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Constituents of human mother milk VI: Non-methylene interrupted cis, cis-octadecadienoic acids

by U. Murawski, H. Egge, P. György, and F. Zilliken

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Inhaltstoffe der Frauenmilch VI: Nicht methylenunterbrochene cis,cis-Octadecadiensäuren.

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SUMMARY

Human Milk, Octadecadienoic Acids, Hydroxylation, Trimethylsilylation

The non methylene interrupted *cis,cis*-octadecadienoic acids from human milk fat were prepared by thin layer chromatography on $AgNO_3$ impregnated silicagel plates. The position of double bonds was determined by hydroxylation of the double bonds with OsO_4 followed by silylation. The corresponding O-trimethylsilylether derivatives of the fatty acid methyl esters were analyzed by gaschromatography mass spectrometry. 14 isomers of the following series could be identified: $C_{18:2}$ Δ9.(12.13.14.15.16.17); $C_{18:2}$ Δ(6.7.8.9.).12; $C_{18:2}$ Δ(10.11.12.).15 (Δ8.15); $C_{18:2}$ Δ5.9; Δ6.10; Δ10.14; (Δ12.16). Possible biosynthetic pathways are discussed.

Institute for Physiological Chemistry, University of Bonn.

For reprints, write to: Professor Dr. H. Egge, Rheinische Friedrich-Wilhelms Universitätll, D-5300 Bonn, Nussallell (West Germany).

* Philadelphia General Hospital, Philadelphia, U.S.A.

Ordinarily the double bonds in naturally occurring dienoic as well as polyenoic fatty acids maintain the divinylmethane rhythm (1). Furthermore, as earlier investigations have shown, mother milk also contains isomeric monoenoic and dienoic fatty acids (2). Mass spectrometric analysis of derivatives of synthetic isomeric octadecadienoic acids (3) revealed that the arrangement of double bonds in the isomeric octadecadienoic acids of mother-milk does not exclusively follow the divinylmethane rhythm (4,5). Non-methylene interrupted octadecadienoic acids of fungi, bacteria, plants and mammals have already been described (6-13). Although the physiological significance of these positional fatty acid isomers is still obscure, some recent research findings on artificial and natural membranes suggest that the position of the double bonds in lipids influences physical parameters, and thus also the physiological properties of biological membranes (14,15). The position of double bonds in monoenoic fatty acids is of importance with respect to their further desaturation (16), and affects the hydrolysis of triglycerides by pancreatic lipase (17).

METHODS

The preparation of mother-milk fat and the concentration of unsaturated fatty acids as methyl esters by urea fractionation have already been described earlier (18,19). The C_{18} fatty acid methyl esters were separated by preparative gas chromatography. Quantities of 50 mg of fatty acid methyl ester were injected directly into a glass column (2 m x 15 mm diam.) with 15% SE 30 on chromosorb Q. Instrument: Carlo Erba GV; carrier gas N_2 150 ml/min.; temperature: oven 200°C isothermic; evaporation block 280°C, detector FID 280°C, discharger 230°C. The separated components were collected in U-shaped glass tubes filled with glass-wool and methylene chloride, through a firmly adjusted split 1:50, under N_2. The recovery rate for the C_{18}-fatty acid methyl ester was approximately 90%. Separation by number and position of double bonds by thin layer chromatography on silica gel plates, impregnated with 35% silver

nitrate (11, 20, 21). Preparation of silver nitrate plates: Dissolve 14 g $AgNO_3$ in 72 ml of distilled water; add slowly, under stirring, 40 g silica gel (Machery + Nagel, G-HR + 5% gypsum), stir until a homogeneous pulp is obtained. Plate immediately on a fat-free plate (20x40 cm) with a plastic spatula (thickness of layer: 1 mm). Activate plates for 1/2 hr. at 110°C and store in a dry box. Develop twice in a longitudinal direction using benzene as solvent, and then spray the test material placed to the right and left of the sample with dichlorofluorescein (0.1% methyl alcohol solution), and mark the fractions under ultraviolet light. Subsequently, the separated fatty acid methyl esters are scraped off, extracted with methylene chloride and distilled in a high vacuum. The fractions of the cis,cis-non-methylene interrupted fatty acid methyl esters are hydroxylated with OsO_4 (22), converted with MSTFA (Macherey + Nagel, Duren) to their tetra-O-triphenylsilyl ethers and subjected to gas chromatographic-mass spectrometric analysis (LKB 9000). Gas chromatogr. conditions are listed in the legends to Figs. Mass spectrometric conditions: temperature of ion source 250°C; ionization energy 20 eV; trapping current 60 μA; accelerator current 3.5 KV; magnetic scan approximately 10 sec. in a mass range m/e = 1 - 700. Recording of gas chromatograms through total ionization current.

RESULTS AND DISCUSSION

Fatty acid methyl esters were separated into four fractions by thin-layer chromatography (Fig. 1). The ester mixtures thus obtained were examined on capillary columns (DEGA S.C.O.T.). Fraction I contains octadecanoic and octadecenoic acid methyl esters, fraction II contains linoleic acid methyl esters as its principal component, while fractions III and IV are ester mixtures of dienoic and trienoic acid methyl esters. The gas chromatogram of fraction IV is illustrated in Fig. 2. The mass spectra of peaks #2,3,4,5 and 6 reveal a molecule peak of m/e = 294 and other characteristic fragments of octadecadienoic acid methyl esters. Peak #7 is produced by an octadecatrienoic

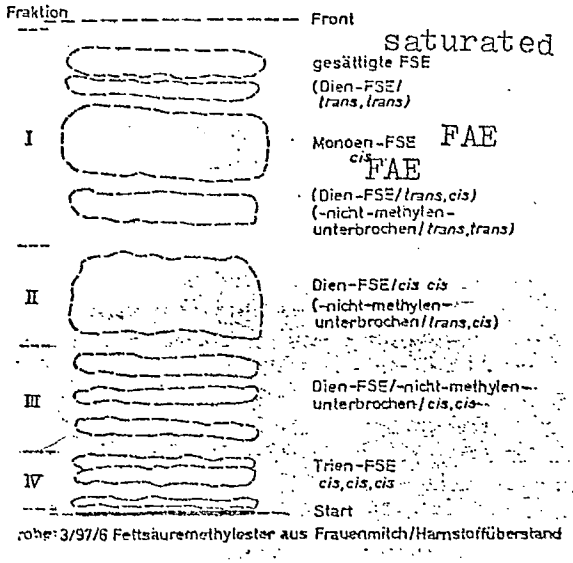


Fig. 1: Thin-layer chromatographic separation of fatty acid methyl esters on silver nitrated imppregnated silica gel. Solvent: benzene.

nicht = not (non);
unterbrochen = interrupted;
FSE = FAE (fatty acid esters)

Sample 3/97/6 Fatty acid methyl ester from mother milk/urea supernatant.

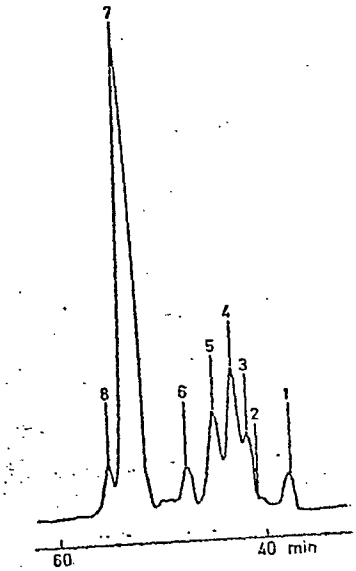


Fig. 2: Gas-chromatogram of fatty acid methyl esters of fraction IV. Peak 1, not identified; peaks 2-6, isomeric octadecadienoic acid methyl esters; peak 7, octadecatrienoic acid methyl esters; peak 8, octadecatrienoic and octadecatetraenoic acid methyl esters. Gas-chromatographic conditions: instrument: LKB 900; column 100' x 0.02" SCOT (Perkin-Elmer); phase: diethylene glycol adipate (DEGA); carrier gas: He; flow: approx. 4 ml/min.; temperature: oven 170°C; injection block 290°C; separator 280°C.

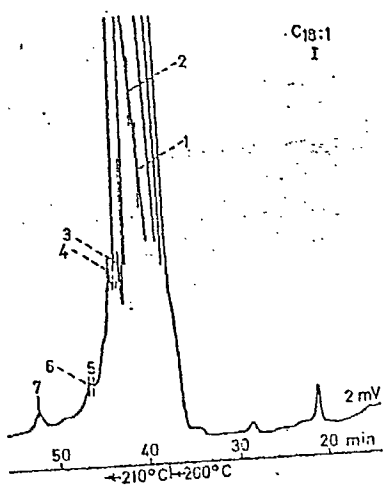


Fig. 3: Gas-chromatogram of fatty acid methyl esters from fraction I following derivatization. The peaks marked by numbers are listed in Table 1. Gas-chromatographic conditions: instrument: LKB 9000; column: 50 ft. x 0.02 inch SCOT (Perkin-Elmer); phase: silicone rubber SE 30; carrier gas: He; flow: approx. 4 ml/min.; temperature: oven step program 200-230°C, injection block 290°C, separator 280°C.

acid methyl ester, while peak #8 shows two molecule peaks, to wit m/e = 292 and m/e = 290; it reflects a mixed spectrum of octadecatrienoic and octadecatetraenoic acid methyl esters. No "trans-bands" could be detected in the urea supernatant of mother milk fatty acid methyl esters (958 cm^{-1} measured as film on the NaCl-plate) nor in fraction IV. According to the intensity of the "trans-bands", fraction III contains approximately 5% of trans-double bonds.

Since cis,trans-octadecadienoic methyl esters would be recovered in fraction II in view of their higher R_f-value, this trans-content must originate from a cis,cis,trans- (or cis,trans,cis or trans,cis,cis)-octadecatrienoic acid methyl ester, or from the oxidation product of a linoleic acid methyl ester, a 9-hydroperoxy-10-trans, 12-cis-octadecadienoic acid methyl ester that will be discussed further below, or the corresponding 9-cis,11-trans,13-hydroperoxy-octadecadienoic acid methyl ester. Although fractions III and IV did contain 3
octadecadienoic acid methyl esters separable by gas chromatography, the position of the double bonds could not be determined from retention times and mass spectra (23). This was only possible after the esters had been converted to the corresponding polyhydroxy-O-TMS compounds. Fig. 3 illustrates the gas chromatographic separation of methyl ester derivatives from fraction I. The identified components are listed in Table 1.

What is remarkable is that in contradistinction to the results of the van Rudloff hydrolysis (2), no double bonds between C-9 and the terminal carboxy group were discovered in mother milk octadecenoic acids through their OTMS derivatives. - The methylene interrupted octadecadienoic acid methyl esters can also be separated as tetra-O-trimethylsilyl ethers by gas chromatography, as indicated by the gas chromatogram of fraction II. Since the distances between the glycol groups in the C-chains are all equal, the isomers have very similar retention times. The problem of separation is further complicated by the large proportion of 9,10,12,13-tetra-OTMS-octadecanoic acid methyl esters. To detect 4
the isomers requires that the column with the major component be greatly over-

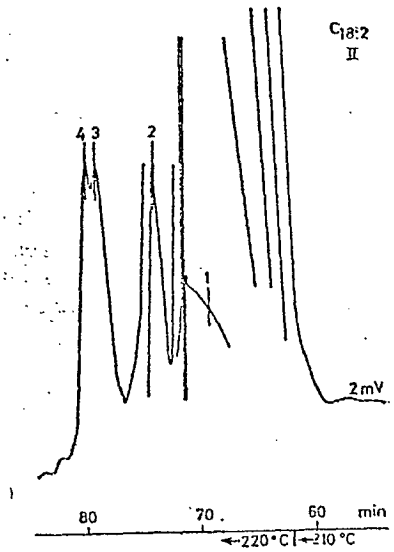


Fig. 4: Gas-chromatogram of fatty acid methyl esters from fraction II following derivatization. The peaks marked by numbers are listed in Table 2. Gas-chromatographic conditions: same as in legend to Fig. 3.

Table 1: Components from fraction I identified by mass-spectrometryl

Mischspektrum = mixed spectrum;

Saure = acid;

nicht identifizierte Komponente= unidentified component.

Peak # (Fig. 3).

Peak Nr. (Abb. 3)	Komponenten-components
1	9.10-Di-OTMS-Octadecansäure-
2	9.10-Di-OTMS-Octadecansäure-
3 Mischspektrum	10.11-, 11.12-, 12.13-, 13.14-Di-OTMS-Octadecansäure-
4 Mischspektrum	10.11-, 11.12-, 12.13-, *13.14*-Di-OTMS-Octadecansäure-
5 Mischspektrum	*13.14*-, 14.15-Di-OTMS-Octadecansäure-
6 Mischspektrum	*14.15-Di-OTMS-Octadecansäure-methylester* + nicht identifizierte Komponente

Peak # (Fig. 4)

Peak Nr. (nach Abb. 4)	Komponente components
1	9.10.12.13-Tetra-OTMS-Octadecansäure-
2	9.10.12.13, 10.11.13.14-, 11.12.14.15-, 12.13.15.16-Tetra-OTMS-Octadecansäure-
3	9.10.12.13-9.10.15.16-Tetra-OTMS-Octadecansäure-, + unidentifizierte Komponente
4	9.10.15.16-Tetra-OTMS-Octadecansäure-methylester, + unidentifizierte Komponente

Table 2: Components of fractinn II identified by mass-spectrometry.

unidentifiziert = unidentified

Säure = acid

stressed which complicates evaluation of mass spectra. Two factors have a bearing on retention time: 1. The distance between TMS-glycol groups and 2. the distance between the first TMS-glycol group and the carboxy group. The tetra-OTMS-octadecanoic acid methyl esters migrate more slowly the further apart the TMS-glycol groups are. In other words, the tetra-OTMS-derivative of $C_{18:2}^{\Delta 9,17}$ has a longer retention time than that of $C_{18:2}^{\Delta 9,16}$, the $C_{18:2}^{\Delta 9,15}$ and so forth. When the distance between TMS-glycol groups is the same, a shorter distance between the first OTMS-group and the carboxy ester group has an accelerating effect. Consequently, there is overlap in complex isomer mixtures; the same has been reported following gas chromatographic analysis of isomeric octadecadienoic acid methyl esters without derivatization (24). In the non methylene interrupted tetra-OTMS-octadecanoic acid methyl esters separating conditions are more favorable (Fig. 5). Since the column was over - charged due to the large quantity of 9,10,12,13-tetra-OTMS-octadecanoic acid methyl esters, the sample was once more diluted 1:50 prior to analysis; subsequently only the major components appear.

In peak #6 the mass spectrum shows that we are apparently dealing with the autoxidation product of $C_{18:2}^{\Delta 9,12}$ where oxidation with the shift of a double bond towards C-10,11 or C-12,11 has apparently occurred either at C-9 or at C-13. Subsequent oxidation with OsO_4 yielded a 9,10,11,12,13-penta-OTMS-octadecanoic acid methyl ester. This is the first case known to us where an autoxidation product of a fatty acid could be identified at intact C-chain (25 - 27). According to Hamberg et al. (27) the formation of hydroperoxides requires a 1,4-pentadiene structure so that no oxidation products should be expected in non methylene interrupted octadecadienoic acids, nor have any been discovered so far. In fraction IVthe quantitative distribution is much more favorable and satisfactory separation in the form of OTMS-derivatives could be achieved.

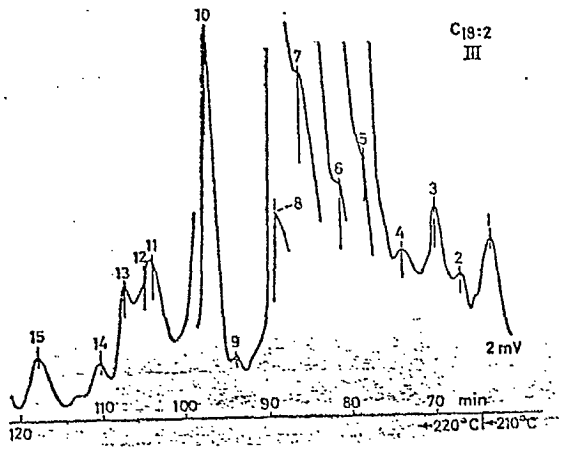
Peak # (Fig. 5)

Peak Nr. (nach Abb. 5)	Komponente components
1	nicht identifiziert
2	nicht identifiziert
3	nicht identifiziert
4	5.6.9.10-Tetra-OTMS-Octadecansäure- 6.7.10.11-Tetra-OTMS-Octadecansäure-
5	8.9.12.13-Tetra-OTMS-Octadecansäure- 9.10.13.14-Tetra-OTMS-Octadecansäure-
6	9.10.13.14-Tetra-OTMS-Octadecansäure- 8.9.12.13-Tetra-OTMS-Octadecansäure-
7	9.10.13.14-Tetra-OTMS-Octadecansäure-
8	9.10.12.13-Tetra-OTMS-Octadecansäure-
9	10.11.14.15-Tetra-OTMS-Octadecansäure-
10	11.12.15.16-Tetra-OTMS-Octadecansäure-
11	9.10.15.16-Tetra-OTMS-Octadecansäure- (12.13.16.17-Tetra-OTMS-Octadecansäure-)
12	9.10.15.16-Tetra-OTMS-Octadecansäure-
13	9.10.15.16-Tetra-OTMS-Octadecansäure-
14 (Mischspektrum)	8.9.15.16-Tetra-OTMS-Octadecansäure- 9.10.15.16-Tetra-OTMS-Octadecansäure-
15 (Mischspektrum)	9.10.11.12.13-Penta-OTMS-Octadecansäure- 9.10.16.17-Tetra-OTMS-Octadecansäuremethylester + nicht identifizierte Komponente

Table 3: Components of fraction III identified by mass-spectrometry.

nicht identifiziert = unidentified;
Säure = acid;
Mischspektrum = mixed spectrum

Fig. 5



Gas-chromatogram of fatty acid methyl esters of fraction III following derivatization. Peaks marked by numbers are listed in Table 3. Gas-chromatographic conditions same as in legend to Fig. 3.

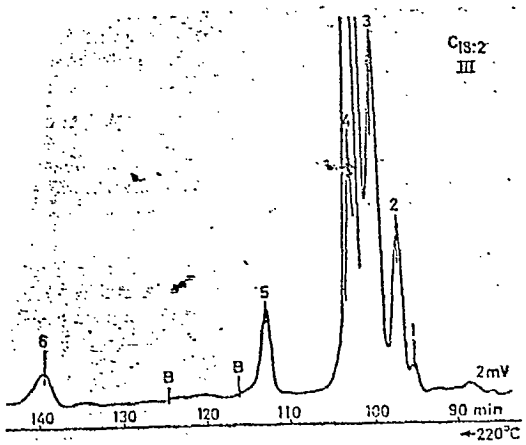


Fig. 6: Gas-chromatogram of fatty acid methyl esters of fraction III. Injected in a 1:50 dilution. Peaks marked by numbers are listed in Table 4. Gas-chromatographic conditions same as in legend to Fig. 3.

Table 4

Peak # (Fig. 6)

Peak Nr. (nach Abb. 6)	Komponente component
1	5.6.9.10-Tetra-OTMS-Octadecansäure- 6.7.10.11-Tetra-OTMS-Octadecansäure-
2	8.9.12.13-Tetra-OTMS-Octadecansäure-
3	9.10.13.14-Tetra-OTMS-Octadecansäure-
4	9.10.12.13-Tetra-OTMS-Octadecansäure-
5	11.12.15.16-Tetra-OTMS-Octadecansäure-
6	9.10.11.12.13-Penta-OTMS-Octadecansäuremethylester

Table 5

Peak # (Fig. 7)

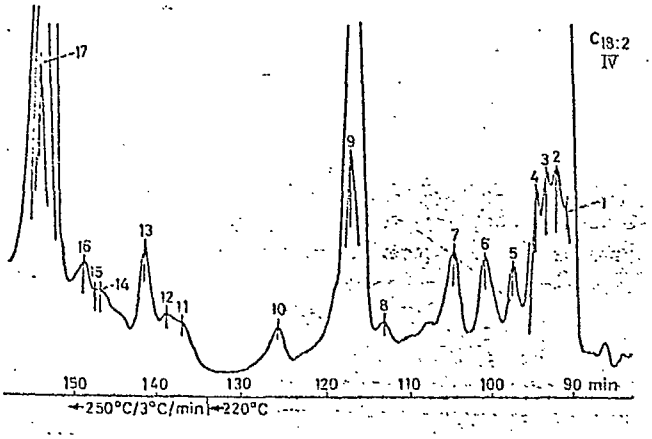
Peak Nr. (nach Abb. 7)	Komponente component
1	8.9.12.13-Tetra-OTMS-Octadecansäure- 9.10.13.14-Tetra-OTMS-Octadecansäure-
2	9.10.13.14-Tetra-OTMS-Octadecansäure- 8.9.12.13-Tetra-OTMS-Octadecansäure-
3	9.10.13.14-Tetra-OTMS-Octadecansäure-
4	9.10.13.14-Tetra-OTMS-Octadecansäure-
5	7.8.12.13-Tetra-OTMS-Octadecansäure-
6	6.7.12.13-Tetra-OTMS-Octadecansäure-
7	9.10.14.15-Tetra-OTMS-Octadecansäure-
8	10.11.15.16-Tetra-OTMS-Octadecansäure-
9	9.10.15.16-Tetra-OTMS-Octadecansäure-
10	9.10.16.17-Tetra-OTMS-Octadecansäure-
11	6.7.9.10.12.13-Hexa-OTMS-Octadecansäure-
12	6.7.9.10.12.13-Hexa-OTMS-Octadecansäure-
13	9.10.17.18-Tetra-OTMS-Octadecansäure-
14/15	8.9.11.12.14.15-Hexa-OTMS-Octadecansäure
16	nicht identifiziert
17	9.10.12.13.15.16-Hexa-OTMS-Octadecansäuremethylester

Table 4: Components of fraction III (at 1:50 dilution) identified by mass-spectrometry.

Table 5: Components of fraction V identified by mass-spectrometry.

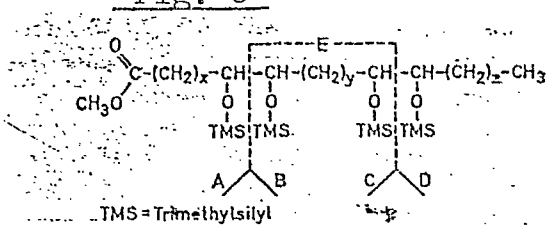
Säure = acid.

Fig. 7



Gas chromatogram of fatty acid methyl esters of fraction IV after derivatization. Peaks marked by numbers are listed in Table 5. Gas-chromatographic conditions same as in legend to Fig. 3.

Fig. 8



Fragmentation scheme of OTMS-octadecanoic acid methyl esters.

Based on the principal investigations on synthetic substances*, interpretation of mass spectra of poly-OTMS-octadecanoic acid methyl esters was simple. While the fragmentation scheme is principally similar in all types of OTMS-octadecanoic acid methyl esters, the intensity of the fragments formed depends on the position of the double bond (Fig. 8). In the mass spectra of methylene interrupted OTMS-octadecanoic acid methyl esters, the base peak is formed by the fragment (B-90)(splitting of TMSOH) or (C-90), depending on the distance of the first double bond from the terminal carboxy group. All other fragments, such as A, A +73, (addition of TMS to carbonyl oxygen of A) B,C,D and subsequent fragments are present. 6

Upon transition from 9,10,13,14-tetra-OTMS-octadecanoic acid methyl esters to 9,10,14,15-tetra-OTMS-octadecanoic acid methyl esters, the base peak of (B-90) changes over to fragment A: apparently the stability of the fragment (B-90) or (C-90) is strongly reduced by the three methylene groups between the original double bonds, a fact evident from the relative elevation of the corresponding fragments (B-180) or (C-180). Naturally, in view of the elevation of fragment A, the fragments depending on A are also enlarged. The abovementioned fragments m/e = 129 and m/e = 191 are now distinctly higher. This trend is further enhanced when two further methylene groups are introduced between the original double bonds: the stability of fragments (B-90) and (C-90) is further reduced while fragment A becomes relatively larger and the base peak remains. If they do not contain too many components, even mixed spectra can be interpreted unequivocally (Fig. 12). This mixed spectrum is the spectrum of two OTMS-octadecanoic acid methyl esters, to wit that of the 5,6,9,10- and of the 6,7,10,11-tetra-OTMS-octadecanoic acid methyl ester. Since an ethylene group lies between the TMS-glycol groups in this case, the fragment m/e = 129 appears; 7

*Tables for the mass spectra of non methylene interrrupted octadecadienoic acid methyl esters as OTMS derivatives may be requested from the author.

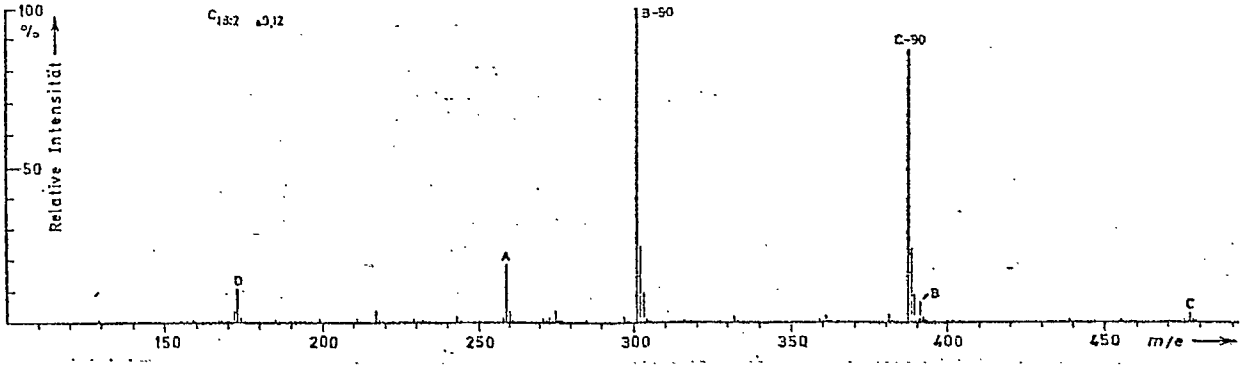


Fig. 9: Mass spectrum of the 9,10,12,13-tetra-OTMS-octadecanoic acid methyl ester (peak #4/fraction III). Mass spectra of fragments m/e = 129 and m/e = 191 - which do not fit directly into the fragmentation scheme - are absent from this mass spectrum. These spectra appear at an intensity depending from the distance between the two TMS-glycol groups.

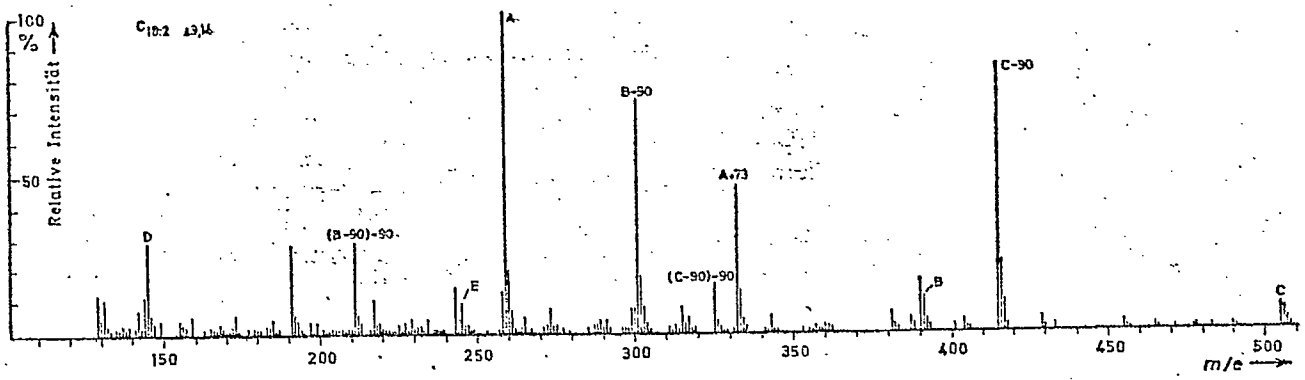


Fig. 10: Mass spectrum of 0,10,14,15-tetra-OTMS-octadecanoic acid methyl ester.

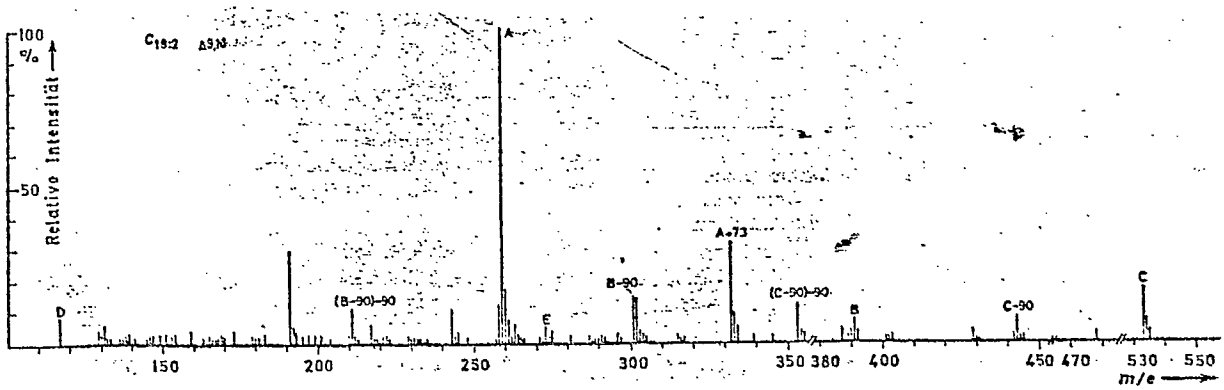


Fig. 11: Mass spectrum of 9,10,16,17-tetra-OTMS-octadecanoic acid methyl ester.

Relative Intensität = relative intensity.

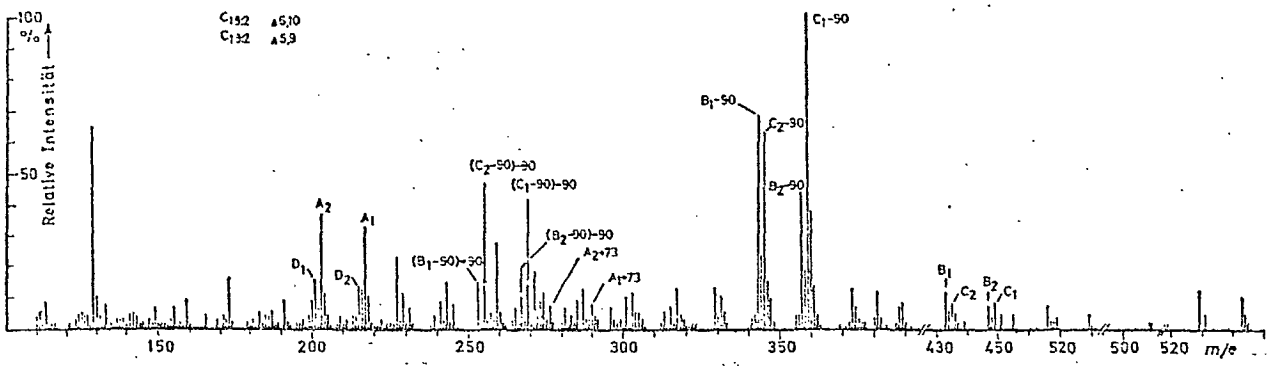


Fig. 12: Mass spectrum of 5,6,9,10 and 6,7,11,11-tetra-OTMS-octadecanoic acid methyl esters.

Table 6

	Äthylen- Propylen- Butylen- Pentylen- Hexylen, unterbrochene Octadecadiensäuren aus Frauenmilch				
$C_{18:2}$	Δ5.9	Δ7.12	Δ6.12	Δ9.16	Δ9.17
	Δ6.10	Δ9.14	Δ9.15	(Δ8.15)	
	Δ8.12	Δ10.15			
	Δ9.13				
	Δ10.14				
	Δ11.15				
	(Δ12.16)				

Table 7

$C_{18:2}$	$C_{18:2}$	$C_{18:2}$	$C_{18:2}$
Δx.9/Δ9.x	Δx.12/Δ12.x	Δx.15	Δ6.x
Δ5.9	Δ9.12	Δ9.15	Δ6.10
Δ9.12	Δ8.12	Δ10.15	Δ6.12
Δ9.13	Δ7.12	Δ11.15	
Δ9.14	Δ6.12	Δ12.15	
Δ9.15	Δ12.15		
Δ9.16	Δ12.16		
Δ9.17			

Table 6: Octadecadienoic acids identified in mother milk.
unterbrochene = interrupted; ...sauren = acids; aus Frauenmilch = from mother milk.

Table 7: Octadecadienoic acids detected by the author and rearranged in a different scheme (see text).

this fragment reaches its highest intensity in the tetra-OTMS derivatives of ethylene interrupted dienoic acid methyl esters. But, the base peak in this group of methyl esters is the fragment (B-90) or (C-90) respectively. Following evaluation of all mass spectra the octadecadienoic acids listed in Table 6 were found to be present in mother milk.

In an analysis of the isomeric octadecadienoic acids, one is struck by the fact that - unlike in findings on $C_{18:1}$ fatty acids from mother milk (2) and cow's milk (28), one finds hardly any isomers with a double bond between C-9 and the carboxy group. Apart from possible biological reasons for this phenomenon, we must first dwell on experimental reasons. Based on the R_f-values of the isomers on $AgNO_3$-impregnated silica gel plates and the retention times of tetra-OTMS-octadecanoic acid methyl esters on a SE-30 column, we should anticipate that - with the exception of a 2,3,9,10- or 3,4,9,10-tetra-OTMS-octadecanoic acid methyl ester - such isomers would behave similar to a $C_{18:2}{}^{\Delta 9,12}$ and its derivatives. Consequently its mass spectrometric demonstration would seem to be thwarted by the large excess in 9,10,12,13-tetra-OTMS-octadecanoic acid methyl ester. Furthermore, one must also reckon with experimental problems when a double bond in position 2 or 3 (as fatty acid ester) is hydroxylated with OsO_4.

The biogenesis of these positional fatty acid isomers is not known so far. When the octadecadienoic acids detected by us are arranged differently (Table 7), one obtains some clues for a possible biosynthetic pathway. For the 8
series $C_{18:2}{}^{\Delta 9,x}$, a second double bond in $\Delta 9$ may be introduced into existing Δx-octadecenoic acids through a $\Delta 9$-desaturase (16). Among the remaining groups one is struck by the fact that - apart from the $C_{18:2}{}^{\Delta 10,14}$ - all other fatty acids have a double bond at the "normal" site. Among these, the series of $C_{18:2}{}^{\Delta 6,x}$ may be attributed to an active $\Delta 6$-desaturase (30). The appearance of $\Delta x,12$ and $\Delta x,15$ is hard to explain since - according to the classic studies

of Klenk (31) and Stumpf (32) Δ12 and 15-desaturases belong to the realm of plants. Despite the fact that these desaturases allegedly occur in plants, we found no such isomers in plant oils, such as maize germ oil and soy lecithin, rich in linoleic acid*(33). In all probability there is a constant alternation between desaturation, α-oxidation,β-oxidation and chain elongation. However, this biosynthetic pathway for positional isomeric fatty acids need not be limited to the mammary glands, as Hay and Morrison reported recently (34), but may take place at all sites with active lipid metabolism.

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REFERENCES

[1] E. Klenk, Advances in Lipid Res. 3, 1 [1965].
[2] H. Egge, U. Murawski, R. Ryhage, F. Zilliken u. P. György, Z. analyt. Chem. 252, 123 [1970].
[3] U. Murawski, H. Egge u. F. Zilliken, Z. Naturforsch. 26 b, 1241 [1971].
[4] U. Murawski, H. Egge, P. György u. F. Zilliken, FEBS-Letters 18, 290 [1971].
[5] U. Murawski, H. Egge u. F. Zilliken, Z. analyt. Chem. 259, 211 [1972].
[6] M. O. Bagby, C. R. Smith Jr., T. K. Miwa, R. L. Lohmar u. I. A. Wolff, J. org. Chemistry 26, 1261 [1961].
[7] F. Davidoff u. E. D. Korn, Biochem. biophysic. Res. Commun. 9, 54 [1962].
[8] E. D. Korn, J. Lipid Res. 5, 352 [1964].
[9] T. Takagi, J. Amer. Oil Chemists' Soc. 41, 516 [1964].
[10] R. W. Miller, M. E. Daxenbichler, F. R. Earle u. H. S. Gentry, J. Amer. Oil Chemists' Soc. 41, 167 [1964].
[11] H. van der Wel u. K. de Jong, Fette, Seifen, Anstrichmittel 69, 279 [1967].
[12] G. Hoffman u. P. W. Beijboom, J. Amer. Oil Chemists' Soc. 46, 620 [1968].
[13] A. J. Fulco, Biochim. biophysica Acta [Amsterdam] 187, 169 [1969].
[14] H. Träubl, Naturwissenschaften 58, 277 [1971].
[15] A. Weiss, J. Spiess, E. Stuke u. G. Lagaly, Z. Naturforsch. 27 b, 317 [1971].
[16] M. I. Gurr, M. P. Robinson, A. T. James, L. J. Morris u. D. Howling, Biochim. biophysica Acta [Amsterdam] 280, 415 [1972].
[17] W. H. Heimerman, R. T. Holman, D. T. Gordon, D. E. Kowalyshya u. R. G. Jensen, Lipids 3, 45 [1973].
[18] H. Egge, U. Murawski, R. Ryhage, F. Zilliken u. P. György, FEBS-Letters 11, 113 [1970].
[19] J. Cason, G. Sumrell, C. F. Allen, G. I. Gillies u. S. Elberg, J. biol. Chemistry 205, 435 [1963].
[20] B. de Vries u. C. Jurriens, Fette, Seifen, Anstrichmittel 65, 725 [1963].
[21] K. de Jong u. H. van der Wel, Nature [London] 202, 553 [1964].
[22] W. G. Niehaus, Jr. u. R. Ryhage, Analytic. Chem. 40, 1840 [1968].
[23] A. Zeman u. H. Scharman, Fette, Seifen, Anstrichmittel 75, 32 [1973].
[24] W. W. Christie, J. Chromatogr. [Amsterdam] 37, 27 [1968].
[25] A. Dolev, W. K. Rohwedder u. H. J. Dutton, Lipids 2, 28 [1967].
[26] A. Dolev, W. K. Rohwedder, T. L. Mounts u. H. J. Dutton, Lipids 2, 33 [1967].
[27] M. Hamberg u. B. Samuelson, J. biol. Chemistry 242, 5328 [1967].
[28] J. D. Hay u. W. R. Morrison, Biochim. biophysica Acta [Amsterdam] 248, 71 [1971].
[29] U. Murawski, H. Egge u. F. Zilliken, unveröffentlicht. unpublished
[30] N. Nicolaides, Hwei, C. Fu., M. N. A. Ansari u. G. R. Rice, Lipids 7, 506 [1972].
[31] E. Klenk, Current Trends in the Biochemistry of Lipids, ed. J. Ganguly u. R. M. S. Smellie, p. 41, Acad. Press.
[32] H. Stumpf, ebenda p. 57.
[33] U. Murawski, H. Egge u. F. Zilliken, unveröffentlicht. unpublished
[34] J. D. Hay u. W. R. Morrison, Lipids 8, 94 [1973]. same as above