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METHYL ENOL ETHERS AS ARTEFACTS IN CAPILLARY GAS CHROMATOGRAPHIC PROFILES OF ALDEHYDE DIMETHYL ACETALS

T. BROSCHE*

Institut für Gerontologie der Universität Erlangen-Nürnberg, Heimerichstrasse 58, D-8500 Nürnberg (F.R.G.)

D. PLATT

Institut für Gerontologie der Universität Erlangen-Nürnberg and 2. Medizinische Klinik, Klinikum Nürnberg, Flurstrasse 17, D-8500 Nürnberg (F.R.G.)

and

O. VOSTROWSKY

Institut für Organische Chemie der Universität Erlangen-Nürnberg, Henkestrasse 42, D-8520 Erlangen (F.R.G.)

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SUMMARY

Methyl enol ethers are detected within the capillary gas chromatographic profiles of aldehyde dimethyl acetals derived from human red cell plasmalogens or from a synthetic aldehyde mixture. They arise from the dimethyl acetals via methanol elimination as artefacts of the injection technique. In a nutritional experiment, the probands showed a rising proportion of dimethyl acetals in the phospholipid fraction of their erythrocyte ghosts; this fact is seen as an effect of the lipid composition of the milk fat administered.

INTRODUCTION

In order to observe diet-induced changes in complex mixtures of fatty acid methyl esters (FAMEs) and dimethyl acetals (DMAs), generated by acidic transesterification of the total phosphatides of human erythrocyte ghosts, we had to define DMA profiles to reveal possible peak overlapping in capillary

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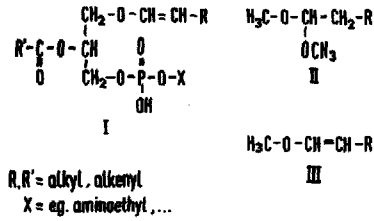

Fig. 1. Structural formulae of 1-alkenyl-2-acylglycerophospholipids (I), dimethyl acetals (II) and methyl alk-1-enyl ethers (III).

gas chromatography (GC). Plasmalogens, 1-alkenyl-2-acylglycerophospholipids (I, Fig. 1), are widespread in lipids of animal tissue [1] and can represent over 30% of the total phospholipids in mammalian organs, especially in heart and nervous tissue. In man, plasmalogens comprise 3—5% of the plasma phosphatides but 12—23% of the total phosphatides in erythrocytes [2]. The lipids present in mature, intact red cells seem to reside exclusively in the cell membrane [3], and, as a consequence of the lack of cell organelles, changes in lipid composition are the result of exchanges with the surrounding milieu [4, 5]. Different dietary fats in nutritional experiments influence the fatty acid and plasmalogen aldehyde composition of erythrocyte phospholipids [6—8].

Several methods have been published to characterize and quantify the alkenyl ethers of red cells [2, 9—12]. Long-chain aliphatic aldehydes, DMAs and the corresponding methyl enol ethers have been synthesized and examined by mass spectrometry [13] and by gas chromatography—mass spectrometry (GC—MS) [14]. The conversion of DMAs to vinyl ethers during gas–liquid chromatography (GLC) on preparative and packed analytical columns has been documented [15, 16]. We wish to report the presence of methyl alk-1-enyl ethers (III, Fig. 1) in capillary GC elution profiles of DMAs (II, Fig. 1), which could also explain the "unknown" assignments in former publications.

EXPERIMENTAL

Materials

The following chemicals were used: hexane, chloroform, methanol, acetone, toluene, diethyl ether (all analytical-reagent grade), boron trifluoride—methanol (20% in methanol) and thin-layer chromatographic (TLC) plates (silica gel 60 without fluorescence indicator, 20 × 20 cm, with a layer thickness of 0.25 mm) from Merck (Darmstadt, F.R.G.), lithium tetrahydridoaluminate ($LiAlH_4$, krist.) from Metallgesellschaft (Frankfurt, F.R.G.), 2,6-di-*tert.*-butyl-4-methyl phenol (BHT) from EGA-Chemie (Steinheim, F.R.G.), 10% palladium on charcoal from Fluka (Buchs, Switzerland) and silica Sep-Pak cartridges from Waters Assoc. (Milford, MA, U.S.A.). Hexadecanal and pentadecanal were synthesized by pyridinium chlorochromate oxidation of the corresponding alcohols [17].

Instrumentation

A Finnigan 3200E gas chromatograph—mass spectrometer interfaced with a Finnigan 6000 data system was used for analysis. GLC was performed on a

WCOT-FSCC SE30 column, 25 m × 0.25 mm I.D. (Chrompack, Müllheim, F.R.G.), with helium at 1.1 bar, split and splitless modes, temperature programmed. The electron-impact spectra were recorded at 70 eV and 2—3 s/scan.

Gas chromatograms were run and integrated with a Perkin Elmer Sigma 1: WCOT-FSCC SE54 column, 50 m × 0.25 mm I.D. (Chrompack), with nitrogen at 0.8 bar, split mode, temperature programmed, flame-ionization detection (FID); and with two Hewlett-Packard 5880A level-four instruments: WCOT-FSCC CPSil88, 50 m × 0.25 mm I.D. (Chrompack), with helium at 2.4 bar; WCOT-FSCC DB210, 15 m × 0.25 mm I.D. (J. & W. Scientific, Rancho Cordova, CA, U.S.A.), with helium at 0.7 bar; and WCOT-FSCC OV-1, 25 m × 0.25 mm I.D. (Hewlett-Packard, Waldbronn, F.R.G.), with helium at 1.0 bar, split and splitless modes, isothermal and themperature programmed. TLC plates were charged with extracts by means of a Linomat III applicator (Camag, Muttenz, Switzerland).

Preparation of samples

Blood was obtained from 27 female donors, aged over 70 years, within the context of a three-week milk-feeding study described elsewhere [18]. Samples were collected into vials containing ammonium heparin as anticoagulant (Sarstedt, Nümbrecht, F.R.G.); red cell ghosts were prepared according to ref. 19 and lyophilized.

Extraction of lipids

The lipids were extracted from the ghosts [20] by three consecutive extraction steps at room temperature: first, 5—60 mg of dry erythrocyte ghosts were stirred with 30 ml of chloroform—methanol (1:2) for 10 h and filtered, then with 15 ml for 1 h and finally with 15 ml of chloroform—methanol (2:1) for 1 h. All preparations were carried out under nitrogen. All solvents contained BHT as an antioxidant. The combined extracts were evaporated under vacuum, redissolved twice with 1 ml of chloroform and transferred to silica Sep-Pak cartridges.

Silica gel column chromatography

First, all the neutral lipids were separated from the total lipid mixture by elution with 10 ml of chloroform, next the glycolipids were separated with 20 ml of acetone—methanol (9:1) and finally the phospholipids were eluted from the cartridges with 25 ml of methanol [21].

Derivatization of phospholipids

The phospholipids of the methanol fraction were concentrated under reduced pressure, and, after addition of 50 μg of BHT, transesterified with 5 ml of boron trifluoride—methanol reagent [22] by heating for 2 h at 100°C in nitrogen-flushed, sealed glass ampoules. The reaction mixture was diluted and neutralized (4 ml of 1 *M* sodium hydroxide), and the methyl esters and DMAs thus generated were extracted from the mixture three times with 2-ml aliquots of hexane. The hexane layer was dried over sodium sulphate, transferred to brown vials and concentrated in a gentle stream of nitrogen. This solution was

then submitted to GLC analysis, lithium aluminum hydride reduction and TLC separation.

Reduction of esters

The reduction of the methyl esters was performed with an excess of $LiAlH_4$ in dry diethyl ether for 1 h at 4°C. After work-up, the resulting mixture of fatty alcohols and DMAs was extracted with diethyl ether and the diethyl ether solution was dried before GLC and TLC analysis.

Hydrogenation of aldehyde dimethyl acetals

The DMAs were hydrogenated by treating in diethyl ether under a slight pressure of hydrogen for 2 h at 4°C with 10% palladium on charcoal as the catalyst. After filtration, the solution was concentrated for GLC analysis.

Preparative thin-layer chromatography

The preparative TLC separations were performed on methanol pre-run plates. Prior to use, they were activated at 100°C for 1 h. Extracts were applied in lines, and the developing solvent was toluene (hR_F 100 = 17 cm). Separated fractions were detected by visualizing a broken part of the plate by spraying sulphuric acid—methanol (1:1) and charring. The appropriate areas of silica gel were scraped off and eluted with diethyl ether.

RESULTS AND DISCUSSION

The human red cell plasmalogen DMAs consist mainly of hexadecanal-DMA 16:0 (9, Table I) and octadecanal-DMA 18:0 (16) [11], as shown in the gas chromatograms (Figs. 2 and 3) of the TLC DMA fractions of twenty donors. The pooled mixtures of red cell total phospholipid FAMEs and DMAs were either directly separated by preparative TLC (hR_F = 41 and 16, respectively), or first reduced with $LiAlH_4$ and then the resulting fatty alcohols (hR_F = 10) were separated from the unchanged DMAs. Both TLC DMA fractions showed an identical composition. Together with the isomeric 18:1 DMAs (region I in Fig. 3), the two saturated acetals 9 and 16 make up over 90% of the gas chromatogram (Fig. 3). However, the GC peak areas varied with the method of injection. When a slow or hot-needle injection was used, some peaks increased in size and showed a slight tailing (e.g. 9e and 16e in Fig. 2), whereas the size of some other peaks decreased (e.g. 9 and 16). This effect was even more pronounced when a splitless-injection method was employed. The same phenomenon was also observed with a mixture of synthetic 16:0 (9) and 15:0 (7), which was analysed before and after derivatization as well as TLC. Depending on the injection conditions, GC—MS analysis also revealed varying amounts of new peaks at the cost of the parent DMA peaks. Fig. 4 presents the total-ion current (TIC) chromatogram of the TLC DMA fraction derived from the synthetic mixture. The corresponding mass spectrum of 16:0 (9, Fig. 5a) shows typical features of fatty aldehyde DMAs [13, 14]; although the molecular-ion peaks are not recognizable, there are the "quasi-molecular ions", $M^+ - 31$, and the typical loss of 64 a.m.u. from the molecular ions. Further characteristic ions are the oxygen-containing fragments at *m/z* 75 (base peak)

TABLE I

PLASMALOGEN ALDEHYDE DIMETHYL ACETAL COMPOSITION OF HUMAN RED CELL PHOSPHOLIPIDS

Pooled TLC DMA fraction of twenty donors (female, mean age 80.2 years). The numbers correspond to the individual peaks in Figs. 2—4.

Component*	No.	Mass chromatogram			Flame-ionization detection** (%)
		m/z 71	*m/z* 75	$M^+ - 31$	
BHT	1				trace
Unknown			+		
Unknown			+		
12:0***	2		+		
br 14:0***	3		+	227	trace
14:0	4		+	227	trace
Unknown			+		trace
br 15:0***	5		+	241	trace
br 15:0***	6		+	241	trace
15:0	7		+	241	0.14
br 16:0***	8		+	255	trace
16:0	9		+	255	22.89
16:0 AME	9e	+			0.31
16:0 AME	9e	+			0.14
Unknown			+		trace
br 17:0***	10		+	269	0.37
br 17:0***	11		+	269	0.81
17:0	12		+	269	1.49
Unknown	13		+		0.28
Z 9-18:1***	14		+	281	8.26
Z 9-18:1 AME	14e	+			trace
Z 11-18:1	15		+	281	2.34
Z 11-18:1 AME	15e	+			trace
18:0	16		+	283	53.69
18:0 AME	16e	+			0.76
18:0 AME	16e	+			0.38
Unknown	17		+		0.28
br 19:0***	18		+		0.13
19:0***	19		+	297	0.26
20:1***	20		+	309	trace
20:1***	21		+	309	0.52
20:0***	22		+	311	trace
20:0	23		+	311	0.90

*BHT 2,6-di-*tert.*-butyl-4-methyl phenol. The shorthand designation for the individual fatty aldehyde DMAs is *X*:*Y*, where *X* is the number of carbon atoms and *Y* is the number of double bonds in the aliphatic chain. Geometry and double-bond position are given as prefixes; br indicates methyl branching of the chain. AME = (*Z*)-1-/(*E*)-1-alkenyl methyl ether(s), artefacts of GLC analysis.

**Values are taken from Fig. 3.

***Tentatively assigned.

and *m/z* 71. Mass chromatograms were generated from these characteristic ions, which permitted a clear differentiation to be made between one parent DMA and the two derived compounds (Fig. 4). The virtually identical mass spectra of the latter are illustrated in Fig. 5b, for the isomeric compounds 9e, which were

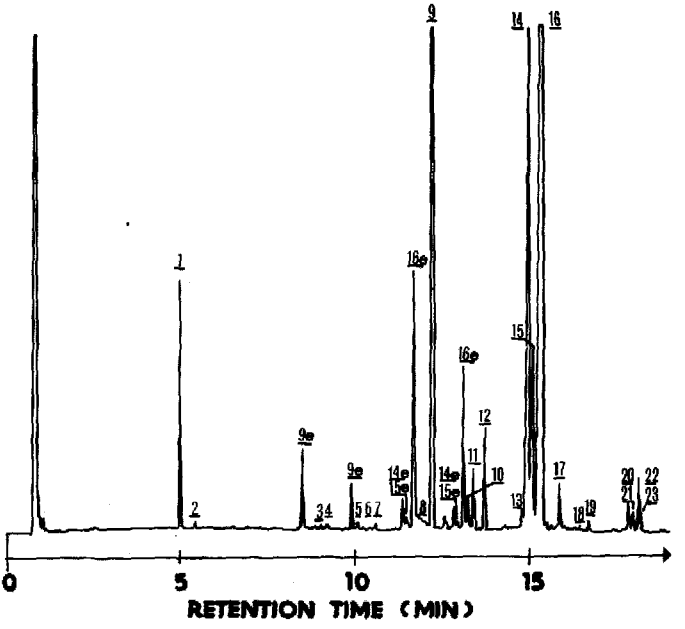

Fig. 2. Gas chromatogram of the red cell plasmalogen aldehyde dimethyl acetals. Pooled TLC DMA fraction of twenty donors. FSCC DB210, temperature programme 1 min 100°C, 5°C/min up to 225°C; hot-needle injection. Numbers refer to Table I.

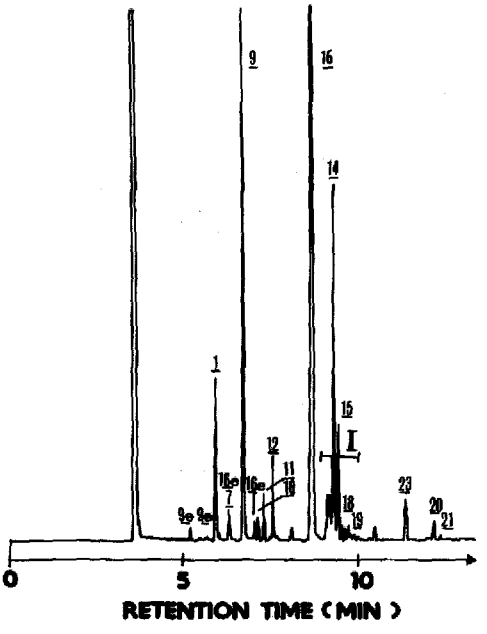

Fig. 3. Gas chromatogram of the red cell phospholipid-derived DMA profile. FSCC CPSil88, temperature programme 1 min 170°C, 2°C/min up to 220°C; cold-needle injection. Numbers refer to Table I. Region I marks the elution of geometric and positional isomers of 1,1-dimethoxy octadecenes.

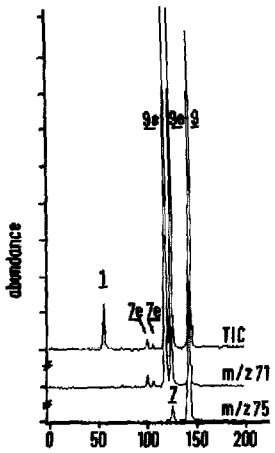

Fig. 4. Total-ion current (TIC) chromatogram and mass chromatograms at *m/z* 75 and 71 of synthetic 16:0 (9) and 15:0 (7) TLC DMA fractions, which demonstrate the presence of (*Z*)-1/(*E*)-1-alkenyl methyl ethers (the e numbers). Numbers refer to Table I.

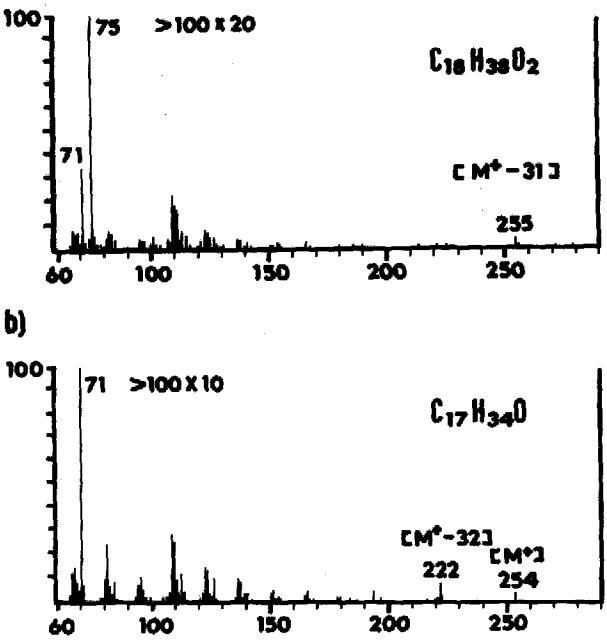

Fig. 5 (a) Mass spectrum of 1,1-dimethoxy hexadecane (9), (b) mass spectrum of (*Z*)-1/(*E*)-1-hexadecenyl methyl ether (9e). Numbers as in Table I.

assigned as those of (*E*)-1/(*Z*)-1-hexadecenyl methyl ethers. The molecular ion, the fragment $M^+ - 32$, the characteristic ion *m/z* 71 that arises from an allyl fragmentation $(CH_3O{-}CH{=}CH{-}CH_2)^+$, and the absence of *m/z* 75, confirm the structure [13, 14]. The equidistant appearance in linearly temperature-programmed gas chromatograms of the methyl enol ethers (III) from the parent DMAs (II) and the constant relative proportion of the isomeric compounds III

(e.g. 16e in Fig. 2) support the idea of (*E*)/(*Z*)-isomerism at the 1-position double bond. This was further confirmed by hydrogenation of the red cell DMA fraction under mild reaction conditions, whereafter only the saturated DMAs and the corresponding enol ethers remained unchanged in GLC analysis. The octadecenyl DMAs and enol ethers were not detectable. The source of the artefacts (III) seems to lie, therefore, in methanol elimination from the DMAs (II) in the injection port, though no clear dependence of the yield on the injector temperature between 180—340°C could be found. In spite of TLC separation and the hydrogenation experiment, we cannot completely rule out some formation of the artefacts during derivatization and subsequent discrimination by poor injection technique.

During the assignment of the DMA species derived from erythrocyte membrane by mass spectrometry and mass chromatography, the literature data on GLC elution behaviour [9] were taken into consideration. The DMA mixtures were also run on FSCC OV-1 and FSCC SE54 columns. The direct comparison of the FAME pattern associated in the same membranes with that of the DMAs revealed clear analogies in the 18:1 DMA isomeric pattern, so we assigned them as *Z* 9-18:1 and *Z* 11-18:1 (Table I). The most volatile isomers (Fig. 3, region I) seem to possess (*E*)-configuration at the olefinic double bonds, though it is reported that (*E*)-isomers do not appear in plasmenyl cholines of milk fat [23] (see below).

No artefacts (< 0.02%) were seen when the cold-needle injection method was applied. Thus, in a nutritional experiment, the influence of orally administered milk fat (35 g per day for three weeks) on the composition of the human red cell membrane was investigated. Total phospholipid FAME- and DMA-profiles were recorded on a CPSil88-coated capillary column (cf. Fig. 3). There was no interfering peak overlap of DMAs and FAMEs. An increase,

TABLE II

DIMETHYL ACETAL COMPOSITION OF HUMAN ERYTHROCYTE GHOST PHOSPHOLIPIDS BEFORE AND AFTER ADMINISTRATION OF 1 l MILK PER DAY FOR THREE WEEKS

Data are measured individually on twenty female donors and analysed three times on a CPSil88 capillary column as percentage area of red cell total phospholipid fatty acid methyl esters and fatty aldehyde dimethyl acetals. The paired *t*-test yielded non-significant values in all cases.

Component*	Percentage area (mean ± S.D., n = 20)		Coefficient of variation (%)		Delta (%)
	Week 0	Week 3	Week 0	Week 3	
16:0	1.94 ± 0.25	2.00 ± 0.24	2.49	1.59	+3.1
18:0	3.51 ± 0.35	3.71 ± 0.32	1.96	1.91	+5.7
E 9-18:0	0.38 ± 0.14	0.38 ± 0.14	7.54	8.23	—
Z 9-18:0	0.66 ± 0.12	0.68 ± 0.15	3.54	4.74	+3.0
Z 11-18:0	0.16 ± 0.05	0.16 ± 0.05	5.50	6.68	—
Total DMA	6.64 ± 0.64	6.92 ± 0.56	1.91	1.76	+4.2

*Abbreviations as in Table I.

though not significant, of the major DMAs and total DMAs was observed (Table II). Surprisingly, the concentration of saturated FAMEs, which, in their native form are the biochemical precursors of DMAs [24], decreased. This contradiction may be explained by the presence of 1-alkyl- and 1-alkenyl ether diacylglycerides [25—27] and 1-alkenyl-2-acyl-phosphoglycerides [23] in the administered milk fat: the 1-alkenyl side-chains of plasmalogens are derived from the corresponding alkyl ether derivatives, which are themselves synthesized de novo or ingested in the diet.

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