Analysis of Isomeric Monoethylenic Fatty Acids in a Partially Hydrogenated Herring Oil by Glass Capillary Gas Liquid Chromatography

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ABSTRACT

The use of a glass capillary column coated with Silar 10 CP for gas liquid chromatographic analysis of geometric and positional isomers of monoethylenic fatty acids of a partially hydrogenated herring oil is reported. The results are in agreement with previous studies performed by different methods and demonstrate the usefulness of this technique in detecting and determining many types of isomeric fatty acids.

INTRODUCTION

The monoethylenic fatty acids originally present in raw herring oil are mainly *cis* isomers with odd "ω"-values (1). Partial hydrogenation of this oil with a nickel catalyst gives a complex mixture of *cis/trans* and positional isomers of monoethylenic fatty acids, the composition of which depends upon the degree of hydrogenation and the composition of the original oil (2-4). Until now this complex fatty acid mixture has been accurately analyzed mainly by using the time-consuming Florisilsilver nitrate chromatography in conjunction with an ozonization technique (5-8). However, the development of new highly polar and temperature stable cyanopropylsiloxane liquid phases, such as Silar 10 CP, Silar 9 CP, Silar 5 CP, and SP 2340 has made the gas chromatographic separation of *cis/trans* isomeric fatty acids possible even on packed columns (9,10). Small but constant differences have been observed in retention data of positional isomers (10) suggesting that these phases could be used in high performance capillary columns which are known to be highly selective for the separation of many types of isomeric fatty acids. The performance of capillary columns coated with SP 2340 (11) and Silar 5 CP (12,13) has recently been tested. In the present work, a 50 m glass capillary column coated with Silar 10 CP has been used to separate the monoethylenic fatty acid isomers of a partially hydrogenated herring oil.

EXPERIMENTAL PROCEDURES

All the fatty acid methyl ester standards (>99% pure) were purchased from Nu-Chek Prep (Elysian, MN). Additional secondary standards for *cis/trans* isomers of known composition were prepared from rapeseed and cod liver oil using the nitrous acid isomerization method of Litchfield and co-workers (14). Partially hydrogenated Norwegian herring oil (mp 39 C) was provided by SOK, Vaajakoski Plant. Fatty acid methyl esters were prepared by base-catalyzed transesterification according to Helme and co-workers (15).

A Varian Model 3700 gas chromatograph equipped with a dual flameionization detector was used for the analyses. The column employed was a 50 m long, 0.3 mm ID glass capillary column coated with Silar 10 CP (Applied Science

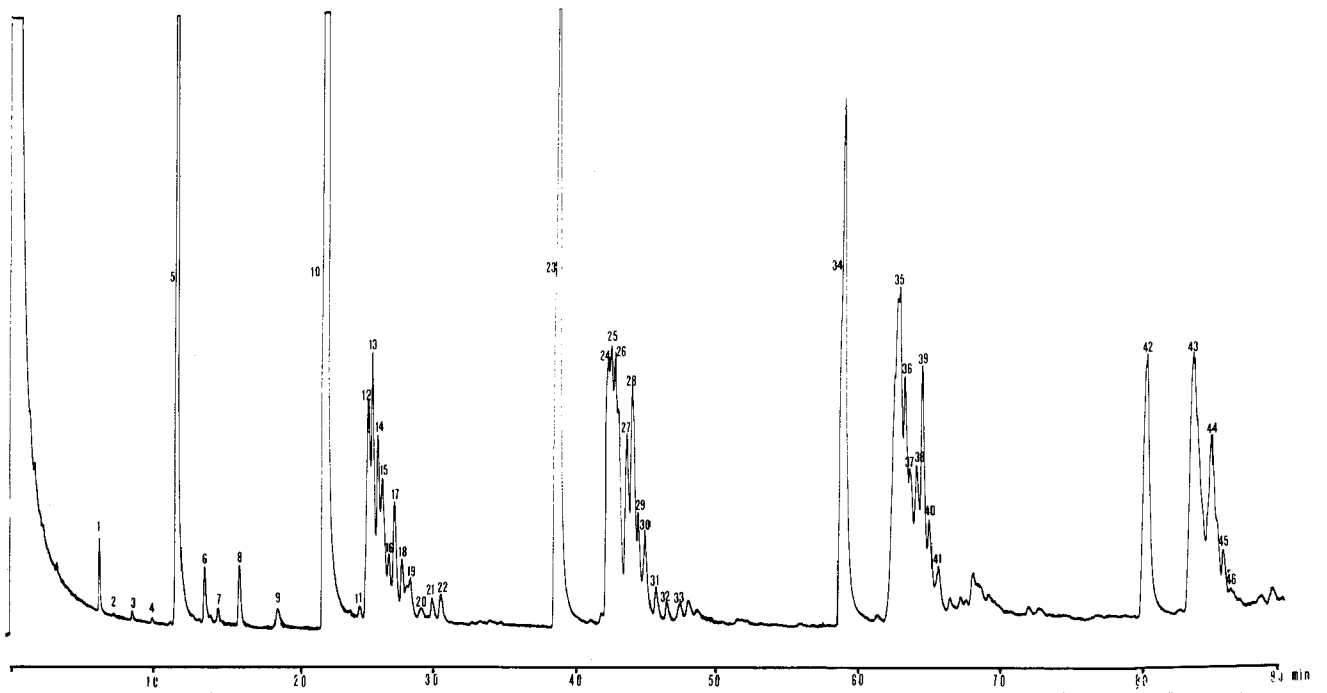

FIG. 1. Typical gas liquid chromatographic pattern of partially hydrogenated herring oil fatty acid methyl esters. Silar 10 CP coated 50 m, 0.3 mm ID glass capillary column, operated in a Varian Model 3700 at 150 C $\xrightarrow[30\ \text{min}]{\text{isot.}}$ $\xrightarrow{0.5\ \text{C/min}}$ 185 C $\xrightarrow[10\ \text{min}]{\text{isot.}}$ and N_2 flow 0.45 ml/min. Peak numbers correspond to the numbers in Table I.

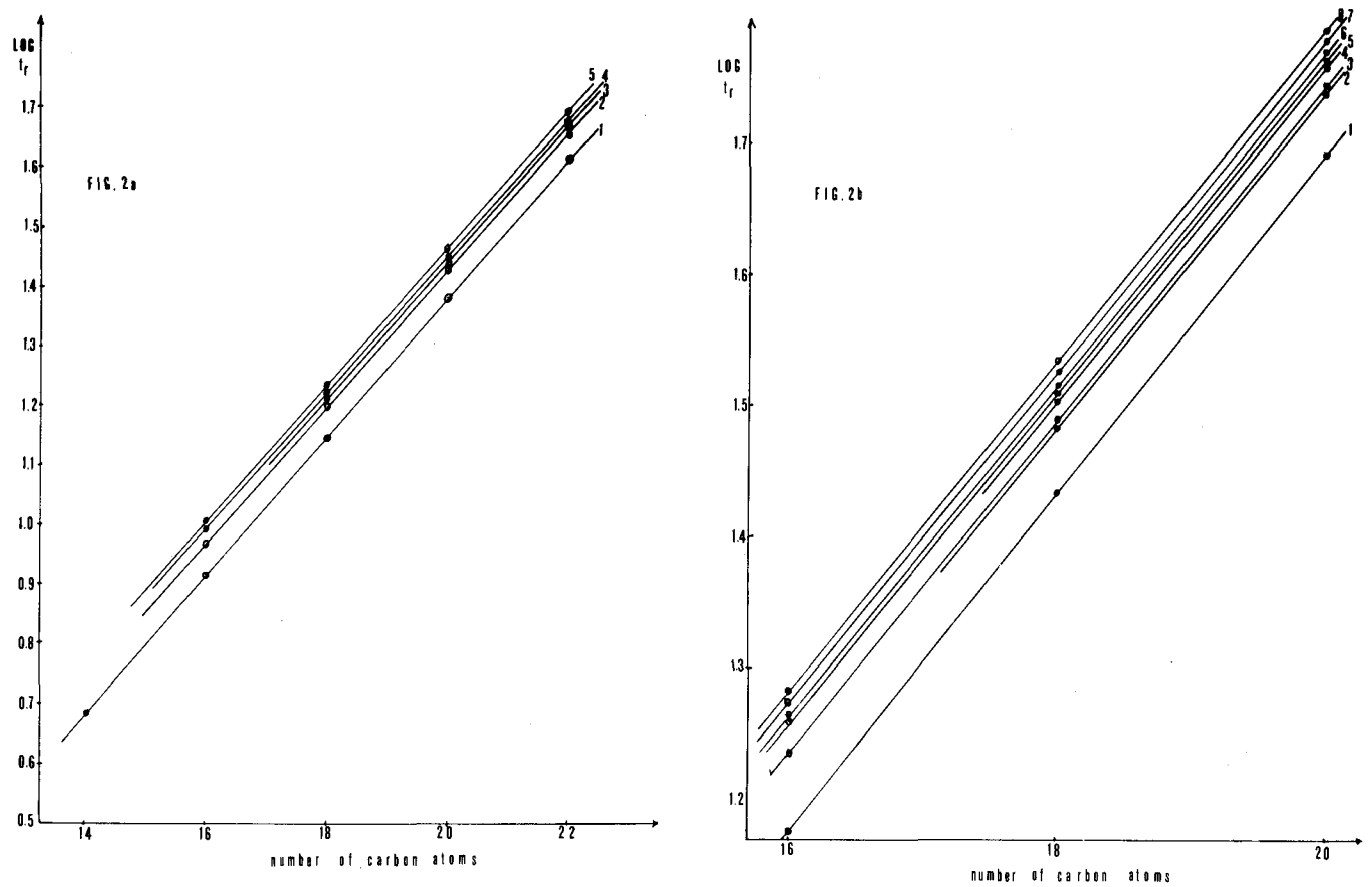

FIG. 2. Plots of log t_r vs. number of carbon atoms for different fatty acid methyl esters; t_r = adjusted retention time. (a) At 175 C; 1 = n-fatty acids, 2 = monoene ω9*trans*, 3 = monoene ω11*cis*, 4 = monoene ω9*cis*, 5 = monoene ω7*cis*. (b) At 160 C; 1 = n-fatty acids, 2 = monoene ω9*trans*, 3 = monoene ω7*trans*, 4 = monoene ω9*cis*, 5 = monoene ω8*cis*, 6 = monoene ω7*cis*, 7 = monoene ω6*cis*, 8 = monoene ω5*cis*.

TABLE I

ECL Values[a] and Fatty Acid Composition of a Partially Hydrogenated Herring Oil

Component[b]		%[c]	ECL 160 C	ECL 175 C	Component[b]	%[c]	ECL 160 C	ECL 175 C
C12:0	(1)[d]	0.4	12.00	12.00	C18:1 ω6c	(31) 0.5	18.73	18.80
	(2) <	0.1	12.48	12.48	C18:1 ω5c	(32) 0.2	18.80	18.88
C13:0	(3)	0.1	13.00	13.00	C19:0	(33) 0.2	19.00	19.00
	(4) <	0.1	13.47	13.48	C20:0	(34) 6.9	20.00	20.00
C14:0	(5)	6.2	14.00	14.00	C20:1 ω10tr			
	(6)	0.4	14.49	14.48	C20:1 ω 9tr	(35) 6.6	20.37	20.43
	(7)	0.1	14.68	14.62	C20:1 ω 7tr	(36) 2.4	20.42	20.46
C15:0	(8)	0.4	15.00	15.00	C20:1 ω11c	(37) 1.5	20.50	20.56
	(9)	0.2	15.48	15.46	C20:1 ω10c	(38) 1.5		20.60
C16:0	(10)	14.5	16.00	16.00	C20:1 ω 9c	(39) 2.5	20.53	20.64
	(11)	0.1	16.26	16.27	C20:1 ω 8c	(40) 1.0	20.56	20.70
C16:1 ω 8tr	(12)	2.0	16.46	16.51	C20:1 ω 7c	(41) 0.5	20.61	20.76
C16:1 ω 7tr	(13)	2.3			C22:0	(42) 4.4	22.00	22.00
C16:1 ω 6tr	(14)	1.8	16.52	16.57	C22:1 ω11tr			22.36
C16:1 ω 5tr	(15)	1.2			C22:1 ω10tr	(43) 6.9		
C16:1 ω 8c	(16)	0.7	16.65	16.68	C22:1 ω 9tr			22.42
C16:1 ω 7c	(17)	1.3	16.70	16.76	C22:1 ω11c	(44) 3.9		22.49
C16:1 ω 6c	(18)	0.6	16.77	16.83	C22:1 ω10c			
C16:1 ω 5c	(19)	0.3	16.84	16.89	C22:1 ω 9c	(45) 0.7		22.57
	(20)	0.2	16.90		C22:1 ω 7c	(46) 0.1		22.63
C17:0	(21)	0.3	17.00	17.00				
	(22)	0.3	17.07					
C18:0	(23)	8.2	18.00	18.00				
C18:1 ω10tr	(24)	2.8	18.39					
C18:1 ω 9tr	(25)	3.1	18.41	18.46				
C18:1 ω 8tr	(26)	2.9	18.44					
C18:1 ω11c	(27)	1.9	18.51					
C18:1 ω10c			18.56	18.60				
C18:1 ω 9c	(28)	3.9	18.58	18.66				
C18:1 ω 8c	(29)	0.9	18.61					
C18:1 ω 7c	(30)	1.0	18.67	18.73				

[a] From at least three measurements, Δ ECL = ±0.02. ECL = equivalent chain length.
[b] tr = *trans*, c = *cis*.
[c] Mean area %, relative standard deviation 5-20% for individual fatty acid methyl esters.
[d] The numbers in parentheses correspond to the numbers in Figure 1.

Laboratories, Inc., State College, PA); coating efficiency[1] CE=73% and the number of theoretical plates, n = 143,000 for linoleic acid methyl ester at 170 C. The oven temperature was kept at 160 C or 175 C, or it was programmed from 150 C to 185 C, heating first isothermally at 150 C for 30 min, then 0.5 C/min up to 185 C, with the temperature maintained for 10 min. The splitting ratio was 1:25 for a 0.2-0.3 μl sample in heptane. The injector and detector temperatures were 250 C, and the flow rate of the nitrogen carrier gas was 0.45 ml/min.

RESULTS AND DISCUSSION

The separation of fatty acid methyl esters of a partially hydrogenated herring oil on a Silar 10 CP coated glass capillary column in temperature-programmed run is shown in Figure 1. Linear plots of log t_r versus number of carbon atoms for different fatty acid methyl esters (Fig. 2.) were obtained when column was operated isothermally. This information was used for further identification of some less common isomers. The quantitative results obtained from the peak areas of the temperature-programmed run and the equivalent chain length (ECL) values from the corresponding isothermal runs are given in Table I.

The same elution order of fatty acid methyl esters normally observed on colums coated with polar cyanopropylsiloxane phases (10,19) was also detected in these systems. The *trans* isomers eluted before *cis*, and the retention times of positional isomers increased with decreasing "ω"-values. The separation of *cis/trans* isomers is 0.15-0.3 ECL units, being the largest for C_{16} monoenes and the least for C_{22} monoenes. The positional isomers of C_{16} monoenes are also best resolved, and major peaks corresponding to add as well as even "ω"-values are visible. All the major isomers of C_{18} and C_{20} monoenes can also be detected at least as shoulders, whereas for the C_{22} monoenes only the major peaks with odd "ω"-values are separated. In general the isomers with low "ω"-values [7,6,5] separate better than the ones with high "ω"-values [9,10, or 11]. If the sample contains mainly the isomers with odd "ω"-values (for instance cod liver oil), the separation between positional isomers is at least 0.04 ECL units which is considered to be sufficient for accurate analysis (18).

The quantitative results obtained from this analysis agree well with the results obtained by using different techniques (5-8). The symmetrical distribution of the double bond position around a maximum at ω7 for C_{16} monoenes, ω9 for C_{18} and C_{20} monoenes, and ω11 for C_{22} monoenes, agrees with the results of Lambertsen et al. (6). The small amount of erucic acid (C22:1ω9*cis*) observed is also in agreement with earlier findings (5). In addition, the analysis shows that the partially hydrogenated herring oil studied has 58% of its monethylenic fatty acids as *trans* isomers and 42% as *cis* isomers. Hydrogenation of polyethylenic fatty acids to corresponding monoethylenic or saturated compounds is also quite complete since only traces are present for instance from C18:2 dienes which have retention times of 50-55 min under the conditions used to record Figure 1.

In conclusion, it can be said that his method is a convenient and accurate method in determining the isomeric monene distribution in catalytically hydrogenated marine and vegetable oils. It can be also used for determination of the erucic acid content of these oils which is currently done by measuring the total amount of C22:1 isomers and assuming that most of it corresponds to erucic acid. With rapeseed oil this type of analysis is generally correct (20), but for marine oils (another source of docosenoic acid in the human diet) the separation of different C22:1 isomers is necessary. The superiority of cyanopropylsiloxane phases in separating fatty acid methyl esters seems obvious, since even the more efficient capillary columns coated with BDS (17) or FFAP (21) do not give good resolution. Provided that technical problems in coating capillaries with Silar 10 CP or SP 2340 can be solved, the laborious ozonization technique may be totally replaced by capillary gas liquid chromatography.

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[1]The following equations were used (16):

$$CE = \frac{n}{n_{max}} 100\%; \quad n = 5.5413\left(\frac{t_r}{w_{1/2}}\right)^2; \quad n_{max} = \frac{1000\ L}{HEPT_{min}}$$

t_r= actual retention time; $w_{1/2}$= peak width from half height; $HEPT_{min}$= height equivalent to theoretical plate; L = column length.

[2]The ECL values were calculated by the following relationship (17,18):

$$ECL = n + (\log t_{r,x} - \log t_{r,n})/(\log t_{r,n+1} - \log t_{r,n})$$

t_r = adjusted retention time; x refers to an unknown fatty acid which eluted between the normal saturated fatty acids with n and n + 1 carbon atoms.