A MODEL FOR FATTY ALDEHYDE DIMETHYL ACETAL GAS–LIQUID CHROMATOGRAPHY
THE CONVERSION OF OCTADECANAL DIMETHYL ACETAL TO METHYL 1-OCTADECENYL ETHER*

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INTRODUCTION

Acid-catalyzed methanolysis of lipid mixtures containing plasmalogens (I) yields dimethyl acetals (II) derived from the 1-alkenyl moiety of these compounds:

$$\underset{\text{(I)}}{R'OCH{=}CHR} \xrightarrow{MeOH_2^{\oplus}} \underset{\text{(II)}}{\begin{matrix} MeO \\ MeO \end{matrix}\!\!>\!CHCH_2R} + R'OH$$

Since gas–liquid chromatography (GLC) is the prime technique for analyzing mixtures of dimethyl acetals obtained in this way, it was somewhat disconcerting to observe that dimethyl acetals chromatographed on various gas–liquid columns used in this laboratory gave peaks whose relative retention times did not agree with literature values[1]. Such anomalous behavior has also been noted by others, dimethyl acetals having been reported to decompose on polyethylene glycol adipate on Celite[1], Apiezon L or M on a variety of supports[2,3] and on diethylene glycol succinate on Anakrom A[4]. Satisfactory columns have been made with alkaline supports[2], although this alone is not necessarily a sufficient condition for the prevention of dimethyl acetal decomposition[3]. The identity of the decomposition product has not been established, although methyl ester[1] and methyl 1-alkenyl ether[5] have been suggested. The methyl 1-alkenyl ether hypothesis was supported by the reported isolation of 1-cyclohexenyl methyl ether after GLC of cyclohexanone dimethyl acetal[6].

To gain more understanding of problems connected with plasmalogen analysis, a quantitative and qualitative appraisal was made of the GLC behavior of a representative acetal, octadecanal dimethyl acetal.

METHODS AND MATERIALS

Six mixtures of octadecanal dimethyl acetal (see below) and methyl arachidate** (purity > 99 %) were dissolved in redistilled cyclohexane (Table I) and analyzed

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TABLE I

COMPOSITION OF MIXTURES OF OCTADECANAL DIMETHYL ACETAL AND METHYL ARACHIDATE

Sample	*Octadecanal dimethyl acetal*	*Methyl arachidate*	*Acetal/Ester ratio*		
			Weight	*Mole*	*GLC peak area*[a]
	(*g*)	(*g*)			
A-1	0.0086	0.0610	0.141	0.146	0.132
A-2	0.0092	0.0308	0.299	0.310	0.262
A-3	0.0089	0.0162	0.549	0.571	0.503
A-4	0.0080	0.0081	0.988	1.02	0.866
A-5	0.0153	0.0088	1.74	1.81	1.67
A-6	0.0470	0.0071	6.62	6.88	6.21

[a] Obtained on column 1, Table III.

quantitatively on various gas–liquid columns. Six similarly prepared and examined mixtures (Table II) of methyl *trans*-1-octadecenyl ether (see below) and methyl arachidate were employed to assess production of methyl 1-octadecenyl ether observed during GLC of the dimethyl acetal. The six GLC columns used for chromatographing the standard samples were contained in Barber Colman Model 10 Chromatographs, and are described in a footnote of Table III. The chromatographic peak areas calculated from the peak height and width at one-half peak height, are given in Table III. The hydrogen flame cell used with columns 1 to 4 was calibrated with a standard containing equal weights of methyl palmitate, methyl stearate, methyl arachidate,

TABLE II

COMPOSITION OF MIXTURES OF METHYL *trans*-1-OCTADECENYL ETHER AND METHYL ARACHIDATE

Sample	*Methyl trans-1-octadecenyl ether*	*Methyl arachidate*	*Ether/Ester ratio*		
			Weight	*Mole*	*GLC peak area*[a]
	(*g*)	(*g*)			
E-7	0.0092	0.0623	0.148	0.170	0.152
E-8	0.0092	0.0294	0.313	0.361	0.317
E-9	0.0096	0.0170	0.565	0.654	0.555
E-10	0.0096	0.0075	1.28	1.48	1.28
E-11	0.0187	0.0080	2.34	2.70	2.28
E-12	0.0504	0.0074	6.81	7.85	6.98

[a] Obtained on column 1, Table III.

and methyl behenate*, and the areas for all peaks were within ±3 % (relative) of known values. The argon detectors used with columns 5 and 6 were not calibrated. The retention times of the compounds used in this investigation are listed in Table V. GLC detector response of each of the ratios, octadecanal dimethyl acetal/methyl arachidate and methyl 1-octadecenyl ether/methyl arachidate, was plotted against its

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known weight composition (as in Fig. 1), and the slope of the best line relating these points was determined by the method of least squares. The slopes obtained from the standard samples on the various columns employed and the *t*-values, indicating the significance of their differences, are given in Table IV.

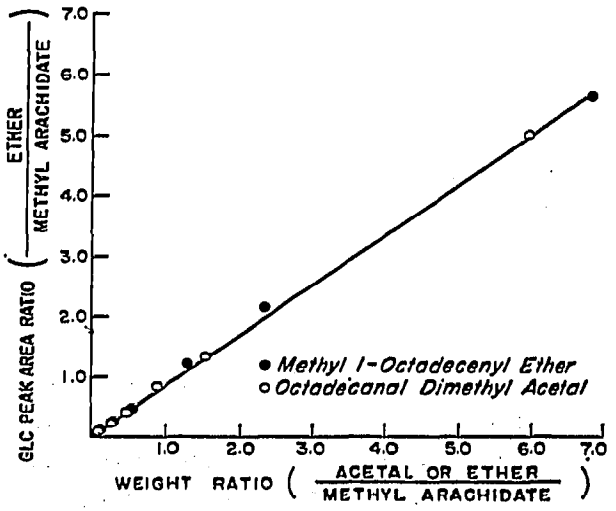


Fig. 1. The efficiency of the conversion of octadecanal dimethyl acetal into methyl 1-octadecenyl ether on GLC column 4. Octadecanal dimethyl acetal–methyl arachidate and methyl *trans*-1-octadecenyl ether–methyl arachidate mixtures were chromatographed, and the resulting methyl 1-octadecenyl ether/methyl arachidate GLC peak area ratio was determined for each sample.

Adsorption column chromatography was used to purify the compounds synthesized for this study. Alumina columns (Merck, active, neutral, or Harshaw, catalyst grade Al-0109 P, 90 % Al_2O_3) were packed by pouring an acetone slurry into the column. Large columns (3.4 × 10 cm) were activated by successive washing with 200 ml each of acetone, ether and pentane. Small columns (2.0 × 10 cm) were activated by successive washing with 100 ml each of the three solvents. Silicic acid (J. T. BAKER's "powder") columns were packed and activated in the same manner. Occasionally benzene was used as one of the chromatographic solvents, and in this case the order of solvent activation was acetone, ether, benzene, pentane. Pentane was sometimes replaced by distilled, reagent grade, 30–60° petroleum ether.

Octadecanal dimethyl acetal

n-Octadecanal (synthesized by the method described below for heptadecanal) was stirred with methanol containing 5.7 % (w/w) HCl for one day at room temperature, and the acid was then neutralized with 5 % aqueous NaOH. The reaction product was extracted into pentane, and the solution was washed with aqueous Na_2CO_3 and dried over anhydrous $MgSO_4$. The dimethyl acetal was eluted by pentane from an alumina column and by 50–75 % benzene in petroleum ether (30–60°) from a silicic acid column.

n-Heptadecanal

This was prepared according to the method of KORNBLUM *et al.*[7]. Warm 1-bromoheptadecane (Eastman Organic Chemicals) (9.94 g) was added in one portion to a suspension of 12 g of $NaHCO_3$ in 75 ml dimethyl sulfoxide heated in a Wood's

TABLE III

GLC PEAK AREA RATIOS METHYL 1-OCTADECENYL ETHER/METHYL ARACHIDATE OBTAINED FROM THE STANDARD SAMPLES

Column 1: 37 in. 0.29% SE-30 on 80–120 mesh glass beads; 181°, 265°, 237° (column, detector and flash heater, respectively); 25 p.s.i.g. argon; H_2 flame detector. *Column 2*: 37 in. 0.23% ethylene glycol succinate on 80–120 mesh glass beads; 144°, 265°, 242°; 15 p.s.i.g. argon; H_2 flame detector. *Column 3*: 36 in. 15% ethylene glycol succinate on acid-washed Chromosorb; 177°, 262°, 244°; 25 p.s.i.g. argon; H_2 flame detector. *Column 4*: 4 ft. 10% Apiezon L on 60–115 mesh siliconized West Coast fire brick; 245°, 257°, 265°; 25 p.s.i.g. argon; H_2 flame detector. *Column 5*: 40 in. 10% ethylene glycol succinate on 70–80 mesh Anakrom ABS; 162°, 223°, 252°; 10 p.s.i.g. argon; argon ionization detector. *Column 6*: 6 ft. 15% ethylene glycol succinate on 90–100 mesh Anakrom ABS; 188°, 220°, 230°; 20 p.s.i.g. argon; argon ionization detector.

Sample	*Column*					
	1	*2*	*3*	*4*	*5*[a]	*6*[a]
A-1	[b]	[b]	0.069	0.114	0.118	0.078
A-2			0.114[c]	0.230	0.292	0.202
A-3			0.251	0.410	0.600	0.310
A-4			0.508	0.831	1.16	0.931
A-5			0.943	1.32	2.07	1.37
A-6			4.37[a]	5.00[c]	10.1	5.60
E-7	0.152	0.133	0.082	0.132	0.183	0.127
E-8	0.317	0.315	0.191	0.281	0.386	0.253
E-9	0.555	0.543[c]	0.355	0.478	0.762	0.530
E-10	1.28	1.35	0.811	1.20	1.66	1.09
E-11	2.28	2.19[c]	1.52	2.12	2.60[c]	2.29
E-12	6.98	6.87	5.59	5.64	13.6	8.24

[a] These data were obtained in other laboratories.
[b] Methyl 1-octadecenyl ether was not formed.
[c] Average of two or three values.

TABLE IV

SLOPE OF GLC PEAK AREA *vs.* WEIGHT RATIOS OF OCTADECANAL DIMETHYL ACETAL–METHYL ARACHIDATE (A-SERIES) AND METHYL *trans*-1-OCTADECENYL ETHER–METHYL ARACHIDATE (E-SERIES) MIXTURES

Column	*Decomposition of dimethyl acetal*	*A-series*	*E-series*	*t-Value*[a]	*Significant difference at 5% level*[b]
1 SE-30/glass	no	0.940	1.025	8.03	yes
2 EGS/glass	no	0.944	1.005	4.05	yes
3 EGS	yes	0.752	0.830	7.26	yes
4 Apiezon	yes	0.839	0.826	0.47	no
5 EGS	yes	(1.734)[c]	(2.026)[c]	(2.95)[c]	—
6 EGS	yes	0.952	1.227	6.30	yes

[a] $t = \dfrac{\text{slope ether} - \text{slope acetal}}{\sqrt{(s^2 \text{ ether} + s^2 \text{ acetal})}}$, where s is the standard deviation of the slope.

[b] $t_{0.05}$ for 4 degrees of freedom = 2.78. Values exceeding this indicate the statistical probability that the A and E-series slopes are different.

[c] Values in parentheses were obtained with an argon ionization detector and are included for comparison. The relation was curvilinear, and application of least squares procedure is not strictly valid.

TABLE V

RETENTION TIMES (MIN) OF METHYL STEARATE, METHYL ARACHIDATE, OCTADECANAL, AND RELATED COMPOUNDS[a]

Compound	*Column*[b]					
	1	*2*	*3*	*4*	*5*	*6*
Methyl stearate	2.6	3.4	9.4	7.6	5.2	—
Methyl arachidate	6.1	9.2	18.6	14.9	9.3	8.6
Octadecanal	1.7	2.2	7.2	6.0	4.4	—
Octadecanal dimethyl acetal	3.3	3.6	—	—	—	—
Methyl *trans*-1-octadecenyl ether	2.0	1.8	4.3	6.0	2.3	2.6

[a] The retention time is measured from the solvent front to the apex of the peak.
[b] These columns are described in a footnote to Table III.

metal bath at 160°. Nitrogen was constantly bubbled through the solution. Some frothing occurred during the reaction. After 5 min the mixture was poured into 200 ml of saturated aqueous $NaHCO_3$. The product was extracted into pentane and the solution was washed with aqueous $NaHCO_3$ and dried over anhydrous $MgSO_4$. After evaporation of the solvent, the aldehyde was separated on a large silicic acid column. Pentane eluted unreacted starting material first and then aldehyde (after about 300 ml). The remaining aldehyde was eluted by 1 and 2 % (v/v) ether in pentane. The yield was 1.35 g of heptadecanal, m.p. 32–33°. (In subsequent chromatography on a smaller silicic acid column, aldehyde was not eluted with pentane so that its appearance from the original column in this fraction was probably due to a column overload.)

Methyl 1-octadecenyl ether

This was prepared by way of a Wittig reaction according to a method of LEVINE[8]. Triphenyl-(methoxymethyl)-phosphonium chloride (4.88 g, 0.0143 mole) was stirred with 100 ml of dry ether in a nitrogen atmosphere. Phenyllithium (0.0145 mole) in benzene–ether solution* was injected through a rubber septum into the suspension of the phosphonium salt. The very intense orange color of the ylid developed immediately. After 10 min, *n*-heptadecanal (1.85 g, 0.0073 mole) in 50 ml of dry ether was added dropwise over a 30-min period, and then about 5 ml of acetone was added to react with the excess ylid. The reaction mixture was poured into aqueous $NaHCO_3$ solution and extracted with pentane. The pentane extract was washed with aqueous $NaHCO_3$ and dried over anhydrous $MgSO_4$. The crude product was chromatographed on a large alumina column. Pentane eluted a total of 1.50 g of 1-octadecenyl ether (72.6 % yield based on heptadecanal) from two columns. GLC (described below) showed that the product consisted of two components, which were identified as the *cis* and *trans* isomers of methyl 1-octadecenyl ether. In two synthetic runs the *cis*:*trans* ratio was 1:3 and 1:2. The leading and trailing portions of the product peak eluted by pentane from the alumina column contained the *cis* and *trans* isomers, respectively. The last portion of the peak also contained some biphenyl, which was present in the phenyllithium reagent. The middle fraction was rechromatographed to obtain additional amounts of the isomers. The infrared spectrum of each compo-

* Foote Mineral Company, Route 100, Exton, Pa.

nent and the relative order of elution from the alumina column was consistent with characteristics of the *cis* and *trans* isomers of methyl 1-dodecenyl ether reported earlier[9]. Catalytic hydrogenation in ethyl acetate over 10% palladium on charcoal reduced both of these compounds to a saturated ether, which had an infrared spectrum and GLC behavior identical to authentic methyl octadecyl ether. The *cis* and *trans* isomers of methyl 1-octadecenyl ether were not separated by thin-layer chromatography on Silica Gel H (Merck) using 1:9 (v/v) ether in pentane as solvent. On an ethylene glycol succinate GLC column (12.2% on Chromosorb G, 80/100 mesh, 4 1 in. long, operated at 177° and 25 p.s.i.g. A), the retention time relative to methyl octadecyl ether was 1.07 for the *cis* isomer and 1.41 for the *trans*. On an SE-30 GLC column (column 1, Table III), the retention time relative to the saturated ether was 0.92 for the *cis* isomer and 1.06 for the *trans*.

RESULTS

GLC of octadecanal dimethyl acetal on various columns routinely used for methyl esters of fatty acids showed that the acetal is unchanged on some while on others it is converted into methyl 1-octadecenyl ether or octadecanal. One sufficient condition for the formation of the aldehyde on the column was the presence of a small amount of HCl in the sample. An acetal sample dissolved in dry HCl/methanol contained sufficient residual HCl after removal of the solvent by evaporation with nitrogen to effect the conversion. This could be prevented by shaking an ether solution of the treated acetal with sodium bicarbonate or by washing the ether solution with aqueous sodium hydroxide.

The quantitative evaluation of the behavior of the dimethyl acetal and 1-alkenyl ether on the GLC columns is based on a comparison with methyl arachidate. It is assumed that there is no loss of the ester on any of the columns. The SE-30-glass bead and EGS-glass bead columns (columns 1 and 2) did not cause loss of the 1-alkenyl ether. This can be seen from the slope of linear relationship between weight and GLC peak area (Table IV) which is essentially 1 for these columns. Columns 3 and 4 which involved diatomaceous supports, decreased the apparent amount of 1-alkenyl ether, the slope of the linear relationship between weight and GLC peak area decreasing in both cases to 0.83. Columns 5 and 6 were used each with a different argon detector, and showed an apparent gain in 1-alkenyl ether, which probably results from a lack of proportionality between detector response and mass.

The comparison of the GLC ratios with the weight and mole ratios of the standard samples (Tables I and II) shows a further example of the well-documented fact that the response of the hydrogen flame ionization detector is more closely related to carbon content than it is to number of molecules.

Chromatography of the dimethyl acetal standard samples on various columns (series A, Table III) reveals amounts of 1-alkenyl ether formed from the acetal. The glass bead GLC columns did not convert the acetal to 1-alkenyl ether. On all the other columns, which were packed with a diatomaceous support, the acetal disappeared and 1-alkenyl ether was formed. How efficiently this conversion occurred may be seen from the slopes recorded in Table IV. Column 3 (Apiezon on firebrick) gave a conversion of acetal to 1-alkenyl ether with the same loss as that observed for the 1-alkenyl ether itself. These data are plotted in Fig. 1. On all of the other columns

the 1-alkenyl ether was formed in less than theoretical amounts (established by chromatography of 1-alkenyl ether standards). The GLC peak area for 1-alkenyl ether formed from dimethyl acetal was corrected for a decrease in the molecular weight due to the loss of methanol.

DISCUSSION

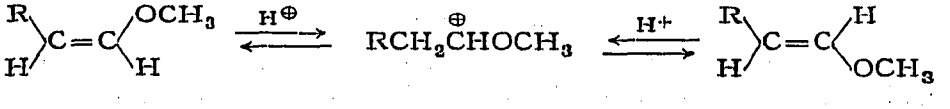
This study shows that octadecanal dimethyl acetal, representative of acetals formed when plasmalogens are transmethylated with acidic methanol, is converted on several types of gas–liquid chromatographic columns to methyl 1-octadecenyl ether. Appearance of the ether as a well-defined chromatographic peak indicates that this conversion occurs at the top of the column. If the conversion occurred all along the column and the sample molecules migrated through the first part of the column as acetal and the last part as ether, the sample would emerge from the column over the entire time range between ether and acetal. The conversion can occur in the first 1 cm of the column. The addition of 1 cm of Chromosorb P, W, or silicic acid, or 10 cm of 20–60 mesh aluminum on the top of a column that did not otherwise change the acetal into the 1-alkenyl ether, effected a complete conversion. One cm of Chromosorb G brought about a partial conversion. The minimum amount of material needed on the top of the column to achieve complete conversion was not established. Presumably this will depend on the number of catalytic sites available. A GLC solid support coated with a liquid phase may behave differently from an uncoated support, and its behavior may be altered with redistribution of the liquid phase on the support after extended use.

Octadecanal was not normally apparent in the chromatograms obtained from columns that caused dimethyl acetal decomposition. However, it was detected in some with very high amplification of the detector signal.

Collection of the effluent from column 4 when dimethyl acetal or 1-alkenyl ether was chromatographed, and rechromatography on column 1 showed that some octadecanal was present (up to 10 %).

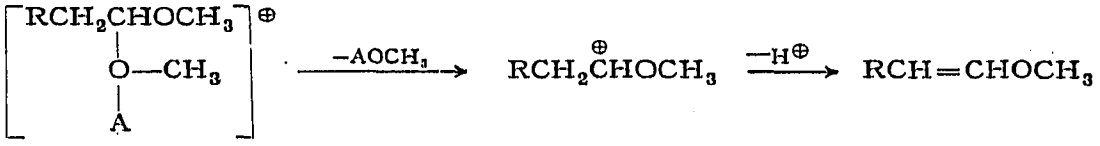
The 1-alkenyl ether collected from column 4 was rechromatographed (GLC column described under methyl 1-octadecenyl ether synthesis) and found to be a mixture of *cis* and *trans* isomers. The 1-alkenyl ether which was collected had a *cis* to *trans* ratio of 0.90 when it came from *trans*-1-alkenyl ether, and 1.0 when it came from the *cis* isomer. Similar examination of samples collected from column 1 showed that no *cis–trans* isomerization occurred on this column.

The mechanisms for the elimination reaction and the isomerization are undoubtedly different in detail but may converge at a common intermediate. The isomerization probably involves a reversible addition of a proton to the 1-alkenyl ether to give a carbonium ion which would have no stereospecific memory.



The source of the proton might be a silanol group from glass or the diatomaceous support. This protonation reaction has recently been shown to be the rate determining step in the acid hydrolysis of vinyl and 1-alkenyl ethers[10].

A mechanism for the alcohol-elimination process may be pictured as involving an attack on acetal oxygen by a Lewis acid and the subsequent formation of a carbonium ion which would lose a proton and form the 1-alkenyl ether.



The identity of the Lewis acid implicated here is not clear, but Al^{3+} in the crystal of the silicate solid support is suspected. In this regard, the only column of those made with diatomaceous solid support that caused relatively little decomposition was made with Chromosorb G, which contains 1.3 % Al_2O_3 compared with the 4.0 and 4.4 % for Chromosorbs W and P.

The significance of these findings with respect to GLC analysis of mixtures of dimethyl acetals derived from lipids is two-fold. First, the dimethyl acetals may, under conditions commonly employed, appear as vinyl ethers, aldehydes or unchanged. Second, quantitative calibration of a GLC detector and a given column with a dimethyl acetal or methyl 1-alkenyl ether standard will not necessarily be valid when different columns are used.

The problems of GLC analysis of dimethyl acetals brought into focus by this research can be satisfactorily solved by qualitative and quantitative calibration of the GLC columns. In this connection, it cannot be too strongly emphasized that a substance emerging from a GLC column is not necessarily the same as that which was introduced. A less empirical solution must await improved knowledge of the catalysts causing the transformations encountered here so that the product obtained from the GLC of dimethyl acetals can be controlled.

SUMMARY

Gas–liquid chromatography of fatty aldehyde dimethyl acetals, produced by methanolysis of plasmalogenic lipids, is shown to be complicated on some columns by more or less extensive conversion of dimethyl acetal into the corresponding methyl 1-alkenyl ether (of both *cis* and *trans* configuration) and/or the parent aldehyde. Interpretation of the resulting chromatograms may be further complicated by less than quantitative detection of such products. Studies of the gas–liquid chromatographic behavior of synthetic octadecanal dimethyl acetal and methyl 1-octadecenyl ether (both isomers) and aldehydes and esters of known structure and purity serve to assess the uncertainties of this analytical technique. Mechanisms involved in the alteration of dimethyl acetals in the course of gas–liquid chromatography on certain columns are discussed.

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