MINOR CONSTITUENTS OF HUMAN MILK
II. IDENTIFICATION OF DODECADIENOIC-, TETRADECADIENOIC- AND HEXADECADIENOIC ACID IN HUMAN MILK FAT

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1. Introduction

Dienoic acids found in mammals are predominantly of the linoleic acid type. This acid is synthesized in plants and lower animals. The dietary intake of this acid is essential for the development and survival of mammals [1, 2]. Besides the octadeca-dienoic acid hexadeca-dienoic acids were found in menhaden oil [3], fish oils [4], algae [5], beef testis [6] and other sources [7, 8]. Dodeca-3,6-dienoic acid, tetradeca-5,8-dienoic acid and hexadeca-7,10-dienoic acid have been synthetized chemically [9]. To the best of our knowledge dienoic acids with chain length shorter than C_{16} have not been detected before in natural sources. In this communication we wish to report on the occurrence of C_{12}, C_{14} and C_{16} dienoic acids in human milk fat.

2. Methods

The preparation of fatty acid methyl esters from human milk fat has been described previously [10]. The unsaturated fatty acid methyl esters were enriched by urea fractionation [11] or mercury adducting [7]. Gas-liquid chromatography was performed on a Carlo Erba Fractovap GV. Mass spectra were taken on a LKB 9 000. The conditions for gas chromatography and mass spectrometry are given in the legend of fig. 1.

3. Results

The mixture of FA found in human milk fat is extremely complex. Starting from different samples of milk more than 200 compounds were totally or partially separated by gas-liquid chromatography on a capillary column in the range of C_6 to C_{20} fatty acids. In the chromatogram shown in fig. 1 special attention has been focussed on the peaks following the even numbered saturated straight chain FA (no. 1–6). The mass spectra taken from components following methyl decanoate (no. 3) contained no fragments pointing to the occurrence of a decadienoic acid. Due to the rather high volatility of this acid, it cannot be entirely excluded that traces of this acid may have been lost during the preparation. The C_{12}-, C_{14}- and C_{16}-dienoic acids constitute 0.02, 0.05 and 0.10% (moles) of the total mixture obtained after urea fractionation as determined by quantitative gas-liquid chromatography. Calculated on the basis of the amount of linoleic acid present after urea fractionation the dienoic acids have been enriched by the factor of 10.

The complexity of the FA mixture did not permit a complete separation of all components. Therefore the mass spectra obtained from the C_{12}-, C_{14}-, C_{16}-dienoic acids contained also fragments belonging to slow moving positional isomers of the corresponding monoenoic acids [12]. From data obtained after hydrogenation of the FA mixture the mass spectra may contain in addition fragments belonging to branched chain acids. Without knowing the complete fragmentation pattern and possible recombinations of all the components simultaneously recorded in the

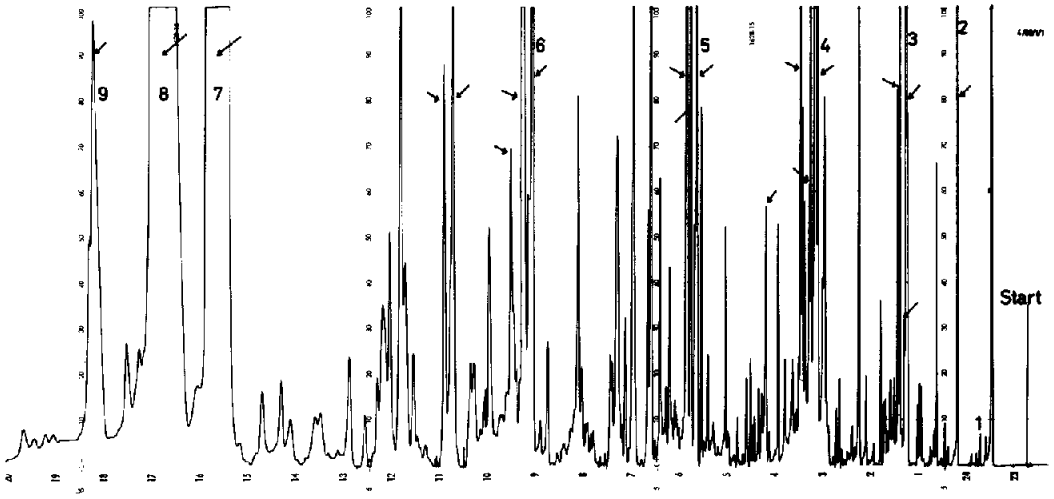

Fig. 1. Gas-chromatogram of fatty acid methylester of human milk fat after saponification and urea fractionation. Mass spectra were taken at points indicated by arrows. Peak no. 1 caproic acid methylester; 2 caprylic acid methylester; 3 caprinic acid methylester; 4 lauric acid methylester; 5 myristic acid methylester; 6 palmitic acid methylester; 7 oleic acid methylester, 8 linoleic acid methylester; 9 linoleinic acid methylester.

Capillary column: 0.25 mm × 50 m
Liquid phase : polyphenylether OS 138
Temp. program : 160° → 220°; 1°/min
Carrier gas : He, 1.3 ml/min
Injection block : 300°

Separator : 250°
Ion-source : 250°
Accel. voltage : 3.5 kV
Electron energy : 70 eV
Scan. speed : ∿3 sec in mass range m/e = 12–350

mass spectra a complete reduction to the pure spectra of the dienoic acids by substraction of the appropriate m/e-values seems to be beyond our possibilities. Therefore only the prominent peaks pertaining to the corresponding mono- and dienoic acids are reported.

Table 1 gives the m/e and relative abundance (R.A.) for the major peaks of the monoenoic (M_1) and dienoic (M_2) acids found. Only a few data have been published on the mass spectra of dienoic acids [13, 14]. In the spectra reported for linoleic methylester and its positional isomers hydrocarbon peaks are predominant. In the spectra reported here, all typical fragments for dienoic acids including the molecule ions are present. In contrast to linoleic acid with a base peak m/e = 67 for the shorter chain homologs the lower fragments m/e = 41 and 55 are more prominent. In the spectrum of the mixture $C_{12:2}$ and $C_{12:1}$ the m/e = 41 accounts for 94% of the base peak, whereas in $C_{16:1}$ and $C_{16:2}$ m/e = 55 represents the base peak. The values for $C_{14:2}$ and $C_{14:1}$ mixture are in between $C_{12:2}$ and $C_{12:1}$, $C_{16:2}$ and $C_{16:1}$. Dodeca-3,6-dienoic acid and tetradeca-5,8-dienoic acid do not serve as a precursor for the synthesis of octadeca-9,12-dienoic acid [9]. This is only the case with hexadeca-7,10-dienoic acid [9, 15]. From these data it is likely that the lower dienoic acids found in human milk are produced by β-oxidation of dietary linoleic acid.

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Table
Major fragments and their relative abundances (R.A.) for the $C_{12:1}$, $C_{12:2}$, $C_{14:1}$, $C_{14:2}$ and $C_{16:1}$, $C_{16:2}$ fatty acid methyl-ester.

	$C_{12:1}$ M = 212	$C_{12:2}$ M = 210	$C_{14:1}$ M = 240	$C_{14:2}$ M = 238	$C_{16:1}$ M = 268	$C_{16:2}$ M = 266
	R.A.		R.A.		R.A.	
M	3.0	2.3	2.8	8.8	3.1	1.8
M-31	1.4	5.1	–	–	1.8	2.2
M-32	1.6	2.0	2.8	8.8	4.2	1.3
M-74	23.0[a]	2.3	9.1	11.0[a]	6.6	4.6
m/e						
180	1.6		3.5		5.2	
178	2.0		4.2		3.1	
164	2.7		11.0[a]		3.3	
163	7.3		8.8		5.5	
152	4.1		3.9		9.4	
151	4.6		5.6		9.1	
150	1.6		7.0		5.0	
138	23.0[a]		4.2		25.0	
137	10.0		11.0		17.0	
124	5.1		8.8		10.5	
123	12.0		16.9		24.0	
111	10.0		9.5		31.0	
110	11.5		20.0		16.0	
109	12.5		14.0		27.0	
101	9.6		12.0		10.5	
97	27.0		29.0		52.0	
96	36.0		26.0		17.0	
95	6.9		16.0		36.0	
87	60.0		62.0		55.0	
84	39.0		17.0		35.0	
83	24.0		12.0		67.0	
81	17.5		48.0		49.0	
74	100.0		100.0		93.0	
69	48.0		44.0		93.0	
67	18.0		46.0		39.0	
59	3.7		27.0		25.0	
55	76.0		57.0		100.0	
43	22.0		63.0		68.0	
41	94.0		77.0		76.0	

[a] These unusually high fragments may arise also from an unidentified impurity.

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