Isomers of Monoethylenic Fatty Acids in Some Partially Hydrogenated Marine Oils

H.B.S. CONACHER, B.D. PAGE and **R.K. CHADHA**, Research Laboratories,
Health Protection Branch, Department of National Health and Welfare,
Ottawa, Ontario, Canada

ABSTRACT

An analytical study of the monoethylenic isomers in commercial samples of partially hydrogenated herring, whale and seal oils is presented. The results show that with hydrogenated herring oil there is a slight decline in monoene *trans* content from 37% in C_{16} through to 32% in C_{22}. With both whale and seal oils, monoene *trans* contents were constant at 54% and 59%, respectively, throughout all chain lengths. In general the *cis* and *trans* positional isomers from hydrogenated whale and seal oils were more scattered than those from hydrogenated herring oil; however in each oil the major *cis* isomers of each chainlength were indicative of original *cis* fatty acid isomers in the raw oils.

INTRODUCTION

In conjunction with nutritional studies in our laboratories on partially hydrogenated oils containing C_{20} and C_{22} monoenoic acids (1,2), it was of interest to determine, as far as possible, the detailed fatty acid composition of these oils.

In a recent report (3) we described the experimental techniques developed and the results obtained in a study of the monoenoic isomers in rapeseed and partially hydrogenated rapeseed oils. We now present data on the monoenoic isomers in partially hydrogenated herring, whale and seal oils.

Although there is information on the monoethylenic isomers present in raw herring (4), and whale and seal oils (5), only herring has been examined in detail after partial hydrogenation (6,7). Data have also been reported on the isomers present in hydrogenated fish oils (8) and in a commercial margarine based on a hydrogenated marine oil (9).

MATERIALS AND METHODS

Samples of partially hydrogenated herring (HHO, IV 76), whale (HWO, IV 78) and seal (HSO, IV 84) oils were provided by Canada Packers Ltd. Methyl esters of each oil were separated by preparative gas liquid chromatography (prep GLC) into five fractions of chainlengths 14, 16, 18, 20 and 22; small amounts of odd and branched chain acids being incorporated with the previous major fraction.

TABLE I

General Fatty Acid Composition of Partially Hydrogenated Herring, Whale and Seal Oils

	(Area % Composition[b])				
		Monoene			
Fraction[a]	Saturated[c]	*trans*	*cis*	Polyene	IR[d]
Hydrogenated herring oil					
14 (7.4)	95.0		5.0	—	—
16 (19.2)	60.5	14.8	24.7	—	15.1
18 (17.5)	14.0	26.2	48.8	11.0	31.5
20 (23.9)	8.5	27.6	55.6	8.3	31.0
22 (31.0)	6.4	29.5	64.1	—	33.0
			trans (Original)		27.9%
			trans (Summed)		26.4%[e]
Hydrogenated whale oil					
14 (6.0)	92.0		8.0	—	—
16 (25.8)	60.0	21.6	18.4	—	22.6
18 (36.9)	15.5	43.5	36.5	4.5	45.2
20 (16.2)	6.2	36.4	31.6	25.8	73.4
22 (14.6)	3.8	25.5	22.3	48.4	102.4
			trans (Original)		50.2%
			trans (Summed)		49.4%
Hydrogenated seal oil					
14 (4.8)	90.0		10.0	—	—
16 (27.5)	38.7	36.1	25.2	—	33.9
18 (30.7)	15.5	50.0	34.5	—	48.3
20 (22.0)	6.3	40.3	28.7	24.7	71.7
22 (15.0)	4.2	25.3	16.3	54.2	101.8
			trans (Original)		54.7%
			trans (Summed)		55.2%

[a]Figures in parentheses after chainlengths are area % composition on SE30 column. Small amounts of odd and branched chain acids are incorporated with previous major fraction. HHO and HWO also contain 1% and 0.5% C_{24}.

[b]From preparative gas liquid chromatography fractions. Saturated-monoene-polyene from gas liquid chromatography (DEGS); *cis-trans* from argentation thin layer chromatography.

[c]Incorporates branched and odd chain acids.

[d]*trans* content from IR measurements.

[e]*trans* summed = (19.2 x 15.1) + (17.5 x 31.5) + (23.9 x 31.0) + (31.0 x 33.0) = 26.4%.

TABLE II

Composition of Raw and Partially Hydrogenated Herring, Whale and Seal Oils[a]

	HO[b]			WO			SO			HHO			HWO			HSO		
Fraction chainlength	S[c]	M	P	S	M	P	S	M	P	S	M	P	S	M	P	S	M	P
14	7	—	—	7	1	—	6	1	1	7	1	—	6	—	—	5	—	—
16	12	9	2	15	12	—	10	19	1	12	7	—	16	10	—	11	17	—
18	1	13	4	3	32	3	1	26	2	3	13	2	6	29	2	5	26	—
20	—	15	8	—	10	4	—	12	7	2	20	2	1	11	4	1	15	5
22	—	23	6	—	7	6	—	4	11	2	29	—	1	7	7	1	6	8
Total	20	60	20	25	62	13	17	62	21	26	70	4	30	57	13	23	64	13

[a]Data for raw herring oil summarized from Ackman and Eaton (10), and for raw whale and seal oils from Ackman et al. (5).
[b]HO = Herring oil, WO = whale oil, SO = seal oil; HHO, HWO and HSO = above oils partially hydrogenated.
[c]S = Saturates, M = monoenes, P = polyenes.

TABLE III

Isomers of Major Monoenoic Acids in Hydrogenated Herring Oil

	Composition of aldehyde-esters, mole %										
Fraction	5	6	7	8	9	10	11	12	13	14	15
16 (7.6%)[a]											
trans (37%)[b]	1	12	4	14	45	14	6	3	1	—	—
cis (63%)	—	2	3	5	80	5	4	1	—	—	—
18 (13.2%)											
trans (35%)	—	—	5	11	40	17	17	7	3	—	—
cis (65%)	—	—	6	3	66	3	16	3	3	—	—
20 (19.9%)											
trans (33%)	—	—	—	3	9	15	50	15	5	2	1
cis (67%)	—	—	—	1	10	4	75	5	5	—	—
22 (29.0%)											
trans (32%)	—	—	—	—	2	15	57	17	6	2	1
cis (68%)	—	—	—	—	2	3	82	4	9	—	—

[a]Per cent in parentheses after fractions refers to monoene content of total oil.
[b]Per cent in parentheses after *trans* and *cis* refers to per cent within monoene fractions.

Fractions were subsequently studied by gas liquid chromatography (GLC) and infrared spectroscopy (IR); separated by argentation thin layer chromatography (Ag^+/TLC), and geometric isomers of monoenes examined by micro-ozonolysis. These techniques were as described previously (3), except that prep GLC fractionations were performed with manual temperature programing from 185C to 220C.

RESULTS AND DISCUSSION

The general fatty acid compositions of the oils under investigation are summarized in Table I. These values for chainlength composition fall in the same range as those reported previously for liquid (10) and hydrogenated herring oil (7) and for liquid whale and seal oils (5). The essential differences between the three oils are the relatively high proportion of longer chain acids ($C_{20} + C_{22}$) in HHO (54.9%) compared to that in HWO (30.8%) and HSO (37.0%), together with the correspondingly higher levels of shorter chain acids ($C_{16} + C_{18}$) in the whale and seal oils.

For each oil the total *trans* content, calculated by summation of IR measurements of each fraction, shows excellent agreement with that of the original methyl esters. Also, in those fractions of polyene content less than 5%, there is good correlation between total *trans* content measured by IR and monoene *trans* content (Ag^+/TLC). In fractions of higher polyene content, the IR *trans* values are considerably higher than those from Ag^+/TLC, indicating significant *trans* contribution from polyenes. From a consideration of the amounts of polyene involved, it is

TABLE IV

Isomers of Major Monoenoic Acids in Hydrogenated Whale Oil

	Composition of aldehyde-esters, mole %											
Fraction	5	6	7	8	9	10	11	12	13	14	15	16
16 (10.3%)[a]												
trans (54%)[b]	2	6	6	19	44	16	4	3	—	—	—	—
cis (46%)	—	2	6	12	67	8	3	2	—	—	—	—
18 (29.5%)												
trans (54%)	—	1	5	14	40	18	13	6	3	—	—	—
cis (46%)	—	—	6	9	57	9	15	3	1	—	—	—
20 (11.0%)												
trans (54%)	—	—	1	5	12	15	39	15	7	3	2	1
cis (46%)	—	—	1	4	16	8	53	8	6	3	1	—
22 (7.0%)												
trans (53%)	—	—	—	1	5	15	47	19	9	4	—	—
cis (47%)	—	—	—	—	5	10	62	9	11	3	—	—

[a]Per cent in parentheses after fractions refers to monoene content of total oil.
[b]Per cent in parentheses after *trans* and *cis* refers to per cent within monoene fractions.

TABLE V

Isomers of Major Monoenoic Acids in Hydrogenated Seal Oil

Fractions	Composition of aldehyde-esters, mole %										
	5	6	7	8	9	10	11	12	13	14	15
16 (16.8%)[a]											
trans (59%)[b]	—	3	5	18	46	19	6	2	1	—	—
cis (41%)	—	1	5	11	65	13	4	1	—	—	—
18 (25.9%)											
trans (59%)	1	4	8	14	31	17	14	7	3	1	—
cis (41%)	—	2	12	8	46	9	16	4	3	—	—
20 (15.2%)											
trans (58%)	—	—	3	5	11	17	39	15	7	3	—
cis (42%)	—	—	1	6	14	12	49	10	6	2	—
22 (6.2%)											
trans (61%)	—	—	3	5	8	13	29	17	14	7	4
cis (39%)	—	—	3	4	5	8	41	12	19	5	3

[a]Per cent in parentheses after fractions refers to monoene content of total oil.

[b]Per cent in parentheses after *trans* and *cis* refers to per cent within monoene fractions.

evident that some polyenes must contain more than one *trans* double bond per molecule. These polyenes were not investigated further.

The composition of each oil in terms of saturates, monoenes and polyenes in each fraction is given in Table II. Although the raw oils were not available for comparison, previous analyses of 12 herring (10) and three, each, of whale and seal oils (5) have given the average values also included in this table. Comparison of these data would indicate that only 7-8% of the original or newly formed monoenes and polyenes are fully hydrogenated under the commercial hydrogenation conditions employed. It can also be observed that in HHO there is considerable reduction in polyene content, whereas with both HWO and HSO significant amounts of polyene are still present. These results probably reflect differences in the processing of each oil.

The proportions of monoethylenic isomers present in each oil are shown in Tables III, IV and V. It can be observed with HHO (Table III) that there is a slight decline of percentage *trans* from the C_{16} monoene (37%) through to the C_{22} monoene (32%). Similar observations have been made previously with a partially hydrogenated herring oil (7). These results are somewhat unusual, as it might have been predicted that the C_{20} and C_{22} monoenes in this oil would contain higher *trans* percentages than the C_{16} and C_{18} monoenes, since the former fractions in raw herring oils (Table II) contain most of the polyunsaturates. However previous studies on a partially hydrogenated rapeseed oil (3) have indicated that isomerization of *cis* monoenes to *trans* isomers may not be independent of chainlength and, neglecting *cis* and *trans* formation by polyene reduction, that isomerization may occur in the order $C_{18} > C_{20} > C_{22}$. Thus in HHO we have the apparent case where polyene contribution to *trans*, in the long chain fractions, is offset by a reduced monoene contribution, resulting in slightly less *trans* percentage in these fractions.

In both HWO and HSO, the monoene *trans* percentages are relatively constant at 54% and 59%, respectively throughout all chainlengths. With these two oils predictions and observations are more difficult to correlate, since high amounts of polyene remain. Nevertheless, with HSO, the C_{22} monoene *trans* percentage is slightly higher (61%) than that of other chainlengths, as would be predicted from comparison of the raw and hydrogenated oil data given in Table II.

Examination of the *cis* monoethylenic isomers in each chainlength shows that in general these isomers are more scattered in HWO and HSO than in HHO. In the latter oil the main isomer generally represents 70-80% of each monoene fraction, whereas in whale and seal oils it approximates only 40-65%. Also, as found previously with hydrogenated rapeseed oil, the major *cis* isomers in the various fractions are indicative of monoenoic isomers in the raw oils. These isomers, summarized in Table VI, are in agreement with those reported for herring (4), whale and seal oils (5).

In all cases the *trans* isomers are more widely scattered than corresponding *cis* isomers. The major isomerizations are similar to those found previously in partially hydrogenated rapeseed oil, viz., a conversion from original *cis* monoene to *trans* with the double bond retaining its position, accompanied by the formation of *trans* isomers on either side of the original double bond position.

The one apparent anomaly in these data is the relatively high percentage of a *trans* hexadec-6-enoic acid in HHO (Table III), compared to that of the *cis* isomer. This could indicate the presence of this acid in the raw herring oil. A similar acid has been characterized in sea turtles (11).

The values presented in Tables III, IV and V permit the percentage of any isomer to be calculated. Thus the major C_{22} *cis* monoenoic acid in HHO represents 82% x 68% x 29.0% = 16.4% of the total methyl esters. Similarly the major C_{22} *cis* monoenes in HWO and HSO are 2.0% and 0.9%, respectively.

TABLE VI

Possible Monoethylenic Isomers in Raw Herring, Whale and Seal Oils

Monoenoic acid chainlength	Isomer[a]		
	Hydrogenated herring oil	Hydrogenated whale oil	Hydrogenated seal oil
16	9	9	9
18	9,11,7	9,11	9,11,7
20	11,9,13	11,9	11,9
22	11,13	11,13	11,13

[a]Isomers presented in order of decreasing magnitude. Figures refer to position of double bond from carboxyl group.

REFERENCES

1. Beare-Rogers, J.L., E.A. Nera and H.A. Heggtveit, Can. Inst. Food Technol. 4:120 (1971).
2. Beare-Rogers, J.L., "Cardiac Lipids in Rats and Gerbils Fed Oils Containing C_{22} Fatty Acids," AOCS Meeting, Atlantic City, October 1971, Abstr. 75.
3. Conacher, H.B.S., and B.D. Page, JAOCS 49:283 (1972).
   Ackman, R.G., and J.D. Castell, Lipids 1:341 (1966).
5. Ackman, R.G., S. Epstein and C.A. Eaton, Comp. Biochem. Physiol. 40B:683 (1971).
6. Holmer, G., and E. Aaes-Jorgensen, Lipids 4:507 (1969).
7. Ackman, R.G., S.N. Hooper and J. Hingley, JAOCS 48:804 (1971).
8. Lambertsen, G., H. Mykelstad and O.R. Braekkan, Ibid. 48:389 (1971).
9. Ackman, R.G., S.N. Hooper and J. Hingley, J. Chromatogr. Sci., in press.
10. Ackman, R.G., and C.A. Eaton, J. Fish. Res. Bd. Can. 23(7):991 (1966).
11. Hooper, S.N., and R.G. Ackman, Lipids 5:288 (1970).

[Received May 26, 1972]