The Structure of Polyenoic Odd- and Even-Numbered Fatty Acids of Mullet *(Mugil cephalus)*

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Abstract

Mullet oil contains more than 25% straight-chain odd-numbered fatty acids. Odd- and even-numbered components of chain lengths C_{15} to C_{20} were isolated and their structures determined. The vinylmethane rhythm prevails in all polyunsaturated acids. Numerous homologs have their double bonds in identical positions, relative to the carboxyl group, as for example, $\Delta^{9,12}-$ and $\Delta^{6,9,12}-$ C_{15}, $-C_{16}$, $-C_{17}$ and $-C_{18}$ acids. The terminal structures which are characteristic for oleic, linoleic, etc., families are not found in the unsaturated odd-numbered acids.

The results show: that the proximal structure has greater influence than the terminal structure on the biosynthesis of unsaturated odd-numbered acids; that chain lengths of 17 and 18 carbon atoms with double bonds in position 9 are crucial for synthesis of the polyenoic C_{19} and C_{20} acids; that chain lengths C_{15} and C_{16} with double bonds in position 9 are suitable for desaturation but that they are not suitable for desaturation after elongation.

These specifications bring all acids of mullet into a rational order and reflect their possible interconversions. Presently, such classification has only limited predictive value in regard to the physiological properties of polyunsaturated acids. However, the new definitions for grouping the polyunsaturated fatty acids lead to interesting working hypotheses.

Introduction

THE OCCURRENCE of straight-chain odd-numbered fatty acids has been reported during recent years from ruminant, fish and many other animal lipids, and they can be considered as normal constituents of such fats. However, the odd-numbered acids represent only a very small percentage of the total acids and, therefore, their isolation meets with considerable difficulty. So far, only saturated and some monounsaturated straight-chain, odd-numbered acids have been isolated and their structure established beyond the tentative identification furnished by gas-liquid chromatography (GLC).

Mullet *(Mugil cephalus)* oil is a promising source for studying unsaturated odd-numbered fatty acids. Gruger, Nelson and Stansby have reported, in a survey of numerous marine oils, that the fatty acids of mullet are distinct from others inasmuch as they contain about 25% of odd-chain length acids (1). The isolation, identification and assay of mullet fatty acids is described here. As with polyunsaturated even-numbered acids, data on the structures of odd-numbered acids should reflect principles which govern their biosynthesis. A comparison of the two series is of particular interest in the present period of fatty acid research where the biosynthesis and physiological activity of even-numbered polyenoic acids is under intense investigation.

The mullet fatty acid methyl esters were fractionated essentially by distillation and by liquid-liquid chromatography (LLC). However, crystallization or preparative GLC had to be employed before, or instead of, the final separations by LLC. GLC, hydrogenation followed by GLC (H-GLC), UV and IR spectroscopy were utilized to monitor the fractionation procedures and to characterize the esters by chain length and number of double bonds. The individual esters or the mixtures of isomers were subjected to ozonization-hydrogenation and the aldehydic proximal and terminal fragments identified by GLC. The results from the ozonization were in agreement with the conclusions drawn from the intermediary analytical procedures.

Experiments and Results

Mullet Fatty Acid Methyl Esters. The fish had been caught in the coastal area of the Gulf of Mexico near Pascagoula, Miss. They were shipped in dry ice and were stored at −18C until used. Seasonal differences of fatty acid compositions of catches in March and August, 1961, were not observed; the work described here was carried out with the latter shipment.

The frozen fish were diced and then immediately worked through a meat grinder. The pulp-like material was then extracted three times by reflux with chloroform + methanol, 2:1. After suction filtration on a Buchner funnel, the solvents were removed in a rotary evaporator under reduced pressure. The chloroform phase yielded a light-brown oil. A minor amount of lipid was recovered from the aqueous methanol phase and the two portions were combined.

The oil was saponified by mild reflux with alcoholic KOH. Part of the ethanol was removed under vacuum and water was added to the concentrated soap solution. Unsaponifiables were removed by three extractions with low-boiling petroleum ether (Skellysolve F). The fatty acids were recovered in the usual manner, and 560 g acids was esterified in methanol containing about 5% sulfuric acid under gentle reflux for 6 hr. The esters were isolated after dilution with water by extraction with petroleum ether, washing and drying. The yield was 530 g esters.

Distillation. The amber-colored esters were distilled in a column, 90 cm × 25 mm (SemiCal high temperature unit, Podbielniak, Inc.), first at ca. 2 mm, and from the C_{18} fraction on at ca. 0.5 mm pressure. Highest temp were 250C in the pot and 170C at the take-off. The distillation lasted about 13 hr and 18 fractions were taken. They were characterized by GLC and H-GLC on β-cyclodextrin acetate (2). The results which provided some tentative identifications are listed in Table I. According to these analyses, fractions were selected as indicated in Table I for further isolation procedures.

The equivalent chain lengths (3) after hydrogenation (H-ECL) show that the fractionation was not completely successful. This is due to the broad variety of unsaturation which causes an inefficient separation of chain lengths by distillation. The difficulty is ag-

TABLE I

GLC Analysis of Distilled Methyl Esters

Fraction No.	Grams [a]	H-ECL % Area	ECL % Area	Fractions used for isolation of esters
1	1.8		9.6 (60) 11 (14) 12 (14) 13 (13)	
2	1.5	11 (3) 12 (21) 13 (44) 14 (33)	11 (3) 12 (22) 13 (42) 14 (33)	
3	2.0	12 (1) 12.7 (tr.) 13 (32) 13.6 (tr.) 14 (65)	12 (5) 13 (38) 14 (58)	
4	18.0	13 (tr.) 14 (100)	14 (100)	
5	5.0	14 (34) 14.6 (6) 15 (60)	14 (31) 14.6 (5.2) 15 (46) 15.4 (16) 15.7 (2)	C_{15}
6	15.5	14 (6) 15 (94)	14 (3) 14.6 (0.4) 15 (90) 15.7 (7)	
7	38.5	14 (2) 15 (62) 16 (36)	14 (2) 15 (63) 15.4 (8) 15.9 (8) 16.5 (19) 17.2 (2) 17.8 (4)	
8	41.0	15 (24) 16 (76)	15 (27) 16 (30) 16.5 (37) 17.2 (2) 17.8 (4)	
9	48.0	15 (12) 16 (86) 17 (1)	15 (16) 15.7 (1) 16 (42) 16.6 (32) 17.3 (0.2) 17.6 (4.1) 18.1 (4.7) 19.05 (0.3)	
10	41.0	15 (6) 16 (85) 17 (8)	15 (7) 16 (42) 16.4 (35) 17.3 (10) 17.7 (4) 18.2 (2) 18.6 (1)	
11	14.0	15 (3) 16 (84) 17 (13)	15 (6) 16 (47) 16.4 (30) 17.3 (10) 17.8 (5) 18 (2) 18.8 (1)	
12	47.0	15 (3) 16 (76) 17 (22)	15 (1) 16 (47) 16.4 (31) 17.3 (15) 17.8 (2) 18 (2) 18.8 (2)	C_{16}
13	6.0	15 (1) 16 (68) 17 (33)	16 (40) 16.4 (28) 17.3 (19) 18 (7) 18.8 (4)	
14	41.0	16 (42) 17 (58)	16 (28) 16.4 (15) 17 (4) 17.3 (31) 18 (12) 18.8 (9)	
15	48.0	16 (tr.) 17 (25) 18 (75)	17 (5) 17.8 (12) 18 (5) 18.7 (46) 19.4 (15) 20 (4) 20.3 (4) 20.9 (10)	C_{17}, C_{18}
16	28.0	18 (78) 19 (22)	18 (25) 18.5 (38) 19.1 (10) 19.3 (1) 19.6 (6) 20.3 (6) 20.8 (14)	C_{19}
17	13.0	18 (8) 19 (46) 20 (46)	18 (4) 18.3 (5) 18.8 (6) 19.2 (14) 19.6 (5) 20.2 (4) 20.8 (27) 21.6 (17) 22.4 (17)	
18	35.0	19 (3) 20 (97)	20.4 (5) 21 (4) 21.8 (27) 22.6 (63)	C_{20}

[a] Starting material: 501 g; total distilled: 444 g; residue: 43 g.

gravated here with a mixture of homologous rather than the usual *bis*-homologous esters.

Preparation of Samples for LLC. Enrichment of unsaturated esters or further purification to obtain fractions of more uniform chain length was necessary in most cases to make more efficient the final separations by LLC. The intermediary procedures are outlined in the following.

C_{15} Methyl Esters: Fractions 5 and 6 were combined and distilled in a spinning band distillation apparatus, 60 cm × 8 mm (Podbielniak, Inc.). The subfractions were analyzed by GLC and H-GLC as above. Suitable portions were combined and 8 g material dissolved in 120 ml acetone. About 4 g saturated esters could be eliminated by crystallization at −12C (4). The unsaturated C_{15} esters were enriched in the mother liquor and used in three portions for LLC.

C_{16} Methyl Esters: Fractions 12 and 13 were combined and redistilled as above. Eleven g of proper subfractions were subjected to crystallization from 165 ml acetone at −5C. About 6 g esters remained in the filtrate and an aliquot of them was used for LLC.

C_{17} and C_{18} Methyl Esters: Samples suitable for LLC were obtained by redistilling fraction 15 as described before. Enrichment of unsaturated components was not necessary.

C_{19} Methyl Esters: The combined fractions 16 and 17 were redistilled and suitable subfractions selected for separation of the components by LLC.

C_{20} Methyl Esters: An aliquot of fraction 18 was used for LLC without further purification.

Table II gives the GLC analyses of the samples which were subjected to LLC. UV spectroscopy did not indicate conjugated unsaturation in any of the samples. IR spectroscopy revealed 5% *trans* double bonds in the sample of C_{19} and 15% in that of C_{20}

TABLE II

GLC Analysis of Esters Before Separation by LLC

Chain length	H-ECL % Area	ECL % Area
C_{15}	14.6 (5) 15 (95)	14.6 (4) 15 (70) 15.8 (21) 16.1 (1) 17.3 (3)
C_{16}	15 (2) 16 (98)	15 (2) 16 (30) 16.7 (51) 17.4 (8) 18.1 (9)
C_{17}	16 (2) 17 (96) 18 (2)	16 (3) 17 (3) 17.6 (42) 18.4 (24) 19.0 (13) 19.3 (1) 20.2 (3) 20.8 (2)
C_{18}	18 (100)	18 (7) 18.7 (46) 19.3 (20) 19.9 (7) 20.3 (9) 20.8 (11)
C_{19}	18 (11) 19 (90) 20 (<1)	18 (6) 19.1 (2) 19.7 (9) 20.4 (5) 21.1 (13) 21.6 (64) 22.3 (2)
C_{20}	19 (3) 20 (97)	20.4 (5) 21 (4) 21.8 (27) 22.6 (63)

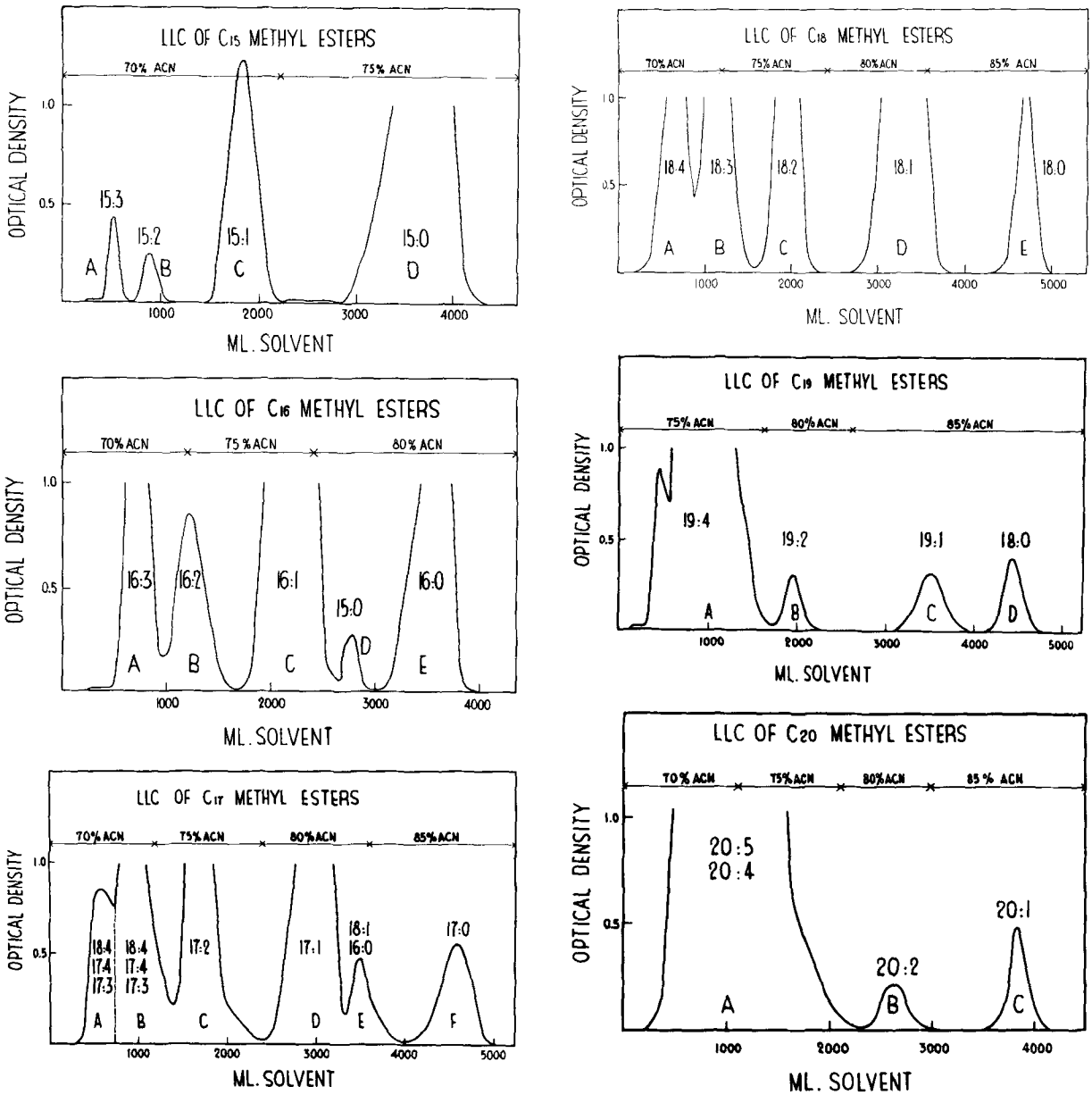

FIG. 1. LLC of mullet fatty acid methyl esters.

methyl esters. The *trans* isomers appear only in fractions which are collected at the end of the distillations and, very likely, they are artefacts due to thermal rearrangement.

Unsaturated fatty esters have slightly lower boiling points than the corresponding saturated esters. Accordingly, the saturated component of a certain chain length is enriched towards the end of that fraction and may contaminate the unsaturated components of the next higher chain lengh. The H-ECL show that only the C_{19} fraction contains an appreciable amount of esters having other chain length contaminants and they were found to be mainly stearate. The C_{17} fraction contains C_{16} and C_{18} esters in minor amounts; enrichment by LLC and subsequent procedures showed them to be mainly palmitate and octadecatetraenoate.

Only the C_{15} methyl esters contain a marked amount of a branched isomer. By earlier experience with GLC of isomeric fatty esters (2), the compound is tentatively identified as 13-methyltetradecanoate, but it was not further investigated. The presence of branched isomers is also indicated in some fractions of higher fatty esters but their amount is insignificant.

Liquid-Liquid Chromatography of Esters. The system of aqueous acetonitrile and silicone oil was employed as previously described (5,6) using columns of 90-cm length and 27 mm diam. The monitoring curves are shown in Figure 1. Table III lists the pertinent analytical data of the peak fractions.

Most peak fractions are pure in regard to chain length and number of double bonds and the esters can be easily identified in these terms. Fractions $C_{17}A$ and $C_{20}A$ need further comment.

Fraction $C_{17}A$ contains three components, ECL 20.8 (40%), ECL 20.2 (47%) and ECL 19.0 (12%). H-GLC shows C_{18} (40%) and C_{17} (60%) chain lengths. ECL 20.8 is an 18:4 ester since it was identified as such in the fraction $C_{18}A$. ECL 19.0 is a 17:3 ester since it was identified as such in the fraction $C_{17}B$, where it represents more than 95% of the material. The third component of fraction $C_{17}A$, ECL 20.2, must have a chain length of C_{17} according to the quantification by H-GLC which shows 60% C_{17} ester but only 12% of it is accounted for by the 17:3 ester, ECL 19.0. UV absorption after isomerization (7) does not indicate the presence of a pentaene. The difference between ECL 20.2 and ECL

TABLE III

Analysis of LLC Fractions by GLC, H-GLC and Alkaline Isomerization

Peak fraction	A		B		C		D	E	F
Chain length of methyl esters	ECL H-ECL	λ_{max} mμ	ECL H-ECL	λ_{max} mμ	ECL H-ECL	λ_{max} mμ	ECL H-ECL	ECL H-ECL	ECL H-ECL
C_{15}	17.3 15	268	16.5; 16.1 15	232	15.8 15	.....	15; 14.6 15; 14.6		
C_{16}	18.1 16	268	17.6; 17.3 16	232	16.7 16	.....	15 15	16 16	
C_{17}	20.8; 20.2; 19.0 18; 17	268, 300, 315 [a]	20.8; 19.0 17, tr. 18	268	18.3 17	232	17.6 17	18.7; 16 18; 16	17 17
C_{18}	20.8 18	232, 286, 300, 315	20.3; 19.9 18	268	19.3 18	232	18.7 18	18 18	
C_{19}	21.5; 21.1 19	238, 286, 300, 315	20.4 19	232	19.7 19	.....	18 18		
C_{20}	22.6; 21.8 20	234, 285, 300, 315 [b] 233, 285, 300, 315, 328, 346 [b]	21.0 20	232	20.4 20	.....			

[a] Mixture of tetraene and triene (see text).
[b] Isomerization after separation of pentaene and tetraene (see text).

19.0 rules out an isomeric 17:3 acid. A heptadecatetraenoate remains as the most likely identification and, according to LLC fractionation patterns, such compound should appear here. Ozonization-hydrogenation of fraction C_{17}A and identification of the fragments verified the above conclusions.

Fraction C_{20}A consists of two components which have identical chain length. However, the considerable difference in their ECL indicates that they are not isomeric. The mixture was separated by preparative GLC on β-cyclodextrin acetate (8). The faster migrating component, ECL 21.8, had λ_{max} 315 mμ and 300 mμ after isomerization and, therefore, must be a tetraenoate. The other component, ECL 22.6, showed λ_{max} 346 mμ and 328 mμ after isomerization and is a pentaenoate (9).

The small portion running ahead of fraction C_{19}A has not been investigated.

Ozonization-Hydrogenation. A semi-micro ozonizer according to Bonner was used (10). Amounts between 5 and 30 mg esters were allowed to react with O_3 at −10C in 2 ml methyl acetate or 1 ml ethyl caprate. Ozone was generated in a stream of O_2, 80 ml/min. The reaction times were 1–2 min, varying with amount of ester and number of double bonds per molecule. A rimless test tube, 15 × 125 mm, was placed into the ozonization vessel originally described and served for the reaction. Both inner and outer vessel were precooled and then attached to the equilibrated ozonizer. After the timed period of ozonization, the test tube was removed and immediately flushed at 0C with a stream of nitrogen. Lindlar catalyst (11,12) was added and a slow stream of H_2 bubbled through the suspension at 0C for ca. 10 min. The fragments were identified by comparison of their GLC retention values with those of authentic compounds which had been obtained either commercially or by ozonization-reduction of unsaturated fatty esters with known structures.

Both the carboxylate and the methyl end fragments of the unsaturated esters were analyzed. Methyl acetate was the solvent for ozonization when the former were to be identified. This solvent appears in GLC ahead of C_4 ald-ester. Ethyl caprate was used when aldehydes were to be identified (13); it emerges after C_{10} aldehyde so that the peaks of short-chain aldehydes can be interpreted without interference of the solvent. The identification of acetaldehyde by GLC was verified by TLC of the 2,4-dinitrophenylhydrazone.

The identification of proximal and terminal fragments suffices for locating the double bonds in polyenoic fatty acids which have the usual methylene-interrupted structure. When chain length, number of double bonds and their non-conjugation are ascertained, it appears unnecessary to demonstrate the three-carbon fragments which result from the center of the molecule by ozonization. No indication for double bond systems, other than methylene-interrupted, was encountered with the fatty esters of mullet.

Malondialdehyde or, possibly, a product derived from it appears in GLC as a peak earlier than propionic aldehyde and is distinct from it. This peak is missing when monoenoic esters had been ozonized, but its size does not increase proportionately with the predicted amount of C_3 fragments of higher unsaturated esters.

Qualitative results of the ozonization-hydrogenation and the structures of the unsaturated fatty esters are listed in Table IV.

Assay of Fatty Esters by GLC and Supplementary Methods. Quantifications by GLC require resolution of peaks so that areas of individual compounds can be measured. Our GLC methods did not resolve the total esters efficiently enough for quantification of the components. Satisfactory separations by GLC were achieved after prefractionation by other methods. The peaks could be identified once the major components had been preparatively separated, identified by chemical degradation and their ECL determined. However, GLC of model mixtures consisting of saturated esters, oleate, linoleate, linolenate and arachidonate had shown that the response of the thermoconductivity detector is markedly below proportionality when compounds contain more than two double bonds. The correction factor of each compound can be determined and is reproducible for a given stationary phase and set of chromatographic conditions. However, such a procedure would have required greater amounts of pure components than we were ready to sacrifice for precise quantifications. Therefore, the assay is based on peak areas without correction for any of the highly unsaturated esters and, consequently, the values are too high for saturated and too low for polyunsaturated esters.

Although ald-ester and aldehyde peaks were identified after ozonization-reduction in all cases, the ratios of isomers were calculated from the ald-ester peaks only. Aldehydic esters of different mole wt

TABLE IV

Results of Ozonization-Hydrogenation of Mullet Fatty Esters

LLC fraction	Carbon atoms of aldehyde fragments	Carbon atoms of ald-ester fragments	Structure of fatty esters [a]
$C_{15}C$	8;6	7;9	7- and 9-Pentadecenoate
$C_{15}B$	6;3	6;9	6,9- and 9,12-Pentadecadienoate
$C_{15}A$	3	6	6,9,12-Pentadecatrienoate
$C_{16}C$	7	9	9-Hexadecenoate
$C_{16}B$	7;4	6;9	6,9- and 9,12-Hexadecadienoate
$C_{16}A$	4	6	6,9,12-Hexadecatrienoate
$C_{17}D$	8	9	9-Heptadecenoate
$C_{17}C$	8;5	6;9	6,9- and 9,12-Heptadecadienoate
$C_{17}B$	5	6	6,9,12-Heptadecatrienoate
$C_{17}A$ [b]	2(3;5)	6	6,9,12,15-Heptadecatetraenoate (C18:4, see Fraction $C_{18}A$; C17:3, see Fraction $C_{17}B$) [(b)]
$C_{18}D$	9;7	9;11	9- and 11-Octadecenoate
$C_{18}C$	6	9	9,12-Octadecadienoate
$C_{18}B$	6;4;3	6;8;9	6,9,12-, 8,11,14- and 9,12,15-Octadecatrienoate
$C_{18}A$	3	6	6,9,12,15-Octadecatetraenoate
$C_{19}C$	8	11	11-Nonadecenoate
$C_{19}B$	8;7;5	8;9;11	8,11-, 9,12- and 11,14-Nonadecadienoate
$C_{19}A$	5	5	5,8,11,14-Nonadecatetraenoate
$C_{20}C$	9;7	11;13	11- and 13-Eicosenoate
$C_{20}B$	6	11	11,14-Eicosadienoate
$C_{20}A$ [b]	6;3	5;8	5,8,11,14- and 8,11,14,17-Eicosatetraenoate
$C_{20}A$ [b]	3	5	5,8,11,14,17-Eicosapentaenoate

[a] For quantification of isomers see Table V.
[b] See text.

arise from isomeric unsaturated fatty esters, and the area percentages were corrected in proportion to their mole wt. The validity of such procedure was established with several model mixtures; moreover, quantitative data obtained by ozonolysis were in agreement with data of GLC when isomeric esters were sufficiently separated for quantification of areas. However, the ratios of isomers were determined only with selected fractions and an analysis of the isomers in the total material has not been made. Accordingly, there is some uncertainty in these ratios, inasmuch as distillation and crystallization may involve selective enrichment in the course of isolation.

Complete results of the quantifications and an assay of the individual components are found in Table V.

Discussion

Propionic acid was recognized some time ago as precursor of odd-numbered long-chain acids in animals (14,15,16). More recently, Horning et al. (17, 18) have shown that propionyl-S-CoA serves as primary unit for synthesis of odd-numbered acids by enzymes of rat adipose tissue. The biosynthetic system formed mainly pentadecanoic acid while palmitic acid was the major product when acetyl-S-CoA was the substrate. In mullet, as well as menhaden oil (5), the maximum amounts of odd acids are found with chain lengths C_{15} and C_{17}, while C_{16} acids represent the greatest portion of the even-numbered acids.

It is characteristic for fish acids that unsaturation is increased with chain length. Odd- and even-numbered fatty acids of mullet follow this rule.

The fish had been caught from the natural habitat. Therefore, endogenous, exogenous and semi-endogenous (necessarily exogenous but further converted by the fish) acids can not be distinguished. The question of endogenous or exogenous origin of fatty acids usually pertains to dienoic or higher unsaturated acids. The odd-numbered acids in mullet present an additional problem.

Evidence has been brought forward that fish can elongate and desaturate fatty acid chains and synthesize some of the polyunsaturated acids, but very

TABLE V

Composition of Mullet Fatty Acids

Acid	Chain lengths, % of total: By H-GLC of total esters	Chain lengths, % of total: By H-GLC of distilled fractions	Components, % of one chain length: By GLC of distilled fractions	Components % of unsat. isomers: By O_3–H_2–GLC of isomers after LLC	Individual components % of total: Assay by combined results
C14	6	5	100		5.3
C15	15	11	87		11.2
Δ^9—C15:1			10	58	0.8
Δ^7—C15:1				41	0.5
$\Delta^{6,9}$—C15:2			1	35	0.05
$\Delta^{9,12}$—C15:2				64	0.1
$\Delta^{6,9,12}$—C15:3			1.5		0.2
C16	42	38	80		32.0
Δ^9—C16:1			15		6.0
$\Delta^{6,9}$—C16:2			2	71	0.1
$\Delta^{9,12}$—C16:2				83	0.7
$\Delta^{6,9,12}$—C16:3			3		1.2
C17	10	11	12		1.2
Δ^9—C17:1			45		4.6
$\Delta^{6,9}$—C17:2			25	7	0.2
$\Delta^{9,12}$—C17:2				93	2.5
$\Delta^{6,9,12}$—C17:3			12		1.2
$\Delta^{6,9,12,15}$—C17:4			5		0.5
C18	8	12	20		2.0
Δ^9—C18:1			51	49	2.5
Δ^{11}—C18:1				41	2.1
$\Delta^{9,12}$—C18:2			15		1.5
$\Delta^{6,9,12}$—C18:3			8	38	0.3
$\Delta^{6,11,14}$—C18:3				15	0.1
$\Delta^{9,12,15}$—C18:3				48	0.4
$\Delta^{6,9,12,15}$—C18:4			6		0.6
C19	2	3	5		0.1
Δ^{11}—C19:1			15		0.4
$\Delta^{8,11}$—C19:2			10	33	0.08
$\Delta^{9,12}$—C19:2				26	0.06
$\Delta^{11,14}$—C19:2				41	0.1
$\Delta^{5,8,11,14}$—C19:4			70		1.7
C20	10	9	2		0.2
Δ^{11}—C20:1			12	37	0.45
Δ^{13}—C20:1				63	0.75
$\Delta^{11,14}$—C20:2			10		1.0
$\Delta^{5,8,11,14}$—C20:4			25	91	2.3
$\Delta^{8,11,14,17}$—C20:4				9	0.2
$\Delta^{5,8,11,14,17}$—C20:5			51		5.0
C21 (total)	0.7	1			0.9
C22 (total)	6	5			5.7

likely not all of them (19,20). Klenk and Kremer (21) incubated livers of a variety of fish with acetate-C^{14} and studied its incorporation into highly unsaturated fatty acids. They concluded that there is no indication for total synthesis of fatty acids of linoleic or linolenic type (22).

We are not aware that mullet eats a diet which is unique among fish; still, the fatty acid composition is unusual. Small amounts of odd-numbered acids have been found in the fresh water algae, *Chlorella pyrenoidosa* (23), and their occurrence may be expected also in marine organisms. However, straight-chain odd-numbered acids have never been reported from potential maritime feeds at a level which would, without further assumption, account for the large amount which is found in mullet. When such fatty acids occur in a natural source, they are often associated with similarly small amounts of branched acids. In mullet, the portion of straight-chain odd-numbered acids is drastically increased, while branched acids are present only in the usual non-spectacular amount.

Alternatives to explain the origin of the odd-numbered acids are that mullet is distinct from other fish either by synthesizing such acids at an unusually high rate or by catabolizing them after ingestion at an unusually low rate. In both cases, the result would be prominent storage. The first hypothesis, i.e., total synthesis of odd-numbered acids, including polyunsaturated ones, is unlikely

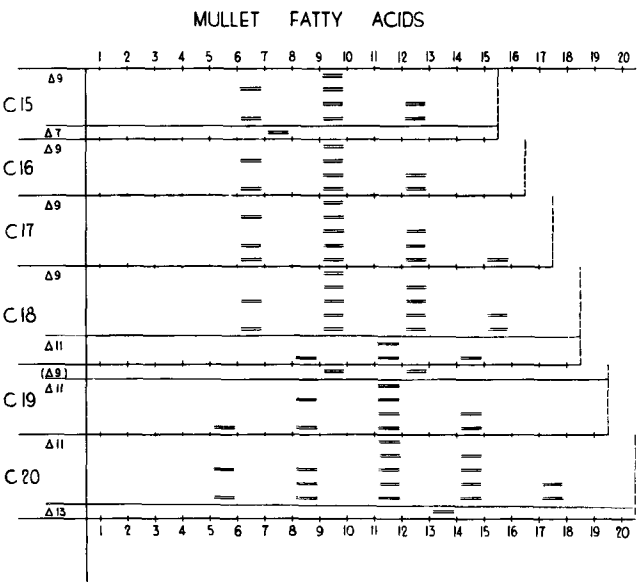

FIG. 2. The structures of unsaturated fatty acids in mullet oil.

in view of the findings on restricted synthesis of polyunsaturated acids in fish. The second hypothesis, i.e., a low catabolic rate of ingested acids, finds meager support in the fact that rats have a slower catabolic rate for odd- than for even-numbered acids (24). This may be the case also in fish, but mullet would have to be outstanding in this respect.

The structures of the unsaturated acids make apparent some principles of correlation regardless in which organism desaturation and elongation take place. The discussion of the double bond systems is facilitated by Figure 2 which presents the structures in condensed form for easy comparison. The numbering of carbon atoms in Figure 2 is according to the official nomenclature; for clarity, only the double bonds are symbolized; the chain lengths are indicated by vertical broken lines. Saturated acids are omitted and the sequence of acids within one chain length is different from that in Table V.

The pattern of double bonds shows that a direct transition from unsaturated even- to odd-numbered acids is most unlikely, since the positions of double bonds coincide in many cases when counting from the carboxyl group. The principle of methylene interruption prevails in all cases, which indicates that similar or identical enzyme systems act upon even- and odd-numbered acids.

Breusch (25) postulated carbons 9 and 10 as the preferred site of initial desaturation of a saturated fatty acid. The monoenoic C_{15} to C_{17} acids of mullet oil bear this out except for a minor amount of Δ^7—C_{15}. Most likely, the latter arises from Δ^9—C_{17} by a two-carbon degradation; similarly, the presence of Δ^{11}—C_{18}, —C_{19}, —C_{20} and Δ^{13}—C_{20} can be explained by two-carbon additions to Δ^9—C_{16}, —C_{17} and —C_{18}. Chain elongation of polyunsaturated acids is established for fish and the correlations, Δ^7—C_{16} ⟷ Δ^9—C_{18} and Δ^9—C_{16} ⟷ Δ^{11}—C_{18}, have been proven for the rat (26). It is reasonable to assume that such conversions occur also in biogenesis of the acids which are found in mullet.

Monoenoic odd-numbered fatty acids of natural sources have their double bond preferentially in position 9, and Appel et al. (27) showed that in the rat desaturation of dietary saturated odd-numbered fatty acids takes place in the same position.

All polyunsaturated acids of mullet can be derived from the monoenes, Δ^9—C_{15} to Δ^9—C_{19}, by the vinylmethane and the two-carbon pattern; the importance of the different monoenes in such scheme is discussed in the following.

Δ^9—C_{15} is of consequence only for desaturation but not for elongation. The structures of polyunsaturated C_{15} acids conform fully with those of the polyunsaturated C_{16} acids.

Δ^9—C_{16} represents the largest amount of monoenes. Elongation leads to Δ^{11}—C_{18} and some Δ^{13}—C_{20}, but the only product of desaturation combined with elongation is a small amount of $\Delta^{8,11,14}$—C_{18} acid. Klenk and Eberhagen (28) pointed out the same trend for the structures of unsaturated acids in herring oil and in plankton. Similarly, Stoffel and Ahrens (29) reported Δ^9—C_{16} acid as a major component in menhaden oil, but an acid which may derive from it by elongation and subsequent desaturation was not found. Quite generally, Δ^9—C_{16} or Δ^{11}—C_{18} is not an efficient precursor for longer chain acids with higher unsaturation.

Although not being readily elongated and then desaturated, Δ^9—C_{15} and Δ^9—C_{16} acids are amenable to immediate desaturation towards the carboxyl group as well as towards the methyl group.

Δ^9—C_{17} and Δ^9—C_{18} acids resemble each other but are in contrast to the foregoing in that they are particularly suitable for elongation and desaturation. By far the majority, in number and amounts, of the highly unsaturated acids in mullet oil can be derived from them.

Δ^9—C_{19} has not been found but a corresponding diene, $\Delta^{9,12}$—C_{19}, is present. C_{21} esters have not yet been investigated so that the importance of the C_{19} acids as precursors is uncertain. It is noted from Table V that the amounts of C_{19} and C_{21} acids are much less than the C_{20} and C_{22} acids although the presumable precursory chain lengths, C_{17} and C_{18}, are present in equal amounts. Apparently, the elongation of the odd-numbered acids does not take place as efficiently as that of the even ones.

Δ^9—C_{20} acid has not been identified nor has any polyunsaturated C_{20} acid been encountered which may be derived from it.

Klenk et al., in further investigation of polyenoic C_{16} acids, administered to fat-deficient rats $\Delta^{9,12}$—, $\Delta^{6,9,12}$— and $\Delta^{6,9,12,15}$—C_{16} acids from fish (30) and $\Delta^{4,7,10,13}$—C_{16} acid from algae (31). Only the latter acid was converted into C_{20} and C_{22} acids, and they were of the linolenic type like the precursor. The former C_{16} acids do not belong to one of the common fatty acid families. Their non-conversion can be ascribed to one or several of the following criteria: their terminal structure, their chain length, or their proximal structure.

Experiments in this laboratory showed that $\Delta^{10,13}$—C_{19} acid (synthetic) is not converted into higher unsaturated acids by the fat-deficient rat (26); the same criteria may be quoted to explain this lack of conversion.

When considering the unsaturated acids of mullet, it is seen that all groups, C_{15} to C_{18}, possess the same proximal structures but different terminal structures. On the other hand, C_{15} and C_{16}, as well as C_{17} and C_{18} acids, resemble each other in regard to potential desaturation and elongation. Therefore, it is suggestive to admit a structure which has a double bond in position 9 as one of the prerequisites for

efficient further conversions. An additional specification is that chain lengths C_{15} and C_{16} are amenable mainly to desaturation but much less to elongation, while chain lengths C_{17} and C_{18} are amenable to both processes.

In the above terms, the conversion of $\Delta^{4,7,10,13}$—C_{16} acid by the rat (31) can be explained by elongation into a C_{18} acid which has a double bond system implying the favorable position 9. When the same elongation occurs with the other C_{16} acids, their double bond system involves position 11, i.e., the first prerequisite for efficient subsequent conversions is not fulfilled. The non-conversion of $\Delta^{10,13}$—C_{19} acid is explained similarly by the unfavorable distance between double bond and the carboxyl group.

The conventional classification of fatty acid families is based on the distance between double bond system and terminal methyl group. It had been deduced from the overwhelming majority of polyunsaturated fatty acids which occur in fish or liver and brain of other higher animals (32). The classification was found to reflect interconversions of the most common even-numbered acids with 18 or more carbon atoms, and it has the advantage to afford predictions about their physiological importance for higher animals.

The classification deduced from odd- and even-numbered acids of mullet takes reference to the carboxyl group. It specifies the 9–10 position as crucial for interconversions which may involve plant or vertebrate type desaturation, and it distinguishes the chain lengths of 9-monoenes in regard to their ability to elongate in the course of further desaturation. The fatty acids of mullet fit well into such scheme and certain other data on interconversions can be explained. It is uncertain to what extent such classification will lead to positive predictions about the physiological importance of unsaturated fatty acids for higher animals. So far, there are only negative results which can be explained by reference to the carboxyl group but not by reference to the terminal group (26). The different concept might contribute to better understanding of the earlier definition of structures prerequisite for essentiality.

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• *Letters to the Editor*

Re: The Pancreatic Hydrolysis of Natural Fats. III

I HAVE RECENTLY reported (1) that during the hydrolysis of lard with pancreatic lipase, the residual triglycerides, and the diglycerides formed, become progressively more saturated as hydrolysis proceeds; the monoglyceride composition remains virtually constant throughout. I have suggested that these observations may be explained on the assumption that the fatty acid distribution in lard follows that suggested by R. J. VanderWal (2).

However, though these observations are consistent with the occurrence of the VanderWal distribution in lard, they are not demonstrative of it, since other distributions can give rise to the same results. The VanderWal distribution implies:

a) The fatty acids of the 2-positions are randomly distributed with respect to those of the 1- and 3-positions.
b) The acids of the 1-positions are randomly distributed with respect to those of the 3-positions.
c) The fatty acid composition of the acids of the 1-positions is the same as that of the acids of the 3-positions.

Now to obtain the results described above, it is only necessary for condition (a) to be fulfilled. It does not matter how the acids of the 1- and 3-positions are distributed, if thereafter those of the 2-positions are distributed randomly: the same experimental results will be obtained in all such cases.

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