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The Triglycerides of Sable Fish (*Anaplopoma fimbria*). II. Fatty Acid Distribution in Triglyceride Fractions as Determined with Pancreatic Lipase[1]

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Abstract

Sable fish muscle lipids were fractionated on a silicic acid column with mixtures of chloroform and methanol as eluting solvents. Three main peaks containing only triglycerides were isolated; 11 additional peaks contained phosphorous. Each of the 3 triglyceride peaks was separately fractionated into 300 fractions on silica gel columns impregnated with silver nitrate. Mixtures of petroleum ether and ethyl ether were the eluting solvents. About 25 distinct fractions were isolated from each column. The fractions were characterized for fatty acid content by gas chromatography of the methyl esters. The results showed that the fractionation did not depend upon the presence of single fatty acids but upon total unsaturation. Fatty acid distribution within each fraction was determined with the use of hog pancreatic lipase, followed by thin-layer chromatography and gas chromatography.

Introduction

PREVIOUS ATTEMPTS to characterize triglycerides (1) have depended upon the use of total carbon number with gas-liquid chromatography (2,3), according to partition number on rubber columns (4,5) and liquid-liquid column partition chromatography (6). The development of adsorption chromatography on silicic acid impregnated with silver nitrate (7,8) for fractionation of the triglycerides was a significant break-through. This combined with the use of pancreatic lipase to determine the positional distribution of the fatty acids (9), were the methods used in the present study. While it was in progress, several other papers based on the same combination of methods were published (10,11).

In this study, we describe the nature of the triglycerides of a marine oil that contains 21 different fatty acids.

Experimental

The methods used for extraction of the lipids, and the fractionation of the different triglycerides by column chromatography, were those described previously (12).

Separation of Neutral Lipids and Phospholipid Fractionation

Neutral lipids were separated from the rest of the lipid components by column chromatography on silicic acid gel column using a modified method of that described by Shuster et al. (13). The silica gel (Mallinckrodt, 100 mesh) was washed three times with chloroform and two identical columns were packed wet (4.0 cm I.D., 55 cm long, packed silica gel height 46 cm). Chloroform was run continuously through the columns for 24 hr before application of the lipid. A sample containing 11.30 g of sable fish muscle lipids mixed with 15 ml chloroform was applied to one of

[1] Presented at the AOCS Meeting in Houston, Texas, 1965.

TABLE I

Extraction of Sable Fish Oil

Extract	Method	Amount of lipid extracted			Phospholipid classification [a]
		% of dry weight	% of wet weight	% Phospholipids	
1st	Blended twice 3 min with $CHCl_3$: CH_3OH 95 : 5	60.3	19.2	0.48	Cephalins only
2nd	Refluxed for 94 hr with $CHCl_3$: CH_3OH 2 : 1	4.2	1.1	50.2	Cephalins, phosphatidyl inositol, lecithin, lysolecithin, sphingomyelin
3rd	Refluxed for additional 170 hr with $CHCl_3$: CH_3OH 2:1	0.2	0.1	21.3	Lecithin, lysolecithin, sphingomyelin
TOTAL		64.7	20.4	2.44 (based on dry weight)	

[a] By thin layer chromatography.

the columns. The flow (by gravity only) was 81 ml/hr. A Van Waters liquid chromatograph differential refractometer was used continuously to measure and record the refractive index, hence two identically packed columns had to be used simultaneously. No solvent changes were made until the refractive index had returned to its baseline. Solvent changes were made simultaneously on both columns. The columns were run continously for 120 hr, during which 690 fractions were collected (14 ml/fraction).

Column Chromatography of the Triglycerides

Essentially the same procedure as that described in our previous paper (12) was used. A 2.0 cm I.D. column was used, height of packed material was 31.0 cm, flow was by gravity only at a rate of 102 ml/hr, the entire fractionation was run in a constant temperature room at 12C, and fractions were collected on a Gilson model VL fraction collector, 10 ml/fraction. Two separate runs were made. In the first, 1.99 g triglyceride was applied on the column and collected in 280 fractions. In the second, 1.45 g triglyceride was applied on the column and collected in 307 fractions.

Extraction of Residual Lipids

In order to determine how much lipid was left in the fish meal after the extraction, the extracted fish meal was freeze-dried, then a 50 g aliquot of the dehydrated meal was placed in an extraction thimble of Soxhlet extractor (laid out in 4 layers between 5 layers of glass wool) and extracted with 1350 ml of chloroform-methanol 2:1, at 83C. After 92 hr the solvent was removed, and fresh solvent was placed in the extractor and the extraction continued for another 170 hr. The solvent was removed in a rotatory evaporator, and the residual lipids were stored under nitrogen at −18C.

Phosphorous Determination

The method described by Bartlett was used (14).

Thin-Layer Chromatography of Phospholipids

Skipski's method (15) for preparation of the plates was modified as follows: Thirty grams of silica gel H (according to Stahl, by E. Merck A. G. Damstadt, Germany) was mixed well in a mortar with 60 ml lmM Na_2CO_3 and then with another 5 ml lmM Na_2CO_3, spread on five 20 × 20 cm glass plates, allowed to dry at room temperature and stored in a desiccator. Lipid mixtures were applied as solutions in chloroform-methanol 1:1, approximately 50 mg/ml, 1–3 μl, every 2 cm along a baseline 2.5 cm from the lower edge of the plate. Development was by ascending chromatography in a closed tank, 2–23C, 70 min, using a mixture of chloroform-methanol-glacial acetic acid-water, 25:15:4:2, respectively, by volume. After development the plates were dried under a nitrogen stream and placed in a tank containing iodine vapors for 5 min. Spots were identified by comparing them with standard phospholipids.

Thin-Layer Chromatography of Neutral Lipids

The method of Bowyer (16) for preparation of the plates was modified as follows: Thirty grams silica gel H was mixed well in a mortar with 60 ml water and then with another 5 ml water, spread on five 20 × 20 cm glass plates, allowed to dry at room temperature for 30 min, activated for 2 hr at 110C, cooled to room temperature and stored in a desiccator.

Development of Plates. All solvents and equipment were equilibrated to 4C. The neutral lipid fractions were applied to the plates in the same manner as before but in petroleum ether. Development was by ascending chromatography in closed tanks, 40 min, using a mixture of petroleum ether-diethyl ether-glacial acetic acid, 80:20:1, respectively by volume. After development the plates were dried under a stream of nitrogen. Two strips, one on each side, 2 cm wide, were sprayed with 0.2% 2′7′-dichlorofluorescein in methanol, and observed under ultraviolet light without exposing the rest of the plate to the light. The neutral lipids migrated in the following order (from bottom to top): Monoglycerides, diglycerides, free fatty acids, triglycerides. Each band was scraped off separately, placed in a sintered glass funnel and the lipid component washed off successively with petroleum ether, chloroform and methanol. Solvents were evaporated in a stream of nitrogen and the residual lipid was saponified and methylated for GLC.

Pancreatic Lipase Hydrolysis of the Triglycerides

Micro-techniques as described by Luddy (17) and the pH stat method as described by Jacobson (18) were used. Sample size ranged from 1 to 8 mg. Con-

TABLE II

Fatty Acid Composition of the 3 Triglyceride Peaks Obtained During the Separation from Phospholipid

	A Fractions 33–50 43.3% of total triglycerides	B Fractions 51–62 31.4% of total triglycerides	C Fractions 63–80 25.3% of total triglycerides	Total triglycerides
C_{12}	T	T	T	T
C_{14}	4.9	4.5	3.4	4.4
$C_{14:1}$	0.3	0.2	0.2	0.2
C_{16}	13.0	10.6	8.6	11.1
$C_{16:1}$	10.2	8.9	7.3	9.1
C_{18}	2.4	1.8	1.4	2.0
$C_{18:1}$	31.4	25.7	21.5	27.1
$C_{18:2}$	1.7	1.4	1.2	1.5
$C_{18:3}$	15.0	10.4	9.7	12.2
$C_{20:2}$	1.2	1.4	0.6	1.1
$C_{20:3}$	12.3	7.7	8.0	9.8
$C_{22:3}$	—	3.3	1.6	1.4
$C_{22:4}$	5.3	5.2	4.3	5.0
$C_{22:5}$	1.4	0.6	0.8	1.0
$C_{22:6}$	0.5	—	0.4	0.3
$C_{24:6}$	1.5	3.8	4.9	3.1
$C_{26:4}$	—	14.3	25.8	11.0

TABLE III

Fatty Acid Distribution Between α, α', and β Positions of the Major Fatty Acid Components of Fraction A of the Original Triglycerides

Carbon number	Fraction number																													
	68		79		87		94		109		114		126		140		154		169		183		190		197		212		226		241		256		266	
	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β
C_{14}	14	9	11	9	17	4	5	15	4	14	3	13	4	13	3	11	3	10	1	2	1	3	1	3	1	2	1	1	2	1	2	1	3	4	2	5
C_{16}	26	27	27	28	22	22	13	25	12	25	10	24	10	22	9	25	9	25	4	7	3	8	7	14	4	6	6	5	11	7	7	5	9	13	8	24
$C_{16:1}$	13	12	14	12	19	10	5	8	8	10	6	12	10	14	13	15	15	17	9	10	10	22	7	11	16	20	18	11	13	5	11	4	11	10	10	15
C_{18}	7	8	7	7	3		5	3	5	3	5	16	4	2	3	3	3	4	2	1	2	2	5	5	2	8	3	48	8	59	5	67	6	24	5	12
$C_{18:1}$	18	16	18	15	23	4	26	19	34	20	34	22	42	23	46	26	42	26	35	27	40	33	41	25	40	40	37	20	33	9	28	7	29	24	38	25
$C_{18:3}$	3	2	2	3	8	6	20	12	20	11	21	8	17	8	14	8	13	8	24	27	23	12	19	12	20	13	17	7	17	3	18	4	19	13	16	8
$C_{20:3}$	1	1	1	1	1	1	20	8	20	4	15	5	11	4	9	4	10	4	23	22	18	5	16	5	13	7	11	2	11	2	15	2	15	3	14	2

TABLE IV

Fatty Acid Distribution Between α, α' and β Positions of the Major Fatty Acid Components of Fraction C of the Original Triglycerides

Carbon number	Fraction number																													
	76		90		106		126		142		154		265		296		300		216		234		247		169		185		200	
	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β	α α'	β
C_{14}	3	11	3	13	2	11	4	14	3	11	3	11	3	11	1	5	1	2	1	1	1	2	1	3	1	3	2	3	2	4
C_{16}	9	27	12	30	15	26	12	27	9	24	8	24	7	22	4	10	3	7	7	4	9	5	8	11	5	9	10	11	13	12
$C_{16:1}$	1	3	1	3	2	3	4	5	5	7	8	11	9	17	9	18	14	22	12	11	9	13	12	15	11	13	7	7	6	9
C_{18}	8	24	12	11	16	16	9	12	6	21	5	6	3	3	2	3	1	3	4	3	8	10	5	4	3	14	5	2	8	2
$C_{18:1}$	8	7	14	8	19	8	22	17	21	19	22	25	20	31	35	29	33	41	40	19	35	26	34	29	28	28	35	21	38	16
$C_{18:3}$	7	5	16	6	17	5	19	9	23	9	24	12	37	8	35	11	24	13	19	5	19	6	14	9	24	11	19	10	16	5
$C_{20:4}$	9	3	22	3	21	3	23	5	27	5	26	6	18	4	21	5	19	5	12	2	14	2	18	5	20	5	16	2	14	T
$C_{20:5}$	52	2	16	T	4	1	1	1	1	T	T	3	1	T	1	1	1	T	1	T	1	T	1	1	1	5	1	2	2	T
$C_{22:3}$									4	T	3	1	2		2		2		1		1		3		1		1	1	1	1
$C_{22:4}$	1		3		2		3	1																						
$C_{22:6}$							T		T	1	T	1	T	T		1		5	1	53		33		16		5		2		3
$C_{24:6}$		15		26		26		9		2		1		2																

ditions for lipase hydrolysis were chosen with special emphasis on proper mixing to assure at least 50% hydrolysis as quickly as possible in order to minimize acyl migration (19,20).

The apparatus used was a Radiometer Type TTTlc pH-Stat coupled with a Radiometer scale expander type PHA 630P, a Radiometer automatic titration assembly type TTA31 and a Radiometer recorder type SER2c. The water-jacketed reaction chamber was kept at constant temperature with a Haake Circulator type F. The concentrations and volumes of the reagents, were adjusted to satisfy the reaction chamber measurements, and to assure satisfactory stirring by a round magnetic stirrer inside the chamber. The triglyceride sample was transferred into the pH-stat reaction chamber with the solvent, in which it was eluted off from the column and the solvent was removed in a stream of nitrogen. All subsequent operations were performed under nitrogen. To the triglyceride in the cell the following reagents were added: 0.25 ml 0.05% bile salts (bile salts No. 3 by DIFCO), 1.0 ml 0.002 M Tris buffer pH 8.2, 0.1 ml 2.2% $CaCl_2$, 0.5 ml 2 mg/ml sodium taurocholate (by DIFCO) (21). All reagents had been adjusted to pH 8.2 at 24C. The reagents with the substrate were allowed to equilibrate at 37C, with stirring. The automatic titrator kept the pH at 8.0 by adding 0.05N NaOH when necessary. The baseline for the titration of the lipase reaction was thus obtained. After 5 min, 0.25 ml of 1 mg/ml hog pancreatic lipase (Worthington) in 0.002 M Tris buffer (pH 8.2 at 24C) was added from a syringe through the nitrogen aperture of the reaction chamber. The activity of the lipase was 116 units/mg enzyme. Fifteen minutes after the enzyme was added the reaction was stopped by the addition of 1.0 ml 1.0 N HCl.

The rate of hydrolysis, as measured by the rate at which NaOH was added (the slope of the curve traced by the recorder), indicated that 50% hydrolysis of the substrate was achieved after about 15 min. The mixture was transferred quantitatively into a 50 ml separatory funnel, extracted with 10 and 5 ml of diethyl ether, respectively, followed by extraction with 5 ml chloroform. The ether and chloroform layers

TABLE V

Fatty Acid Composition of the Total Triglyceride Fractions of Fraction A

Carbon number	Fraction number																				
	17	39	51	69	73	78	86	93	108	125	141	155	168	182	191	198	211	227	242	255	265
12	0.4	0.3	0.1	0.2	0.2	0.3	0.2	0.1	0.1	0.1	T	T									
	T	0.1	0.1	T	0.1	0.1	0.1	T	T	T	T	T									
14	10.9	11.7	12.3	11.5	10.4	10.7	10.7	9.9	9.3	7.9	7.4	6.1	1 3	1.3	1.5	1.0	2.0	2.9	2.6	3.7	3.5
14:1	1.1	0.7	0.7	0.6	0.6	0.5	0.6	0.4	0.5	0.4	0.5	0.8	0.3	0.5	0.7	0.7	1.2	0.8	0.5	0.5	0.6
16	31.5	31.2	31.7	28.3	26.3	25.3	21.9	20.8	19.5	17.6	16.9	13.6	4.5	2.8	3.0	2.2	4.1	6.5	6.3	8.6	7.8
16:1	8.9	6.6	6.5	6.1	6.4	5.8	6.6	6.8	8.8	10.5	14.0	15.9	9.2	18.2	18.9	23.0	21.9	18.9	13.1	12.7	15.2
16:2	T	T			T		0.7	0.4	0.7	T	T	T	0.3	0.5	1.0	0.5					
18	9.2	7.8	7.3	6.0	5.4	5.0	3.7	3.4	2.8	2.3	1.8	1.2	1.3	0.9	2.2	0.8	2.5	5.5	9.8	8.2	7.5
18:1	10.2	14.2	14.7	18.3	17.5	18.4	20.6	22.9	28.3	34.8	40.2	39.0	32.0	37.9	37.0	46.0	39.9	44.5	33.5	33.5	37.6
18:2	1.1	0.7			T	T	T	T		0.3	T		0.5	1.2		T	4.4	5.4	9.5	6.2	5.7
20		T	0.6	0.5	0.4	T	0.3	T												T	
20:1																					
18:3	9.6	11.2	10.2	12.4	13.8	14.8	17.5	17.5	17.2	15.1	11.9	13.5	25.0	24.0	23.7	17.9	16.8	9.7	13.2	15.7	13.9
18.4																T	T	0.8	1.2	0.6	T
22	0.4	T	0.3		T																
20:3	13.9	13.3	11.3	13.2	16.0	15.9	15.1	15.1	12.2	10.5	6.9	9.1	22.7	13.0	12.1	7.9	7.0	5.0	8.1	7.6	6.7
20:4		T	2.5	0.8	0.5	0.9	0.8	1.4		T											
22:3											T		2.1	0.6	0.5	0.2	0.2		2.0	2.2	2.3
20:5	2.9	2.0	1.6	2.1	2.4	2.1	1.5	1.3	0.9	0.7	0.4	0.7		T		T	T	T	0.5	0.4	0.3
22:4																T	T	T	T	T	T
20:6																		T	T	T	T
22:6																		T	T	T	T

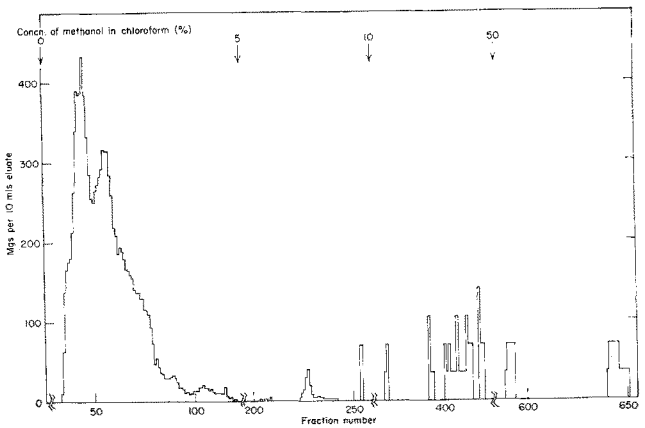


FIG. 1. Fractionation of whole muscle lipid extract of sable fish on silicic acid column.

were combined and reduced in volume for application to the thin-layer plates in order to separate the hydrolysis products.

Fatty Acid Analysis

Fatty acid composition of the different components (total triglycerides, fractionated triglycerides, monoglycerides, diglycerides and free fatty acids) was determined by gas chromatography following methylation by transesterification with methanol containing 1% sulfuric acid after TLC separation. An F & M model 810, equipped with dual columns with hydrogen flame detectors was used. Two 6 ft glass columns, 2 mm I.D., packed with 10% DEGS (diethylene glycol succinate, Applied Science (22)) on siliconized chromosorb P (mesh 100–120) were employed. Conditions were as follow: oven temperature 190C, injection port temperature 230C, detector temperature 220C. Nitrogen pressure at head of columns, 40 lb/in^2 producing a flow of 75 ml/min, hydrogen pressure 12 lb/in^2, oxygen pressure 10 lb/in^2. Peak areas were estimated by the method described by Carrol (23). The fatty acid esters were identified by comparison with those of NIH Standard Mixes (24), and by hydrogenation according to Farquhar et al. (25). Fatty acid compositions are reported in weight %.

Results and Discussion

The results obtained by the additional extraction of residual lipids from the extracted sable fish meal

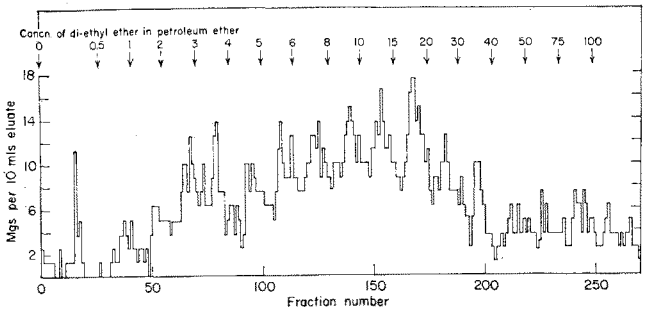


FIG. 2. Fractionation of sable fish triglyceride fraction A (fractions 33–50 on Fig. 1) on a silica gel-$AgNO_3$ column. Solvent gradient started with 100% petroleum ether and ended with 100% diethyl ether. Column washed with 100% methanol after fraction 266.

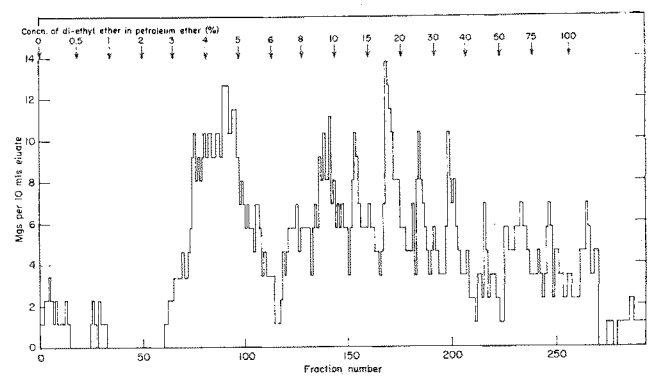


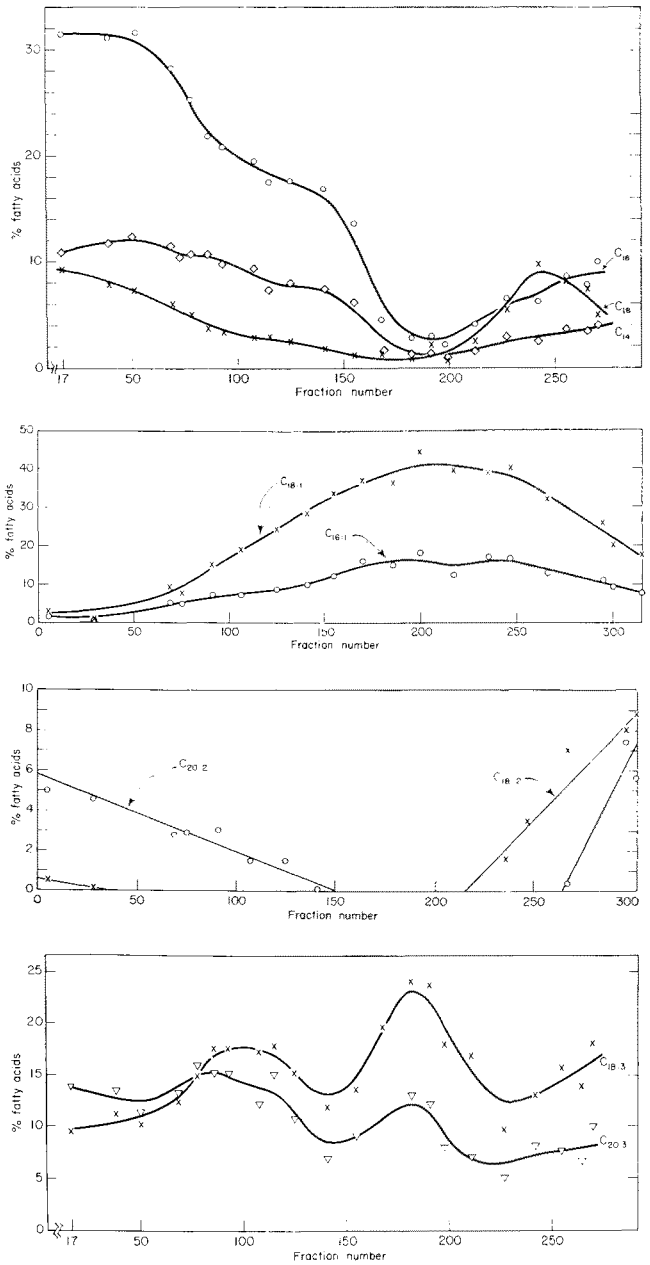
FIG. 3. Fractionation of sable fish triglyceride fraction C (fractions 63–80 on Fig. 1) on a silica gel-$AgNO_3$ column. Solvent same as Fig. 2. Column washed with 100% methanol after fraction 287.

are presented in Table I. Those results raise questions concerning the accuracy of total lipid extraction by the methanol-chloroform tissue lipid extraction procedure used. Only 20% of the phospholipids extractable by the three steps employed was extracted at first and included in what is usually called total lipid extracts. Of the phospholipids present in sable fish muscle, 80% were extracted by continuous Soxhlet extraction for 264 hr. The distribution of the different classes of phospholipids within the 3 extracts raises some interesting points as to the possibility of a preliminary fractionation of phospholipids by virtue of the methods employed for extraction. Cephalins

TABLE VI

Fatty Acid Composition of the Total Triglyceride Fractions of Fraction C

Carbon number	Fraction number 5	28	69	75	91	107	125	141	155	170	186	200	217	235	246	266	295	300	Column Cut No. 1
12				T			T				T					T	T		
14	0.6	0.3	7.9	6.7	10.4	10.7	12.1	11.2	8.6	7.5	2.5	1.6	0.9	2.2	2.0	2.5	4.1	3.6	3.4
14:1	0.2	0.2	0.4	0.40	0.5	0.6	0.7	0.6	0.5	0.8	0.8	0.6	0.3	0.6	0.7	0.6	T	0.4	0.2
16	3.5	1.7	17.1	12.3	24.4	24.4	24.2	19.8	17.0	16.0	5.3	3.0	1.8	6.2	5.1	5.6	8.9	9.5	8.4
16:1	1.4	0.5	5.0	4.7	6.9	6.9	8.6	9.7	12.1	16.0	15.0	18.1	12.2	17.0	16.5	12.8	10.9	9.2	7.5
16:2																			
18	80.5	90.3	7.3	4.6	9.2	7.9	6.0	3.4	3.5	2.1	1.1	1.0	0.3	2.6	3.3	9.0	2.2	1.3	2.3
18:1	2.8	0.8	9.0	7.7	15.1	19.0	24.5	28.6	33.7	37.2	36.6	44.4	30.1	38.7	39.7	31.6	25.6	19.9	17.6
18:2	0.4	0.1				T								1.6	3.5	7.0	8.0	8.8	4.1
20		0.1				T													
18:3	1.0	1.1	7.3	6.5	10.8	12.1	12.1	14.1	13.6	11.7	18.3	13.8	7.8	9.1	11.8	14.7	9.2	5.5	6.3
20:2	5.0	4.6	2.8	2.9	8.2	5.1										0.4	7.4	5.6	3.5
18.4		0.1																	
20:3	0.6	0.2	6.1	5.6													9.6	4.2	4.1
20:4	3.9		34.2	47.6	10.0	10.5	9.4	10.8	8.5	6.6	11.5	6.4	3.8	4.2	7.2	8.8	4.6	2.5	
20:5					2.6		0.9	0.9	0.4	0.5	0.7				0.9	0.7	4.5	24.1	30.8
22:3			0.8	0.4	1.4	1.0	0.6	0.5	0.6	1.1	1.2	1.4	2.0	1.0	1.2	1.1	0.8	1.5	1.5
22:4																	0.4		
22:5																			
22:6			0.2	0.1		0.4	0.3	0.5	0.5	0.2	1.8	4.7	39.3	14.8	6.6	3.5	1.0	2.4	2.2
24:4			1.3	0.2								2.2	1.3	2.0	1.4	1.6	0.7	0.6	8.2
24:6			0.4	0.2	1.2	1.4	0.4	0.5	0.9	1.4	0.4	2.5							
26:4											3.5	0.2					1.6	0.4	



FIGS. 4–7. Per cent distribution of fatty acids in the fractionated triglycerides of fraction A (original fractionation presented in Fig. 2).

only were present in the first extract and none at all in the third. Phosphatidyl inositol was present in the second extract only, in which over 50% of the total phospholipids were extracted. Those preliminary observations, while noteworthy, require further detailed study.

The triglyceride peak as shown in Figure 1 seemed to be composed of 3 adjacent peaks. The refractive index monitor revealed also that 3 peaks were involved. Fatty acid composition of the 3 peaks revealed a different composition as shown in Table II. Since the middle peak, peak B, was most probably contaminated by both peak A and peak C, only the first and last peaks were chosen for fractionation on the silica gel impregnated with silver nitrate columns.

An aliquot of fraction A was refractionated into 280 fractions on the silica gel impregnated with silver nitrate (column A), and an aliquot of fraction C was similarly fractionated into 307 fractions (column B). The results are presented in Figures 2 and 3. Twenty-two distinct peaks were observed with column A, and 15 distinct peaks with column B. The distribution of the major fatty acids between the α and β positions as found by the lipase procedure is presented in Tables III and IV. The total fatty acid composition of the triglyceride fraction is found in Tables V and VI. The lipase reactions were not carried to completion. Hence the free fatty acid component represents the α positions from the triglyceride hydrolyzed to the monoglyceride and also from those hydrolyzed only to the diglyceride. Therefore total quantization of the results is difficult. That is, one cannot add the results of the α positions with those of the β position and expect the sum to approximate the total fatty acid composition of the same fraction. Therefore the method introduced by Coleman (26) was used, in which the composition of α, α' positions was computed from the composition of the β'-monoglycerides and the original triglycerides. The fatty acid composition of the triglyceride fraction recovered after the lipase hydrolysis was identical to the fatty acid composition of the triglyceride of the fraction adjacent to the peak fraction, providing evidence that the attack of the lipase was not selective with respect to triglyceride structure. This was true only when no long chain polyunsaturated fatty acids were present in the original triglyceride. It is very interesting to note the trend that the different fatty acids follow in the triglycerides after the column fractionation. The curves showing the per cent distribution of saturated fatty acids in the fractionated triglycerides are similar (Fig. 4). The same is true about the monoenoic, dienoic and trienoic acids (Fig. 5–7). These also are consistently alike but each differ from the other families of curves. Several of the polyunsaturated fatty acids, e.g. $C_{22:6}$, were present in the total triglyceride, in the monoglyceride and in the diglycerides, but never in the free fatty acid component after lipase hydrolysis. These specific fatty acids ester linkages may resist lipase action. Reiser (27) has also observed that pancreatic lipase did not liberate long-chain polyunsaturated acids of the type $C_{20:5}$, $C_{22:5}$, $C_{22:6}$ from the α positions of whale and menhaden oil.

It has usually been considered that most of the unsaturated fatty acids of triglycerides are in the β position. This is especially true with $C_{18:1}$ and $C_{18:2}$ of plant and mammalian triglycerides (11,28,29). However, the same distribution does not hold for the unsaturated fatty acids of sable fish triglycerides. As can be observed from Tables III and IV,[2] $C_{18:3}$, $C_{20:3}$, and $C_{20:4}$ occur mainly in the α, α' positions in contrast to C_{14}, C_{16}, and $C_{16:1}$ which occur mainly in the β position. C_{18} and $C_{18:1}$ seem to be distributed evenly.

The highly unsaturated $C_{26:4}$ which was present in fraction C was not seen in any of the products of the lipase hydrolysis step except the triglycerides. This leads us to assume that the intermediate substrate-enzyme complex may not form when $C_{26:4}$ is present as part of the substrate (triglyceride).

Considering only the 14 different fatty acids that account for 1% or more of the total fatty acids, and assuming that isomers are not separable there is still a possibility that 560 different triglycerides were present (30) in sable fish oil. Separation of all 560 individual triglycerides was not the object of this study.

[2] The data presented in Tables III, IV, V and VI are available as graphs in which the interrelationships can be more readily seen. Copies are available to those who request reprints.

However the observations made point to the value of continuing effort in solving the problems of triglyceride structures.

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Relation of Triglycerides to Phosphoglycerides in Fungi

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Abstract

Lipids of the fungi *Phycomyces blakesleeanus, Lipomyces lipoferus, Glomerella cingulata* and *Coprinus comatus* have been analyzed by physical and chemical methods. Triglycerides were the largest fraction of all the lipids in these fungi but significant amounts of phosphoglycerides were also present. The presence of relatively large amounts of triglycerides and phosphoglycerides, and the fatty acid patterns of these glycerides, suggests that formation of the tri- and phosphoglycerides involves participation of key intermediates from a common pathway of synthesis. The triglycerides of *Glomerella cingulata* have been studied in more detail than those of the other species. It has been found, using preparative thin-layer chromatography and analytical gas-liquid chromatograhpy, that *G. cingulata* triglycerides comprise one fraction of saturated and monoenoic fatty acids, another fraction of saturated, mono-, and dienoic acids and two fractions containing varying proportions of saturated, mono-, di-, and trienoic fatty acids.

Introduction

PREVIOUS WORK from this laboratory (2,3) has shown that triglycerides constitute the predominant class of lipids in conidia of the fungus *Glomerella cingulata*. In addition, these studies have shown that phosphoglycerides are the predominant phosphatides in the conidia. Arising from these results is the question, does the mycelium of *G. cingulata* and that of other fungi also contain large amounts of tri- and phosphoglycerides, and, if so, is there any relationship between the composition and structure of fungal triglycerides and the composition and structure of fungal phosphoglycerides?

The work presented in this paper is a preliminary report on studies that were undertaken to investigate the distribution of fatty acids among triglycerides and phosphoglycerides obtained from the fungi *Phycomyces blakesleeanus, Lipomyces lipoferus, Glomerella cingulata* and *Coprinus comatus*.

Materials and Methods

Growth of Fungi

The fungi used in this study were *Phycomyces blakesleeanus* (ATCC 6200), *Lipomyces lipoferus* (ATCC 10742), *Glomerella cingulata* (originally provided by Dr. Jack Ziffer, Pabst Laboratories, Milwaukee, Wis.) and *Coprinus comatus* (ATCC 12640). These fungi were maintained on potato dextrose agar slants. The composition of the nutrient medium (in grams per liter) for obtaining cells of *L. lipoferus* and *G. cingulata* was as follows: sucrose, 20; ammonium tartrate, 5; NH_4NO_3, 1; KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.5; NaCl, 0.1; $CaCl_2$, 0.1; and trace element solution, 1 ml. The composition of the trace element solution (in milligrams per liter) was: $Na_2B_4O_7.10H_2O$, 88; $(NH_4)_6MoO_{24}$, 64; $FeCl_3.6H_2O$, 960; $ZnSO_4.7H_2O$, 8,800; $CuCl_2$, 270; $MnCl_2.4H_2O$, 72; biotin, 5. The composition of the nutrient medium (in grams per liter) for obtaining mycelium of *P. blakesleeanus* was: glucose, 50; asparagine, 2; KH_2PO_4, 1.5; yeast extract, 1; $MgSO_4.7H_2O$, 500; thiamine, 50μg. The composition of the nutrient solution (in grams per liter) for obtaining mycelium of *C. comatus* was: glucose, 100; K_2HPO_4, 3; $(NH_4)_2SO_4$, 1; yeast extract, 1; $MgSO_4.7H_2O$, 0.5; $CaCl_2.2H_2O$, 0.5; $MnSO_4.7H_2O$, 0.1; $FeSO_4.7H_2O$, 0.01; $ZnSO_4.7H_2O$, 0.01. These nutrient solutions are essentially the same as those given in references 1, 6, and 8, respectively. They were sterilized at 15 psig (125C) for 15 min. Conidia of *G. cingulata* were obtained by culturing the fungus on potato dextrose agar as described previously (2). Mycelial pellets of *P. blakesleeanus* and *G. cingulata* were harvested after 6 days' growth, and cells of *L. lipoferus* and pellets of *C. comatus* after 10 days' growth.

Standard Lipid Compounds

Cholesterol stearate, ergosterol, phosphatidylethanolamine and cardiolipin, bis(phosphatidyl)glycerol were obtained from Nutritional Biochemicals Corporation. Phosphatidylserine, phosphatidylcholine and fatty acid methyl esters were obtained from Applied Science Laboratories. Tripalmitin was obtained