Hydrogenation of a Menhaden Oil: I. Fatty Acid and C_{20} Monoethylenic Isomer Compositions as a Function of the Degree of Hydrogenation[1]

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ABSTRACT

US menhaden oil is rich in long-chain polyethylenic fatty acids, chiefly C_{20} (eicosapentaenoic) and C_{22} (docosahexaenoic) fatty acids, unlike Canadian herring oil, which is rich in long-chain (C_{20} and C_{22}) monoethylenic fatty acids. An examination of the product fatty acids from hydrogenation of menhaden oil therefore complements studies previously published for herring oil. During a commercial hydrogenation of menhaden oil, iodine value (IV) 159.0, on nickel catalyst, samples were collected at IV 150.0, 140.0, 131.5, 120.5, 96.5, 90.0 and 84.5. The fatty acid compositions were determined using a combination of mercuric adduct fractionation and gas liquid chromatographic (GLC) analyses, and the total *trans* content by infrared spectroscopy. The partial hydrogenation resulted in the disappearance of the pentaenoic and hexaenoic fatty acids, a decrease in tetraenes, and a definite increase in trienes, 8.3% at IV 84.5 compared to 4.2% at IV 159.0. The dienoic fatty acids increased to 13.2% at IV 84.5 compared to 4.1% at IV 159.0, and the monoenoic fatty acids increased to 34.2% from 24.0%. No important changes in the saturated acids were observed, 43.8% at IV 84.5 compared to 41.6% at IV 159.0. The total *trans* content varied from 3.4% at IV 150.0 to 45.1% at an IV of 84.5. The isomer compositions of the *cis* and *trans* C_{20} monoethylenic fatty acids were determined using a combination of preparative GLC, $AgNO_3$ thin layer chromatography and ozonolysis. The *cis* 20:1 acids at IV 84.5 still retained 27.5% of the major isomer (Δ11) originally present at 72%. The parent Δ5, Δ8, Δ11, Δ14 and Δ17 bonds of the 20:5 originally present could be detected in the *cis* 20:1 isomers at IV 96.5 but not at IV 84.5. At IV 84.5, 58% of the 20:1 was *trans*, the major isomer being Δ11 (9.4% of total 20.1), accompanied by important quantities of Δ10 and Δ12, respectively, 6.9% and 6.6% of the total 20:1.

INTRODUCTION

Canadian studies on the heterogeneous hydrogenation of a low iodine value (IV) marine oil (herring oil) have shown that there was little or no increase of saturated and monoethylenic fatty acids during the process (1,2). The original two major polyethylenic fatty acids in this oil of IV 119 were 20:5 and 22:6, respectively, 5.6% and 6.0% of the total fatty acids (1). They made only minor contributions of newly formed monoethylenic fatty acids to the total 20:1 and 22:1 in the course of reduction of the IV from 119 to 79, since these, respectively, totalled 13.8% and 21.1% of the original fatty acids.

In the case of high IV marine oils low in 20:1 and 22:1, such as a menhaden or anchovy oils of IV 165-185 (3), some of the 20:1 and 22:1 acids finally accumulated are clearly formed by the progressive hydrogenation of the highly unsaturated fatty acids, again mostly 20:5 and 22:6 (4). However, less data is available for our understanding of the hydrogenation process in high IV marine oils (3-7) compared to that for the hydrogenation of the low IV marine oils (1,2,8,9). To differentiate the monoethylenic isomers formed by positional and geometrical isomerization of the preexisting C_{20} and C_{22} monoenes, similar to those of herring oil (1), from those formed by partial hydrogenation of the polyenes, we have followed the hydrogenation of a high iodine value menhaden oil. This oil, of US origin, is widely used in margarines and shortenings in Europe. At some future time an application to place partially hydrogenated fish oils on the generally recognized as safe (GRAS) list will require detailed knowledge of fatty acid composition. In this and a companion study on dienes and trienes (10), we have used current "state-of-the-art" analytical technology to investigate the fatty acids of products such as may be projected as typical of those produced from menhaden oil.

MATERIAL AND METHODS

Hydrogenation of Menhaden Oil

A batch of menhaden oil (150 kg, 1978-79 Gulf of Mexico production, iodine value 159, 1.8% free fatty acid) was first degummed by washing with a 0.2% phosphoric acid solution at room temperature (11). The oil was then stirred with a 6.6% (w/w) sodium hydroxide solution at 55 C followed by overnight settling. After removal of foots, the oil was washed with water. Activated clay at 1% was mixed with the oil at 104 C for 30 min followed by filter press clarification. This refined and bleached oil was then hydrogenated with 0.2% Calsicat nickel catalyst at 175-215 C, and 138 kPa hydrogen pressure. Samples were collected at intervals during this pilot scale hydrogenation. Wijs iodine values on these were determined by AOCS Method Cd-8-25 (Fig. 1).

Gas Liquid Chromatography

All analytical gas liquid chromatography was performed on stainless-steel open-tubular columns, 47 m in length and 0.25 mm id, coated with SILAR-5CP, SILAR-7CP, or Apiezon-L and operated in a Perkin-Elmer Model 910 GLC unit fitted with a flame ionization detector (FID). Preparative GLC was executed on a stainless steel column, 2 m in

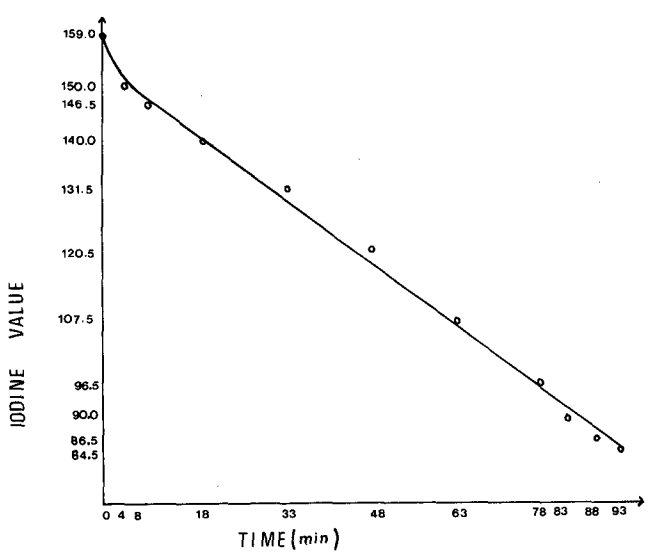

FIG. 1. Iodine values of samples collected during the hydrogenation of a refined and bleached menhaden oil (IV 159.0) over nickel catalyst (0.2%).

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length and 4 mm id, packed with 5% SE-30 on Chromosorb W (100-120 mesh). The apparatus was a Varian Autoprep with thermal conductivity detector.

Fatty Acid Composition of the Partially Hydrogenated Menhaden Oil Samples

Eight of the samples collected during the hydrogenation process (respective IV of refined starting oil 159.0, of partially hydrogenated oils 150.0, 140.0, 131.5, 120.5, 96.5, 90.0 and 84.5) were saponified (AOCS Method Ca 6b-53), the unsaponifiables removed and the recovered fatty acids converted to the methyl esters by refluxing in a solution of 7% BF_3 in MeOH for 15 min (12). The methyl esters were then converted to the corresponding methoxy-bromomercuric adducts (MBM) as described elsewhere (4). These adducts were then fractionated into groups of isomers of different degrees of unsaturation by thin layer chromatography (TLC) (11,12) using a mixture of hexane/dioxane (60:40) as solvent system (~10 mg/plate, Prekotes; Adsorbosil-5, Applied Science Laboratories). The total fatty acid quantitative analysis of each hydrogenated sample was effected by recovery of the methyl esters from the MBM adduct TLC bands by HCl, addition of 17:0 methyl ester as an internal standard, followed by GLC analysis of the different fractions on an open-tubular GLC column coated with Silar-5CP (13).

Determination of the Total *trans* Fatty Acid Content

The evolution of the total *trans* content as a function of the hydrogenation degree was followed using the official AOCS Method Cd-14-61. The results were obtained with reference to methyl elaidate.

Study of the C_{20} Monoethylenic Isomer Composition

The C_{20} acids were first isolated by preparative gas liquid chromatography (1) and then fractionated into the different fatty acid classes via their methoxy-bromomercuri adducts (4). The recovered monoethylenic fatty acid methyl esters were separated into *cis* and *trans* isomers by $AgNO_3$-TLC (14). Each fraction was then ozonized in BF_3/MeOH and the resulting monoesters and diesters analyzed on a BDS column (15,16).

trans Content of the C_{20} Monoethylenic Fatty Acids

The *trans* content of the C_{20} monoethylenic fatty acids was determined by the $AgNO_3$-TLC/FID method elaborated elsewhere (17,18). Clean Chromarods were immersed in a 2.5% solution of silver nitrate in acetonitrile for 15 min and then activated at 120 C for 3 hr. The rods were spotted and developed for 25 min in a 1:1 mixture of hexane/benzene. The rods were then scanned by a flame ionization detector in an Iatroscan TH-10 (19,20). After use, the rods were cleaned by soaking overnight in concentrated nitric acid, rinsing with water and then acetone, and passage through the flame.

RESULTS AND DISCUSSION

Usually, menhaden oils with iodine values ranging from 150 to 180 contain a large quantity of polyunsaturated fatty acids, dependent on the area where the fish were caught (3,21). The two major polyunsaturated fatty acids (21,22) are 20:5Δ5,8,11,14,17 and 22:6Δ4,7,10,13,16,19, ca. 13% and 6% of the total fatty acids in the menhaden oil at our disposal. The proportion of 20:5 in menhaden oil is 3-4 times that of herring oil, and we have focused our study on the C_{20} acids as it seemed easier to follow the large contribution of the preexisting polyethylenic fatty acid into the isomer content of the monoenes formed after partial hydrogenation for C_{20} than for C_{22}.

Transformation of Fatty Acids During Hydrogenation

As observed in the hydrogenation of herring oil (1), there were differences between the Wijs IV of the partially hydrogenated samples (Fig. 1) and those calculated from the GLC runs of total methyl esters (Table I) by a computer method (13). These differences could be due to three factors: the precision in the identification of peaks for measurements; the formation of polymers during hydrogenation, as these components would not appear in GLC analyses, or the choice of correction factors for the GLC analysis of the newly formed fatty acids (13). Studies in progress (J.-L. Sebedio, T. F. Farquharson and R. G. Ackman, unpublished results) have shown that the amount of polymers formed in these hydrogenation conditions is less than 0.5% of the total lipid classes, which consist of ca. 98% of triglyceride, the balance being a mixture of cholesterol, diglycerides, free fatty acids, and traces of hydrocarbons, vitamins, etc. This small amount of polymers is confirmed by calculating the chain length totals before and after total hydrogenation (Table II). A very good agreement was obtained for the important chain lengths (C_{16}, C_{18}, C_{20} and C_{22}) between the refined oil and the seven partially hydrogenated samples. It is therefore very likely that these differences in the iodine values are due in part to the underestimation of GLC peak areas, but principally arise from the choice of number of ethylenic bonds in broad peaks for fatty acids measured by GLC. One of the effects of partial hydrogenation is to transform the natural methylene-interrupted fatty acids of *cis* geometry into a variety of nonmethylene-interrupted acids containing *trans* ethylenic bonds. However, due to the complexity of the GLC analyses of the partially hydrogenated oils (Fig. 2), it was initially impossible to identify all the fatty acids formed in the mixture of total oil methyl esters on either polar or nonpolar columns. The experimental and calculated equivalent chain lengths (23,24) predict limits within which groups of certain structures occur, but there are overlaps, e.g., between esters of fatty acids with two and three ethylenic bonds, even in the case of all-*cis* polyethylenic acid isomers (4).

In anticipation of this problem of overlap, the TLC separation of the MBM adducts into fatty acid classes (monoene, diene, triene, etc.) was applied. Once each class was isolated, the correct iodine value could be applied to the mass indicated by GLC.

Although comprehensive in scope, this approach left a deficiency of ca. 10 IV units (Table I). In the raw oils, or those only mildly reduced, the deficiency could be described to the sum of numerous small polyunsaturated fatty acids not specifically identified. In the more heavily reduced oils (e.g., IV 100) the IV deficiency was the same, although most polyethylenic acids with five and six bonds were gone. We believe that this deficiency is due to further analytical corrections required by fatty acids including *trans* ethylenic bonds. This has been demonstrated experimentally (17). As yet, we have not examined this question in depth as the correction factor is dependent on the number of ethylenic bonds, the number of carbon atoms and also on the geometry of the unsaturation (17,25,26).

Only a slight increase in total saturated acids was observed (41.6% at IV 159 compared to 43.8% at IV 84.5). The major effects of hydrogenation to IV 84.5 were a large increase in total dienes (3.9% at IV 159 compared to 13.2% at IV 84.5) and in total monoenes (24% at IV 159 compared to 34.2% at IV 84.5). A large increase was observed for the eicosenoic acids (1.3% at IV 159, 4.9% at IV 84.5). As found in the catalytic hydrogenations of herring oil (1),

TABLE I

Total Fatty Acid Methyl Esters (wt %) of a Refined and a Sequence of Partially Hydrogenated Menhaden Oil Samples

Menhaden oil	Refined	Partially hydrogenated						
iodine value (Wijs)	159.0	150.0	140.0	131.5	120.5	96.5	90.0	84.5
Fatty acids								
14:0	10.8	9.9	10.2	10.8	11.3	10.2	10.4	10.5
15:0	0.6	0.8	0.8	0.8	0.9	0.8	0.7	0.8
16:0	23.2	23.0	23.6	23.4	23.8	23.2	24.0	24.1
17:0	0.6	0.7	0.8	0.8	0.8	0.6	0.7	0.5
18:0	4.2	4.5	4.4	4.3	4.4	4.7	4.9	5.2
20:0	0.4	0.4	0.4	0.5	0.5	0.5	0.6	0.7
22:0	<0.1	<0.1	0.1	0.2	0.2	0.2	0.2	0.3
Minor[a]	1.8	1.4	1.4	1.3	1.2	1.6	1.7	1.7
Σ Saturate	41.6	40.7	41.7	42.1	43.1	41.8	43.2	43.8
16:1	11.4	12.0	12.5	12.9	13.7	15.5	15.0	15.0
18:1	10.6	11.0	11.2	10.8	11.9	13.4	13.0	12.5
20:1	1.3	1.5	1.7	2.1	2.6	3.6	4.0	4.9
22:1	0.2	0.2	0.3	0.4	0.7	1.1	1.0	1.7
Minor[b]	0.5	0.4	0.2	0.1	0.1	0.1	0.1	0.1
Σ Monoene	24.0	25.1	25.9	26.3	29.0	33.7	33.1	34.2
16:2	1.5	1.5	1.7	2.3	1.9	1.5	1.0	0.9
18:2	1.8	2.0	2.3	2.8	1.6	2.0	1.7	2.4
20:2	0.6	0.7	1.0	1.9	2.9	4.7	5.5	6.6
22:2	–	<0.1	0.1	0.7	1.6	2.4	2.3	3.3
Σ Diene	3.9	4.2	5.1	7.7	8.0	10.5	10.5	13.2
16:3	2.2	2.1	1.9	2.2	1.6	0.3	0.1	–
18:3	1.7	1.9	1.7	2.1	0.9	0.4	0.4	0.2
20:3	0.3	0.6	1.7	3.0	4.1	5.4	5.4	3.8
22:3	–	0.2	0.8	1.6	2.1	4.6	5.0	4.3
Σ Triene	4.2	4.8	6.1	8.9	8.7	10.7	10.9	8.3
16:4	1.0	0.3	0.1	–	–	–	–	–
18:4	2.1	1.8	1.2	0.5	0.3	–	–	–
20:4	2.3	2.9	3.5	4.0	3.7	1.3	0.8	0.1
22:4	0.2	0.4	0.9	2.0	2.0	1.7	1.3	0.3
Σ Tetraene	5.6	5.4	5.7	6.5	6.0	3.0	2.1	0.4
20:5	11.9	10.5	7.9	3.6	1.9	–	–	–
21:5	0.5	0.5	0.3	0.1	–	–	–	–
22:5 + 22:6	8.8	8.8	7.1	5.1	3.0	0.1	trace	trace
Σ Pentaene + hexaene	21.2	19.8	15.3	8.8	4.9	0.1	trace	trace
Σ Conjugated acids[c]	0.7	1.2	1.5	1.3	1.1	0.7	–	0.5
IV Calculated	148	142	130	115	102	84	80	79

Analyses reconstituted from MBM adduct fractions with GLC on SILAR-5CP.

[a]Includes 12:0, 19:0, iso 14:0, iso and anteiso 15:0, iso 16:0, iso and anteiso 17:0, iso 18:0.

[b]Includes 7 Me-16:1, 17:1, 19:1 and 24:1.

[c]Determined by AOCS Method Cd 7-58.

the details of the fatty acid compositions (Table I) show a preferential absorption of the polyunsaturated fatty acids on the catalyst surface with a rapid decrease in pentaenes and hexaenes in the early stages of the reaction. This monopolization of the catalyst surface is dependent on the hydrogenation coverage (27,28). A low hydrogen coverage (low H_2 pressure, high catalyst concentration, high temperature and low rate of stirring) would favor the preferential absorption of these polyunsaturated fatty acids. However, these decreases did not correspond to an increase in C_{20} tetraene until the IV decreased to 131.5. Instead, there was an increase in C_{20} trienes and dienes. This phenomenon is due to the rapid reduction of some of the bonds in adsorbed molecules of the 20:5Δ5,8,11,14,17 in the starting oil. The conjugated acids first increased and then decreased with a maximum of 1.5% at IV 140.0 which could indicate that this hydrogenation reaction can proceed via conjugated intermediates as is usually accepted (23,29,30).

However, the low amount of conjugated material found in the partially hydrogenated samples shows that the conjugated isomer probably reacts and hydrogenates more rapidly than the unconjugated acids. Therefore, these compounds are not desorbed from the catalyst surface to accumulate in the oil. The conjugated intermediates are reported to desorb only if the hydrogen pressure is very low (31).

TABLE II

Even Chain Lengths (C_{16-22}) for Fatty Acid Methyl Esters in Refined and Partially Hydrogenated Menhaden Oils

Chain lengths (wt %)	Refined IV 159.0	Partially hydrogenated IV 150.0	IV 140.0	IV 131.5	IV 120.5	IV 96.5	IV 90.0	IV 84.5
Σ C_{16}	39.3	38.9	39.8	40.8	41.0	40.5	40.1	40.0
	38.9	**40.8**	**41.2**	**40.1**	**39.7**	**38.3**	**40.0**	**40.7**
Σ C_{18}	20.4	21.2	20.8	20.5	19.1	20.5	20.0	20.3
	19.9	**20.5**	**20.6**	**20.1**	**20.1**	**20.6**	**20.1**	**19.7**
Σ C_{20}	16.8	16.6	16.2	15.1	15.7	15.5	16.3	16.1
	16.1	**15.1**	**14.7**	**15.4**	**15.2**	**16.1**	**15.5**	**15.2**
Σ C_{22}	9.2	9.6	9.3	10.0	9.6	10.1	9.8	9.9
	10.6	**9.5**	**8.8**	**9.9**	**9.9**	**10.5**	**10.0**	**9.4**

Data in medium type is summed from total analysis of Table II and that in bold type is from analysis of fatty acid methyl esters completely hydrogenated on PtO_2 in the laboratory.

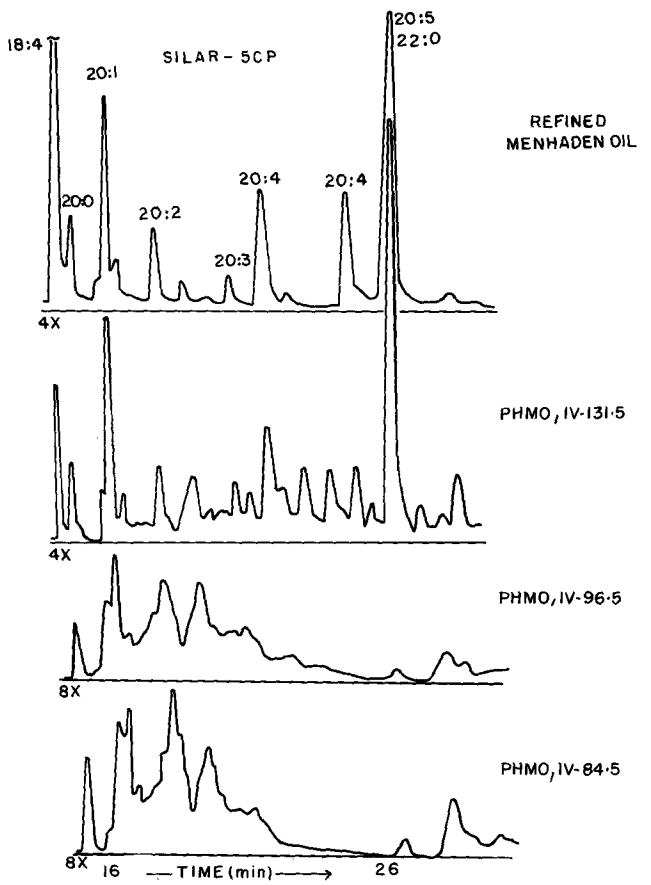

FIG. 2. Comparison of parts of gas liquid chromatographic charts for fatty acid methyl esters from refined and bleached menhaden oil (IV 159.0) and from three partially hydrogenated menhaden oils of Wijs iodine values of 131.5, 96.5 and 84.5. Column, open-tubular, 47 m × 0.25 mm id, 175 C, SILAR-5CP.

trans Acid Content

The *trans* contents of the original oil and of seven partially hydrogenated samples are given in Table III. As the IV decreased, the *trans* content increased (3.4% at IV 150.0 compared to 45.1% at IV 84.5). The isomerization index (32), described as the percentage of *trans* ethylenic bonds per unit decrease of IV, increased progressively (Table III) to reach a stable value of 65 between an IV of 131.5 and an IV of 120.5 (between 33 and 48 min of hydrogenation). These results indicate that at the beginning of the hydrogenation process there is a preferential addition of hydrogen to the ethylenic bonds in 20:5, 22:5 and 22:6, and a limited isomerization of individual ethylenic bonds. The isomerization process is therefore only of importance when 70% of the polyunsaturated fatty acid is transformed to less unsaturated analogues (Table I).

TABLE III

Total *trans* Content of Selected Partially Hydrogenated Menhaden Oils (Determined by AOCS Infrared Spectroscopy Method Cd-14-61), and the Isomerization Index Derived from this Data

Time (min)	Wijs iodine value	% *trans*	Isomerization index
0	159.0	ND[a]	
4	150.0	3.4	38
18	140.0	9.3	49
33	131.5	15.9	58
48	120.5	24.9	65
63	107.5	33.4	65
78	96.5	40.4	65
93	84.5	45.1	61

[a]ND: Not detected under analytical conditions.

Distribution of Isomers of the Eicosenoic Acids

As mentioned above, the hydrogenation process with nickel catalyst resulted in a large increase of the C_{20} monoethylenic fatty acids (from 1.3% at IV 159 to 2.1% at IV 131.5, 3.6% at IV 96.5 and 4.9% at IV 84.5). The amount of 20.0 increased only slightly (from 0.4% at IV 159 to 0.5% at IV 96.5 and 0.7% at IV 84.5). The *trans* 20.1 content (Table IV) increased rapidly as the IV decreased (21% at IV 131.5 compared to 58% at IV 84.5). The increases in the total 20:1 isomers (Table I) were 0.8% from IV 159.0 to IV 131.5, 1.5% from IV 131.5 to IV 96.5 and 1.3% from IV 96.5 to IV 84.5. The increase of *trans* 20:1 (as percentage of the total fatty acids) calculated from Tables I and IV were, respectively, 0.4%, 1.2% and 1.2% for the same intervals of iodine values. These results indicate that, from IV 159.0 to IV 131.5, the total 20:1 acids include new *trans* isomers formed by isomerization of the original 20:1, and also some new *cis* positional isomers formed during the progressive transformation of the preexisting all-*cis* 20:5Δ5, 8,11,14,17. From the modest increase of *trans* 20:1 (0.4% of the total fatty acids), compared to the increase of the total 20:1 acids (0.8%, one can also deduce a significant contribution from de novo *trans* isomers formed during the stepwise transformation of the original 20:5 into tetraenes, trienes, dienes and finally monoenes. From IV 131.5 to IV 96.5 and from IV 96.5 to IV 84.5, the similar increases in total 20:1 (respectively, 1.5 and 1.3%) and in *trans* 20:1 (respectively, 1.2 and 1.2%) seem to indicate that most of

TABLE IV

Total Percentage and Distribution of *trans* 20:1 Isomers in Mole % of Total 20:1 for Refined and Partially Hydrogenated Menhaden Oil Samples

Ethylenic position Δ	ω	Partially hydrogenated IV 131.5	IV 96.5	IV 84.5
Σ%	*trans*	21.0	45.0	58.0
3	17	0.9	0.5	trace
4	16	2.5	2.7	0.9
5	15	1.2	0.6	1.0
6	14	2.6	6.8	2.9
7	13	0.8	1.0	2.7
8	12	1.8	3.9	3.6
9	11	2.0	5.5	5.4
10	10	1.6	4.2	6.9
11	9	2.1	5.5	9.4
12	8	1.5	3.9	6.6
13	7	1.5	3.4	5.6
14	6	0.7	2.6	4.4
15	5	0.8	2.3	4.0
16	4	0.6	1.3	2.9
17	3	0.4	0.8	1.7

TABLE V

Total Percentage and Distribution of *cis* 20:1 Isomers in Mole % of Total 20:1 for Refined and Partially Hydrogenated Menhaden Oil Samples

Ethylenic position Δ	ω	Refined IV 159.0	Partially hydrogenated IV 131.5	IV 96.5	IV 84.5
Σ%	*cis*	100.0	79.0	55.0	42.0
3	17	trace	trace	0.1	trace
4	16	0.5	6.2	0.4	0.3
5	15	0.5	0.5	0.9	0.3
6	14	2.5	3.6	0.8	1.4
7	13	0.6	0.5	0.2	0.8
8	12	1.8	1.6	2.1	0.7
9	11	2.3	1.8	2.4	1.0
10	10	1.8	1.5	2.4	1.7
11	9	72.7	50.1	29.6	27.5
12	8	0.6	1.0	2.3	1.6
13	7	15.1	10.2	7.6	2.7
14	6	0.6	1.0	3.0	1.7
15	5	0.9	0.8	1.1	0.7
16	4	0.1	0.2	0.6	0.6
17	3	ND[a]	ND	1.5	1.0

[a]ND: Not detected under analytical conditions.

the additional 20:1 isomers were formed continuously and only from the more numerous highly modified dienes, trienes and tetraenes which are (Fig. 2) successors of the all-*cis*-20:5Δ5,8,11,14,17, but now include a high proportion of *trans* ethylenic bonds.

The original oil (IV 159, Table V) had several minor *cis* 20:1 isomers with ethylenic bonds in odd-number positions such as Δ13 (15.1%) accompanying the major Δ11 isomer, and some unexpected isomers of quantitative significance in even-numbered positions (e.g., Δ6, Δ8 and Δ10, respectively, at 2.5%, 1.8% and 1.8% of the total 20:1 isomers). The proportions of isomers among the *cis* acids (Table V) in the three partially hydrogenated samples showed a strong retention of the original isomer mixture. However, the *trans* acids in the hydrogenated samples showed a different trend (Table V), with a random variety of isomers even at IV 131.5 and IV 96.5. At these moderate iodine values, a large quantity of isomers with ethylenic bonds closer to the carbonyl group than the original major Δ11 isomer was observed (Δ4, Δ6, Δ8 and Δ9), Δ6 being the larger isomer (at IV 96.5, 6.8% *trans* Δ6 compared to 5.5% *trans* Δ11). As the iodine value decreased from 96.5 to 84.5, the relative proportion of these *trans* isomers with low numbers decreased (at IV 84.5, 2.9% *trans* Δ6 compared to 9.4% *trans* Δ11).

From iodine values 159.0 to 96.5, more *trans* isomers with ethylenic bonds in Δ3 to Δ10 than in Δ12 to Δ17 were observed. The opposite phenomenon was detected at an IV of 84.5 (23.4% of Δ3 to Δ10 compared to 25.2% of Δ12 to Δ17). This change in the isomer distribution corresponds to an increase in the contribution of the polyethylenic fatty acids to the total of 20:1 isomers. This could either reflect a migration of the ethylenic bonds towards the methyl group or more probably a preferential hydrogenation of some ethylenic bonds with high Δ numbers present in the original highly unsaturated acids at the beginning of the process. As the reaction proceeds, the reduction of the ethylenic bonds closer to the carboxyl group would then occur. In this event, the isomers with residual *trans* ethylenic bonds close to the carboxyl group (low Δ numbers) should also be found in acids with higher degrees of unsaturation, such as in the 20:2 or 20:3 fractions. It is generally believed that the position of the ethylenic bond on the carbon chain has an influence on its reactivity towards hydrogenation (33). For methyl esters, the rate of hydrogenation usually increases as the ethylenic bonds move away from the carboxyl group. However, it has also been shown that in processes with a high degree of isomerization, the ethylenic bonds very close to the carboxyl group hydrogenate faster than others near the methyl end of the molecule (34).

These *cis* and *trans* isomer distributions were different from those determined for the hydrogenation of a herring oil (1). In the case of herring oil, the hydrogenation process was characterized largely by a geometrical and positional isomerization of the 20:1 acids. In this process, the three major $C_{20:1}$ *trans* fatty acids formed were *trans* Δ11, *trans* Δ10 and *trans* Δ12 (77% of *trans* 20:1). In the case of the hydrogenation of a menhaden oil, where a large proportion of the new isomers was formed from the 20:5 polyethylenic acid, a wide range of *trans* isomers was formed. In this process, a high proportion of novel *trans* 20:1 isomers with ethylenic bonds in Δ6 to Δ16 positions were observed. In the initial oil, the major polyunsaturated fatty acid is the all-*cis* 20:5 with ethylenic bonds in the Δ5,8,11,14 and 17 positions. As the hydrogenation proceeds, the original *cis* ethylenic bonds of this acid are detectable at IV 96.5 (i.e., Δ5, Δ8, Δ14, Δ17) but not at IV 84.5. However, the *trans* 20:1 isomer composition (Table IV) suggests a more extensive positional isomerization during the early stages of hydrogenation of the 20:5Δ5,8,11,14,17 to give the novel 20:1 isomers. No major difference was observed for the final pattern of *cis* 20:1 isomers between the results for partially hydrogenated herring oil (1) and the menhaden oil sample of corresponding IV in this study. However, the proportion of Δ11 was less important in the menhaden oils than in the herring oils. European practice in hydrogenation differs from that in Canada (4), and a partial analysis of 20:1 isomers in oil from capelin *(Mallotus villosus)* hydrogenated in Europe (35), shows that more extensive production of *trans* 20:1 (75%) not only produces randomization of *trans* isomers, but also of *cis* 20:1 isomers. The *cis* Δ11 20:1 is still the dominant isomer, but *cis* Δ12 20:1 is half again as much (compare with Table V).

The major difference between the hydrogenation of a low and a high iodine value marine oil is the greater extent of the geometrical and positional isomerizations which occur during the progressive hydrogenation of the highly unsaturated fatty acids present in high IV marine oils. It is therefore of interest also to study the isomer composition of the more unsaturated fractions (diene and triene) in order to characterize the isomerization reactions which

occur during the hydrogenation of the highly unsaturated fatty acids. These details will be found in another paper (10).

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REFERENCES

1. Sebedio, J-L., M.F. Langman, C.A. Eaton and R.G. Ackman, JAOCS 58:41 (1981).
2. Ackman, R.G., S.N. Hooper and J. Hingley, JAOCS 48:804 (1971).
3. Ackman, R.G. in Nutritional Evaluation of Long-Chain Fatty Acis in Fish Oil, edited by S.M. Barlow and M.E. Stansby, Academic Press Inc. (London) Ltd., 1982, pp. 25-88.
4. Sebedio, J-L., and R.G. Ackman, Lipids 16:461 (1981).
5. Ackman, R.G., in Fishery Products, edited by R. Kreuzer, FAO, 1974, pp. 112-131.
6. Kudrawcew, W., and H. Niewiadomski, Acta Aliment. Poloni. 2:53 (1976).
7. Zschau, W., Fette Seifen Anstrichm. 83:527 (1981).
8. Conacher, H.B.S., B.D. Page and R.K. Chadha, JAOCS 49:520 (1972).
9. Holmer, G., and E. Aaes-Jorgensen, Lipids 4:507 (1969).
10. Sebedio, J-L., and R.G. Ackman JAOCS 60:1992 (1983).
11. Teasdale, B.F, in Oilseed and Pulse Crops in Western Canada, edited by J.T. Harapiak, Western Co-Operative Fertilizers, 1975, pp. 551-85.
12. Morrison, W.R., and L.M. Smith, J. Lipid Res. 5:600 (1964).
13. Ackman, R.G., and C.A. Eaton, Fette Seifen. Anstrichm. 80:21 (1978).
14. Gunstone, F., I.A. Ismail and M. Lie Ken Jie, Chem. Phys. Lipids 1:376 (1967).
15. Ackman, R.G., Lipids 12:293 (1977).
16. Sebedio, J-L., and R.G. Ackman, Can. J. Chem. 56:2480 (1978).
17. Sebedio, J-L., and R.G. Ackman, J. Chromatogr. Sci. 19:552 (1981).
18. Ackman, R.G., Chem. Ind. Oct. 19, 715 (1981).
19. Sipos, J.C., and R.G. Ackman, J. Chromatogr. Sci. 16:443 (1978).
20. Ackman, R.G., in Methods in Enzymology, Vol. 72, edited by J.M. Lowenstein, Academic Press, New York, 1981, pp. 205-253.
21. Ackman, R.G., in Advances in Fish Science and Technology, Jubilee Conference of the Torry Station, Aberdeen, Scotland, 1979, edited by J.J. Connell, Fishing News Books, England, 1980, pp. 86-98.
22. Stansby, M.E., JAOCS 56:793A (1979).
23. Ackman, R.G., A. Manzer and J. Joseph, Chromatographia 7:107 (1974).
24. Sebedio, J-L., and R.G. Ackman, J. Chromatogr. Sci. 20:231 (1982).
25. Sheppard, A.J., S.A. Meeks and L.W. Elliott, J. Gas Chromatogr. 6:28 (1968).
26. Sheppard, A.J., A.R. Prosser and L.W. Elliott, Ibid. 6:34 (1968).
27. Coenen, J.W.E., JAOCS 53:382 (1976).
28. Coenen, J.W.E., and H. Boerma, Fette Seifen Anstrichm. 70:8 (1968).
29. Allen, R.R., JAOCS 58:166 (1981).
30. Schofield, C.R., JAOCS 49:583 (1972).
31. Coenen, J.W.E., Riv. Ital. Sostanze Grasse LVIII:445 (1981).
32. El-Shattory, Y., L. deMan and J.M. deMan, J. Food Technol. 18:519 (1981).
33. Dutton, H.J., in Progress in the Chemistry of Fats and Other Lipids, Vol. 9, edited by R.T. Holman, Pergamon Press, New York, 1969, pp. 351-375.
34. Allen, R.R., JAOCS 41:521 (1964).
35. Svensson, L., L. Sisfontes, G. Nyborg and R. Blomstrand, Lipids 17:50 (1982).

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