Isomeric Monoethylenic Fatty Acids in Herring Oil

R. G. Ackman, Fisheries Research Board of Canada, Halifax Laboratory, Halifax, Nova Scotia, and **John D. Castell**,[1] Chemistry Department, Dalhousie University, Halifax, Nova Scotia

ABSTRACT

Monoethylenic fatty acids from herring oil were concentrated by chromatography by chromatography on silver nitrate-silicic acid columns. Examination of consecutive fractions by open tubular gas chromatography confirmed the preferential elution of longer chain length esters and of esters within one chain length with the double bond closer to the terminal methyl group. Isomeric monoethylenic fatty acids with double bonds in the positions closer to the carboxyl group than the approximate midpoint of the even-numbered fatty acid chains could not be adequately separated by gas chromatography and were determined by ozonolysis. The isomers observed are consistent with primary formation from saturated acids through the action of an enzyme specifically removing hydrogen atoms in positions Δ^9 and Δ^{10} relative to the carboxyl group. Chain extension of particular monoethylenic isomers by two carbon atoms in the C_{20} and longer chain lengths is apparently influenced by the position of the double bond.

INTRODUCTION

A NUMBER OF MONOETHYLENIC fatty acids of various chain lengths from marine lipids have been characterized by classical procedures (1–3). With the development of recent techniques for chromatographic separation and oxidative fission, supplemented by gas chromatography, further research has indicated that mixtures of isomers would probably occur in each of the C_{16}, C_{18}, C_{20} and C_{22} chain lengths (2,4–11). The recognition of different isomers in gas chromatographic analyses has, however, not been possible with conventional packed columns, since the column efficiencies are too low. The separation of certain monoethylenic isomers of one chain length has recently been shown to be feasible with high efficiency open tubular (capillary or Golay) polar columns (12,13). The present report shows that it is possible to determine the occurrence of most, but not all, of the isomers of monoethylenic fatty acids of marine lipids in the even chain lengths through separation of the monoethylenic fatty acids as a class followed by open tubular gas chromatography. Several previously unreported fatty acids have been tentatively identified in herring oil by this procedure combined with ozonolysis.

EXPERIMENTAL

Methyl esters (ca. 200 mg) from a British Columbia herring oil (a commercial oil principally from *Clupea pallasii* Valenciennes) were subjected to column chromatography on silicic acid impregnated with silver nitrate as previously described (14). Following elution of the major part of the saturated acid esters with 4% diethyl ether in petroleum ether (40–60C bp) three further fractions (3.6, 4.7 and 10.0 ml) were usually collected for these studies. No detectable *trans* material was found in an examination of the infrared spectra of these fractions.

Open tubular gas chromatography was carried out with a column 150 ft in length and 0.01 in. I.D., coated with butanediol-succinate polyester. The apparatus was a Perkin-Elmer Model 226, operated isothermally at 170C, with an injection port temperature of 260C and helium as the carrier gas at 40 psig. Output was recorded on a Honeywell ElectroniK 16 (−0.05 to +1.05 mv) recorder, fitted with a Model 227-S Disc Instruments Inc. integrator, operated at a chart speed of 15 in./p hr. Other gas chromatographic equipment used in this work included an Aerograph A-90 (semipreparative, SE-30 column) and an Aerograph Hy-FI with both polyester and SE-30 columns. Areas of peaks of different chain lengths were corrected to weight percent (15).

RESULTS

The three fractions usually obtained from the chromatography on silver nitrate-silicic acid columns represented a gradation from a fraction containing only a modest proportion of monoethylenic fatty acids to a fraction containing traces of saturated fatty acids but no polyethylenic fatty acids (Analysis 1, Table I). The saturated fatty acids were present in similar relative proportions in the three fractions, but the composition of monoethylenic fatty acids differed markedly for the several chain lengths, the longer chain materials eluting first as reported by Bhatty and Craig (16) and Kishimoto and Radin (17). The total recoveries of various monoethylenic fatty acids

[1] This work was carried out in partial fulfillment of MSc requirements at Dalhousie University.

TABLE I
Proportions of Monoethylenic Fatty Acids of Even Chain Lengths Recovered in Fractions from Silver Nitrate-Silicic Acid Chromatography

	Fractions from Analysis 1			Totals from this study		Totals from complete oil
	1	2	3			
Weight recovery (mg) each fraction	25	90	42	Analysis		analysis
Percent monoethylenic acids each fraction	14.8	58.6	98.2	1[a]	2[b]	(18)
16:1	......	7.1	28.0	15.7	12.9	14.1
18:1	9.8	40.4	54.0	44.9	45.0	41.9
20:1	27.3	23.2	11.5	18.4	18.5	19.8
22:1	60.2	28.5	6.5	20.5	23.5	22.3
24:1	2.7	0.7	......	0.5	......[c]	1.8

[a] From 3 separate fractions, open tubular GC.
[b] From pooled fractions, packed column GC.
[c] 24:1 not determined.

were approximately in agreement with the results of a previous analysis of the esters of whole herring oil carried out by conventional means (18).

Interpretation of Gas Chromatograms

The preliminary identification of the components indicated by gas chromatography was based in part on the known isomers of monoethylenic fatty acids isolated by classical techniques (1–3). These procedures, including extensive crystallization and other purification steps, generally indicated only one component in each chain length. More reliable data (Table II) based on modern chromatographic procedures, but also usually including some concentration steps, clearly showed that at least two isomeric monoethylenic fatty acids could be expected in each chain length.

Each of the three fractions was cochromatographed with rapeseed oil esters to identify the acids of ω9 and ω7 series[2] (13,19).

[2] The shorthand notation of chain length:number of double bonds is used in this paper. The addition of an "ω" value defines the double bond position through the number of carbon atoms from the center of the double bond to and including the terminal methyl group. The symbol Δ is conventionally used to indicate the position of the double bond relative to the carboxyl group where this is discussed. In monoethylenic fatty acids chain length = Δ + ω.

A plot of log retention time against chain length was drawn based on these components (20). Additional lines then joined points for other components of presumed common *w* values, resulting in a system of lines for esters of the ω11, ω9, ω7, ω5 and ω3 monoethylenic fatty acids. The 24:1 acid was identified as the ω9 isomer both by the log plot and by the mixed chromatogram with rapeseed oil esters.

The lines in the linear log plot were all virtually parallel, although subsequent detailed study with more efficient columns has indicated that some divergence may be observed with increasing chain length and larger ω values (21). There was in the present instance very little deviation of the points from the idealized lines and the separations between lines were adequate to place the point for a 17:1 ester (largely in fraction 3, not shown in Figure 1) between the ω9 and ω7 lines in agreement with presumption of an ω8 structure (9). The average separations between lines (longer retention times divided by shorter) for the series of ratios of end carbon chains 9/11, 7/9, 5/7 and 3/5 were respectively 1.022, 1.032, 1.052 and 1.077. The progressive nonlinear increase in these *average* values is similar to those for isomers of polyethylenic fatty acids (20,22).

TABLE II
Proportions of Isomers of Monoethylenic Fatty Acids of Even Chain Lengths from Marine Sources Reported in the Literature

Fatty acid	Isomer structures	Isomer ratios	Origin of sample and reference
16:1	ω7, ω8	6:1	Menhaden oil (5)
	ω7, ω9, ω10	11:1:2	European herring oil (4)
	ω7, ω9 (trace)	?	North Sea plankton (10)
18:1	ω9, ω7	5:1	Tuna meat (7)
	ω9, ω7	2.5:1	Dogfish liver oil (8)
	ω9, ω7	1.2:1	Mullet oil (9)
20:1	ω11, ω9	1.2:1	Tuna meat (7)
	ω11, ω9	0.8:1	Dogfish liver oil (8)
	ω9, ω7	0.6:1	Mullet oil (9)
22:1	ω11, ω9	14:1	Tuna meat (7)
	ω11, ω9	4:1	Dogfish liver oil (8)
	ω11, ω9	?	Dogfish liver oil (11)

TABLE III

Proportions of Isomeric Monoethylenic Fatty Acids of Even Chain Lenghts in Silver Nitrate-Silicic Acid Chromatographic Fractions (Analysis 1) as Indicated by Open Tubular GC and in Fraction 3 by Ozonolysis

Chain length and isomer	Isomer (GC) area percent in fraction			Isomer weight percent by ozonolysis in fraction 3 only
	1	2	3	
16:1 ω9	......	...	1.7	1.7
16:1 ω7	......	14.7	96.5	97.0
16:1 ω5	......	82.4	1.7	2.3
16:1 3	......	2.9	0.1	......
18:1 ω11				1.0
}	33.3	69.5	89.8	
18:1 ω9				87.3
18:1 ω7	54.5	26.9	8.5	10.3
18:1 ω5	11.1	3.1	1.7	1.4
18:1 ω3	1.0	0.5	0.2	......
20:1 ω13				1.6
}	7.3	29.7	66.7	
20:1 ω11				65.0
20:1 ω9	83.9	66.1	30.6	29.9
20:1 ω7	7.3	3.3	2.8	2.9
20:1 ω5	1.5	0.9	......	0.6
22:1 ω13				29.8
}	83.3	92.2	95.5	
22:1 ω11				65.2
22:1 ω9	13.6	5.9	4.5	3.3
22:1 ω7	3.1	1.9	......	1.7
24:1 ω9?	100	100	. .	......

The actual values for the 7/9 and 5/7 separations are very close to those averaged from data for bacterial fatty acids separated on a Carbowax open tubular column (12,13,21).

The results of the preliminary identifications based on GC separations only (Table III) appeared reasonable in view of the data in the literature. The major isomers were qualitatively and quantitatively similar to those previously reported and the additional isomers could be fitted reasonably into known metabolic pathways for monoethylenic fatty acids (see discussion). In subsequent work of this type on the same sample, traces of 18:1ω6 have been tentatively identified by GC only (21).

The observations of Bhatty and Craig (16) that the isomers of monoethylenic fatty acids of common chain length are further subfractionated in the course of chromatography on silver nitrate-silicic acid to give an initial enrichment in isomers with the shorter end carbon chains (ωvalues) supported these identifications. This effect is clearly illustrated in Figure 1 and quantitatively assessed in Table III but since they would not be well defined on reproduction the C_{16} acids and the trace 18:1ω3 acid have been omitted from Figure 1.

Oxidative Fission Studies

Subsequent to these preliminary identifications qualitative and quantitative ozonolysis studies (Table III) were carried out on the materials of even chain length isolated from fraction 3 of Analysis 1 by semipreparative gas chromatography (23). The results from oxidative fission generally confirmed the tentative identifications based on GC alone, but with some minor differences and one major difference. The failure of ozonolysis to detect the tentatively identified 16:1ω3 and 18:1ω3 acids is probably primarily due to the structure and to the very low proportions in fraction 3. Thus in the ozonolysis product GC the esters of the product monoacid would be masked by solvents, and the ester of the diacid product would have a very inconspicuous peak with a long retention time. Conversely the recognition in fraction 3 of 20:1ω5 and 22:1ω7 by ozonolysis but not in the open tubular GC is due to the relatively minor amounts of the latter isomers in terms of the low proportion of 22:1 sample analyzed by GC (compare Tables I and III, Figure 1). The failure of of 22:1ω13 and 22:1ω11 to separate was unexpected, but close scrutiny of the peak in question failed to show any evidence of two materials both on the column used in the present study and on more efficient columns subsequently employed in detailed studies of the effect of double bond position on such separations (21). The latter work does show that there are grounds for predicting that separation will probably be very slight in the pairs 18:1ω11 and 18:1ω9, and 20:1ω13 and 20:1ω11.

Owing to the effect of subfractionation within each chain length it was possible that the

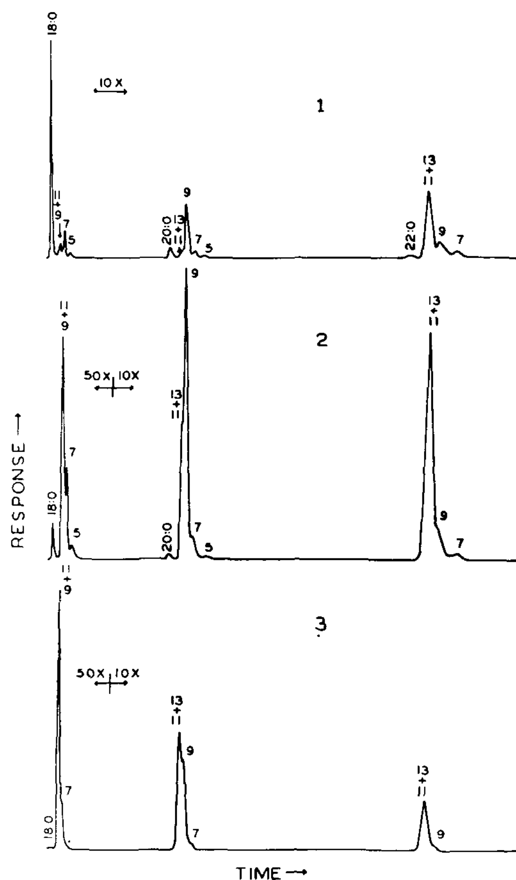


FIG. 1. Partial gas-liquid chromatograms (C_{18}-C_{20}-C_{22}) of fractions 1, 2 and 3 from herring oil methyl ester chromatography. Open tubular column 150 ft × 0.01 in I.D., BDS coated, isothermal operation at 170 C and 40 psig helium. Saturated acids are labelled as to chain length, monoethylenic peaks (excepting trace 18:1 ω3) denoted by ω values. Attenuations as marked.

22:1ω13 acid was particularly concentrated in fraction 3, but the bulk (75%) of the 22:1 acids had been in fraction 2, which was no longer available for oxidative fission. Accordingly a fresh isolation (Analysis 2) of the monoethylenic fatty acid esters of herring oil was carried out by chromatography on silver nitrate-silicic acid. The three fractions were pooled and the proportions of saturated and monounsaturated acids in the various chain lengths determined by conventional packed-column GC (Table I). Preparative GC separation of each chain length and ozonolysis were carried out as before (Table IV). This established the overall content of 22:1ω13 as about 13% of the 22:1 acids. On this basis the composition of fraction 2 in analysis 1 would be approximately 12% 22:1ω13, as compared with approximately 30% in fraction 3. This degree of enrichment appears reasonable in view of the proportions in each fraction and the nature of the isomers involved.

DISCUSSION

Analytical Techniques

The overall results of the analyses of the sample of herring oil (Table IV) by two somewhat different approaches are in reasonable quantitative agreement for the proportions of most components. The subfractionation effect of the silver nitrate-silicic acid technique (initial elution of the longer chain lengths and of the isomers with the lesser ωvalues) is particularly advantageous in the open tubular GC analyses where minor components such as 18:1ω3 are readily recognized when thus concentrated. In terms of convenience, the employment of open tubular GC alone has obvious advantages, but the failure to separate those isomers of even chain length with double bonds occurring from approximately the midpoint of the fatty acid chain to a Δ^6 position would be a handicap if such isomers were significant (21). The study of isomers of monoethylenic fatty acids of odd chain length, which are very minor components of most marine lipids, could also be largely accomplished by open tubular GC.

It should be noted that monoethylenic fatty acids with double bonds sufficiently close to the carboxyl group for interaction to occur (e.g., *trans* 16:1ω13) have increased retention times and therefore could be confused with isomers with low ωvalues (21, cf 24). This is a further limitation on the use of open tubular GC alone, and the risk of confusion is compounded by the observation that in chromatography on silver nitrate-silicic acid columns (elution with benzene in petroleum ether) the *trans* isomers precede the *cis* isomers of the same chain length (16,25). In open tubular GC on polar substrates the separation of *trans* and *cis* fatty acids with the same chain length and double bond positions may be negligible, although if such a separation occurs the *trans* isomer will appear first (21,26). In the present study, infrared analysis of the esters of whole herring oil, and of the individual fractions (Analysis 1) did not show any detectable *trans* absorption. *Trans* material of unspecified nature has been concentrated from menhaden oil (27), and appears in certain fractions from mullet oil where it is indicated that this is probably due to artifact formation during distillation (9).

TABLE IV
Proportions of Isomers of Monoethylenic Even Chain Length Fatty Acids in Herring Oil as Indicated by Open Tubular GC and Partial Ozonolysis (Analysis 1) and by Packed Column GC and Ozonolysis (Analysis 2)

Chain length and isomer	Isomers as percent of chain length		Isomers as percent of net C_{16}-C_{24}[a] monoethylenic acids	
	Analysis 1	Analysis 2	Analysis 1	Analysis 2
16:1 ω9	1.3	3.2	0.2	0.4
16:1 ω7	76.5	76.7	12.0	9.9
16:1 ω5	21.6	17.3	3.4	2.2
16:1 ω3	0.6	2.8	0.1	0.4
18:1 ω11 }	75.7	3.2	34.9	1.4
18:1 ω9 }		70.4		31.6
18:1 ω7	21.6	24.2	9.9	10.9
18:1 ω5	2.4	2.8	1.1	1.3
18:1 ω3	0.3	......	0.1	......
20:1 ω13 }	38.3	1.5	6.9	0.3
20:1 ω11 }		36.7		6.8
20:1 ω9	57.2	59.0	10.3	10.9
20:1 ω7	3.3	2.8	0.6	0.5
20:1 ω5	1.2	......	0.2	......
22:1 ω13 }	91.6	13.1	18.4	3.1
22:1 ω11 }		80.9		19.0
22:1 ω9	6.5	6.0	1.3	1.4
22:1 ω7	1.8	......	0.4	......
24:1 ω9 ?	100	.....	0.5	......

[a] 24:1 not determined in Analysis 2.

Isomers of Monoethylenic Fatty Acid Isomers in Other Animals

Of the less well-known isomers of monoethylenic fatty acids the widespread occurrence of 18:1ω7 in animal lipids is well documented (28–31). In most lipids the proportions of 18:1ω9 and 18:1ω7 are about 10/1, although in specialized lipids of rats the ratio may be lower (29,31). The data from the literature (Table II) and from the present analysis of herring oil (Table IV) suggest that in depot fats of fish a ratio of 3/1 is typical for these two isomers.

Very little is known about the occurrence of other unusual isomers of monoethylenic fatty acids. The detailed study of fatty acids of rat lipids by Sand et al. (28) does provide evidence for the natural occurrence in animal lipids at low levels of some of the acids found in the present study. These authors report, in addition to the major C_{16} and C_{18} monoethylenic fatty acids, 14:1ω7 and 14:1ω5 (ratio of 10/1), 16:1ω10 and 16:1ω8 (cf. Table II), 18:1ω11 and 18:1ω10. From certain organs of the rat the acids recovered were 20:1ω13, 20:1ω9 and 20:1ω7, with very little 20:1ω11. The livers of fat deficient rats are also reported to contain 20:1ω9 and 20:1ω7 acids (32). These results may reflect the dietary status of the rat, since the abolition by fasting of desaturation, but not of elongation, would lower the proportion of 20:1ω11 acid (31). Other studies with rats suggest the presence of 16:1ω5 as well as 16:1ω6 and 18:1ω8 acid (31). In fatty acids of pig brain lipids 22:1ω9 and 22:1ω7 are accompanied by some 22:1ω13, but not by 22:1ω11, and the absence of 20:1ω11 (see above) should also be noted (33).

Origin of Isomers of Monoethylenic Fatty Acids

The composition distribution, rearranged in terms of proportions of isomers (Table V) shows clear trends with both chain length and ωvalue, yet poses a number of problems, particularly since the longer chain acids are not significant in depot fats of mammals and therefore have not received much attention. These acids are probably formed largely in the herring, since the limited literature on the fatty acid composition of zooplankton suggests that the fat of the primary food of the herring would be low in 20:1 and 22:1 acids (34). This is a generalization and may not hold in some instances (35).

It is possible to explain the various isomeric fatty acids found in the C_{16}-C_{22} even chain lengths of herring oil on the basis of one desaturation enzyme removing two hydrogens from the Δ^9 and Δ^{10} positions of saturated fatty acids. This was originally proposed by Breusch (36) and has been specifically

TABLE V
Comparison of Percent Isomers in Each Chain Length with Structure

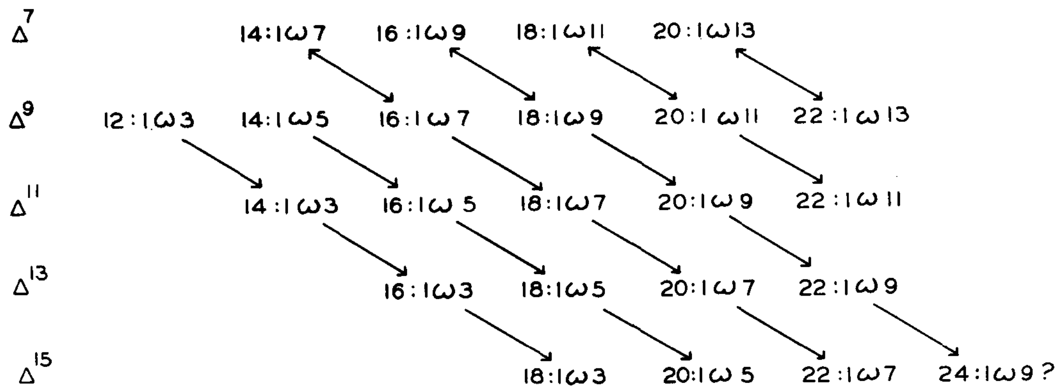
Double bond position from carboxyl	Isomer percentage in each chain length and ω value					
	Herring oil acids[a]					
	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}
Δ^7	1.3(ω9)	3.4(ω11)	1.5(ω13)	....		
Δ^9	76.5(ω7)	72.3(ω 9)	36.8(ω11)	12.8(ω13)		
Δ^{11}	21.6(ω5)	21.6(ω 7)	57.2(ω 9)	78.8(ω11)		
Δ^{13}	0.6(ω3)	2.4(ω 5)	3.3(ω 7)	6.5(ω 9)		
Δ^{15}	....	0.3(ω 3)	1.2(ω 5)	1.8(ω 7)	100(ω9)?	
	Pig brain sphingolipid acids (17)					
Δ^{13}				15 (ω 9)	....	....
Δ^{15}				85 (ω 7)	78(ω9)	....
Δ^{17}				....	20(ω7)	40(ω9)
Δ^{19}				....	....	49(ω7)

[a] Composite percentages from Analyses 1 and 2.

demonstrated to apply to 16:0 and 18:0 in rats (29,31). Numerous other workers in this field have discussed this concept (8,9,28,33,37,38,cf.39).

The saturated fatty acids commonly recognized in marine oils normally range from 12:0 to 20:0, and 22:0 has also been noted in concentrates but evidently occurs in very small proportions (14,40,41). The overall reaction, as shown in Scheme I conveniently accounts for the majority of observed isomers by the normal process of two-carbon chain extensions. It is stated that longer chain acids are normally completely broken down to acetate (42). However, formation of 16:1ω12 (as well as 20:1ω12) in small amounts in rats fed on 18:1w12 clearly indicates that in addition to two carbon chain extensions there may be limited selective removal of two carbon atoms as well as complete chain breakdown (28,cf.31,43).

An anaerobic pathway operative in bacteria can produce the same isomers, including the 16:1ω5 acid (39). The specificity of the Δ^9-Δ^{10} dehydrogenation is also illustrated by the presence of 17:1ω8 and apparent absence of other 17:1 isomers in significant amounts in the fatty acids of the herring oil under study, and also in the unusual oil of the striped mullet (9). In the latter oil 15:1ω6 is also found, with only slightly less 15:1ω8. However, 19:1ω8 is the only isomer reported in the mullet oil. Since 15:0 and 17:0 are present, and 19:0 is virtually absent from this oil, the correlation proposed for the desaturation is excellent, accounting for 15:1ω6, 17:1ω8 and the absence of 19:1ω10. The 15:1ω8 and 19:1ω8 evidently arise by respective shortening and lengthening of the chain of 17:1ω8. There must be considerable metabolic activity in the saturated and monounsaturated acids of these shorter chain lengths, since 16:1ω9 must arise from 18:1ω9 and is fairly common (28,29). This may also account for the 14:1ω9 reported in addition to 14:1ω5 in a number of marine oils (1). There has recently been considerable interest in plant fatty acids with unsaturation in a Δ^5 position relative to the carboxyl group (44). It is therefore possible that 14:1ω9 or a homologous acid could be found in some unknown marine plant source, particularly as 12:1ω7 has been isolated from a marine oil (1). The contrast between the complex mixture of isomers found in the fatty acids of herring oil (Table IV) and in other oils (Table II), and the apparent simplicity of marine oils based on earlier isolations of single



SCHEME I.

fatty acids by classical techniques, suggests that speculation on the shorter chain fatty acids should be limited pending further data.

To account for the wide range of isomers of monoethylenic fatty acids in pig brain lipids an additional dehydrogenation enzyme has been suggested operating on the carbon atoms Δ^6 and Δ^7 relative to the carboxyl group (33). This could account for the 16:1ω10 reported for herring oil (4) and in lipids of the rat (28), for the 17:1ω11 accompanying 17:1ω8 in menhaden oil (27), and if operating on 15:0 and 13:0 could also be invoked to explain the 19:1ω9 and 19:1ω7 observed in minor amounts in a shark liver oil (45).

The apparent poor recovery of 24:1, as compared with the analysis of the whole oil (Table I), may be deceptive as this acid was previously estimated by difference from coincident components, and the level found was somewhat higher than found in other herring oil analyses (18). The presence of isomers other than 24:1ω9 is reported in lipids of brain tissue (17,33,46). They may also occur in the herring oil although this is the only known isomer from marine sources (1). The origin of 24:1ω9 by chain extension from 18:1ω9 has been shown in rat brain, and 20:1ω9 and 22:1ω9 were indicated as intermediate precursors (46), although direct multistep elongation from 18:1ω9 is a significant process in this particular system (cf.47). In herring oil, a depot fat, it is quite possible that the stepwise two carbon chain extension process is the principal synthetic route to 24:1.

Some support for this view is provided by the fatty acids of shark liver oils, which were reported as very rich (10%) in 24:1 (1,48). However, more recent analyses of some species indicate 24:1 levels of 1–3% varying roughly in proportion to the amount of 20:1 and 22:1 acids present (11,49,50). It will be noted that the proportion of 22:1ω9, the potential precursor to 24:1ω9, is higher relative to 22:1ω11 in dogfish liver oil than in tuna lipids (Table II) or in the herring oil under study (Table IV). Studies with rat liver enzymes have explored the conditions for the conversion of 22:1ω9 into 24:1ω9 in this animal (47).

Structure and Chain Elongation in Isomers of Monoethylenic Fatty Acids

The proportions of the isomers of different chain lengths (Table V) indicate that chain length and structure influence the probability of either two carbon chain extension or direct multistep elongation to a given end product. The presumed stoppage of chain extension at 22:1ω11 and 22:1ω13 suggests that either the proximal Δ^{11} or terminal ω11 and ω13 structures make this step less probable. The relatively high levels of 20:1ω11 and 22:1ω13 in comparison with the low levels of 20:0 and 22:0, as related to total saturates, point to these ω11 and ω13 structures as relatively inert. Monoethylenic acids with ωvalues greater than 11 are found in the even chain acids of pig brain lipids, but only as minor components (33). On the other hand the progressive accumulation of the Δ^{11} acids as chain length increases in the herring oil suggests that in the longer chain lengths the Δ^{11} acids are increasingly inert. The high proportion of 20:1ω11 could then be a feedback effect from accumulated 22:1w11 due to the nonreactive Δ^{11} structure of the latter.

If Δ^{11} or ω11 and ω13 give effective blockage of chain extension in the C_{22} acids, then preferential structures might behave similarly in the C_{24} acids. It is in fact indicated by the proportions of these acids in pig brain sphingolipids (Table V) that 22:1ω9 is possibly elongated in preference to 22:1ω7, but 24:1ω7 in preference to 24:1ω9. The comparison is not specific since the monoethylenic fatty acids of longer chain length in the pig brain sphingolipids play a particular role which may not be duplicated in a depot fat and they may be of different origin (see above). The complete pig brain fatty acid analysis for C_{18}-C_{22} monoethylenic acids also shows an increasing disproportionation between ω9 and ω7 isomers in favor of increasing amounts of the latter (33). However, proportions in the C_{22} chain length for fatty acids of whole pig brain are 1.7/1, whereas in the sphingolipid fatty acids (Table V) the proportions are 0.18/1. More than one lipid system is represented in the whole brain analysis and this difference in ratios emphasizes the possibilities of different routes to a given acid in different lipids. The pig brain data, however, support the view that low "ω" values promote chain elongation.

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