Conversion of fatty aldehyde dimethyl acetals to the corresponding alk-1-enyl methyl ethers (substituted vinyl ethers) during gas–liquid chromatography

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ABSTRACT The behavior of palmitaldehyde and linolealdehyde and of their dimethyl acetals during gas–liquid chromatography on β-cyclodextrin acetate (β-CDX acetate) and ethylene glycol succinate polyester–phosphoric acid (EGSP) columns was studied. The aldehydes were well separated from their dimethyl acetals on the β-CDX acetate column. However, on the EGSP column the retention times of palmitaldehyde and its dimethyl acetal were identical; a mixture of linolealdehyde and its dimethyl acetal gave a split peak.

The aldehydes were recovered unchanged in 80–85% yield by preparative GLC from both columns, but the dimethyl acetals were quantitatively converted to the corresponding alk-1-enyl methyl ethers. The structure of these compounds was elucidated by infrared spectroscopy, mass spectrometry, and chemical means. Upon hydrolysis at low temperatures with 100% H_2SO_4 they yielded the corresponding aldehydes, which were identified as 2,4-dinitrophenylhydrazones.

KEY WORDS separation · gas–liquid chromatography · fatty aldehydes · dimethyl acetals · instability · conversion · alk-1-enyl methyl ethers · low temperature hydrolysis

Methanolysis of tissue lipids containing plasmalogens yields a mixture of methyl esters of fatty acids and dimethyl acetals (DMA). The plasmalogen aldehydes are identified and analyzed by GLC of the DMA (1–4).

Several investigators have reported abnormal behavior of DMA during GLC, including partial decomposition. Gray (4) reported that when DMA were chromatographed on a poly(ethylene glycol adipate) column, they reacted in some way with the polyester to produce a mixture of compounds which included unchanged DMA and some of the corresponding acid methyl esters. Farquhar (1), on the other hand, found the DMA to be stable when chromatographed on ethylene glycol adipate polyester and ascribed the findings of Gray to residual acid catalysts present in the polyester during its preparation. Marcus, Ullman, Safier, and Ballard (5) found that DMA did not emerge from Apiezon L or M columns when commercial solid supports were coated with these liquid phases. They found that DMA were eluted from the columns if the supports were first treated with 1% methanolic KOH, dried, and washed until they gave a pH of 7.4 when suspended in distilled water. Morrison and Smith (3) found partial decomposition of DMA on poly(diethylene glycol succinate) columns but found satisfactory separations on SE-30 (methylpolysiloxy gum, General Electric) columns. The products of decomposition in the former column were not identified.

In view of these divergent results, we studied the behavior of pure fatty aldehydes and their DMA on two stationary phases commonly used in GLC of lipid materials. This paper reports the behavior of pure DMA of palmitaldehyde and linolealdehyde, as well as of the free aldehydes, during GLC on β-cyclodextrin acetate (β-CDX acetate) and ethylene glycol succinate–phosphoric acid (EGSP) columns. The eluted materials from both columns were collected by preparative GLC and

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; DMA, dimethyl acetal(s); β-CDX acetate, β-cyclodextrin acetate; EGSP, ethylene glycol succinate polyester–phosphoric acid.

analyzed by TLC, IR spectroscopy, mass spectrometry, and other techniques. In what follows, only the behavior of palmitaldehyde and its DMA during GLC will be described in detail; essentially identical phenomena were observed with linolealdehyde and its DMA.

MATERIALS AND METHODS

Pure fatty aldehydes and DMA were prepared as described previously (6, 7). The purity of the compounds was established by TLC and other techniques (6, 8).

For TLC, glass plates (20 × 2.5 cm) were coated with a well-stirred suspension of Silica Gel G (E. Merck A. G., Darmstadt, Germany) to give a layer about 250 μ thick. The plates were air-dried for 15 min and activated at 110°C for 1 hr.

GLC

GLC analyses of the aldehydes and DMA were carried out with two gas chromatographs. A Beckman GC-2A instrument equipped with a hydrogen flame ionization detector contained a 366 × 0.4 cm I.D. (0.64 cm O.D.) aluminum column packed with 20% β-CDX acetate on Gas-Chrom R, 30–60 mesh. Helium was used as carrier gas at a flow rate of 60 ml/min and the column temperature was 230°C. A dual column Beckman GC-4 apparatus contained 183 × 0.16 cm I.D. (0.32 cm O.D.) aluminum columns and was also equipped with a hydrogen flame ionization detector. The columns were packed with Gas-Chrom P, 80–100 mesh, impregnated with 20% EGS and 2% phosphoric acid. The column packing was prepared as described by Metcalfe (9). The temperature of the column was 175°C. Helium was the carrier gas at a flow rate of 30 ml/min.

Preparative GLC was carried out with the Beckman GC-2A gas chromatograph equipped with a thermal conductivity detector and the GC-4 apparatus equipped with a 10:1 downstream splitter. The columns used were similar to the analytical columns except that the preparative EGSP column was 0.64 cm O.D. Samples which were solid were dissolved in the minimum quantity of diethyl ether and injected. Others were injected without dilution. The effluents from the columns were collected by means of the gradient cooling technique described by Schlenk and Sand (10).

The IR spectra of samples were taken with a Perkin-Elmer Model 21 double-beam spectrophotometer equipped with sodium chloride optics.

The mass spectra of the eluates from the preparative columns were obtained on a Hitachi Perkin-Elmer RMU-6D single focusing instrument. A liquid injection system was used, with an oven heating the sample to 155°C to produce the vapor. Spectra were recorded at both high (70 ev) and low (11 ev) ionization potentials.

RESULTS

Palmitaldehyde DMA and the free aldehyde had retention times of 10.6 and 18.1 min, respectively, on β-CDX acetate. Linolealdehyde and its DMA were also well separated (retention times 44.7 and 25.3 min, respectively). On EGSP, palmitaldehyde and its DMA had identical retention times (2 min) and were not separated; linolealdehyde and its DMA gave a split peak (retention times 5.7 and 5.2 min, respectively). During preparative GLC, the aldehydes and DMA were injected singly in 20–25 mg quantities and recovered in 80–85% yields. Both palmitaldehyde and its DMA partly crystallized as needles in the collecting tube, whereas linolealdehyde and its DMA condensed as liquid droplets.

Analysis of the Eluates from Preparative Columns

Identical products were obtained from the β-CDX acetate and from the EGSP columns. The products collected after interchanging the columns between the two instruments were also identical. In what follows, the decomposition product of palmitaldehyde DMA is described; exactly analogous results were obtained for linolealdehyde DMA.

TLC. Fig. 1 shows the TLC analysis of the products collected after the injection of palmitaldehyde and its DMA. Palmitaldehyde (R_f in this TLC system 0.6) emerged from the GLC columns unchanged, but the DMA was completely converted to a substance with R_f 0.95. Similar results were obtained with linolealdehyde and its DMA.

Infrared Spectroscopy. Fig. 2 shows the infrared spectra of palmitaldehyde DMA and of the products obtained by preparative GLC of palmitaldehyde and the DMA. The IR spectrum of the product obtained from the aldehyde was identical with that of the original aldehyde. For the product obtained from the DMA, bands characteristic of aldehyde (3.7 and 5.78 μ) (6) and acetal functions (8.37, 8.89, 9.26, and 9.46 μ) (11) were absent. Instead, the infrared spectrum showed strong doublet absorption centered at 6 μ which indicated the presence of a —O—CH=CH-grouping (12) and out-of-plane deformation vibrations of the hydrogens attached to the double bond (10.7 μ), which indicated that the transformation product has a *trans* configuration (13). In addition, the compound exhibited a strong absorption band at 8.22 μ characteristic of enol ethers (14).

Mass Spectrometry. In Fig. 3, the spectra of the decomposition product of palmitaldehyde DMA at high (70 ev) and low (11 ev) ionization potentials are presented. The most important features of the spectra are the peaks at m/e = 254 (corresponding to the molecular weight of hexadec-1-enyl methyl ether) and at m/e = M − 32 (possibly due to the hydrocarbon fragment produced by loss of methanol from the ether). Studies on mass spec-

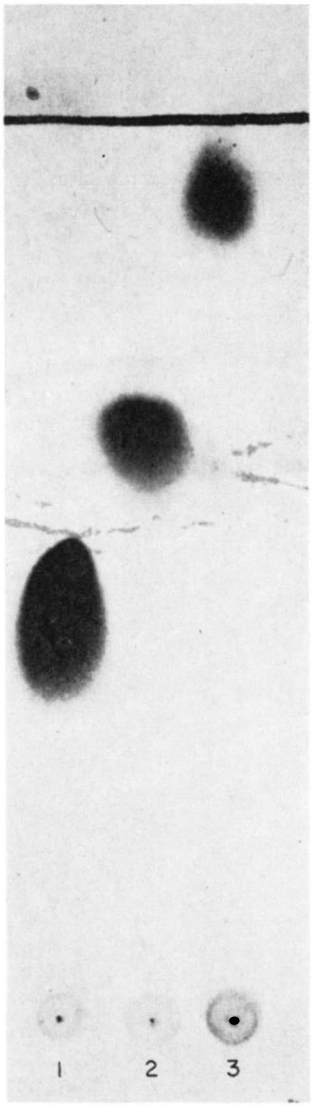

FIG. 1. Thin-layer chromatogram of the products recovered by preparative GLC of palmitaldehyde (lane 2) and of its DMA (lane 3). Lane 1, unchromatographed DMA. Plate developed with toluene and the spots located by charring after the plate had been sprayed with 50% sulfuric acid.

trometry of fatty aldehydes, DMA, and substituted vinyl ethers to elucidate their fragmentation patterns are in progress and will be published later.

Chemical Means. The substituted vinyl ether linkage was easily hydrolyzed by 100% H_2SO_4 by modifications of the procedure used for interesterification of cholesterol esters and phospholipids[1] as described below. The product (30 mg) was dissolved in 20 ml of dry, peroxide-free diethyl ether and cooled to −30°C. To this solution was added 2 ml of 100% H_2SO_4 while the contents were agitated. The mixture was stirred for 20 min at 10°C

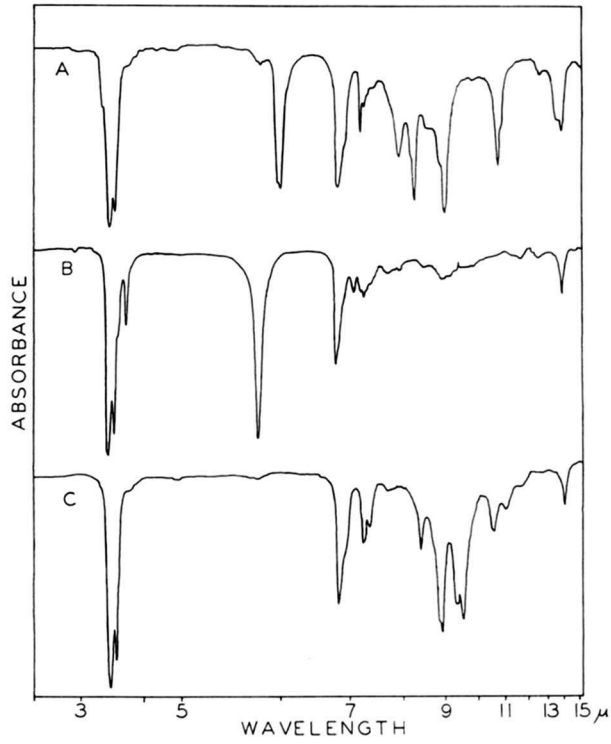

FIG. 2. Infrared spectra of the products recovered by preparative GLC from palmitaldehyde DMA (*A*) and palmitaldehyde (*B*) and of unchromatographed palmitaldehyde DMA (*C*). For *A*, spectrum for a liquid film between salts was obtained. Solution spectra of *B* and *C* were obtained for 10% solutions in carbon disulfide (2.0–4.2 μ, 5–6.1 μ, and 7.2–15.0 μ) and in tetrachloroethylene (4.2–5.0 μ and 6.1–7.2 μ).

under nitrogen and poured into cold water. The precipitated aldehyde was extracted with diethyl ether, the ether extract washed free from acid and dried over anhydrous sodium sulfate, and the ether evaporated off. The conversion to the aldehyde was almost quantitative, as tested by TLC (Silica Gel G–toluene system as described in Fig. 1) and by GLC. The aldehyde was also identified as its 2,4-dinitrophenylhydrazone derivative. The melting point and TLC behavior of this derivative agreed well with previously reported values (6, 8).

Upon hydrogenation of the eluate in the presence of PtO_2 as catalyst, the bands characteristic of the —O—CH═H— grouping (6 and 8.22 μ) described earlier disappeared from the IR spectrum of the hydrogenated product and there appeared instead a strong C—O—C band at 8.8 μ (ether). The spectrum was identical with that of an authentic sample of *n*-hexadecyl methyl ether.

Analysis[2] of the eluate: $C_{17}H_{34}O$ (254.4);

calculated: OCH_3, 12.2

found: OCH_3, 12.0

[1] Viswanathan, C. V., and V. Mahadevan, manuscript in preparation.

[2] Microanalysis was performed by Clark Microanalytical Laboratory, Urbana, Ill.

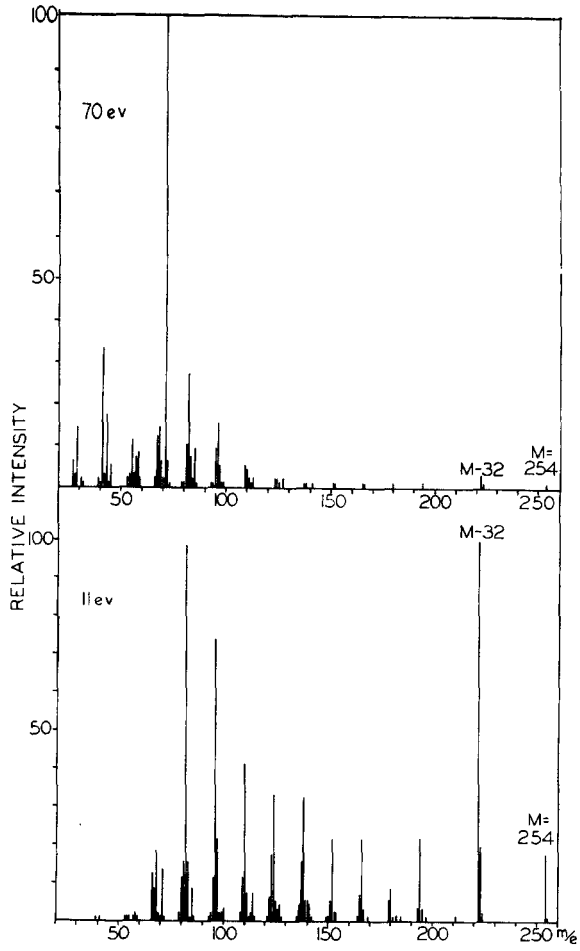

FIG. 3. Mass spectra of the product recovered by preparative GLC from palmitaldehyde DMA at 70 and 11 ev.

We conclude from the above results that the decomposition product on the GLC column was hexadec-1-enyl methyl ether.

DISCUSSION

Since impurities present in the DMA of fatty aldehydes could have given rise to the divergent GLC results of earlier workers, we took care to establish the purity of the aldehydes and DMA used in the present study by a technique other than GLC. They were free from one another and also free from the corresponding methyl esters of the fatty acids as tested by TLC (8).

GLC analysis showed that when tested singly such purified aldehydes and DMA gave single peaks on both the liquid phases used. The solid supports for both columns were used as received from the commercial sources. When 1 g each of the column fillings (supports impregnated with the liquid phases) were suspended in distilled water, the pH (7.2) of the water was unaltered by β-CDX acetate, whereas it was lowered to 3 by EGSP. With such column packings, no peaks corresponding to methyl esters of the respective fatty acids were found nor were any difficulties encountered in the emergence of peaks following injections of DMA during GLC, as reported by other investigators under different conditions (4, 5). The DMA and its parent aldehyde were well separated on the β-CDX acetate column but were not separated on the EGSP column. Since the EGSP column contained 2% phosphoric acid, the identical retention times of the aldehyde and its DMA could have arisen as a result of the decomposition of the DMA to the aldehyde. Subsequent preparative GLC and analysis of the products showed that the DMA was indeed decomposed in both the columns—not, however, to the aldehyde but to the corresponding alk-1-enyl methyl ether.

The mechanism by which a substituted vinyl ether is formed from a DMA during GLC is not known. Temperature, the chemical nature of the solid support, the composition of the liquid phase and the aluminum column itself, or a combination of these factors might influence this reaction. Since identical products were obtained from both preparative GLC columns either using a thermal conductivity detector or using a 10:1 splitter with the hydrogen flame detector, the possibility that the detector catalyzed this decomposition was discounted. Also, keeping the dimethyl acetal in a sealed glass ampule at 230°C for 15 min did not bring about any decomposition, as was shown by TLC and IR spectroscopy of the heated sample. It is reasonable to conclude that the DMA has reacted with the aluminum, the solid support, the liquid phase, or all of them to produce a quantitative conversion to the substituted vinyl ether. Studies on the influences of columns, metal and glass, column supports, polar and nonpolar liquid phases, and temperature during GLC of DMA and other acetals of various fatty aldehydes are in progress.

The findings reported here offer a convenient route to the preparation of substituted vinyl ethers. If they can be obtained without the aid of a gas chromatograph, i.e. if the DMA can be decomposed to the corresponding vinyl ether in the presence of aluminum metal, solid supports, or the liquid phases outside the gas chromatograph, a valuable synthetic route toward the biologically important substituted vinyl ethers, the plasmalogens, may result. Experiments along this line are in progress.

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