Studies on Monoene Fatty Acid Isomers in Hydrogenated Fish Oils[1]

GEORG LAMBERTSEN, HAKON MYKLESTAD and **OLAF R. BRAEKKAN**
Government Vitamin Laboratory, P.O. Box 187, 5001 Bergen, and Institute of Physiology, University of Bergen, Norway

ABSTRACT

An analytical study of the geometrical and positional isomerisation of the monoenoic acids of partially hydrogenated fish oil is presented. The results showed that the monoene fatty acids of chain lengths 16, 18, 20 and 22 consisted of 75% in the *trans*-form and 25% in the *cis*-form. The double bonds were distributed symmetrically over the chain length, with well defined maxima in position Δ-9 for the fatty acids of chain length 16 and 18, and in position Δ-11 for the fatty acids of chain length 20 and 22. *Trans*- and *cis*-isomers showed the same positional distribution. Geometric as well as positional isomerization seemed to have reached an equilibrium state in the sample investigated.

INTRODUCTION

Fish oils produced by reduction of whole fish of the species herring, mackerel, capelin and some of the smaller species of the cod family from the North Atlantic area are used as raw material for the production of margarine. Lambertsen and Braekkan (1) determined the fatty acid composition of selected types of these fish oils and found a content of 45-65% of monoene fatty acids of the chain lengths 16, 18, 20 and 22 with each acid representing 5% to 20% of the total oil. Similar findings are reported by Ackman et al. (2,3) for species caught off the Canadian east coast. After industrial hydrogenation of these oils to melting points between 30 C and 40 C the percentages remain approximately the same, whereas polyenes decrease and saturates increase proportionally (4). The monoene fatty acids are, however, extensively isomerized as to configuration and position of the double bonds. The theory behind these conversions has been discussed by Dutton (5). The present paper reports results from an analytical study of the content of such isomers in hydrogenated fish oils. The methods are those generally applied for the analysis of hydrogenated vegetable oils.

METHODS

An outline of the analytical procedure is given in Figure 1.

Five grams of a refined and hydrogenated fish oil (melting point 38 C) were saponified, the fatty acids isolated and methylated in methanol containing BF_3. Details of these steps were reported in a previous publication (4). The methyl esters were analysed for fatty acid composition by gas liquid chromatography (GLC) and the results corresponded well with the findings previously reported for this type of fat.

The methyl esters were fractionated into groups by partition chromatography on columns 9 x 1000 mm. The stationary phase was silanized celite (Kieselguhr Merck) saturated with a purified paraffin of boiling point 200 C. The eluent was acetonitril-methanol (85:15) saturated with the paraffin. On this column, 200-400 mg could be separated and 100-200 fractions of 1 ml each were collected in a fraction collector. The fractions were checked by thin layer chromatography (TLC) and corresponding fractions were pooled. In a few cases fractions were rechromatographed to obtain better purity.

The four fractions (Fig. 1) were each further fractionated by preparative argentation TLC. Glass plates (20 x 20 cm) were prepared with Silica Gel G (Merck) containing 25% silver nitrate. The methyl esters were spotted along the base line. Each plate was loaded with 15-20 spots each containing 0.8 to 1 mg methyl esters. The plates were developed with 15% ethyl ether in hexane. After visualizing the spots with 0.01% 5,6-dichlorofluorescein in water, the *trans*- and *cis*-fractions were scraped off and extracted from the silica gel with ethyl ether and purified by chromatography through small columns of silica gel. By this procedure eight fractions weighing from 20 to 135 mg were collected, corresponding to the *trans*- and *cis*-monoenes of the chain lengths 16, 18, 20 and 22 carbon atoms. The total

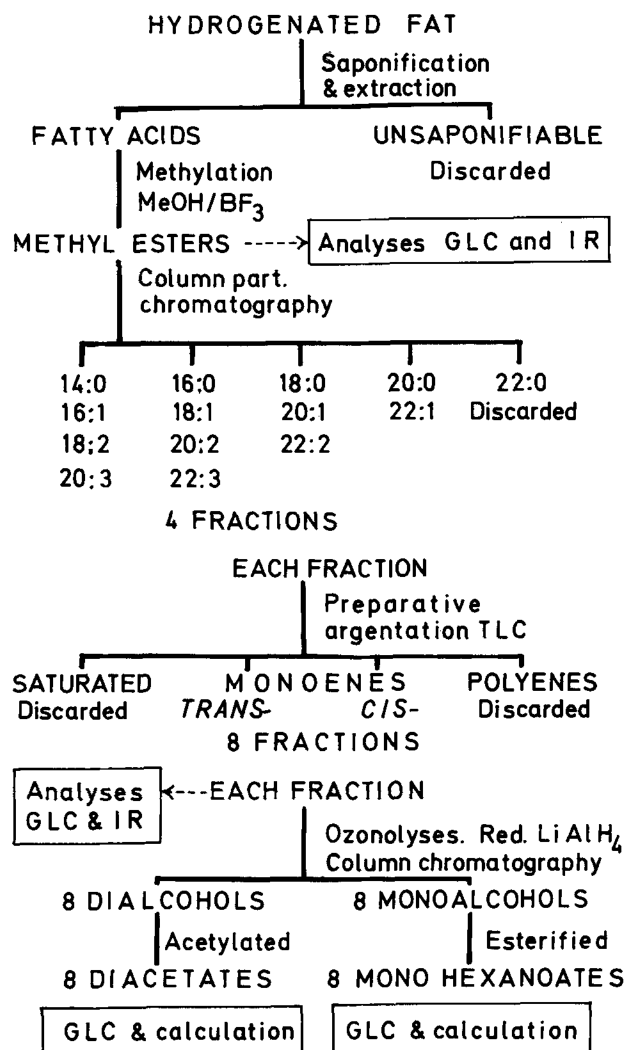

FIG. 1. Analytical procedure.

[1]Presented at the ISF-AOCS World Congress, Chicago, October 1970.

TABLE I

Relative Peak Areas From GLC of Diacetates From Monoenoic Acids in Hydrogenated Fish Oil Cleaved by Reductive Ozonolysis[a]

Chain length of monoenoic acid	Geometric isomer	Chain length of dialcohol									
		7	8	9	10	11	12	13	14	15	16
16	*cis*	15	60	100	80	52	27	16	10		
	trans	10	56	100	90	54	38	21	12		
18	*cis*	18	60	100	94	80	55	36	25		
	trans	12	67	100	98	88	73	45	--		
20	*cis*	14	37	61	78	100	71	42	25	13	8
	trans	8	18	46	73	100	73	47	29	20	13
22	*cis*		20	49	77	100	67	41	20	13	9
	trans		12	30	68	100	76	43	24	16	12

[a]Maximal peak area, 100.

weight of the fractions was 500 mg.

The final fractions, as well as the four fractions before TLC, were dissolved in hexane and their *trans* content determined in an Unicam SP 200 G IR-Spectrophotometer. The *trans* peak was measured at 965 cm^{-1} and calculated compared with the carbonyl peak at 1720 cm^{-1}. Mixtures of methyl esters of elaidic acid and oleic acid in graded proportion were used as reference. In a recent publication Allen (6) has applied the same principle. All eight fractions were further analyzed for their contents of monoene fatty acids of each chain length by GLC.

The methyl esters in the eight fractions were cleaved at the double bond by means of reductive ozonolysis. To the fractions in pentane was added a saturated solution of ozone in pentane (7). The ozonides were reduced by adding a small amount of solid lithium aluminium hydride, and the complexes hydrolyzed by careful addition of dilute sulfuric acid. This hydrolysis was carried out at a temperature around 0 C. The mixture was diluted with water and the mono- and dialcohols extracted with ethyl ether. Hexane was added to this extract to give a final mixture of ethyl ether-hexane 20:80, followed by chromatography through a small column of alumina which retained the dialcohols giving a solution of the monoalcohols. The dialcohols were subsequently eluted with pure ethyl ether.

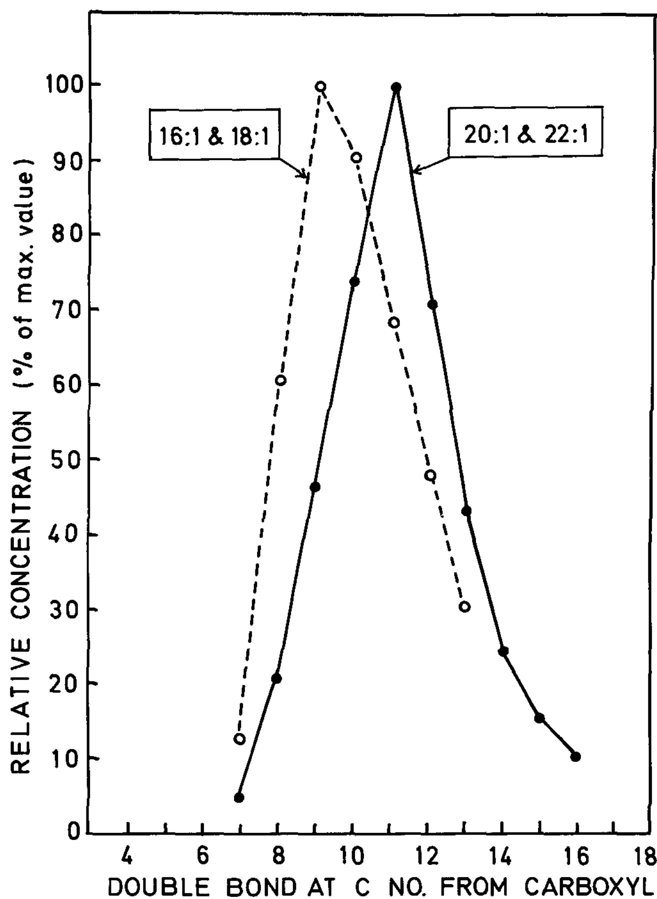

FIG. 2. Double bond positions in monoene acids of hydrogenated fish oils from dialcohol GLC. Mean concentrations for *cis* and *trans*-isomers.

The eight dialcohol fractions were acetylated by acetic anhydride in purified pyridine and dissolved in hexane.

The eight monoalcohol fractions were esterified with hexanoyl chloride in pyridine, and the resulting hexanoates purified and dissolved in hexane.

These final 16 ester fractions were analyzed by GLC in a Perkin Elmer 900 Gas Chromatograph. All separations were carried out on 6 ft 1/8 in. stainless steel columns with 80-100 mesh silanized celite to which was added 15% butanediol succinate polyester as a stationary phase. The temperatures were monitored to obtain a 45 min run on each type of fraction, usually around 170-190 C. All GLC runs were isothermal to give exact "semilog plots" for chain length identification. Weight percentages were calculated from peak areas as height x width at half height.

RESULTS AND DISCUSSION

To achieve fractions of sufficient quantities for the subsequent ozonolyses two major chromatographic steps had to be used. The first of these, the column partition chromatography, does not provide a complete separation according to the scheme outlined in Figure 1. In our experience a purity around 80% was achieved but quantities up to 500-1000 mg could be prepared within a few days. The recently introduced high pressure columns may give improved separation if applied to this problem.

The agentation chromatographic step is mostly used to separate according to unsaturation. Our problem was to achieve a separation between *cis*- and *trans*- isomers. Attempt to apply columns to this operation failed to give a good enough separation and TLC was applied. The high load of 25% silver nitrate to the silica gel gave a sufficiently clear separation of *cis*- and *trans*- monoenes (8). As determined by GLC, 80.9% monoenoic acids together with 12.8% saturated acids and 6.3% dienoic acids could be recovered totally. Small amounts of saturated acids of chain lengths 14 and partly 16 had moved with the *trans* acids but were not considered since they did not ozonolyse. A part of the dienoic acids moved with the *cis*-acids and resulted in a high *trans* value for these acids. A calculation of this value taken together with the gas chromatographic results showed that they were *trans-trans* dienes of the next higher chain length. UV absorption of the fraction only showed the presence of minor amounts of conjugated

double bonds. This systematic error could only be observed and taken into consideration. Summing up, the monoenoic acid analyses gave the values. 6.4% 16:1, 17.4% 18:1, 11.5% 20:1 and 11.1% 22:1, sum 46.4%. This result corresponded well with the original GLC analysis of the fat.

The percentage of *trans*-isomers in the monoene fractions could be measured by two different methods. First, the *trans* values were measured by IR spectrophotometry. A value of 75.4 ± 3% was found for the four chain lengths. Secondly, trans values could be calculated from the gas chromatographic data of the eight fractions. By this method a value of 74.4 ± 1.2% was found for the four chain lengths. As these variations are within the analytical accuracy of the methods, it may be concluded that the hydrogenated marine fat studied had 75% of its monoenoic acids as *trans* isomers and 25% as *cis* isomers, regardless of chain length. These results are within the equilibrium values found by previous investigators, from 2:1 for oleic acid-elaidic acid up to 7:1 for hydrogenated vegetable oils (9).

Ozonolysis of the double bond is the preferred method for establishing its position (7). Usually the fragments are determined after reduction to aldehydes. Our working routine and the number of different fractions made it necessary to consider more stable fragments than the aldehydes. Complete reduction with lithium aluminum hydride gives the corresponding alcohols and further a reduction of the ester groups takes place. Thus a homologous set of *n*-1-alcohols and a corresponding homologous set of *n*-1-ω-dialcohols are obtained. These groups were easily separated by column chromatography on alumina followed by esterification to obtain suitable and stable compounds for GLC analysis. The diacetates gave very clear series of peaks. Relative values for the chainlengths of these alcohols are given in Table I. The results for *cis*- and *trans*-isomers were closely related and one set from chain lengths 16 and 18 and one from 20 and 22 was averaged and are shown in Figure 2. The figure shows an even distribution of the double bond position around a maximum at Δ-9 for the two lower chain lengths and at Δ-11 for the two higher chain lengths. This corresponds to the ω-positions 7, 9, 11, 13 for respectively chain lengths 16, 18, 20, and 22. Ackman and Castell (10) have determined the positions of the double bond in monoenoic acids of herring oil, and found the same pattern for the major isomers.

The monoalcohol esters showed some losses and additional peaks in the chromatograms. This made calculations for the monoalcohols less precise but, at least for the chain lengths 20 and 22, the same distribution as for the dialcohols could be established.

The even distribution of the double bond position after hydrogenation corresponds with the results of Scholfield et al. (11) for the *trans* acids. The results with regard to geometric as well as positional isomerization indicate that an equilibrium state has been reached during the hydrogenation of the sample investigated. A recent report by Hølmer and Aaes-Jørgensen (12) gives results from the analysis of partially hydrogenated herring oil. Their values correspond to the present findings with regard to the chain length composition of monoenes, but their *trans* values range from 21 to 49 F%. This may reflect differences in the processing of the oil, resulting in less extensive isomerization.

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