basic TLC separation was purified by TLC and converted initially to a TMS (trimethylsilyl) derivative. However, TMS ether derivatives did not separate well on gas–liquid chromatography (GLC) on an Omegawax-320 column (Supelco, Bellefonte, PA). Accordingly, isopropylidene derivatives were prepared by a modification of the method of Wood (12). Briefly, 1 mL of acetone containing 0.1 μL of 70% perchloric acid was added to the sample. The mixture was allowed to stand at room temperature for 20 min. The reaction was terminated by adding 1–2 drops of concentrated NH_4OH, then water, and the product was extracted three times with hexane/diethyl ether (50:50, vol/vol). Further purification of the extracted derivatives was achieved by TLC using hexane/diethyl ether/acetic acid (40:60:1, vol/vol/vol) prior to GLC analysis.

Preparation of methyl esters. Up to 20 mg of lipid, 1.0 mL hexane, and 1.0 mL 8% BF_3-methanol solution were placed in a screw-capped (Teflon-lined) centrifuge tube. The tube was flushed with nitrogen, sealed, and heated for 1 h at 100°C. After cooling, 3 mL of water was added to the tube. After vortexing, the hexane layer was removed to another tube. The aqueous layer was again extracted two times with hexane (2 mL each). The combined hexane extract was washed once with 3 mL water, dried over anhydrous Na_2SO_4, and concentrated as required for GLC.

GLC. The fatty chain compositions of the 1-*O*-alkyl-2,3-isopropylidene glycerol derivatives were determined by GLC using a Perkin-Elmer 8240 GC (Perkin-Elmer, Norwalk, CT) fitted with FID and a flexible fused-silica open-tubular column (30 m × 0.32 mm i.d., with a bonded polyglycol phase of Omegawax-320). The injector and detector were held at 250 and 270°C, respectively, the column was at 210°C, and the split ratio was 1:50. Helium was used as the carrier gas at a constant inlet pressure of 90 kP.

Fatty acid compositions of the methyl esters were analyzed by GLC using the same instrument but with a programmed column temperature, initially 185°C for 8 min, then an increase of 3°C/min to 230°C, and a final hold for 10 min. Helium was used as the carrier at a pressure of 90 kP. The injection port and detector were maintained at 250 and 270°C, respectively. Peaks were identified using authentic fatty acid methyl esters, total hydrogenation, GLC–mass spectroscopy (MS), and by plotting techniques, and areas were converted to wt% by correcting for the FID response of different structures and chainlengths (13).

TABLE 1
Lipid Class Composition (w/w%) of Atlantic and Pacific Dogfish Liver Oils from Thin-Layer Chromatography–Flame-Ionization Detection[a]

Component	Atlantic	Pacific
1-*O*-Alkyl diacylglycerol ethers	18.2 ± 1.37	41.24 ± 0.36
Triacylglycerols	80.9 ± 1.37	58.76 ± 0.36
Free fatty acid	0.3 ± 0.02	n.d.
Diacylglycerols	n.d.	n.d.
Sterols	0.5 ± 0.0	Trace
Unknowns	0.1 ± 0.07	Trace

[a]Standard deviation based on five determinations. n.d., not detected.

Hydrogenation. An aliquot of 10 mg or less of lipid derivative was placed in a round-bottomed flask and freed of solvent under nitrogen. Approximately 70 mL methanol and a few mg of platinum oxide catalyst (Sigma, St. Louis, MO) were added, and the suspension was stirred gently for 2 h under hydrogen at a flow rate of 0.01 L/min. The remaining methanol was evaporated to a volume of 2 mL, and the flask contents were dissolved in hexane and transferred to a large screw-capped test tube using three 5-mL rinses of hexane. The hexane was warmed in the flask before transfer by pipet

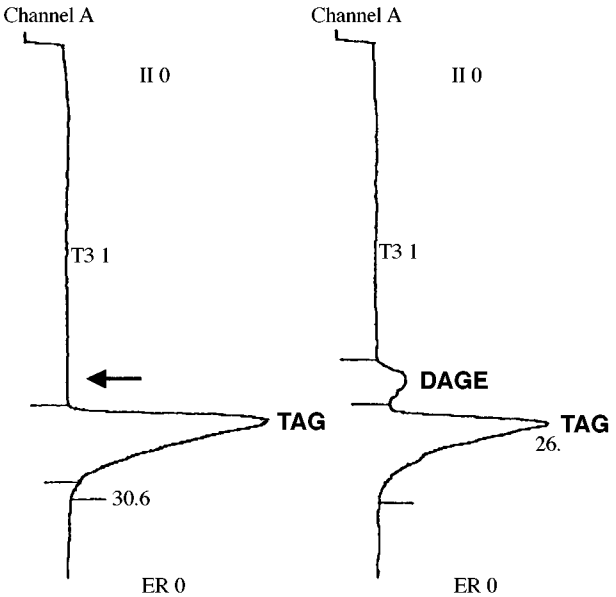

FIG. 1. Comparison of chromatograms of an Atlantic dogfish liver oil and an Atlantic herring oil on Chromarods SIII. Sample is applied below ER O marking. Arrow indicates absence of DAGE in herring oil. For development conditions see text. TAG, triacylglyderols; DAGE, 1-*O*-alkyl-2,3-diacylglycerol ethers.

to the test tube to ensure that the longer-chain methyl esters would be dissolved in the hexane. The combined hexane rinses were washed once with 5 mL water and transferred to another tube for concentration under nitrogen to an appropriate concentration.

GLC–MS analysis. The 1-*O*-alkylglycerol ether peak GLC identification was confirmed by GLC–MS using an ion trap detector (ITD) (Finnigan MAT 700, San Jose, CA) operated with an IBM-compatible personal computer. The ITD was connected to a Perkin-Elmer 990 gas chromatograph (Norwalk, CT) by a heated transfer line so that the column exited directly into the ITD. The column and isothermal conditions for GLC were the same as for the GLC–FID analysis of isopropylidene derivatives.

RESULTS AND DISCUSSION

Characterization of oils. The liver from the Atlantic dogfish yielded 54.8% lipid, of which 99.9% consisted of neutral lipids and such minor amounts (0.1%) of unknown compounds (Table 1) that these were not visible under the normal load conditions of Figure 1.

The liver oil of the Atlantic dogfish was characterized (Table 1) by a high percentage of TAG (80.9%) and small amounts of FFA (0.3%) and sterol (presumably the 0.5% is all cholesterol), and a trace of unknown compounds. The livers for production of Atlantic dogfish liver oil were stored for a longer time prior to extraction than those of Pacific origin, accounting for the production of FFA. No DAG or MAG were observed in either case, so an origin of the FFA in phospholipids is less likely than general TAG/DAGE enzymatic hydrolysis. The TAG content in Atlantic dogfish oil was greater than in the Pacific oil; the balance of DAGE in the Pacific oil was proportionately more. In other commercial samples of Atlantic dogfish oil DAGE were as high as 25%, and in other samples of commercial Pacific oil DAGE approached 50% (4).

Our GLC separations of fatty acid methyl esters from Atlantic dogfish liver total lipids, before and after hydrogenation,

TABLE 2
Fatty Acid Composition (w/w%)[a] of Total Oil and of Isolated Triacylglycerols of Atlantic and Pacific Dogfish Livers

	Total lipid		Triacylglycerol			Total lipid		Triacylglycerol	
Fatty acid	Atlantic	Pacific	Atlantic	Pacific	Fatty acid	Atlantic	Pacific	Atlantic	Pacific
12:0	0.09 ± 0.01	0.04 ± 0.02	0.07 ± 0.06	0.11 ± 0.06	18:3n-6	0.31 ± 0.04	0.11 ± 0.00	0.23 ± 0.04	0.10 ± 0.01
13:0	0.07 ± 0.04	Trace[b]	0.06 ± 0.02	0.05 ± 0.03	18:3n-4	0.14 ± 0.02	0.12 ± 0.01	0.13 ± 0.03	0.12 ± 0.01
14:0 Iso	0.03 ± 0.01	n.d.	0.02 ± 0.01	Trace	18:3n-3	0.61 ± 0.01	0.42 ± 0.01	0.68 ± 0.00	0.48 ± 0.01
14:0	3.34 ± 0.06	1.93 ± 0.04	3.80 ± 0.15	2.39 ± 0.06	18:4n-3	0.90 ± 0.03	0.55 ± 0.03	0.93 ± 0.03	0.64 ± 0.01
14:1n-9	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.02 ± 0.01	18:4n-1	0.22 ± 0.00	0.20 ± 0.05	0.25 ± 0.01	0.18 ± 0.01
14:1n-7	0.03 ± 0.01	n.d.	0.05 ± 0.02	0.07 ± 0.01	19:0	0.04 ± 0.00	0.10 ± 0.01	n.d.	0.10 ± 0.00
14:1n-5	0.05 ± 0.01	n.d.	n.d.	n.d.	20:0	0.14 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
15:0 Iso	0.16 ± 0.01	0.08 ± 0.00	0.17 ± 0.01	0.08 ± 0.01	20:1n-(11 + 9)	15.91 ± 0.06	5.70 ± 0.12	15.67 ± 0.14	4.84 ± 0.06
15:0 Anteiso	0.08 ± 0.01	0.05 ± 0.01	0.05 ± 0.04	0.08 ± 0.04	20:1n-7	1.05 ± 0.00	1.18 ± 0.02	1.13 ± 0.02	0.98 ± 0.01
15:0	0.30 ± 0.01	0.25 ± 0.00	0.36 ± 0.00	0.28 ± 0.01	20:1n-5	0.03 ± 0.00	n.d.	n.d.	n.d.
15:1n-8	0.05 ± 0.01	Trace	0.06 ± 0.01	0.02 ± 0.00	20:2NMID	0.04 ± 0.01	0.18 ± 0.04	0.37 ± 0.05	0.14 ± 0.01
15:1n-6	n.d.	n.d.	n.d.	0.02 ± 0.01	20:2n-6	0.14 ± 0.09	0.28 ± 0.01	0.28 ± 0.05	0.24 ± 0.01
16:0 Iso	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	0.08 ± 0.00	20:3n-4	0.03 ± 0.00	n.d.	0.03 ± 0.00	n.d.
16:0 Anteiso	0.06 ± 0.05	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	20:3n-6	0.17 ± 0.10	0.13 ± 0.00	0.07 ± 0.00	0.12 ± 0.01
Pristanic	0.04 ± 0.01	0.18 ± 0.04	0.04 ± 0.01	0.16 ± 0.00	20:3n-3	0.15 ± 0.01	0.14 ± 0.04	0.17 ± 0.01	0.14 ± 0.01
7-Methyl 16:0	0.22 ± 0.00	0.17 ± 0.00	0.23 ± 0.01	0.19 ± 0.00	20:4n-6	0.33 ± 0.01	0.48 ± 0.04	0.36 ± 0.05	0.48 ± 0.02
16:0	12.16 ± 0.39	20.15 ± 0.04	12.62 ± 0.35	19.48 ± 0.06	20:4n-3	0.77 ± 0.02	0.46 ± 0.01	0.71 ± 0.00	0.42 ± 0.02
16:1n-11	0.05 ± 0.01	n.d.	n.d.	n.d.	20:5n-3	3.67 ± 0.00	4.12 ± 0.12	3.61 ± 0.01	4.32 ± 0.20
16:1n-7	5.15 ± 0.16	5.51 ± 0.14	5.72 ± 0.22	6.60 ± 0.01	21:5n-3	0.30 ± 0.01	0.34 ± 0.04	0.27 ± 0.00	0.36 ± 0.07
16:1n-5	0.28 ± 0.01	0.04 ± 0.00	0.33 ± 0.05	0.04 ± 0.00	22:0	n.d.	n.d.	0.06 ± 0.01	n.d.
16:2n-4	0.29 ± 0.01	0.30 ± 0.01	0.32 ± 0.00	0.36 ± 0.01	22:1n-(13 + 11)	19.13 ± 0.70	4.04 ± 0.20	17.70 ± 0.51	2.90 ± 0.02
16:3n-4	0.11 ± 0.01	0.90 ± 0.07	0.11 ± 0.00	1.44 ± 0.01	22:1n-9	3.56 ± 0.04	2.91 ± 0.00	2.87 ± 0.11	1.98 ± 0.01
16:3n-3	0.05 ± 0.01	n.d.	n.d.	n.d.	22:1n-7	0.28 ± 0.00	0.42 ± 0.05	0.28 ± 0.03	0.30 ± 0.06
16:4n-3	0.26 ± 0.00	0.06 ± 0.01	0.27 ± 0.01	0.08 ± 0.01	22:1n-5	0.05 ± 0.00	n.d.	0.05 ± 0.01	n.d.
16:4n-1	0.23 ± 0.00	0.26 ± 0.00	0.22 ± 0.01	0.28 ± 0.01	22:4n-6	0.31 ± 0.09	0.37 ± 0.07	0.04 ± 0.03	0.21 ± 0.06
17:0 Anteiso	0.12 ± 0.00	0.18 ± 0.01	0.11 ± 0.01	0.19 ± 0.01	22:5n-6	0.23 ± 0.01	0.34 ± 0.04	0.15 ± 0.02	0.28 ± 0.03
17:0 + Phytanic	0.24 ± 0.00	0.64 ± 0.01	0.33 ± 0.01	0.28 ± 0.01	22:4n-3	0.15 ± 0.02	0.05 ± 0.03	0.10 ± 0.02	0.06 ± 0.04
18:0	1.81 ± 0.03	3.66 ± 0.03	2.06 ± 0.05	3.94 ± 0.03	22:5n-3	1.59 ± 0.05	2.12 ± 0.03	1.20 ± 0.01	1.31 ± 0.01
18:1n-9	11.90 ± 0.26	25.19 ± 0.17	12.62 ± 0.26	27.40 ± 0.19	22:6n-3	6.05 ± 0.06	5.10 ± 0.11	5.76 ± 0.06	4.93 ± 0.16
18:1n-7	3.06 ± 0.11	5.80 ± 0.14	3.44 ± 0.00	6.70 ± 0.15	24:1	0.53 ± 0.01	0.31 ± 0.01	0.48 ± 0.03	0.20 ± 0.02
18:1n-5	0.50 ± 0.01	0.31 ± 0.00	0.56 ± 0.01	0.31 ± 0.00	24:1n-9	1.20 ± 0.04	1.17 ± 0.04	1.11 ± 0.07	0.78 ± 0.08
18:2n-9	0.05 ± 0.00	0.08 ± 0.01	0.03 ± 0.03	0.06 ± 0.00	Σ Saturated	19.51	27.73	20.76	27.61
18:2n-7	0.08 ± 0.01	0.08 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	Σ Monoenoic	62.15	52.63	61.72	53.16
18:2n-6	0.97 ± 0.01	0.82 ± 0.01	1.02 ± 0.02	0.92 ± 0.02	Σ Polyenoic	18.34	18.23	17.52	17.99
18:2n-4	0.19 ± 0.02	0.22 ± 0.01	0.14 ± 0.01	0.25 ± 0.00	Mono/poly	3.39	2.89	3.5	2.95

[a]Mean and standard deviation of two determinations. Trace, trace amount; n.d., not detected; NMID, non-methylene interrupted dienoic acid.

TABLE 3
Fatty Acid Compositions (w/w%) of the Lipid Classes DAGE[a] and FFA of Atlantic and Pacific Dogfish Livers

	DAGE		FFA			DAGE		FFA	
Fatty acid	Atlantic	Pacific	Atlantic	Pacific	Fatty acid	Atlantic	Pacific	Atlantic	Pacific
12:0	0.11 ± 0.06	0.28 ± 0.02	0.64 ± 0.04	0.33 ± 0.11	18:2n-4	0.04 ± 0.03	0.18 ± 0.01	0.47 ± 0.09	0.32 ± 0.02
13:0	0.03 ± 0.01	0.04 ± 0.01	0.62 ± 0.02	0.12 ± 0.02	18:3n-6	0.13 ± 0.00	0.08 ± 0.01	1.56 ± 0.01	0.18 ± 0.02
14:0 Iso	0.02 ± 0.00	0.03 ± 0.01	0.72 ± 0.12	0.12 ± 0.02	18:3n-4	0.07 ± 0.01	0.13 ± 0.01	0.40 ± 0.05	0.20 ± 0.01
14:0	1.44 ± 0.13	1.82 ± 0.21	3.49 ± 0.03	1.86 ± 0.04	18:3n-3	0.31 ± 0.07	0.40 ± 0.04	1.55 ± 0.04	0.54 ± 0.01
14:1n-9	0.09 ± 0.01	0.04 ± 0.00	0.69 ± 0.02	0.12 ± 0.05	18:4n-3	0.23 ± 0.01	0.32 ± 0.02	1.05 ± 0.09	0.83 ± 0.03
14:1n-7	0.03 ± 0.01	0.06 ± 0.00	0.43 ± 0.03	0.02 ± 0.04	18:4n-1	0.01 ± 0.00	0.10 ± 0.00	0.72 ± 0.05	0.34 ± 0.08
14:1n-5	0.02 ± 0.02	n.d.	n.d.	n.d.	20:0	0.17 ± 0.01	0.17 ± 0.03	1.25 ± 0.24	0.32 ± 0.07
15:0 Iso	0.11 ± 0.01	0.12 ± 0.01	0.62 ± 0.03	0.16 ± 0.01	20:1n-(11 + 9)	18.01 ± 0.19	5.72 ± 0.77	9.13 ± 0.07	4.70 ± 0.05
15:0 Anteiso	0.06 ± 0.01	0.08 ± 0.01	0.46 ± 0.03	0.34 ± 0.04	20:1n-7	1.12 ± 0.02	1.24 ± 0.15	0.80 ± 0.01	1.61 ± 0.01
15:0	0.26 ± 0.02	0.34 ± 0.04	1.34 ± 0.11	0.48 ± 0.11	20:1n-5	Trace	n.d.	Trace	n.d.
15:1n-8	0.05 ± 0.0	0.04 ± 0.01	1.03 ± 0.03	0.12 ± 0.01	20:2NMID	0.12 ± 0.01	0.14 ± 0.02	2.42 ± 0.03	0.22 ± 0.05
15:1n-6	n.d.	n.d.	n.d.	0.06 ± 0.00	20:2n-6	0.32 ± 0.04	0.28 ± 0.03	0.31 ± 0.00	0.38 ± 0.02
16:0 Iso	0.08 ± 0.01	0.11 ± 0.00	0.53 ± 0.02	0.14 ± 0.01	20:3n-4	0.11 ± 0.10	n.d.	0.07 ± 0.05	n.d.
16:0 Anteiso	0.02 ± 0.01	0.03 ± 0.00	0.38 ± 0.12	0.14 ± 0.01	20:3n-6	0.09 ± 0.02	0.14 ± 0.04	1.69 ± 0.02	0.22 ± 0.01
Pristanic	0.17 ± 0.01	0.32 ± 0.06	0.40 ± 0.01	0.18 ± 0.01	20:3n-3	0.19 ± 0.05	0.11 ± 0.01	0.47 ± 0.01	0.16 ± 0.00
7-Methyl 16:0	0.13 ± 0.03	0.20 ± 0.01	0.62 ± 0.01	0.26 ± 0.00	20:4n-6	0.23 ± 0.13	0.27 ± 0.00	0.55 ± 0.01	1.30 ± 0.06
16:0	13.86 ± 1.01	29.30 ± 2.02	12.31 ± 0.15	18.86 ± 0.01	20:4n-3	0.54 ± 0.15	0.48 ± 0.01	1.29 ± 0.03	0.76 ± 0.02
16:1n-11	0.04 ± 0.00	n.d.	0.48 ± 0.02	n.d.	20:5n-3	1.15 ± 0.04	2.26 ± 0.18	4.55 ± 0.03	6.49 ± 0.47
16:1n-7	2.23 ± 0.14	4.94 ± 0.54	4.88 ± 0.09	4.75 ± 0.06	21:5n-3	0.21 ± 0.05	0.54 ± 0.16	0.24 ± 0.03	0.46 ± 0.01
16:1n-5	0.04 ± 0.00	0.07 ± 0.00	0.83 ± 0.06	0.06 ± 0.01	22:0	0.06 ± 0.06	n.d.	1.10 ± 0.85	n.d.
16:2n-4	0.01 ± 0.01	0.20 ± 0.02	0.67 ± 0.04	0.32 ± 0.04	22:1n-(13 + 11)	24.90 ± 0.39	3.40 ± 0.88	9.98 ± 0.53	3.14 ± 0.09
16:2n-6	0.36 ± 0.03	n.d.	n.d.	n.d.	22:1n-9	6.02 ± 0.34	2.68 ± 0.61	1.36 ± 0.02	2.08 ± 0.06
16:3n-4	0.03 ± 0.01	0.53 ± 0.06	0.19 ± 0.01	0.54 ± 0.01	22:1n-7	0.48 ± 0.05	0.40 ± 0.08	0.15 ± 0.02	0.36 ± 0.16
16:3n-3	0.04 ± 0.04	n.d.	0.27 ± 0.03	n.d.	22:1n-5	0.17 ± 0.03	n.d.	n.d.	n.d.
16:4n-3	0.21 ± 0.01	0.08 ± 0.01	0.37 ± 0.03	0.07 ± 0.01	22:4n-6	0.80 ± 0.17	0.62 ± 0.01	0.31 ± 0.02	0.18 ± 0.00
16:4n-1	0.07 ± 0.01	0.21 ± 0.00	0.54 ± 0.00	0.50 ± 0.04	22:5n-6	0.32 ± 0.04	0.33 ± 0.03	0.10 ± 0.07	0.32 ± 0.02
17:0 Anteiso	0.12 ± 0.02	0.24 ± 0.02	0.41 ± 0.02	0.32 ± 0.00	22:4n-3	0.36 ± 0.04	0.10 ± 0.01	0.20 ± 0.07	0.10 ± 0.06
17:0 + Phytanic	0.28 ± 0.00	0.72 ± 0.00	0.51 ± 0.03	0.51 ± 0.03	22:5n-3	2.92 ± 0.10	2.88 ± 0.14	0.74 ± 0.01	1.48 ± 0.08
18:0	1.43 ± 0.01	2.88 ± 0.17	3.99 ± 0.07	4.86 ± 0.04	22:6n-3	4.34 ± 0.14	3.60 ± 0.00	3.07 ± 0.48	4.94 ± 0.29
18:1n-9	9.76 ± 0.24	22.58 ± 0.09	9.77 ± 0.15	21.12 ± 0.09	24:1	1.15 ± 0.02	0.26 ± 0.07	0.45 ± 0.04	0.16 ± 0.01
18:1n-7	1.61 ± 0.01	4.18 ± 0.08	3.15 ± 0.07	6.62 ± 0.06	24:1n-9	1.87 ± 0.10	0.78 ± 0.22	1.32 ± 0.01	0.92 ± 0.02
18:1n-5	0.22 ± 0.16	0.31 ± 0.00	0.49 ± 0.20	0.46 ± 0.02	Σ Saturated	19.50	36.68	29.85	29.00
18:2n-9	n.d.	0.06 ± 0.01	0.27 ± 0.06	0.09 ± 0.00	Σ Monoenoic	66.72	46.70	44.49	46.30
18:2n-7	0.04 ± 0.04	0.06 ± 0.01	0.29 ± 0.05	0.08 ± 0.01	Σ Polyenoic	13.78	14.94	25.66	22.38
18:2n-6	0.53 ± 0.02	0.84 ± 0.04	1.30 ± 0.03	1.36 ± 0.23	Mono/poly	4.84	5.09	1.73	2.07

[a]DAGE, 1-*O*-alkyl-2,3-diacylglycerol ether; FFA, free fatty acid. See Table 2 for other abbreviations.

confirmed the minor methyl-branched fatty acids commonly found in fish oils (14). No remarkable fatty acids were observed, the GLC baseline of the hydrogenated sample being reasonably clean and similar to other open-tubular GLC analyses of marine oil fatty acid methyl esters. This confirmed that there were no significant amounts of unusual methyl-branched fatty acids present in either the total fatty acids or those of the TAG. The compositions of fatty acid methyl esters of total oil and of TAG recovered from plate TLC are given in Table 2.

The unsaturated individual fatty acids of both oils were unremarkable in most respects. The dogfish is an omnivore and relatively long-lived (5,6). The fatty acids are accumulated from fats in a variety of fish such as herring and mackerel, for which similar details have been published (15–17). An exception was the rather low proportions of 20:1n-(11+9) and of 22:1n-(13+11) relative to 20:1n-7 and 22:1n-9 in the Pacific liver oil. The 18:1n-9 and 18:1n-7 were twice as high in the Pacific as in the Atlantic oil, while total 20:1 and 22:1 were lower because the totals for monounsaturated fatty acids were similar at about 60%. That there could be differences in the two oils in this respect was shown by a different sample of commercial Pacific dogfish liver oil with 17.7% 18:1n-9, 4.3% 20:1n-(11+9), and 1.9% 22:1n-9. In a direct comparison of Atlantic and Pacific herring oils, the average w/w% of total 22:1 was 10.1% in Pacific oils vs. 21.1% in Atlantic oils (18). There is therefore little doubt that the figures for 20:1 and particularly 22:1 reflect fatty acid proportions in major dogfish food items such as herring. The proportion of 18:1n-7 to 18:1n-9 was, however, quite similar in the dogfish oils studied for this report. The Atlantic dogfish liver oil contained less palmitic acid (16:0) (12.16%) than that of the Pacific species (20.15%), but the proportions of myristic acid (14:0) were reversed. The fatty acid compositions of the TAG portion of Atlantic and Pacific dogfish liver oils were similar to those of the total lipid, since TAG served as the main source of the fatty acids. The total liver lipids of Atlantic dogfish contained almost exactly the same total of polyunsaturated fatty acids (18.34%) as the liver oil from the Pacific species (18.23%).

TABLE 4
Alkyl Chain Compositions (w/w%) of the GE[a] of Atlantic and Pacific Dogfish Liver Oils

	Alkyl chain compositions	
Alkyl chain structure	Atlantic	Pacific
14:0	4.20 ± 0.04	3.32 ± 0.52
14:1	0.20 ± 0.16	0.64 ± 0.23
15:0 Iso	0.56 ± 0.01	0.36 ± 0.05
15:0 Anteiso	0.28 ± 0.01	0.14 ± 0.01
15:0	0.71 ± 0.02	0.58 ± 0.06
16:0 Iso	0.33 ± 0.01	0.49 ± 0.14
16:0	13.70 ± 0.41	14.60 ± 0.05
16:1n-7	11.87 ± 0.50	10.38 ± 0.81
17:0 Iso	1.14 ± 0.05	0.88 ± 0.08
17:0 Anteiso	0.38 ± 0.06	0.38 ± 0.07
17:0	0.79 ± 0.32	0.43 ± 0.03
17:1	0.91 ± 0.05	1.10 ± 0.05
18:0 Iso	0.52 ± 0.05	0.46 ± 0.02
18:0	4.33 ± 0.59	4.42 ± 0.33
18:1n-(11+9)	42.36 ± 0.50	50.74 ± 0.74
18:1n-7	4.38 ± 0.29	4.77 ± 0.10
18:1n-5	1.20 ± 0.10	0.50 ± 0.04
18:2n-6	1.94 ± 0.12	0.92 ± 0.23
19:1	0.97 ± 0.03	0.85 ± 0.30
19:1	0.38 ± 0.01	n.d.
20:0	Trace	n.d.
20:1n-11	10.55 ± 0.03	0.46 ± 0.08
20:1n-9	2.87 ± 0.41	0.72 ± 0.06
20:1n-7	4.48 ± 0.33	1.28 ± 0.42
22:1	0.95 ± 0.03	n.d.
Σ Saturated	26.94	26.06
Σ Monoenoic	71.12	70.80
Σ Polyenoic	1.94	0.92
Mono/poly	36.66	76.96

[a]GE, 1-*O*-alkylglycerol ether. See Table 2 for other abbreviations.

FFA. The FFA of the Atlantic dogfish oil (Table 3) were higher in both saturated and polyunsaturated fatty acids than the fatty acids of the TAG (Table 2) or of the DAGE (Table 3). This suggests an origin in marine phospholipid fatty acids (19). However, the more abundant 16:0 and 18:1 and lower 22:1 FFA in the Pacific dogfish liver oil show a similarity in composition to the TAG fatty acids of Table 2, indicating a more general lipolysis.

Fatty acids of DAGE. The fatty acids recovered from the diacyl groups of the DAGE (Table 3) differed only in minor ways from the corresponding TAG fatty acids (Table 2). The relative proportions for the totals were, in fact, relatively similar for the saturated, monoenoic, and polyenoic fatty acids from these two lipid classes in the respective oils. The 18:2n-6 and 18:3n-3 were unimportant (<1.0%) components in both lipid classes of both oils and 20:4n-6 was even lower.

Composition of glyceryl ethers. The typical GLC analysis of the glyceryl ethers is shown in Table 4. The structures of the major components were confirmed by hydrogenation and by GLC–MS. The relative intensities of the principal peaks in the mass spectrum of any one of these components included the protonated molecular ions. In addition, such fragments as *m/z* 101 (base peak, 100%) and M – 15, corresponding to cleavage within the molecule, were characteristic for isopropylidene derivatives of GE (20). Glyceryl ethers of both Atlantic and Pacific oils were very similar in composition. The predominant compounds were 14:0, 16:0, and 18:0 for saturated compounds, and 16:1 and 18:1 for monoenoic contents. The dienoic and minor branched compounds of the GE occurred only in small amounts. The monoethylenic isomers are listed in detail by analogy to the GLC structures given for fatty acids. In the Atlantic oil 18:1n-(11+9) was lower than in the Pacific oil, but was offset by far more 20:1n-11. However, an unusual feature of the GE alkyl chain of both oils was the order of magnitude for the other 20:1 isomers, with n-9 < n-7. Although poorly resolved for GLC–MS, scans of ion fragments recorded across this group and the protonated molecular ion confirmed that only 20:1 isomers were present, and not a possible non-methylene-interrupted diene (21). Confirmation of this group of isomers by hydrogenation and quantitative reanalysis was also successful.

Methodology for the analysis of shark liver oils is generally available (22–24) as part of current interest in the wide occurrence of DAGE in animals (25) and in their biochemistry (26). The occurrence of DAGE in deep-sea sharks and squid is well documented (27–29), although these are not necessarily of biochemical relevance since the dogfish is usually found close to the surface or in shallower coastal waters. Commercial exploitation of shark liver oils for industry is possible (30–35), especially as the overall characteristics of dogfish liver oils, except for the DAGE, resemble those of herring oil. Our work (36) has also documented the digestion of the oil and its potential suitability for a feed oil in the salmon aquaculture industry, where long-chain omega-3 fatty acids are necessary. To promote utilization, some Canadian fish operations have adopted the term "northern" shark, and for food use of the meat the name grayfish has also been applied (37). We conclude that there are only minor differences in the lipids and fatty acids of the liver oils of *S. acanthias* from the Atlantic and Pacific coasts of North America.

ACKNOWLEDGMENTS

The authors would like to thank Ena J. Macpherson for her technical assistance for this experiment. Pacific dogfish livers were provided by Richard F. Addison, Fisheries and Oceans Canada, Sydney, British Columbia, Canada.

REFERENCES

1. Bailey, B.E., N.M. Carter, and L.A. Swain, *Marine Oils with Particular Reference to Those of Canada,* Bulletin No. 89, Fisheries Research Board of Canada, Ottawa, 1952, pp. 413.
2. Malins, D.A., and A. Barone, Glyceryl Ether Metabolism: Regulation of Buoyancy in Dogfish *Squalus acanthias, Science 167:*79–80 (1970).
3. Malins, D.C., and J.R. Sargent, Biosynthesis of Alkyldiacylglycerols and Triacylglycerols in a Cell-Free System from the Liver of Dogfish (*Squalus acanthias*), *Biochemistry 10:*1107–1110 (1971).
4. Ratnayake, W.M.N., B. Olsson, D. Matthews, and R.G. Ackman, Preparation of Omega-3 PUFA Concentrates from Fish Oils *via* Urea Complexation, *Fat Sci. Technol. 90:*381–386 (1988).

5. Hart, J.L., *Pacific Fishes of Canada,* Bulletin No. 180, Fisheries Research Board of Canada, Ottawa, 1973, pp. 44–47.
6. Leim, A.H., and W.B. Scott, *Fishes of the Atlantic Coast of Canada,* Bulletin No. 155, Fisheries Research Board of Canada, Ottawa, 1966, pp. 46–48.
7. Spiny Dogfish, in *Status of Fishery Resources off the Northeastern United States,* NMFS-F/NEW-95, National Marine Fisheries Service, NOAA, Woods Hole, MA, 1992, pp. 99–100.
8. Ackman, R.G., Flame Ionization Detection Applied to Thin Layer Chromatography on Coated Quartz Rods, in *Methods in Enzymology,* edited by J.M. Lowenstein, Academic Press, New York, 1981, Vol. 72, pp. 205–252.
9. Parrish, C.C., Separation of Aquatic Lipid Classes by Chromarod Thin-Layer Chromatography with Measurement by Iatroscan Flame Ionization Detection, *Can. J. Fish. Aquat. Sci. 44:*722–731 (1987).
10. Fine, J.B., and H. Sprecher, Unidimensional Thin-Layer Chromatography of Phospholipids on Boric Acid Impregnated Plates, *J. Lipid Res. 23:*660–663 (1982).
11. AOCS Official Method, Unsaponifiable Matter, *Official Methods and Recommended Practices of the American Oil Chemists' Society,* 4th edn., AOCS Press, Champaign, 1993, Ca 6b-53.
12. Wood, R., GLC and TLC Analysis of Isopropylidene Derivatives of Isomeric Polyhydroxy Acids Derived from Positional and Geometrical Isomers of Unsaturated Fatty Acids, *Lipids 2:*199–203 (1967).
13. Ackman, R.G., and C.A. Eaton, Some Contemporary Applications of Open Tubular Gas–Liquid Chromatography in Analyses of Methyl Esters of Long Chain Fatty Acids, *Fette Seifen Anstrichm. 80:*21–37 (1978).
14. Ratnayake, W.M.N., B. Olsson, and R.G. Ackman, Novel Branched-Chain Fatty Acids in Certain Fish Oils, *Lipids 24:*630–637 (1989).
15. Ratnayake, W.N., and R.G. Ackman, Fatty Alcohols in Capelin, Herring and Mackerel Oils and Muscle Lipids: I. Fatty Alcohol Details Linking Dietary Copepod Fat with Certain Fish Depot Fats, *Ibid. 14:*795–803 (1979).
16. Ratnayake, W.N., and R.G. Ackman, Fatty Alcohols in Capelin, Herring and Mackerel Oils and Muscle Lipids. II. A Comparison of Fatty Acids from Wax Esters with Those of Triglycerides, *Ibid. 14:*804–810 (1979).
17. Ackman, R.G., V.R. Orozco, and W.M.N. Ratnayake, Aspects of Positional Distribution of Fatty Acids in Triacylglycerols of Skin, White and Dark Muscle of Mackerel *Scomber scombrus* in Relation to Hypertension, *Fat Sci. Technol. 93:*447–450 (1991).
18. Ackman, R.G., and C.A. Eaton, Docosenoic (22:1) Acids in Pacific Herring Oils, New Series Circular No. 54, Fisheries and Marine Service, Environment Canada, Halifax Laboratory, 1975, pp. 1–4.
19. Joseph, J.D., Distribution and Composition of Lipids in Marine Invertebrates, in *Marine Biogenic Lipids, Fats, and Oils,* edited by R.G. Ackman, CRC Press, Boca Raton, 1989, Vol. 2, pp. 49–145.
20. Ratnayake, W.M.N., A. Timmins, T. Ohshima, and R.G. Ackman, Mass Spectra of Fatty Acid Derivatives of Isopropylidenes of Novel Glyceryl Ethers of Cod Muscle and of Phenolic Acetates Obtained with the Finnigan MAT Ion Trap Detector, *Lipids 21:*518–524 (1986).
21. Ackman, R.G., and S.N. Hooper, Non-Methylene-Interrupted Fatty Acids in Lipids of Shallow-Water Marine Invertebrates: A Comparison of Two Molluscs (*Littorina littorea* and *Lunatia triseriata*) with the Sand Shrimp (*Crangon septemspinosus*), *Comp. Biochem. Physiol. 46B:*153–165 (1973).
22. Viswanathan, C.V., Chromatographic Analysis of Alkoxy-Lipids, *J. Chromatogr. 98:*129–155 (1974).
23. Totani, N., Some New Methods for the Quantitative Analysis of Ether Lipids, *Fette Seifen Anstrichm. 84:*70–73 (1982) (in German).
24. Bakes, W.J., and P.D. Nichols, Lipid, Fatty Acid and Squalene Composition of Liver Oil from Six Species of Deep-Sea Sharks Collected in Southern Australian Waters, *Comp. Biochem. Physiol. 110B:*267–275 (1995).
25. Mangold, H.K., Ether Lipids in the Diet of Humans and Animals, in *Ether Lipids. Biochemical and Biomedical Aspects,* edited by H.K. Mangold and F. Paltauf, Academic Press, New York, 1983, pp. 231–238.
26. Sargent, J.R., R.R. Gatten, and R. McIntosh, Metabolic Relationships Between Fatty Alcohol and Fatty Acid in the Liver of *Squalus acanthias, Mar. Biol. 10:*346–355 (1971).
27. Hayashi, K., and T. Takagi, Distribution of Squalene and Diacyl Glyceryl Ethers in the Different Tissues of Deep-Sea Shark, *Dalatias licha, Bull. Jpn. Soc. Sci. Fish. 47:*281–288 (1981).
28. Hayashi, K., Y. Okawa, and K. Kawasaki, Liver Lipids of Gonatid Squid *Berryteuthis magister:* A Rich Source of Alkyl Glyceryl Ethers, *Ibid. 51:*1523–1526 (1985).
29. Bordier, C.G., N. Sellier, A. Foucault, and F. Le Goffic, Purification and Characterization of Deep Sea Shark *Centrophorus squamosus* Liver Oil 1-*O*-Alkylglycerol Ether Lipids, *Lipids 31:*521–529 (1996).
30. Chalmers, W., and A.J. Leaf, A Process for the Preparation and Concentration of Free Hydroxylic Substances from Marine Oils, U.K. Patent 1,076,706 (1967).
31. Chalmers, W., and A.J. Leaf, A Process for the Isolation of Alpha-Glyceryl Ethers from Marine Oils, U.K. Patent 1,076,707, U.S. Patent 3,342,876 (1967).
32. Nichols, P.D., D.S. Nichols, and M.J. Bakes, Marine Oil Products in Australia, *INFORM 5:*254–261 (1994).
33. Davenport, S., and P. Deprez, Market Opportunities for Shark Liver Oil, *Australian Fisheries,* Nov:8–10 (1989).
34. Summers, G., and R. Wong, Cosmetic Products from Semi-Refined Shark Liver Oil, *Infofish Internat.* Feb:55–58 (1992).
35. Urata, K., and N. Takaishi, Ether Lipids Based on the Glyceryl Ether Skeleton: Present State, Future Potential, *J. Am. Oil Chem. Soc. 73:*819–829 (1996).
36. Kang, S-J., S.P. Lall, and R.G. Ackman, Digestion of the 1-*O*-Alkyl Diacylglycerol Ethers of Atlantic Dogfish Liver Oils by Atlantic Salmon *Salmo salar, Lipids 32:*19–30 (1997).
37. Jhaveri, S.N., and S.M. Constantinides, Chemical Composition and Shelf Life Study of Grayfish (*Squalus acanthias*), *J. Food Sci. 47:*188–192 (1981).

[Received March 26, 1998; accepted July 17, 1998]