tion of monoaldehyde oil (Runs B and C) 21.0% of stearate was formed when hydrogenation was conducted in the absence of pyridine. When pyridine was present, the stearate content increased only slightly over that of the original oil (3.4%).

Characteristics of these aldehyde oils are listed in Table III. One product (Run A) had considerably higher molecular weight than expected, probably because aldehyde condensation reactions occurred during work-up. The products obtained when pyridine was present during hydrogenation were, generally, slightly more colored and more viscous than those obtained when pyridine was omitted.

Studies on the reactions of these unsaturated aldehyde oils are under way.

Conclusion

The preparation of a potentially useful chemical intermediate containing both unsaturated and aldehydic functionality by partial ozonization of soybean oil has been demonstrated.

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Incorporation of Linolenic-1-C^{14} Acid into Eicosapentaenoic and Docosahexaenoic Acids in Fish

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Abstract

Following intraperitoneal injection of methyl linolenate-1-C^{14} into kelp bass, *Paralablax clathratus,* the highly polyunsaturated fatty acids of their body fats were concentrated by low temperature crystallization from acetone, and eicosapentaenoic and docosahexaenoic acids were isolated from the concentrate by reversed-phase chromatography and hydrogenated. The resulting arachidic and behenic acids were degraded stepwise to margaric acid, and the distribution of activity was determined. The results indicate that the injected linolenic acid was converted to eicosapentaenoic acid and the latter incorporated into docosahexaenoic acid. A probable conversion pathway is linolenic acid ⟶ 6,9,12,15-octadecatetraenoic acid ⟶ 8,11,14,17-eicosatetraenoic acid ⟶ 5,8,11,14,17-eicosapentaenoic acid ⟶ 7,10,13,16,-19-docosapentaenoic acid ⟶ 4,7,10,13,16,19-docosahexaenoic acid.

Introduction

THE EVIDENCE deduced from many studies of the structure of the polyunsaturated fatty acids of fish lipids leaves little doubt that they resemble those found in mammalian lipids in possessing the divinyl methane structure almost exclusively (1–8). Moreover, previous work from this laboratory (9) indicated that the same types of reactions (i.e. chain elongation and dehydrogenation) contribute to the transformation of exogenous linoleic acid to arachidonic acid in a fish *(Tilapia mossambica),* as previously shown to obtain in the rat (10–13). However, the fatty acids generally found in fish on a natural diet are largely of the linolenic type, having the terminal structure $CH_3-CH_2-CH=$ (7) and thus leading to the assumption that they are derived largely from dietary linolenic acid. This view has been strengthened by studies in which it was shown that in young mullet, dietary linolenic acid gave rise to increased deposition of eicosapentaenoic and docosahexaenoic acids, as indicated by gas chromatographic analysis (14).

The present experiments were designed to test the logical supposition that the fed linolenic acid is incorporated *in toto* into the chains of eicosapentaenoic and docosahexanenoic acids. Such a pathway has been indicated in rats by Steinberg et al. (15) and by Klenk and Mohrhauer (17).

Experimental

Synthesis of methyl linolenate-1-C^{14}. Methyl linolenate-1-C^{14} was synthesized following the method of Nevenzel and Howton (16). In the process of synthesis the by-products formed during the Hunsdiecker decarboxylation and debromination of silver 9,10,12,-13,15,16-hexabromostearate were identified (18).

Treatment of fish. Three mature kelp bass *(Paralablax clathratus),*[3] with an average body weight of 167.3 g and average body length of 20.6 cm, were used as experimental animals. The fish were given an intraperitoneal injection of 600 mg methyl linolenate-1-C^{14} (for a total of 0.25 mc) diluted with pure inactive methyl linolenate,[4] and put into a 24 × 7¼ × 7¾ in. salt water glass aquarium at 19C. The aquarium was then placed in a constant temperature laboratory bath[5] set at 9.6C, since previous experience had indicated that a lower temperature might increase the yield of polyunsaturated acids. The temperature in the aquarium reached that of the bath in 110 min. After 5.5 hr of this cold treatment, when two of the fish were becoming weak and only one remained active, they were killed by freezing.

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[2] This paper based partially on work performed under Contract AT(04-1)GEN-12 between the Atomic Energy Commission and the University of California.

[3] Caught by John Prescott, Marineland of the Pacific, Palos Verdes, Calif.
[4] Obtained from the Hormel Institute, Austin, Minn.
[5] American Instrument Co., Inc., Cat. No. 4-8605.

TABLE I
Weights of Extracted Lipids and Residue of Kelp Bass

Fish No.	Body weight, g	Body length, cm	Fat free dry residue, g	Total lipid g
1	194.9	22.0	43.6(22.4%)	.....
2	130.4	18.7	30.8(23.6%)	.....
3	176.7	21.1	39.6(22.4%)	.....
Total	502.0	.....	114.0(22.7%)	51.14(10.2%)

Extraction of oil. The fish lipid was extracted in a Waring Blendor with chloroform-methanol (2:1), which was filtered and evaporated to give the total fat. Weights of fish, fat-free residue and total oil are listed in Table I.

Saponification of oil. The kelp bass oil (50 g) was dissolved in 700 ml of 5% ethanolic KOH and saponified under nitrogen overnight at room temperature. After extraction of the unsaponifiable matter with petroleum ether, 42.85 g of mixed fatty acids were obtained in the usual manner. A small portion of the mixed acids was methylated and analyzed by gas-liquid chromatography, using a Wheelco Model 10 apparatus with a 6 ft by 6 mm column of ethyleneglycol succinate (20% on 80–100 mesh siliconized celite) and an argon ionization detector. Peaks were identified by comparison of their retention times with those of standards or from log T_r vs. chain-length plots (Fig. 1).

Low temperature crystallization. The mixed fatty acids (41.5 g) thus obtained were dissolved in 350 ml of acetone and were separated by low temperature crystallization at −5C and −60C.

The fatty acid composition of these three fractions was obtained by gas-liquid chromatography and compared with the original composition as shown in Table II.

It is apparent from the table that the desired polyunsaturated fatty acids, especially eicosapentaenoic and docosahexaenoic acids, are concentrated in the −60C soluble fraction. Moreover, since this fraction showed a considerable activity (Table IV), further separation was performed on the reversed-phase column (19,20).

Reversed-phase column chromatographic separation of −60C soluble fraction. The acids (200 mg) were dissolved in 1.6 g of mineral oil and adsorbed on 2.5 g of siliconized celite. The coarse homogeneous powder was deaerated in A-50 (50 volume % acetone in water) on a steam bath and was added to the top of the column. All elution was carried out at 37C. Each eluted fraction of 25 ml was titrated with 0.048 N alcoholic potassium hydroxide giving the results illustrated in Figure 2.

TABLE II
Fatty Acid Composition of Original Oil and Fractions Obtained by Low-Temperature Crystallization of Kelp Bass Fatty Acids

Acid	Original oil	Low temperature crystallization		
		Insol. at −5C (11.35 g)	Insol. at −60C (19.09 g)	Sol. at −60C (9.51 g)
	%	%	%	%
14:0	7.7	4.8	8.1	1.0
14:1 and 2	trace	0.3	.....	0.5
16:0	27.3	55.0	9.3	.....
16:1	9.8	4.5	10.1	9.6
16:2	trace	0.9	.....	1.2
18:0	3.4	10.1	1.2	0.6
18:1	14.5	8.2	32.7	9.7
18:2	1.6	1.2	2.6	4.2
18:3	1.2	1.0	1.1	2.0
18:4 (20:1)	2.5	1.2	3.1	3.8
20:2	0.2	trace	0.5	.....
20:3	0.1	.....	0.2	.....
20:4	1.3	0.7	1.6	2.8
20:4 (isomer?)	1.0	0.5	1.0	1.7
20:5	9.9	4.0	9.8	19.4
22:4	0.6	0.3	0.8	2.2
22:5 (isomer?)	0.2	0.1	0.3	1.0
22:5	1.8	0.9	2.4	4.2
22:6	16.9	6.4	15.1	36.4

It had been expected that the separation between 20:5 and 22:6 acids would be quite difficult in light of the empirical observation that the effect of the elution rate of one added double bond is about two thirds of that produced by a decrease in chain length of two carbons. However, a saddle appeared in the broad peak eluted by the A-50 solvent as can be seen in Figure 2. For this reason, the A-50 fraction was collected in two parts, A-50(1) and A-50(2), as indicated. The fatty acid composition of these fractions was analyzed by gas-liquid chromatography (Table III).

It can be seen that in the first fraction, the main component was eicosapentaenoic acid with no other detectable C_{20} acid component and that in the second, docosahexaenoic acid is the main component and the only C_{22} acid. Thus eicosapentaenoic and docosahexaenoic acids were isolated free from acids with the same

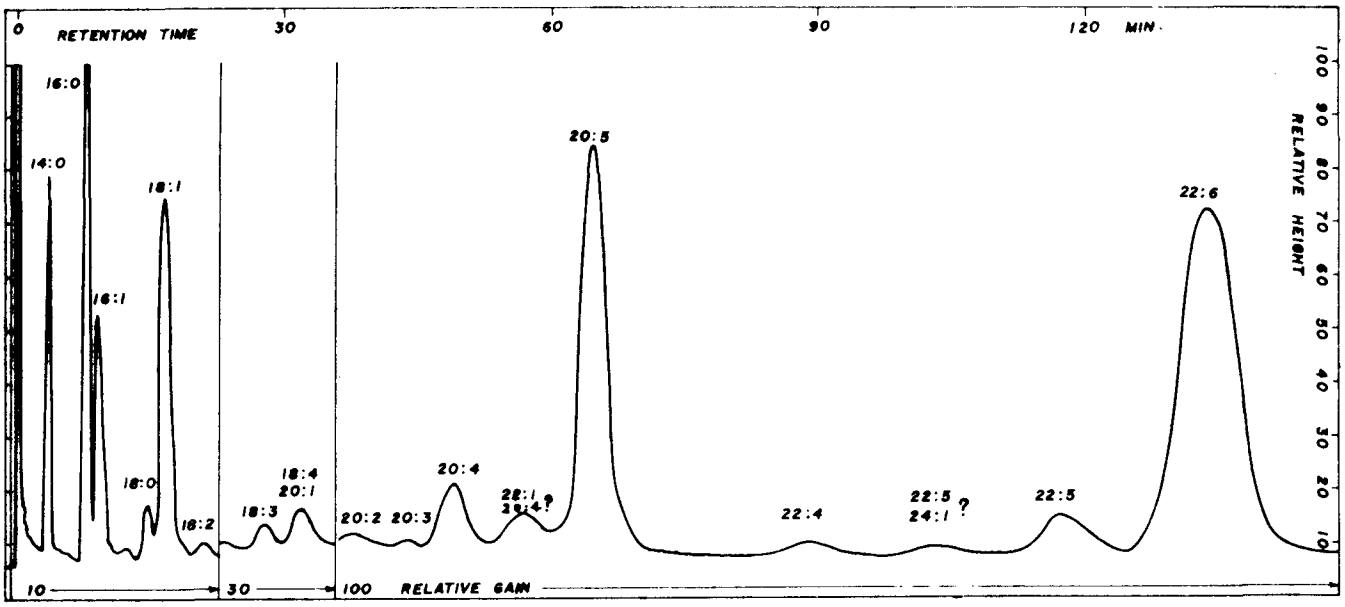

FIG. 1. Gas-liquid chromatogram of kelp bass oil. Temperatures: column 196C; detector cell 229C; flash heater 228C. Gas flow 167 ml/min; detector voltage 750. Only major peaks have been identified.

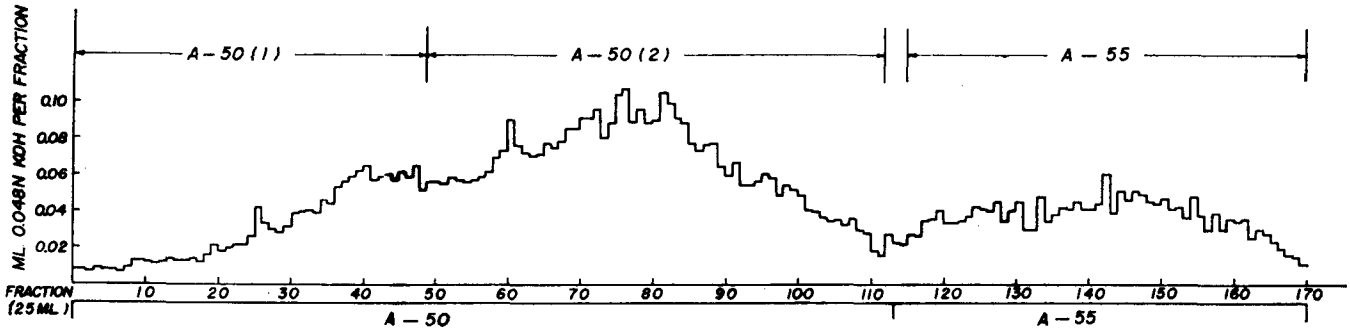

FIG. 2. Reversed-phase chromatogram of the soluble fraction at −60C by low temperature crystallization.

chain lengths, but different amounts of unsaturation. It is also noteworthy that the percentages of 20:5 (9.94) and 22:6 (16.86) in original oil were concentrated to 19.41 and 36.35 by the low temperature crystallization, and then to 74.94 and 71.86, respectively, by the reversed-phase column chromatography.

Hydrogenation and separation of hydrogenated product. The total material from the A-50(1) and A-50(2) fractions was separately hydrogenated over 10% Pd-on-charcoal, and the resulting saturated acids were freed from mineral oil and indicator by chromatography on silicic acid (21).

It is obvious that arachidic acid (20:0) in the hydrogenated A-50(1) acids and behenic acid (22:0) in A-50(2) were derived from eicosapentaenoic (20:5) and docosahexaenoic acids (22:6), respectively. The isolation of 20:0 from A-50(1) and 22.0 from A-50(2) was carried out using reversed-phase chromatography. The 20:0 and 22:0 acids thus obtained were found to be fairly pure (over 99%) with gas chromatography after silicic acid purification.

Determination of activity of various fractions. The radioactivity of the various isolated and purified fractions is listed in Table IV. The highest activity was found in the −60C-soluble fraction obtained by low temperature crystallization, and since the purified arachidic and behenic acids isolated from this fraction still had a fairly high activity, step-wise degradation was carried out on both acids after diluting with the appropriate pure non-labeled acids.

Distribution of C^{14} activity in eicosapentaenoic and docosahexaenoic acids. The purified samples of active arachidic (21 mg; derived from eicosapentaenoic) and behenic (65 mg; derived from docosahexaenoic) acids were diluted to 405 mg and 910 mg, respectively, and degraded by the modified Dauben procedure (11,22,23) down to margaric acid, collecting each successive carbon atom as benzoic acid. The benzoic acid samples and the residual margaric acid were purified and counted, yielding the results shown in Table V.

The following distribution of activity was observed:

For arachidic acid:

Carbon No.	4–20	3	2	1
	$C_{17}H_{35}$–	CH_2–	CH_2–	CO_2H
% Total radioactivity in C_{20}-chain	27	64	2	7

For behenic acid:

Carbon No.	6–22	5	4	3	2	1
	$C_{17}H_{35}$–	CH_2–	CH_2–	CH_2–	CH_2–	CO_2H
% Total radioactivity in C_{22}-chain	19	41	1	6	11	22
% Total radioactivity in terminal C_{20}-chain	28	61	1	9		

The heptadecanoic acid resulting from repeated step-wise degradations was contaminated with analogous substances from over-degradation, necessitating purification by reversed phase chromatography before the radioactivity was determined.

Discussion

From the results obtained in these studies it is obvious that the linolenic (9,12,15-octadecatrienoic) acid molecule is incorporated, at least partially undegraded, into the molecules of eicosapentaenoic 5,8,11,14,17 and docosahexanenoic 4,7,10,13,16,19 acids. The relatively high activity of the even carbons and of the C_{17} terminal ends of these acids is not readily explained at present, and recalls the similar results obtained with rats (15) for linolenic acid. It is possible that this acid, unlike linoleic, undergoes extensive partial degradation and resynthesis before elongation to the final products of the process. Nevertheless, the high activity in the third carbon of arachidic acid and the fifth carbon of behenic acids leaves little doubt that these positions represent the original carboxyl of the administered linolenic acid. Moreover, the very similar distribution of activity in arachidic acid and the terminal 20 carbons of behenic acid indicate the conversion of the former to the latter.

From this evidence, it can be postulated that in the fish a pathway of conversion of linolenic to higher polyunsaturated fatty acids exists very similar to that postulated for the rat (15), with steps similar to

TABLE III

Fatty Acid Composition of A-50(1) and A-50(2) Fractions from the Reversed-Phase Column[a]

Acid	A-50(1)	A-50 (2)
	%	%
14:1 and 2	trace	.....
16:2	trace	14.4
16:3	1.0	.....
16:4	3.2	.....
18:3	4.2	2.3
18:4	11.9	.....
20:4	.....	2.6
20:5	74.9	8.6
22:6	4.8	71.9

[a] Fatty acids are designated by number of carbon atoms followed by number of double bonds. Fatty acids eluted with A-55 are not included here but consisted largely of 16:1, 18:2, and 20:3.

TABLE IV

Radioactivity of Lipid Fractions and Fatty Acids of Kelp Bass

Fraction	Activity, d.p.s./mg[a]
Original oil	61.3
Nonsaponifiable fraction	58.5
Mixed acids	63.7
Insol. at −5C	28.3
Insol. at −60C	72.9
Sol. at −60C	135.6
Arachidic acid	35.2
Eicosapentaenoic acid (calc'd)	36.4
Behenic acid	12.8
Docosahexaenoic acid (calc'd)	13.3

[a] Obtained with the use of a liquid scintillation counter and methods described previously (13).

TABLE V

Radioactivity of Degradation Products of Arachidic and Behenic Acids Derived from Eicosapentaenoic and Docosahexaenoic Acids

Sample	d.p.s./mmole
Arachidic acid (diluted)	871
Benzoic acid (C-1 of arachidic)	57
Benzoic acid (C-2 of arachidic)	18
Benzoic acid (C-3 of arachidic)[a]	557
Margaric acid	235
Behenic acid	758
Benzoic acid (C-1 of behenic)	167
Benzoic acid (C-2 of behenic)	86
Benzoic acid (C-3 of behenic)	46
Benzoic acid (C-4 of behenic)	13
Benzoic acid (C-5 of behenic)	310
Margaric acid	145

[a] Calculated from the difference between the radioactivities of C_{17} and C_{18} acids purified by reversed-phase chromatography.

those shown to occur in the conversion of linoleic to arachidonic acid (11,12,13), and of oleic to 5,8,11-eicosatrienoic acid (23). In this case, the pathway is probably: 9,12,15-octadecatrienoic (linolenic) acid ⟶ 6,9,12,15-octadecatetraenoic acid ⟶ 8,11,14,17-eicosatetraenoic acid ⟶ 5,8,11,14,17-eicosapentaenoic acid ⟶ 7,10,13,16,19-docosapentaenoic acid ⟶ 4,-7,10,13,16,19-docosahexaenoic acid.

The shorter chain more saturated intermediates in this process were not studied in this case, but the structures proposed seem likely not only because of the analogy with the linoleic and oleic conversions but also because some of them have been identified in various fish oils. For example, Klenk and Brockerhoff characterized 6,9,12,15-octadecatetraenoic (2) and 7,10,13,-16,19-docosapentaenoic (1,3) acids in herring and cod liver oils, and Stoffel and Ahrens (7) have identified these and also 8,11,14,17-eicosatetraenoic acid in menhaden body oil. Klenk and Mohrhauer (17), in a very interesting study, have demonstrated the occurrence of steps 2, 3, and 5 in the rat. There is thus ample evidence to suggest that the same pathway occurs in the fish and that the fatty acids of the fish lipids (in analogy with those of mammals) are the result of predictable series of stepwise alterations of a known small number of dietary or biosynthesized acids.

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The Preparation and Properties of Some Synthetic Glycerides. II. Control of the Migration of Acyl Groups[1,2]

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Abstract

In a study of the use of tritylglycerols as starting materials for synthesis of mono- or diglycerides and mixed triglycerides, it was found that acyl migration can be minimized or prevented by detritylation in petroleum ether, avoiding contact with polar solvents or contaminants. Under conditions favoring acyl migration, tetrabromostearoyl radicals were found to migrate less readily than stearoyl radicals.

Introduction

FORMATION OF THE trityl (triphenylmethyl) ether is a convenient method for temporarily blocking one or both of the primary hydroxyls of glycerol. However, the use of tritylglycerols as starting materials for synthesis of mono- or diglycerides has been limited by the facile migration of the secondary acyl radicals during detritylation with dry hydrogen chloride (1).

As a result, it has been necessary to devise relatively complex procedures for the synthesis of 2-monoglycerides or 1,2-diglycerides. In one class of procedures hydroxyl groups are made available in analogs of glycerol by suitable transformations after the desired acylation is complete (2). In another, the hydroxyl-blocking trityl groups are removed by hydrogenolysis under mild conditions (3). In reactions leading to mixtures of 1,2- and 1,3-diglycerides, the two have been separated by fractional crystallization (4).

The need for a more direct procedure for the synthesis of 1,2-diglycerides led to a reinvestigation of the comparatively simple method which uses monotritylglycerol as starting material. It was found that cleavage of the trityl ether with hydrogen chloride does not cause migration of acyl radicals if the tritylglyceride is sufficiently pure and the detritylation is effected in petroleum ether without subsequent exposure of the product to acidic diethyl ether. Thus, by varying the solvent in which detritylation takes place, the same procedure may be used for preparing either symmetrical or unsymmetrical glycerides.

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[2] Publication No. 4265, Agricultural Experiment Station, University of Minnesota.