except that the color indicator crystal violet was employed to determine the end point (4). The percentage of cyclopropenes, calculated as malvalic acid, found in triplicate determinations by each method, were as follows:

Potentiometric titration	Titration to crystal violet end point
0.686%	0.688%
0.696%	0.692%
0.682%	0.684%
Average, 0.691%	Average, 0.688%

Samples of methyl esters derived from refined cottonseed oil and from refined peanut oil were further purified by passage through alumina. The two methyl esters and two mixtures of the methyl esters were analyzed for cyclopropene content. Three of the titration curves obtained are shown in Figure 4, and the amounts of cyclopropenes are recorded in Table I. The titrant required was directly proportional to the amount of methyl ester from the cottonseed oil (Fig. 5).

ACKNOWLEDGMENTS

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REFERENCES

1. Harris, J.A., F.C. Magne and E.L. Skau, JAOCS 40:718 (1963).
2. Harris, J.A., F.C. Magne and E.L. Skau, Ibid. 41:309 (1964).
3. Magne, F.C., J.A. Harris, R.A. Pittman and E.L. Skau, Ibid. 43:519 (1966).
4. Feuge, R.O., Z. Zarins, J.L. White and R.L. Holmes, Ibid. 44:548 (1967).
5. Brown, L.E., Ibid. 46:654 (1969).
6. Feuge, R.O., Z. Zarins, J.L. White and R.L. Holmes, Ibid. 46:185 (1969).
7. Rosie, D.A., and G.G. Shone, Analyst (London) 95:477 (1969).

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Alteration of Long Chain Fatty Acids of Herring Oil during Hydrogenation on Nickel Catalyst[1]

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ABSTRACT

During hydrogenation of a refined herring *(Clupea harengus)* oil iodine value (IV) 119, on a commercial nickel catalyst, samples were collected at IV 108, 101, 88 and 79. In the early stages of the process, IV 119 to IV 101, the positional and geometrical isomerization of the long chain monoenoic fatty acids (20:1 and 22:1) was hindered by the stronger absorption on the catalyst surface of the polyenes with 4, 5 and 6 double bonds. Consequently at IV 101, 70% of these polyenes had been converted to dienoic and trienoic fatty acids, but only 3-4% *trans* 20:1 and 22:1 accumulated. As the hydrogenation proceeded, IV 101 to IV 79, the original *cis* 20:1 and 22:1 isomers (mainly Δ11 with some Δ9 and Δ13) decreased and new positional and geometrical isomers (both *cis* and *trans* in positions Δ6 to Δ15) were formed. The major *trans* isomers were Δ11 accompanied by important proportions of Δ10 and Δ12. At IV 79, more *trans* 20:1 (ca. 36%) than *trans* 22:1 (ca. 29%) was detected. Monoethylenic fatty acids newly formed from polyethylenic fatty acids made only minor contributions to the total 20:1 and 22:1 at these levels of hydrogenation, but a "memory effect" which skews the proportions of minor *cis* and *trans* isomers can be attributed to the proportions of minor *cis* 22:1 isomers (Δ9, Δ13 and Δ15) orginally present.

INTRODUCTION

Vegetable oils dominate world commerce (1) and their hydrogenation has received much attention (2-8). Much less data is available on the subject of fish oil hydrogenations (9-13). The main difference between unprocessed vegetable and marine oils serving as raw materials is the higher degree of unsaturation in some of the fatty acids of the latter. In the marine oils a further difference lies in the position of the ethylenic unsaturation in the eicosenoic (20:1) and docosenoic (22:1) acids relative to the position of bonds in the corresponding polyethylenic fatty acids. The polyethylenic acids of vegetable oils, mainly 18:2 with some 18:3, have bonds in Δ9, Δ12 and Δ15 positions, the former corresponding to the position of the principal octadecenoic acid isomer (18:1Δ9). Similarly in marine oils, the polyethylenic acid 20:5 has bonds in the Δ5, Δ8, Δ11, Δ14 and Δ17 positions, the Δ11 corresponding to the dominant eicosenoic isomer (20:1Δ11). However, in the important 22:6 acid, the positions of the ethylenic unsaturation are Δ4, Δ7, Δ10, Δ13, Δ16 and Δ19, none of which correspond to an ethylenic bond position in the dominant docosenoic isomer (22:1Δ11). Comparisons among products relating to this positional difference could be informative as to the mechanisms of hydrogenation.

Discrepancies exist among the publications of Ackman et al. (9); Conacher et al. (10) and Lund and Hølmer (12) as to the positional distributions of ethylenic bonds in the *cis* and *trans* 20:1 and 22:1 fatty acids of partially hydrogenated marine oils. Accordingly, we have reinvestigated the formation and behavior of these long chain monoethylenic fatty acids during the hydrogenation process. The high content of 20:1 and 22:1 in Canadian herring oil (14), hydrogenated according to commercial practice (practically no change in saturates and monoenes content), facilitates comparison of the original monoethylenic fatty acids with those produced by isomerization only, and with those produced from reduction of the more highly unsaturated fatty acids.

MATERIALS AND METHODS

Samples of herring oil were collected during a pilot scale hydrogenation executed according to Canadian commercial practice with nickel catalyst (0.2%), at 190-225 C, and

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about 70 kPa hydrogen pressure. The Wijs iodine values (AOCS method Cd-8-25) were: refined starting material 119, processed 107, 101, 88 and 79.

Each sample was saponified (AOCS method Ca-6b-53), the unsaponifiables removed, and the recovered fatty acids converted to methyl esters by refluxing in a solution of 7% BF_3 in MeOH for 15 minutes. A nitrogen atmosphere was maintained at all times.

Gas Liquid Chromatography

All analytical gas liquid chromatography (GLC) 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 900 series apparatus with flame ionization detector. Preparative GLC was performed on a column of stainless steel, 2 m in length and 4 mm id, packed with either 5% SE-30 on Chromasorb W (100-120 mesh), or 15% diethyleneglycol succinate (DEGS) on the same support. The apparatus was a modified Varian Autoprep with thermal conductivity detector, and manual collection was in glass tubes containing 20 ml methycyclohexane.

Determination of Geometrical and Positional Monoethylenic Isomers

The eicosenoic (20:1) and docosenoic (22:1) acid methyl esters were isolated by preparative GLC. The 20:1 and 22:1 esters were each separated by thin layer chromatography (TLC) on Adsorbosil-5 silicic acid plates (Applied Science Laboratories) dipped in a solution of 10% $AgNO_3$ in acetonitrile, into *trans* (R_f 0.65) and *cis* (R_f 0.58) bands with benzene:hexane::2:1 and visualized, after spraying with 2,7-dichlorofluorescein, under ultraviolet light. The purity of these geometrical fractions was monitored on open-tubular columns coated with SILAR-7CP (15), with recovery and quantitation based on the addition of 18:0 as

TABLE I

Total Fatty Acid Methyl Esters (wt%) of a Refined and a Sequence of Partially Hydrogenated Herring Oil Samples (SILAR-5CP)

Herring oil sample iodine value (Wijs)	Refined	Partially hydrogenated			
	119	107	101	88	79
Fatty acid					
14:0	7.4	7.8	7.2	6.1	6.7
16:0	15.4	14.0	15.3	15.0	13.9
18:0	0.9	1.1	1.2	1.5	1.9
20:0	0.1	0.2	0.3	0.4	0.8
22:0	tr	tr	0.1	0.2	1.2
Others	2.8	2.9	2.6	2.5	2.1
ΣSaturated	26.6	26.0	26.7	25.7	26.6
16:1	7.5	7.0	6.8	7.8	6.5
18:1	12.3	13.0	14.6	14.2	14.6
20:1	13.8	14.2	14.6	15.3	15.0
22:1	21.1	21.6	23.1	23.8	22.8
Others	1.0	0.7	1.0	1.0	1.0
ΣMonoethylenic	55.7	56.4	60.1	62.1	59.9
16:2Δ8,11	0.3	0.3	0.3	0.5	0.4
18:2Δ9,12	2.3	2.1	1.4	–	–
C_{16}NMID[a]	–	–	–	0.2	0.2
C_{18}NMID	–	–	0.7	3.1	4.1
C_{20}NMID	0.2[b]	0.6[b]	1.0	3.3	3.9
C_{22}NMID	–	0.3	0.9	1.2	2.9
ΣDiethylenic	2.8	3.3	4.3	8.3	11.5
16:3Δ6,9,12 +Δ,7,10,13	0.3	0.3	0.3	–	–
18:3Δ6,9,12 Δ9,12,15 Δ8,11,14	1.4	1.3	–	–	–
C_{18}NMIT[c]	–	–	0.7	0.6	0.8
C_{20}NMIT	0.1[d]	0.1[d]	2.2	2.0	0.6
C_{22}NMIT	–	–	1.2	1.5	1.3
ΣTriethylenic	1.8	1.7	4.4	4.1	2.7
16:4Δ9,7,10,13 +Δ6,9,12,15	0.3	0.2	–	–	–
18:4Δ6,9,12,15 Δ8,11,14,17	2.5	1.9	0.8	0.1	–
20:4Δ5,8,11,14 Δ8,11,14,17	0.5	0.4	0.5	–	–
ΣTetraethylenic	3.3	2.5	1.3	0.1	–
20:5Δ5,8,11,14,17	5.6	4.9	1.7	0.1	–
21:5Δ6,9,12,15,18	0.1	0.1	–	–	–
22:5Δ7,10,13,16,19	0.3	0.3	0.2	–	–
ΣPentaethylenic	6.0	5.3	1.9	0.1	–
22:6Δ4,7,10,13,16,19	4.3	4.4	1.4	0.1	–
IV (Calculated from GLC)	108	105	86	75	74

[a]NMID = non-methylene-interrupted diethylenic.
[b]Total includes (0.2%) unaltered 20:2Δ11,14.
[c]NMIT = non-methylene-interrupted triethylenic.
[d]unaltered 20:3Δ11,14,17.

an internal standard. Further *cis/trans* ratios were compared on Apiezon-L (16).

The ozonolysis of the four fractions (*cis* and *trans* 20:1, *cis* and *trans* 22:1), followed the original BF_3-MeOH method (17), improved by the use of $CHCl_3$ as the product extractant (18). The relative proportions of mono- and dicarboxylic products were determined on SILAR-5CP at 130 C and 180 C, respectively.

RESULTS AND DISCUSSION

Transformation of Fatty Acids during Hydrogenation

The hydrogenation of vegetable or marine oils for use in the production of margarine or shortening is one of the most important technical processes in the fats and oils industry. The process has two objectives, the production of products resistant to oxidation and the production of products possessing desirable physical properties such as plasticity and melting point (19).

In the case of marine oils, the stability of these products is effected by elimination of the oxidation-susceptible all-*cis* methylene-interrupted structures of the polyethylenic fatty acids, especially of those with 4, 5 and 6 bonds in the C_{20} and C_{22} chain lengths, which together constitute at least 20% of the total fatty acids. The required physical properties of the product are attained by the choice of the catalyst, and selection of suitable operating conditions from hydrogen pressure, temperature, rate of agitation and concentration of the catalyst (20-23). A slight increase in the proportion of saturated fatty acids is expected but the physical properties tend to be dictated more by the creation of *trans* ethylenic bonds which give fatty acids of melting points intermediate to those of the natural *cis* ethylenic form and the saturated analogue.

The disappearance of the original polyethylenic fatty acids, their transformations, and the evolution of the fatty acids newly formed during the hydrogenation process are shown in Table I. Differences between the Wijs iodine values and those calculated from the GLC compositions are not unusual (24). This can reflect some polymerization of certain fatty acids, most likely those with the highest number of ethylenic bonds. Although these polymers are not detected in the GLC analyses their residual ethylenic bonds still contribute to the Wijs iodine value (IV). During GLC of total fatty acids the division of the peaks into non-methylene-interrupted diene (NMID) and triene (NMIT) groups is based on retention times for fractions from some preliminary argentation thin layer chromatographic separations. In view of the complexity of the positional and geometrical isomers formed (Fig. 1), resulting in some overlap, these divisions must be regarded as provisional pending further studies. Notwithstanding this problem and that of applying suitable correction factors (15), the chain length totals for the refined oil and the four hydrogenated oils are in reasonable agreement (Table II).

The total fatty acid analyses (Table I) show that there is relatively little increase of saturated and monoethylenic acids during the early stages of the hydrogenation process. The saturated acid content (changes in 18:0, 20:0, 22:0 are readily observed) increases sharply between IV 88 and IV 79. This corresponds to a small decrease in total monoethylenic acids in all chain lengths except C_{18}, whereas down to IV 88 the monoethylenic acids were increasing. This suggests that on nickel catalyst, as long as the original *cis*-polyethylenic acids are present, the availability of sites active for absorption and isomerization of monoethylenic acids is limited (25).

Initially (Fig. 2), there is a decline in polyunsaturated acids with 4, 5 and 6 ethylenic bonds (primarily 18:4Δ6,9,-12,15, 20:5Δ5,8,11,14,17, 22:6Δ4,7,10,13,16,19). This decline corresponds to an increase in both the NMID and NMIT fractions, but after IV 88 is reached, the NMIT also start to decline. This coincides with the total disappearance of the natural all-*cis* polyethylenic fatty acids with 4, 5 and 6 bonds and reinforces the supposition of strong absorption of the latter on the catalyst. Once the NMIT, which presumably still include some methylene-interrupted *cis* ethylenic bonds, become the most highly unsaturated material available, they in turn are reduced to NMID. As the oil IV declines from 118 to 101, the 18:2Δ9,12 falls by only half, whereas 18:3Δ9,12,15 is almost totally eliminated and 18:4, 20:5 and 22:6 are reduced by three-quarters. At IV 79, the total diethylenic acids (initially including 2.3% 18:2Δ9,12) rise to 11.5% of which <0.3% could be 18:2Δ9,12, the balance being NMID.

Distribution of Isomers of Eicosenoic and Docosenoic Acids

As noted above, one of the results of the hydrogenation process on nickel catalyst, terminated at IV 79, is to transform the polyunsaturated fatty acids into non-methylene-interrupted dienes (Table I). Only a slight increase in monoenes (1.5% for 20:1 and 2.7% for 22:1) was observed from IV 119 to IV 88. An increase of saturates occurred with the further reduction from IV 88 to IV 79 (0.4% for 20:0 and 1.0% for 22:0) corresponding to a decrease in monoenes (0.3% for 20:1 and 1% for 22:1). We can therefore consider that most of the new *cis* and *trans* eicosenoic and docosenoic isomers are formed by the positional and geometrical isomerization of the pre-existing *cis* 20:1 and

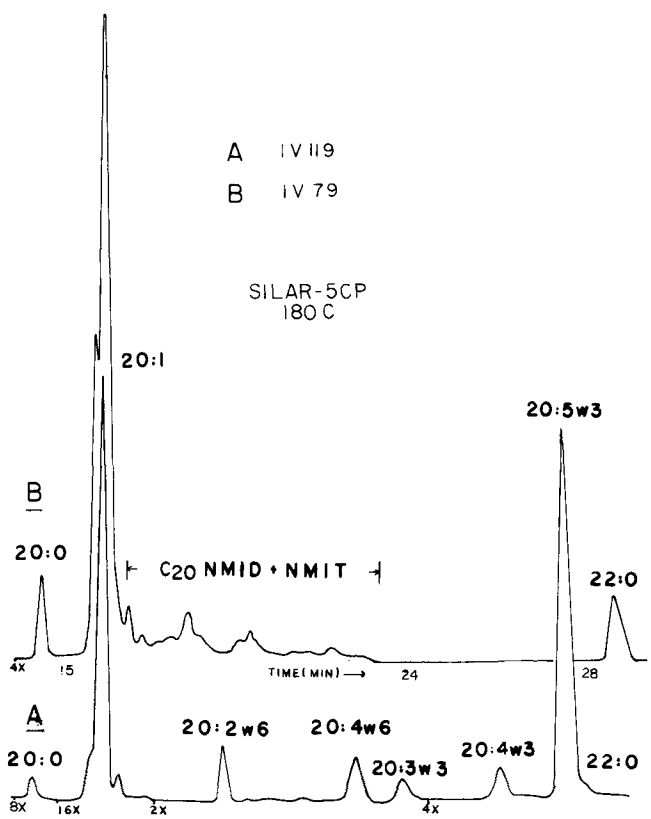

FIG. 1. Comparison of parts of gas liquid chromatographic charts for fatty acids methyl esters from refined herring oil (*below*) and from partially hydrogenated herring oil of Wijs iodine value 79 (*above*). Column, open-tubular, 47 m X 0.25 mm id, 180 C, SILAR-5CP.

TABLE II

Even Chain Lengths (C_{16}-C_{22}) for Fatty Acids Methyl Esters in Refined and Partially Hydrogenated Herring Oils. (Data in roman type are summed from total analyses of Table I and those in *italics* are from analysis of fatty acid methyl esters completely hydrogenated on PtO_2 in the laboratory.)

Chain lengths wt%	Refined herring oil	Partially hydrogenated herring oil			
	IV-119	IV-107	IV-101	IV-88	IV-79
Σ C_{16}	23.9	21.9	22.8	23.6	21.0
	24.8	*22.1*	*22.8*	*21.9*	*22.1*
Σ C_{18}	19.5	19.5	19.4	19.6	21.5
	20.6	*20.3*	*20.5*	*19.5*	*20.3*
Σ C_{20}	20.3	20.5	20.3	21.1	20.4
	19.3	*20.0*	*19.9*	*20.1*	*20.3*
Σ C_{22}	25.7	26.6	27.0	26.8	28.2
	24.7	*27.0*	*27.0*	*27.3*	*27.8*

22:1 and not by the hydrogenation of the highly polyunsaturated fatty acids to monoenes.

The isomeric 20:1 and 22:1 acids were isolated by preparative GLC on SE-30 or DEGS columns. A comparison of the resulting fractions suggested that the DEGS column with the elution order saturated, monoethylenic, diethylenic, gave C_{20} and C_{22} monoethylenic fractions easier to resolve into *cis* and *trans* materials. The SE-30 column, with elution order diethylenic, monoethylenic, saturated, apparently allowed some diethylenic acids to tail into the monoethylenic peak. These were responsible for poor $AgNO_3$-TLC separations and their inclusion led to irregular proportions of isomers from run to run. Each band recovered from $AgNO_3$-TLC had 18:0 added as an internal standard and the proportion of *cis* and *trans* isomers was determined by analyses on a SILAR-7CP open-tubular column. The separation of the *trans* and *cis* isomers on this liquid phase was inadequate for direct quantitation without prior separation (Fig. 3), although very useful for rapid screening of geometric isomerization (15).

The TLC/GLC method with internal standard is especially useful for C_{16} and C_{18} *cis* and *trans* monoethylenic isomers since the separation of geometrical isomers is limited with either polar phases, such as SILAR-10C (26), or non-polar phases, such as Apiezon-L (16). In the C_{20} and C_{22} chain lengths, separations are more favorable and, for mixtures of *cis* and *trans* 20:1 and 22:1 isomers such as encountered in PHHO, the Apiezon-L open-tubular method gives virtually the same results as the combined TLC/GLC method with internal standard (Table III).

The proportion of *cis* and *trans* 20:1 and 22:1 isomers was investigated by two different methods because the sample of IV 79 showed more *trans* 20:1 than *trans* 22:1. The averages from the two methods (Table III) are *trans* 20:1, ca. 36%, and *trans* 22:1, ca. 29%. It is necessary to

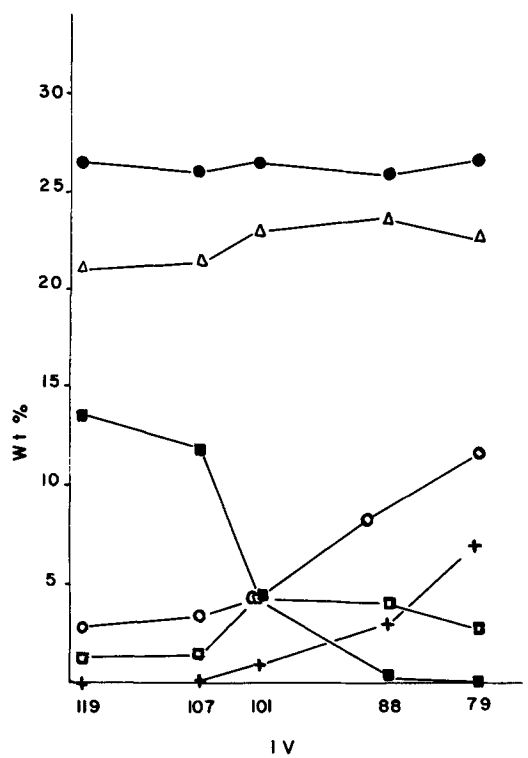

FIG. 2. Transformation of fatty acids observed during hydrogenation of herring oil, • saturates, △ total 22:1, + total *trans* 22:1, ○ dienes, □ trienes, ■ polyenes with 4, 5 and 6 double bonds.

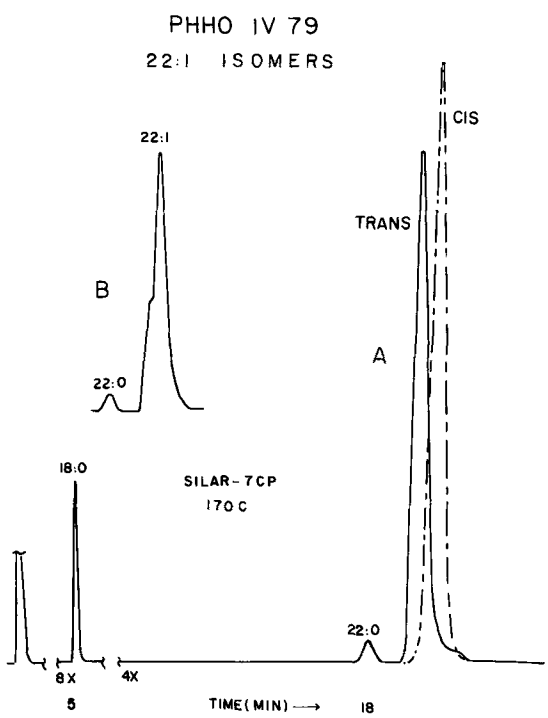

FIG. 3. Comparison of (A) *cis* and *trans* 22:1 isomers (PHHO, IV 79), isolated by preparative GLC followed by silver nitrate thin-layer chromatography, 18:0 added as an internal standard and (B) 22:1 region of total methyl ester including the 22:0, open-tubular column, 47 m × 0.25 mm id, 170 C, SILAR-7CP.

consider the formation of some *trans* monoethylenic isomers from reduction of the highly unsaturated fatty acids 20:5, 22:5 and 22:6. It could be suspected that this *trans* monoene formation should be more important for C_{20} than C_{22}, considering that the C_{20} polyenes slightly exceed the C_{22} polyenes (respectively, 6.2% and 4.6% of refined oil). It has also been demonstrated (27-29) that the position of the fatty acid in a triglyceride affects the hydrogenation rate. The reduction of the unsaturated acids at the 2-position of a triglyceride proceeds more slowly than for those at the 1,3 positions but the geometrical isomerization is greater at the 2-position (29). The triglyceride composition studies of Brockerhoff showed that the monoethylenic acids are predominantly in the 1,3-position, but for herring oil, there is twice the amount of 20:1 than 22:1 (30) available in the 2-position. The extent of geometrical isomerization can therefore be greater for 20:1 than for 22:1. This has been observed in an earlier examination (9) of PHHO and also in partially hydrogenated rapeseed oil (3) where the proportion of *trans* monoethylenic isomer decreased progressively ($C_{18}>C_{20}>C_{22}$) with increasing chain length. In rapeseed oil the 22:1 is primarily in the 1- and 3-positions of the triglyceride molecule.

The ozonolysis products from the respective *cis* and *trans* isolates of 20:1 and 22:1 were analyzed separately by isothermal GLC on SILAR-5CP (diesters at 180 C, monoesters at 130 C). The retention times at 130 C and at 180 C (Figs. 4 and 5) for SILAR-5CP show that there is no coincidence of diesters and monoesters for the conditions employed. It is mandatory that the analyses of the two classes of functional products be complementary to ensure that no differential loss of products due to chain length has occurred. The 22:1 isomers were investigated at all 4 stages of hydrogenation but the 20:1 only in the refined oil and in the last two hydrogenated samples, IV 88 and 79. Tables IV and V, and Figs. 6 and 7, summarize the results of this aspect of the study.

In the refined but unhydrogenated oil, the major isomers of 20:1 (*cis* Δ11>>Δ9>Δ13) were accompanied by small amounts of even-numbered positional isomers (*cis* Δ6,Δ8,-Δ10 and Δ12). The presence of these isomers (Fig. 7) was verified by the agreement between the mono- and diester ozonolysis products. In the 22:1, the corresponding isomers in the even-numbered positions were not detected (Fig. 6). As the hydrogenation proceeded, the pre-existing *cis* 22:1 isomers (Δ9,Δ11,Δ13) decreased while new even-numbered *cis* isomers (Δ8,Δ10,Δ12) were formed (Fig. 6). Similarly, in the 20:1, the original odd-numbered *cis* isomers (Δ9,-Δ11,Δ13) decreased while the minor even-numbered *cis* isomers (Δ8,Δ10,Δ12) increased. An obvious trend, detailed in Tables IV and V, is the progressive development of new positional isomers of both geometries with the migration of the double bond occurring in both directions away from the original Δ11 position. Generally the geometric isomerization is the more important process (Figs. 6 and 7), although evidently hindered by the preferential absorption of polyunsaturated acids on the catalyst in the early stages of the process. After an IV of 101 is reached, these polyunsaturated fatty acids are largely eliminated (Table I) and the positional and geometrical isomerization of monoethylenic acids is then accelerated by the greater availability of

TABLE III

Comparisons between the *trans* 20:1 and 22:1 Contents (%) of Three Partially Hydrogenated Herring Oils Determined Directly from Apiezon-L Analysis or after Silver-Nitrate Thin Layer Chromatographic Separation of cis and *trans* Components and Quantitation by Internal GLC Standard on SILAR-7CP

Chain length and type of analysis	Partially hydrogenated herring oil		
	IV-101	IV-88	IV-79
20:1 Apiezon-L GLC	3	15	35
$AgNO_3$-TLC/GLC[a]	ND[b]	13	38
22:1 Apiezon-L GLC	5	13	30
$AgNO_3$-TLC/GLC[a]	4	13	28

[a]Internal standard (18:0) added after recovery of TLC band from plate. Average of four preparative GLC isolates.

[b]Not determined.

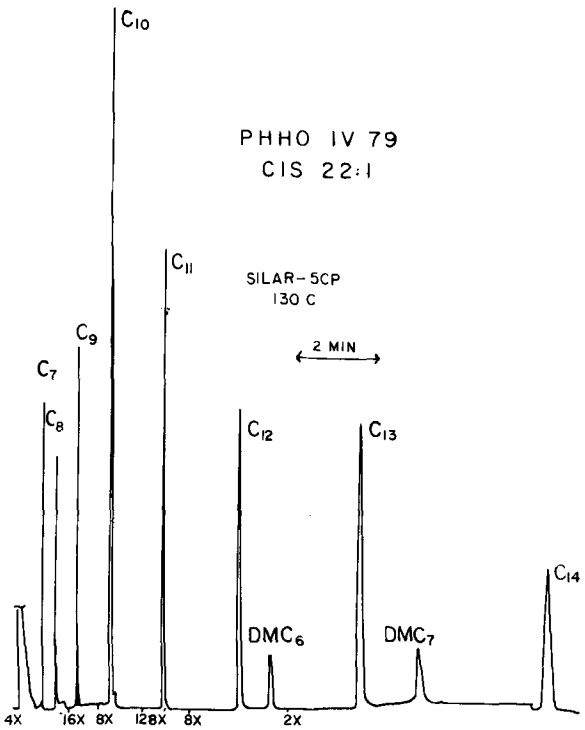

FIG. 4. Monoesters and short-chain diesters from ozonolysis of *cis* 22:1 isomers isolated from herring oil hydrogenated to an iodine value of 79. Open-tubular column, 47 m X 0.25 mm id, 130 C, SILAR-5CP.

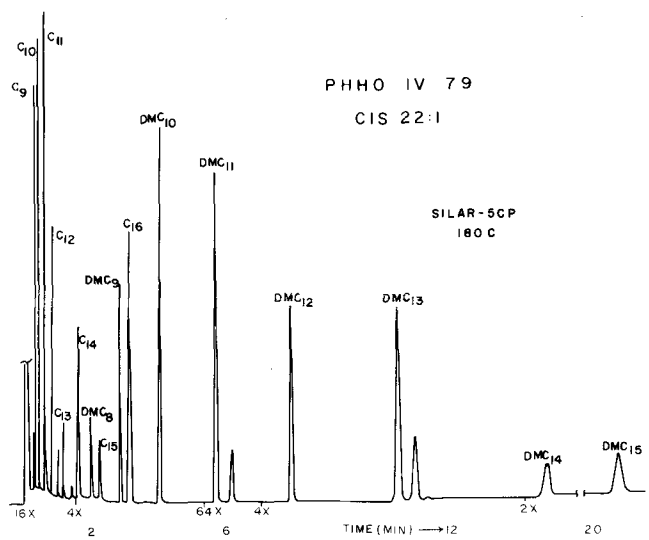

FIG. 5. Mono- and diesters from ozonolysis of *cis* 22:1 isomers isolated from herring oil hydrogenated to an iodine value of 79, open-tubular column 47 m X 0.25 mm, 180 C, SILAR-5CP.

catalyst. Close scrutiny of the isomer proportions in each chain length does not provide any evidence for the presence of isomers which can be connected to original ethylenic positions in the more highly unsaturated fatty acids. In moving through the tetraene and triene stages, the unreduced ethylenic bonds are evidently sufficiently randomized both positionally and geometrically that no original position can be recognized.

In the unhydrogenated oil (Table IV, Fig. 6), there is more of the minor isomer 22:1Δ13 (5.3%) than of the minor isomer 22:1Δ9 (2.6%), and this ratio is not affected in the *cis* products (Fig. 6). In the *trans* (IV 88) products, there is a slight skewing of the even-numbered isomers Δ10 and Δ12 in favor of the latter (1.5% vs 2.9%). At the end of the hydrogenation process (IV 79), this skewing, resulting from the minor *cis* isomers originally present (Δ9 and Δ13), is much less obvious but still present (*trans* Δ12 5.5% vs *trans* Δ10 4.9%). This skewing was not observed for the 20:1 isomers, where the positional and geometrical isomerization is more complex than for the 22:1 acids because of the presence of the even-numbered *cis* isomers (Δ8,Δ10,Δ12) in the starting material.

TABLE IV

Distribution of 22:1 Isomers in Mole % for Refined and Four Partially Hydrogenated Herring Oil Samples

Ethylenic Δ	Position ω	Geometry	Refined herring oil IV-119	Partially hydrogenated herring oil IV-107	IV-101	IV-88	IV-79
6	16	*cis*	ND[a]	ND	ND	0.7	1.5
		trans	*ND*	*ND*	*ND*	*0.3*	*0.3*
7	15	*cis*	ND	ND	ND	0.2	0.3
		trans	*ND*	*ND*	*ND*	*tr*	*0.1*
8	14	*cis*	ND	ND	ND	0.2	0.4
		trans	*ND*	*ND*	*ND*	*tr*	*0.5*
9	13	*cis*	2.6	2.0	2.6	1.5	0.8
		trans	*ND*	*ND*	*0.4*	*0.2*	*0.5*
10	12	*cis*	ND	ND	ND	0.8	2.5
		trans	*ND*	*ND*	*0.4*	*1.5*	*4.9*
11	11	*cis*	91.5	91.7	87.9	78.9	58.7
		trans	*ND*	*ND*	*2.0*	*6.5*	*14.7*
12	10	*cis*	ND	0.3	0.4	0.9	2.6
		trans	*ND*	*ND*	*0.5*	*2.9*	*5.5*
13	9	*cis*	5.3	5.2	4.3	3.4	3.1
		trans	*ND*	*ND*	*0.5*	*1.1*	*1.5*
14	8	*cis*	ND	ND	tr	tr	0.5
		trans	*ND*	*ND*	*0.1*	*0.3*	*0.6*
15	7	*cis*	0.6	0.8	0.8	0.4	0.6
		trans	*ND*	*ND*	*0.1*	*0.2*	*0.4*
Σ%		*cis*	100.0	99.8	96.0	87.0	71.0
		trans	*0.0*	*0.2*	*4.0*	*13.0*	*29.0*

[a]Not detected under analytical conditions.

TABLE V

Distribution of 20:1 Isomers in Mole % for Refined and Two Partially Hydrogenated Herring Oil Samples

Ethylenic Δ	Position ω	Geometry	Refined herring oil IV-119	Partially hydrogenated herring oil IV-88	IV-79
6	14	*cis*	0.9	1.8	0.8
		trans	*ND*[a]	*0.9*	*0.6*
7	13	*cis*	tr	0.5	0.6
		trans	*ND*	*0.2*	*0.3*
8	12	*cis*	0.6	0.8	0.7
		trans	*ND*	*0.4*	*0.7*
9	11	*cis*	8.5	9.7	2.8
		trans	*ND*	*1.6*	*2.6*
10	10	*cis*	1.5	2.4	2.4
		trans	*ND*	*2.2*	*6.5*
11	9	*cis*	84.6	65.7	50.3
		trans	*ND*	*5.0*	*14.6*
12	8	*cis*	0.3	1.1	2.9
		trans	*ND*	*2.1*	*6.7*
13	7	*cis*	3.2	3.2	2.5
		trans	*ND*	*1.0*	*2.2*
14	6	*cis*	ND	0.4	0.6
		trans	*ND*	*0.5*	*1.2*
15	5	*cis*	0.4	0.5	0.4
		trans	*ND*	*0.1*	*0.6*
Σ%		*cis*	100.0	86.0	64.0
		trans	*0.0*	*14.0*	*36.0*

[a]Not detected under analytical conditions.

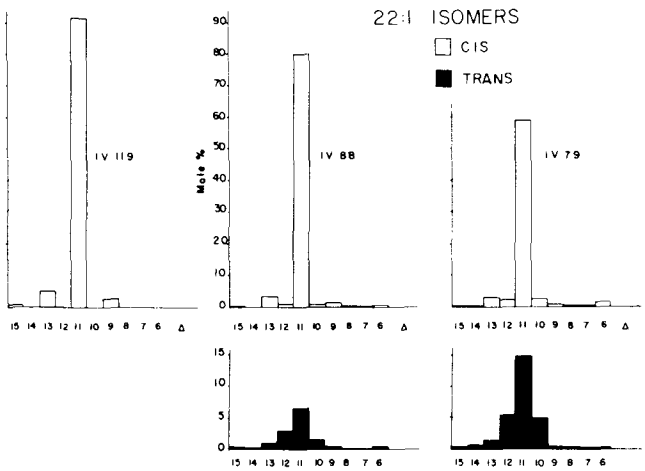

FIG. 6. Distribution of *cis* and *trans* 22:1 isomers in unprocessed herring oil and in samples with iodine values of 88 and 79.

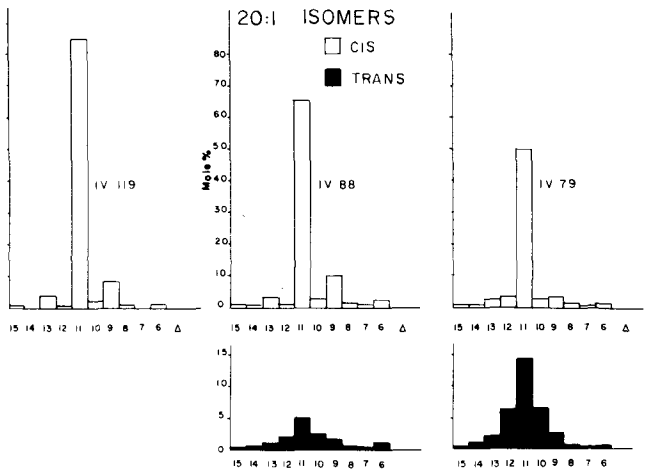

FIG. 7. Distribution of *cis* and *trans* 20:1 isomers in unprocessed herring oil and in samples with iodine values of 88 and 79.

The 20:1 and 22:1 isomer distributions, obtained by oxidative ozonolysis in BF_3-MeOH are similar to those reported by Conacher et al. (10), who used a reductive ozonolysis technique. However, the identification of isomers is extended towards both ends of the fatty acid chains. The details reported here are also more comprehensive than those found in an earlier study on herring oil, hydrogenated to an IV of 76, by Ackman et al. (9). In that study, column chromatography was used for separating the *cis* and *trans* isomers and despite, or perhaps because of, isomer subfractionation effects (16) the study showed only two ($\Delta7 \approx \Delta6$) minor *cis* 22:1 isomers accompanying the *cis* $\Delta11$ isomer at IV 76. The current study shows (Table IV) that *cis* 22:1$\Delta6$ and $\Delta7$ are less obvious than reported earlier. Some of the differences among literature reports of products are evidently due to improvements in methodology but, in the 22:1 isomers in particular, the skewing of product proportions due to the "memory effect" from *cis* monoethylenic isomers originally present is a major consideration. Thus, studies of the effects of hydrogenation on fatty acid structures should include a full analysis of all isomers originally present.

The isomerization of monoenes occurring during catalytic hydrogenation has, in the past, been investigated with vegetable oils rich in 18:1 (2,3) and comparisons have been made in the hydrogenation of methyl esters of C_{18} fatty acids (31-34). For methyl esters, two types of hydrogenation mechanisms have been proposed (35-36). In both cases, the hydrogenation catalyst activates the hydrogen by dissociation into two absorbed hydrogen atoms and the unsaturated fatty acid by the formation of an absorbed intermediate. The first mechanism (Horiuti and Polanyi) (35) postulates the transformation of the absorbed intermediate into a half-hydrogenated intermediate. This half-hydrogenated intermediate either picks up a second hydrogen, desorbs to give a saturated linkage, or gives up a hydrogen and desorbs to form geometrical and positional isomers. In the second (π allyl) mechanism (35,36), the absorbed unsaturated fatty acid gives up a hydrogen atom from an activated methylene group in the α position to the absorbed double bond, thus forming an allylic intermediate. Evidence for the two mechanisms operating simultaneously has been shown by different authors (33, 37). If only the first mechanism existed, it would be difficult to explain the selectivity of the reaction and the monopolization of the surface of the catalyst by the polyunsaturated acids in the form of polybonded species (allylic intermediates) (38), which hinders the isomerization of the monoenes (Fig. 2). If only the second mechanism were functioning, the result would be a minimum $\Delta11$ position in the *trans* monoenes considering that a π allyl mechanism only allows geometrical isomerization via positional isomers. If one accepts the simultaneous existence of these two mechanisms, it is possible to understand the distribution of *cis* and *trans* monoene isomers after hydrogenation. Starting with *cis* 22:1$\Delta11$ (91.5%), *trans* $\Delta11$ could be formed via a half-hydrogenated intermediate. To clarify the alternative π allyl mechanism, the different allylic intermediates proposed by Van der Plank for *cis* and *trans* monoenes are shown in Figure 8. The two allylic intermediates (Fig. 8, 1 and 2) formed from *cis*

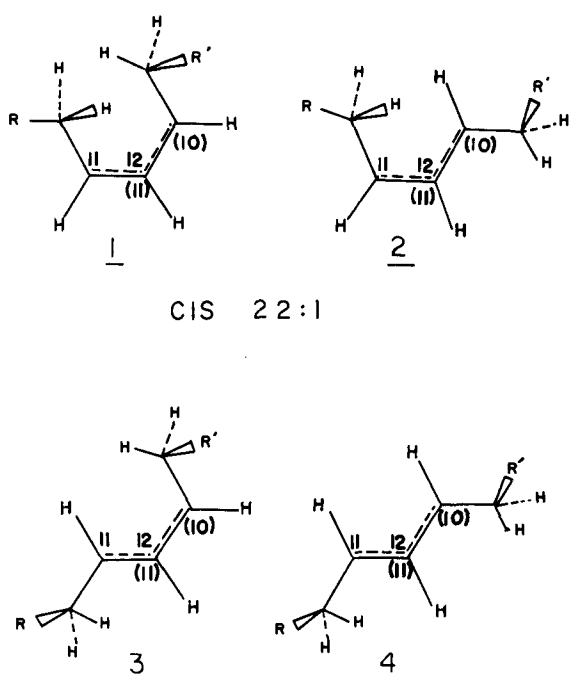

FIG. 8. π Allyl intermediates formed from *cis* and *trans* 22:1$\Delta11$, according to P. Van der Plank. (J. Catalysis, 38:223 [1975]). Numbers refer to carbon atoms. These can be counted from either end of the chain.

22:1Δ11 have different stabilities due to the greater steric effect taking place in intermediate 1. If the formation of the allylic intermediate is the rate determining step (33), and if the activation energy of the isomerization process is lower for intermediate 2 than 1, *cis* 22:1Δ11 will more readily form *trans* Δ10 or *trans* Δ12 (intermediate 2) than *cis* Δ10 or *cis* Δ12 (intermediate 1). Similarly, due to the greater stability of intermediate 4 relative to 3, *trans* Δ11 formed by the geometric isomerization of *cis* Δ11 will more readily form *trans* Δ10 or *trans* Δ12 (intermediate 4) than *cis* Δ10 or *cis* Δ12 (intermediate 3). This reasoning can explain the high proportion in the *trans* isomers of Δ10 and Δ12 formed via the two routes, either by a direct transformation of *cis* Δ11 (intermediate 2) or migration of the *trans* isomers (intermediate 4).

In view of the European Economic Community's regulations for edible fats where erucic acid (*cis* 22:1Δ13) is distinguished from other docosenoic isomers (39), it is important to note that although the total 22:1 is slightly increased during hydrogenation, the proportion of erucic acid (Table IV) is slightly decreased by the hydrogenation process.

Some marine oils, for example menhaden oil, contain relatively little (1-2%) of 22:1, and correspondingly more polyunsaturated fatty acids (40). Studies on the fatty acid modifications produced during hydrogenation of menhaden oil are in progress and may be expected to further our understanding of the competitive nature of the catalyst and the different types of fatty acids of marine oils.

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REFERENCES

1. FAO Food and Nutrition Paper 3. Dietary Fats and Oils in Human Nutrition, Food and Agriculture Organization of the United Nations, Rome, 1978.
2. Vigneron, P.Y., and P. Spicht, Rev. Fse. Corps Gras. 11:631 (1973).
3. Conacher, H.B.S., and B.D. Page, JAOCS 49:283 (1972).
4. Kurucz-Lusztig, E., P. Lukás-Hágony, M. Prepostffy-Jánoshegyi, M. Jerának-Knapecz and P. Biacs, Acta Alimentaria, 3:357 (1974).
5. Pelloquin, A., and E. Ucciani, Rev. Fse. Corps Gras., 7:379 (1975).
6. Koritala, S., JAOCS 45:197 (1968).
7. Scholfield, C.R., R.O. Butterfield and H.J. Dutton, JAOCS 56:664 (1979).
8. Ota, Y., H. Honma and H. Watanabe, Yukagaku 22:206 (1973).
9. Ackman, R.G., S.N. Hooper and J. Hingley, JAOCS 48:804 (1971).
10. Conacher, H.B.S., B.D. Page and R.K. Chadha, JAOCS 49:520 (1972).
11. Kudrawcew, W., and H. Niewiadomski, Acta Alimentaria Polonica, 2:53 (1976).
12. Lund, P., and G. Hølmer, Comparative studies of non-hydrogenated and hydrogenated Danish fish oils, especially with respect to the C_{20} and C_{22} monoene content. Technical University of Denmark, Lyngby, 1976.
13. Lambertsen, G., H. Myklestad and O.R. Braekkan, JAOCS 48:389 (1971).
14. Ackman, R.G., in "Fishery Products," edited by R. Kreuzer, pp. 112-131, FAO, 1974.
15. Ackman, R.G., and C.A. Eaton, Fette. Seifen. Anstrichm. 80:21 (1978).
16. Ackman, R.G., and S.N. Hooper, J. Hingley, J. Chromatogr. Sci. 10:430 (1972).
17. Ackman, R.G., Lipids, 12:293 (1977).
18. Sebedio, J-L., and R.G. Ackman, Can. J. Chem., 56:2480 (1978).
19. Andersen, A.J.C., and P.N. Williams, Margarine, Pergamon Press, London, 1965.
20. Okkerse, C., A. De Jonge, J.W.E. Coenen and A. Rozendaal, JAOCS 44:152 (1967).
21. Allen, R.R., Ibid., 55:792 (1978).
22. Puri, P.S., Ibid., 55:865 (1978).
23. Cecchi, G., and E. Ucciani, Riv. Ital. Sost. Grasse, LVI:235 (1979).
24. Ackman, R.G., and C.A. Eaton, J. Fish. Res. Board Can., 23:99 (1966).
25. Coenen, J.W.E., and H. Boerma, Fette. Seifen. Anstrichm. 70:8 (1968).
26. Conacher, H.B.S., and J.R. Iyengar, J. Assoc. Off. Anal. Chem., 61:702 (1978).
27. Drozdowski, B., JAOCS, 54:600 (1977).
28. Kaimal, T.N.B., and G. Lakshminarayana, JAOCS, 56:578 (1979).
29. Paulose, M.M., K.D. Mukherjee and I. Richter, Chem. Phys. Lipids, 21:187 (1978).
30. Brockerhoff, and R.J. Hoyle, Arch. of Biochem. and Biophys., 102:452 (1963).
31. Allen, R.R., and A.A. Kiess, JAOCS 32:400 (1955).
32. Stefanovic, S., R.H. Price and L.F. Albright. Fette. Seifen. Anstrichm. 12:464 (1978).
33. Van der Plank, P., and H.J. Van Oosten, J. Catal. 38:223 (1975).
34. Van der Plank, P., JAOCS 49:327 (1972).
35. Coenen, J.W.E., Symposium on the Contribution of Chemistry to Food Supplies, held in Hamburg (1973), Butterworth's London, 1974.
36. Rooney, J.J., F.G. Gault and C. Kemball. Proc. Chem. Soc. 407 (1960).
37. Heertje, I., G.K. Koch and W.J. Wosten, J. Catal. 32:337 (1974).
38. Coenen, J.W.E., Chemistry and Industry, 709 (1978).
39. Anon., Council directive of 20 July 1976 relating to the fixing of the maximum level of erucic acid in oils and fats (intended as such for human consumption and in foodstuffs containing added oils or fats). 76/621/EEC. Official Journal of the European Communities No. L 202/35.28.7.76, 1976.
40. Ackman, R.G., JAOCS 43:385 (1966).

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