IDENTIFICATION OF NON-METHYLENE-INTERRUPTED *CIS,CIS*-OCTADECADIENOIC ACIDS IN HUMAN MILK*

U. MURAWSKI, H. EGGE, P. GYÖRGY and F. ZILLIKEN
Institute of Physiological Chemistry, University of Bonn, 53 Bonn, Germany and the Philadelphia General Hospital, Philadelphia, USA

Received 25 August 1971

1. Introduction

In dienoic and polyenoic fatty acids from plants and mammals the double bonds are usually arranged in a divinyl-methane rhythm [1]. During our investigations of human milk fatty acids [2–5] evidence was obtained by combined gas liquid chromatography-mass spectrometry (GLC-MS) for the occurrence of posisional isomers of octadecanoic [4] and also of octadecadienoic acids. Positional isomers of non-methylene-interrupted octadecadienoic acids from mammalian sources other than human milk have been described [6, 7]. In this paper we report the identification of five non-methylene-interrupted *cis,cis*-octadecadienoic acids from human milk fat.

2. Methods

2.1.

The preparation of human milk cream and the enrichment of unsaturated fatty acid methyl esters by urea fractionation after *trans*esterification have been described [2, 8]. The unsaturated fatty acid methyl esters were separated by preparative GLC on a Carlo Erba fractovap GV according to chain length into C_{10}; C_{12}; C_{14}; C_{16}; C_{18} and C_{20} methyl esters. The C_{18} fatty acid methyl esters were further separated by thin-layer chromatography (TLC) on silica gel (Macherey u. Nagel MN-GHR + 5% $CaSO_4$) impregnated with 35% silver nitrate [7, 9–11]. The octadecadienoic acid methyl esters were hydroxylated with OsO_4, converted to their tetra-*O*-trimethylsilyl-derivatives (-O-TMS) and analyzed by GLC-MS [12].

2.2. *Gas-chromatographic conditions*

Column: 50 feet × 0.02 inches S.C.O.T. column (Perkin Elmer); liquid phase: silicone rubber SE 30; carrier gas: helium; gas flow: ~4 ml/min; temperature: oven 240°; injection block 290°; molecule separator 250°.

2.3. *Mass-spectrometric conditions*

Ion source temperature: 250°; ionization energy: 20 eV; trap current: 60 μA; accelerating voltage: 3.5 kV; magnetscan: ~10 sec in the mass range m/e = 10–700.

3. Results and discussion

The separation of C_{12}–C_{20} fatty acid methyl esters on $AgNO_3$ impregnated silica gel obtained after preparative GLC is shown in fig. 1.

The C_{18} fatty acid methyl esters obtained in fractions III and IV after TLC were analyzed by GLC-MS before and after formation of derivatives. Fig. 2 shows a typical gas chromatogram of fraction IV. The experimental conditions are given in the legend.

The mass spectra of positional isomers of octadecadienioc acids show only slight quantitative differences [13]. After hydroxylation and transformation to volatile derivatives, highly characteristic mass spectra show the original position of the double bonds [14]. After thorough inspection of the mass spectra of 16 tetra-*O*-trimethylsilyl-derivatives of synthetic

* Part V of a series: Minor constituents of human milk.

North-Holland Publishing Company – Amsterdam

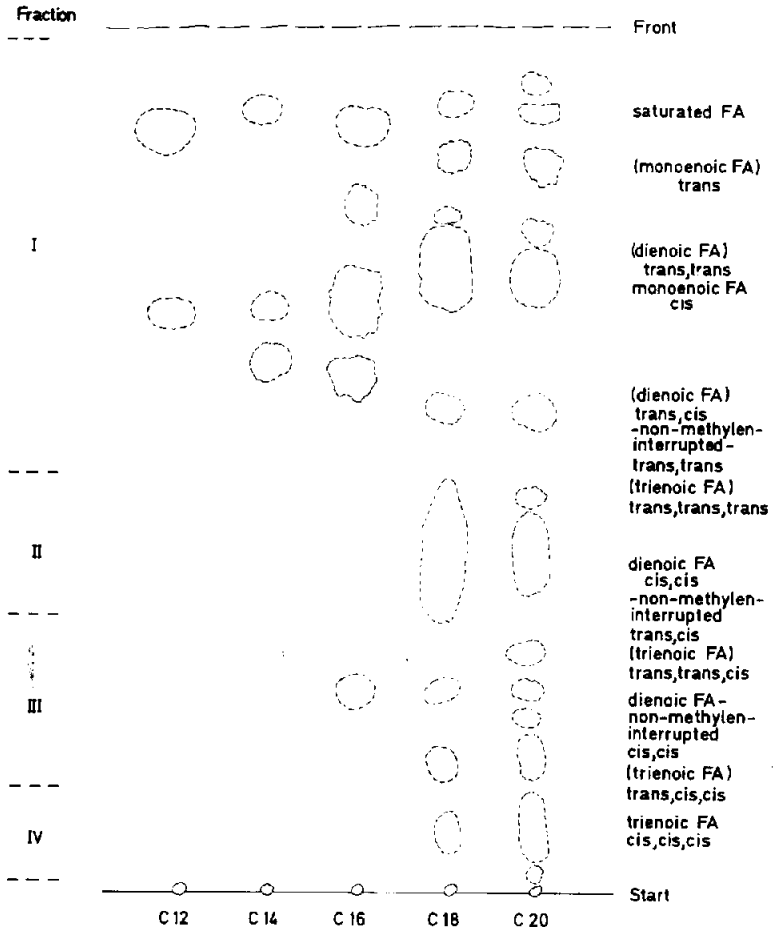
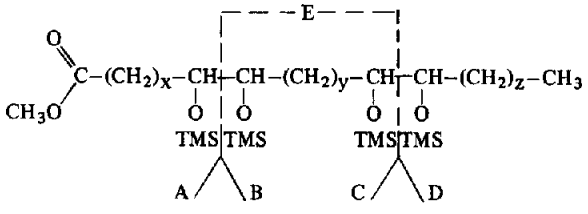


Fig. 1. Separation of C_{12}–C_{20} fatty acid methyl esters from human milk fat on $AgNO_3$ impregnated silica gel. Solvent: benzene. The fractions of C_{18} methyl esters analyzed for positional isomers are indicated.



Scheme 1. Fragmentation pattern of tetra-*O*-trimethylsilyl octadecanoic acid methyl esters. All characteristic fragments and recombinations are indicated.
A; A+73; A+102 and (A+102)–90
B; B–90 and (B–90)–90 two metastable ions
C; C–90 and (C–90)–90 two metastable ions
D; D+102 and (D+102)–90

methylene-, ethylene-, propylene-, butylene- and pentylene-interrupted octadecadienoic acid methyl esters several rules for the fragmentation pattern have been set up [12]. They are summarized in scheme 1.

From their mass spectra five non-methylene-interrupted octadecadienoic acids were identified in fractions III and IV. They belong to the ethylene-, butylene- and pentylene-interrupted type.

As can be seen from the R_f-values shown in fig. 1 all double bonds of these dienoic acids are in *cis* configuration.

Nothing is as yet known about the origin of these acids. If the $C_{18:2}\Delta 11,15$ up to now found only in beef and mutton tallow [6] is of wider distribution in natural fats, it may be assumed that at least a part of

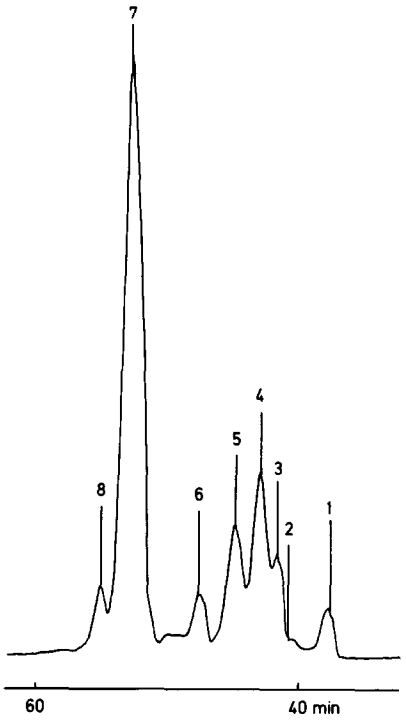


Fig. 2. Gas-chromatographic separation of isomeric octadecadienoic- and octadecatrienoic acid methyl esters. Column: 100 feet × 0.02 inches S.C.O.T. column (Perkin Elmer); liquid phase: DEGA; temperature: oven 170°; other conditions as above. Compounds: 1: $C_{18:1}$; 2: $C_{18:2}$; 3: $C_{18:2}$; 4: $C_{18:2}$; 5: $C_{18:2}$; 6: $C_{18:2}/C_{18:3}$; 7: $C_{18:3}$; 8: $C_{18:3}/C_{18:4}$.

this acid present in human milk fat is of alimentary origin. An endogenous synthesis can however not be excluded. From the fatty acid pattern present in human milk it can be deduced that human mammary gland contains a highly active Δ9-desaturase-system [4]. From the experiments of A.T. James [15] with methyl branched fatty acid CoA esters as substrates for desaturation by goat mammary gland, it may be speculated that positional isomeric octadecanoic acids like $C_{18:1}\Delta 15$ or $C_{18:1}\Delta 13$ present in human milk [4] are further desaturated to the corresponding $C_{18:2}\Delta 9,15$ and $C_{18:2}\Delta 9,13$. For the $C_{18:2}\Delta 11,15$ and $C_{18:2}\Delta 8,12$ acids more complex biosynthetic pathways including partial degradation or chain elongation may be postulated. Compared with classical dienoic acid biosynthesis in plants, this would be a reversal of the desaturation steps. It is without doubt that

Table 1

Non-methylene-interrupted octadecadienoic acids in human milk fat, identified as their tetra-*O*-trimethylsilyl-derivatives.*

a) ethylene-interrupted: $C_{18:2}\Delta 8,12$; $C_{18:2}\Delta 9,13$; $C_{18:2}\Delta 11,15$

b) butylene-interrupted: $C_{18:2}\Delta 9,15$

c) pentylene-interrupted: $C_{18:2}\Delta 9,16$.

* The complete mass spectra can be obtained on request.

these acids, alt[illegible] present only in small quantities, may exert a strong influence on the physical and biological properties of membranes.

Acknowledgement

We thank Mrs. U. Kupietz for skilful technical assistance. This work was supported in part by the German Bundesforschungsministerium.

References

[1] E. Klenk, Adv. Lipid Res. 3 (1965) 1.
[2] H. Egge, U. Murawski, R. Ryhage, P. György and F. Zilliken, FEBS Letters 11 (1970) 113.
[3] H. Egge, U. Murawski, P. György and F. Zilliken, FEBS Letters 2 (1969) 255.
[4] H. Egge, U. Murawski, R. Ryhage, F. Zilliken and P. György, Z. Anal. Chem. 252 (1970) 123.
[5] H. Egge, U. Murawski, R. Ryhage, P. György and F. Zilliken, Chem. Phys. Lipids, in press.
[6] G. Hoffmann and P.W. Meijboom, J. Am. Oil Chem. Soc. 46 (1968) 620.
[7] H. van der Wel and K. de Jong, Fette, Seifen, Anstrichmittel 69 (1967) 279.
[8] J. Cason, G. Surell, C.F. Allers and G.A. Gillies, J. Biol. Chem. 205 (1963) 435.
[9] W.W. Christie, J. Chromatog. 34 (1968) 405.
[10] B. de Vries and G. Jurriens, Fette, Seifen, Anstrichmittel 65 (1963) 725.
[11] K. de Jong and H. van der Wel, Nature 4932 (1964) 553.
[12] U. Murawski, H. Egge and F. Zilliken, in preparation.
[13] R.T. Holman and W.W. Christie, Chem. Phys. Lipids 1 (1967) 407.
[14] G. Wendt and J.A. McCloskey, Biochemistry 9 (1970) 4854.
[15] D. Brett, D. Howling, L.J. Morris and A.T. James, Arch. Biochem. Biophys. 143 (1971) 535.