Monoethylenic Fatty Acids of a Partially Hydrogenated Herring Oil

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ABSTRACT

A Canadian Atlantic herring oil hydrogenated for margarine use to an iodine value of 76 and melting point of 32.5 C was found to have 30% saturated acids and 66% monounsaturated fatty acids. The monounsaturated fatty acids could be analytically determined as *cis* and *trans* isomers by open tubular gas liquid chromatography. *Trans* acids were 33% of the C_{16} and C_{18} monounsaturated acids, and 32 and 28%, respectively, of the C_{20} and C_{22} monounsaturated acids. After separation of geometric isomers by Florisil-silver nitrate chromatography the positional isomers in each class were determined by oxidative fission. The double bond positions of the original *cis* fatty acids were largely retained in both *cis* and *trans* isomers, but additional isomers were observed, especially in the *trans* fatty acids.

INTRODUCTION

The partial hydrogenation of fats and oils to produce a stable, palatable product of suitable plasticity results in the formation of new, structurally altered fatty acids which in most cases differ chemically from familiar natural products. The nutritive aspects of partially hydrogenated fats originally of vegetable origin have been the subject of a recent review (1). In Canada as much as 55 million pounds of hydrogenated marine oils have been used in margarines, shortenings or cooking fats in recent years (2). It has been presumed that the nutritional and physiological qualities of these oils would be similar to those of the partially hydrogenated vegetable oils which are primarily of C_{18} chain length except for rapeseed oil. Recent studies indicated that certain physiological responses of animals to the long chain (C_{20} and C_{22}) monoethylenic fatty acids found in most marine oils in moderate proportions (10-30%) even before hydrogenation, and especially in erucic acid-rich rapeseed oils, required further study (3). This work reports details of the analysis of the partially hydrogenated herring oil used in one such recent study (4,5).

EXPERIMENTAL PROCEDURES

Atlantic herring oil (from reduction of whole *Clupea harengus*), which had been hydrogenated according to Canadian current commercial practice with nickel catalyst at 375-400 F and about 10 psig hydrogen pressure to a Wijs iodine value of 76 and Wiley melting point of 32.5 C, was saponified and nonsaponifiable materials removed by AOCS method C6-6b-53. The soaps were acidified and the fatty acids extracted into diethyl ether, washed and dried. The acids were converted to methyl esters by refluxing with 7% BF_3 in MeOH for 10 min.

The methyl esters were examined initially by gas liquid chromatography (GLC) and after fractionation by thin layer chromatography (TLC) on silver-nitrate impregnated silica gel (Supelcosil 12D) developed in *n*-hexane:benzene 1:1. For oxidative fission studies the monounsaturated methyl esters were separated from the saturates and polyunsaturates and into *cis* and *trans* fractions by column chromatography on a scale of 100-200 mg on acid-washed Florisil impregnated with silver nitrate (6). The solvents were essentially those reported earlier where silica gel-silver nitrate columns were employed (7,8). The esters of saturated fatty acids were almost completely eluted with 75 ml of *n*-hexane. The *trans* and *cis* monoethylenic esters were eluted in sequence with virtually no overlap by successive applications of 75 ml each of 2,4 and 8% diethyl ether-*n*-hexane mixtures. The respective *cis* and *trans* fractions were further separated by preparative scale GLC with recovery of four chain lengths (C_{16}, C_{18}, C_{20}, C_{22}). Ozonolysis of each specific fraction was carried out in methanol with oxidative work-up, the product acids being identified by GLC after in situ esterification with 2,2-dimethoxypropane (9).

Basic determinations of composition were carried out on open-tubular (capillary) GLC columns (150 ft x .01 in ID) coated with butanediol-succinate polyester (BDS) or Apiezon-L grease (AP-L). The columns were purchased ready-coated from Perkin-Elmer and used in Perkin-Elmer Model 226 or Model 900 GLC units.

Preparative gas liquid chromatography was carried out with an Aerograph A-90 (thermal conductivity detector) GLC apparatus fitted with a 6 ft x 5/15 in. OD copper column packed with 5% SE-30 silicone gum on Gas-Chrom Q (100/120 mesh).

Standard *trans* monounsaturated acids were prepared from *cis* monounsaturates of known compositions (8). These were isolated from herring oil methyl esters by preparative TLC on silver nitrate-silica gel plates (Supelcosil 12D). After spraying with 2,7-dichlorofluorescein the monounsaturated esters visualized under UV light were recovered, treated with nitrous acid (10), and separated by TLC into *cis* and *trans* bands. The purity of the *cis* and *trans* materials prepared in this way was verified by both infrared spectroscopy (IR) and by GLC on AP-L. Esters prepared from the commercially hydrogenated sample were examined on a Varian T-60 NMR Spectrometer.

RESULTS AND DISCUSSION

The results of study of this sample of commercially prepared, partially hydrogenated herring oil would indicate the use of a catalyst and conditions favoring selective hydrogenation of the polyunsaturated acids to monounsaturated acids. Only a total of 4% of a variety of artifact polyunsaturated acids remained. The proportions of 22:0, normally absent in herring oil, and 20:0, about 0.1-0.5% in most herring oils, indicate that as little as 8% of the original or newly formed monounsaturated acids may have been hydrogenaged to saturated acids. Total saturated acids were 30% and total monounsaturated acids 66%. Unfortunately the raw herring oil was not available for analysis and the fatty acid composition of herring oil varies with season, age of fish, and catch location. Average figures for 12 Atlantic herring oils of 20% for saturated acids, range 16.4-24.4%, and 60% for monounsaturated acids, range 52.1-71.5%, have been reported (11).

The composition of the saturated fatty acids in this sample is not remarkable for a marine oil. Were it not for the presence of 1.64% 20:0, and 2.04% 22:0, the composition could well be that of the saturated acids of any of

TABLE I

Fatty Acid Composition of Partially Hydrogenated Herring Oil

Principal type of fatty acid	Weight % in total sample	Monoethylenic isomers wt % of isomer in the given chain length			Monoethylenic isomers wt % of isomer in the total sample	
			Cis	*Trans*	*Cis*	*Trans*
Iso 14:0	0.10	16:1ω12	—	trace	—	—
14:0	8.11	16:1ω11	—	4.8	—	0.13
4,8,12 TMTD	0.26	16:1ω10	trace	15.9	—	0.44
14:1	0.08	16:1ω9	6.1	5.8	0.34	0.16
Iso 15:0	0.32	16:1ω8	13.6	17.3	0.76	0.48
Anteiso 15:0	0.12	16:1ω7	74.1	38.4	4.12	1.07
15:0	0.42	16:1ω6	6.1	7.9	0.34	0.22
15:1	0.05	16:1ω5	—	5.1	—	0.14
Iso 16:0	0.03	16:1ω4	—	3.7	—	0.10
Pristanic	0.31	16:1ω3	—	1.0	—	0.03
16:0	12.20					
16:1	8.35	18:1ω13	—	trace	—	—
(*cis*	5.58)	18:1ω12	16.9	3.1	1.39	0.13
(*trans*	2.76)	18:1ω11	8.0	6.2	0.66	0.26
16:2 + ?	0.16	18:1ω10	7.7	9.6	0.63	0.40
Iso 17:0	0.18	18:1ω9	46.6	41.0	3.83	1.69
Anteiso 17:0	0.09	18:1ω8	6.9	17.1	0.57	0.71
17:0	1.36	18:1ω7	13.8	15.7	1.13	0.65
Phytanic	0.21	18:1ω6	—	4.9	—	0.20
Iso 18:0	0.08	18:1ω5	—	2.3	—	0.09
18:0	2.13					
18:1	12.34	20:1ω17	—	trace	—	—
(*cis*	8.20)	20:1ω16	—	trace	—	—
(*trans*	4.13)	20:1ω15	—	trace	—	—
		20:1ω14	2.6	4.4	0.34	0.28
18:2 + ?	1.67	20:1ω13	1.3	3.8	0.17	0.24
19:0 + ?	0.38	20:1ω12	3.7	2.9	0.49	0.18
20:0	1.64	20:1ω11	4.6	11.4	0.61	0.72
20:1	19.49	20:1ω10	2.2	15.9	0.29	1.00
(*cis*	13.20)	20:1ω9	85.6	46.0	11.30	2.89
(*trans*	6.29)	20:1ω8	—	10.4	—	0.65
		20:1ω7	—	4.9	—	0.30
20:2 + ?	1.14					
22:0	2.04	22:1ω16	7.7 ?	1.2	1.45	0.09
22:1	26.52	22:1ω15	7.7 ?	1.1	1.45	0.09
(*cis*	18.87)	22:1ω14	trace	2.7	trace	0.21
(*trans*	7.63)	22:1ω13	trace	5.5	trace	0.42
		22:1ω12	trace	8.2	trace	0.63
		22:1ω11	84.6	57.0	15.96	4.35
		22:1ω10	—	8.9	—	0.68
		22:1ω9	—	14.9	—	1.14

several common marine oils.

The polyunsaturated fatty acids were estimated primarily from GLC analyses on AP-L open-tubular columns where an irregular peak preceding the *cis* monoethylenic fatty acids was shown to represent most of the materials recoverable from argentation TLC plates with mobilities less than the *cis* monoethylenic fatty acids. This figure agreed reasonably well with gravimetric recoveries from the large scale fractionation on Florisil-silver nitrate columns.

In raw fish oils *trans* fatty acids are normally undetectable by application of conventional IR, TLC or GLC technology. It is therefore reasonable to assume that all of the *trans* acids arose by geometrical conversion from pre-existing *cis* unsaturation in the same position, or accompanied formation of new positional isomers. We have drawn attention to positional isomer and chain length enrichment effects in connection with marine oil fatty acid analyses by silver nitrate chromatography (8), and subsequent publication of TLC data for a complete range of *cis* and *trans* octadecenoates (12) further indicates the potential for cross-contamination problems or inadvertent exclusion of a component or components from an otherwise homogenous group of isomeric fatty acids. Without further study we are unable to say whether the absence of a number of *cis* isomers which should have originally been present in the raw herring oil as minor components (specifically 18:1ω5, 20:1ω7, 22:1ω9) arises from deficiencies in the isolation techniques due to such undetected chromatographic mobilities, or to preferential hydrogenation effects such as those recently described for nickel catalyst by Scholfield et al. (13). However analyses reported for several commercial samples of partially hydrogenated vegetable oils of U.S. origin also show the same effect, with the *trans* monounsaturated acids having an appreciably greater proportion of acids of lesser "ω" value (14). The enrichment in *trans* isomers of low "ω" value does not lead to the retention of the vinyl isomers reported for certain hydrogenations with copper chromite catalyst (15), both nuclear magnetic resonance (NMR) and oxidative fission indicating the absence of unsaturation at or near the terminal carbons in the fatty acid chains in the partially hydrogenated fish oil. This also may be an attribute of the nickel catalyst (13). From a hydrogenation point of view, there may be some unknown significance in the accumulation of fatty acids with double bonds in the 6,7 positions (respectively ω12, ω14 and ω16 for 18:1, 20:1 and 22:1) in the various fractions.

The slight decline of percentage *trans* isomers from 33% in the C_{16} and C_{18} chain lengths through 32% in the C_{20} to 29% for the C_{22} chain length (based on GLC area comparisons) is curious as it would be expected that reduction of polyunsaturated acids, mostly of which are in the C_{20} and C_{22} chain length, would favor a higher proportion of *trans* acids. However it should not be overlooked that in herring oils the original monoethylenic acids (*cis*) are normally in proportion to $C_{22} > C_{20} > C_{18} > C_{16}$. There is therefore a greater possibility of unaffected C_{22} and C_{20} than of C_{18} and C_{16} monoethylenic *cis* fatty

acids in this particular analysis.

The reduction of the polyunsaturated acids of Canadian Atlantic herring oil, originally about 2% C_{16}, 4% C_{18}, 8% C_{20} and 6% C_{22} in the total fatty acids (11) perhaps can be detected in the higher "ω" value acids. Thus 20:1ω14 may have arisen from reduction of the 5,6 (ω15) and 8,9 (ω12) bonds of 5,8,11,14,17-eicosapentaenoic (20:5ω3) acid. In this particular case the approximate proportions (10) of 2/3 *trans* and 1/3 *cis* is indicative of new double bond formation.

Gas liquid chromatography on AP-L open-tubular GLC columns is an expeditious means of monitoring *cis-trans* separations achieved by other means (16). A virtually complete separation of the two types of acid was obtained in all chain lengths except the C_{16} on columns of 40,000 theoretical plates. Literature retention data for octadecenoates suggests that some overlap might occur (12), especially for *cis* acids of low "ω" values, but we have no evidence of this in the isomers confirmed by oxidative fission (Table I).

On open-tubular GLC columns coated with BDS the *cis* monounsaturated acids originally present could clearly be discerned as components, albeit with intermediate shoulders in the positions between the peaks regularly separable by this technique (8). The *trans* monounsaturated acids gave less recognizable peak patterns but the locations of the *trans* isomers corresponding to the original *cis* isomers were confirmed through the standards prepared with nitrous acid. For both *cis* and *trans* monounsaturated acids analyzed by GLC on AP-L and BDS semiquantitative confirmation of the individual isomer results listed in Table I was possible. A DuPont curve analyzer was not available but could usefully extend the information obtainable from the GLC envelopes.

The proportions of the various types of fatty acids in partially hydrogenated fish oils, as for vegetable oils, depend on the degree of hydrogenation and type of process as well as on the original oil composition (17,18). Comparison of isomer details in one sample is therefore of limited value. A European analysis of a partially hydrogenated herring oil indicated 41.9% saturated acids and 44.8% monounsaturated acids (19), suggesting less selective hydrogenation conditions. The details of monoethylenic isomers given are also suggestive of severe randomization of geometry and position. These results retain a residual basic relationship to the original double bond positions in the monoethylenic fatty acids of the raw oil, in agreement with our findings (Table I) and another recent study (20).

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