# Synthesis of fatty aldehydes and their cyclic acetals (new derivatives for the analysis of plasmalogens)

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ABSTRACT Saturated and unsaturated fatty aldehydes were prepared in good yield by the reduction of acid chlorides with lithium aluminum tri-*t*-butoxy hydride. Saturated odd and even numbered aldehydes were prepared by the ozonolysis–reduction of 1-alkenes. Ozonides were hydrogenated with a Lindlar catalyst or reduced with dimethyl sulfide.

Several diols, including 1,3-propanediol and ethylene glycol, were used to synthesize cyclic acetals from aldehydes and plasmalogens in quantitative yield. Cyclic acetals were synthesized from 2,4-dinitrophenyl hydrazones when an exchanger such as acetone or acetylacetone was included in the reaction mixture.

A number of physical and chemical properties indicate that cyclic acetals are stable compounds which do not decompose during storage or gas–liquid chromatographic (GLC) analysis. The cyclic acetals have unusually long retention volumes which are probably related to the large dipole moments found with cyclic compounds. These derivatives are, therefore, readily separated from their aldehyde and methyl ester analogues on polar and nonpolar stationary phases. GLC analysis of the aldehydogenic moieties in plasmologens may be conveniently carried out by direct conversion to cyclic acetals without preliminary isolation of aldehydes or removal of methyl esters by saponification.

KEY WORDS fatty aldehyde · lithium aluminum tri-*t*-butoxy hydride · Lindlar catalyst · dimethyl sulfide reduction · 2,4-dinitrophenyl hydrazone · cleavage · dimethyl acetal · cyclic acetal · dioxolane · IR spectra · thin-layer chromatography · gas–liquid chromatography · bovine heart · choline and ethanolamine glycerophosphatides

Abbreviations: GLC, gas–liquid chromatography (or) chromatographic; TLC, thin-layer chromatography; DMA, dimethyl acetal; DNPH, 2,4-dinitrophenyl hydrazone; EGS, ethylene glycol succinate polyester; EGA, ethylene glycol adipate polyester. Aldehydes are designated by number of carbon atoms: number of double bonds.

IN RECENT YEARS, a number of analytical procedures for the characterization of the aldehydogenic moieties of plasmalogens have been described. Van Duin (1) analyzed the aldehydes of butter plasmalogens as their dinitrophenyl hydrazone derivatives. Schogt, Haverkamp Begemann, and Koster (2) and Schogt, Haverkamp Begemann, and Recourt (3) oxidized aldehydes to fatty acids and converted these fatty acids to their methyl esters for GLC analysis. Several investigators have prepared dimethyl acetals (DMA) and used these derivatives in GLC analyses (4–7). Katz and Keeney (8) recently described a quantitative micromethod employing 2,4-dinitrophenyl hydrazone derivatives which were reconverted to aldehydes and then reduced and acetylated for GLC analysis as alcohol acetates.

Several problems have been encountered in different studies employing DMA derivatives for the analysis of the aldehydes in plasmalogens. Gray (4) reported the hydrolysis of DMA during GLC on ethylene glycol adipate (EGA) columns, and Farquhar (5) recommended preparing EGA without an acid catalyst in order to prevent degradation on chromatography. Rapport, Gottfried, and Norton [unpublished experiments, (9)] described the formation of 1-octadecenyl methyl ether during the synthesis of octadecyl dimethyl acetal, and Rapport and Norton (9) suggested that the formation of the 1-tetradecenyl methyl ether from the DMA derivative during GLC on EGA may explain the artifact described by Gray. These studies were recently confirmed by Mahadevan, Viswanathan, and Phillips (10) and Stein and Slawson (11), who showed that DMA derivatives were converted to the corresponding alk-1-enyl methyl ethers during GLC. In addition to the disadvantage that artifacts and degradation products are present in DMA

preparations, DMA derivatives have retention volumes that are similar to the analogous methyl esters. Since methyl esters and DMA are both synthesized in the same reactions, saponification and extraction steps are generally required before GLC analysis of DMA.

Fischer and Smith (12, 13), and Fitton, Pryde, and Cowan (14) have synthesized a number of cyclic acetals by reacting short-chain aldehydes such as acrolein and malonaldehyde with 1,2- and 1,3-glycols. The acetals were stable and obtained in high yields even from aqueous solutions. We have applied their procedure to the synthesis of long-chain saturated and unsaturated cyclic acetals of ethylene glycol and 1,3-propanediol. Reference aldehydes were synthesized either by the reduction of acid chlorides with lithium aluminum tri-*t*-butoxy hydride (15) or the ozonolysis of 1-alkenes followed by reduction with either a Lindlar catalyst or dimethyl sulfide (16). Aldehydes were purified by column chromatography or recrystallized as the 2,4-dinitrophenyl hydrazones. The present investigation describes the synthesis of reference aldehydes and 2,4-dinitrophenyl hydrazones, their conversion to cyclic acetals, and the GLC analysis of these derivatives. Cyclic acetal and dimethyl acetal preparations from the same reference aldehyde are compared. In addition, we describe the direct synthesis of cyclic acetals from the choline and ethanolamine glycerophosphatides of bovine heart lipids and the GLC analysis of these derivatives. A preliminary report of this work has appeared elsewhere (17).

## MATERIALS AND METHODS

### *Materials*

Nonyl aldehyde, dodecyl aldehyde, 1-nonadecene, and 1-heptadecene were purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wis.). Tetradecyl aldehyde, octadecyl aldehyde–bisulfite, and oxalyl chloride were purchased from K & K Laboratories Inc. (Plainview, N.Y.). Methyl myristate, methyl palmitate, and methyl stearate were kindly supplied by Dr. J. B. Brown. These esters were over 97% pure on GLC analysis. Oleic acid was purchased from The Hormel Institute (Austin, Minn.). *Trans* components were not found in this sample by IR analysis. Undecyl aldehyde, ethylene glycol, 1,3-propanediol, and *t*-butyl alcohol were purchased from Matheson Co., Inc. (Cincinnati, Ohio). The *t*-butyl alcohol was dried over lithium aluminum hydride and redistilled. The alkenes 1-hexadecene, 1-octadecene, and 1-eicosene were purchased from Continental Oil Co. (Ponca City, Okla.). Thionyl chloride, 2,4-dinitrophenyl hydrazine, and *p*-toluenesulfonic acid were purchased from Eastman Organic Chemicals (Rochester, N.Y.). Diglyme (diethylene glycol dimethyl ether) was purchased from The Ansul Co. (Marinette, Wis.) and distilled first over calcium hydride and then over lithium aluminum hydride. Calcium hydride, lithium aluminum hydride, and lithium aluminum tri-*t*-butoxy hydride were purchased from Ventron Corp. (Beverly, Mass.). Platinum oxide was purchased either from Baker Catalysts Inc. (Newark, N.J.) or Engelhard Industries, Inc. (Newark, N.J.). Pararosaniline hydrochloride, lot 14659, was purchased from Allied Chemical and Dye Corp. (New York). Analytical reagent grade methanol, chloroform, and diethyl ether were purchased from Mallinckrodt Chemical Works (St. Louis, Mo.).

Bovine heart lecithin was purchased from Sylvana Chemical Co. (Orange, N.J.). Thin-layer chromatography (TLC) showed that this material contained small amounts of sphingomyelin and lysolecithin. Choline glycerophosphatides were isolated from a chloroform–methanol extract of fresh bovine heart. In the isolation procedure, neutral lipids were separated from phospholipids by elution from a Unisil silicic acid (Clarkson Chemical Company, Inc., Williamsport, Pa.) column with chloroform. Phospholipids were eluted with methanol and crude choline and ethanolamine glycerophosphatide fractions separated by chromatography (18) on basic alumina (M. Woelm, Eschwege, Germany). The method of Gray and Macfarlane (19) was then used to obtain purified choline and ethanolamine glycerophosphatides. Column fractions were monitored and purified fractions were judged homogeneous by TLC on Silica Gel G (Brinkmann Instruments Inc., Westbury, N.Y.) with a chloroform–methanol–water 65:25:4 solvent system.

### *Thin-Layer Chromatography*

Acetals were readily separated from aldehydes and impurities or side products by TLC on Silica Gel G. When plates were developed with xylene (20), aldehydes ($R_f$ 0.48), DMA ($R_f$ 0.24), and cyclic acetals ($R_f$ 0.18) were identified by both $R_f$ and a color reaction. Only aldehydes became visible as a magenta spot which appeared approximately 1 min after the plate was sprayed with a fuchsin–sulfurous acid solution used in the estimation of plasmals (21). Pararosaniline hydrochloride was used without recrystallization to prepare this color reagent. Other organic compounds were also seen as magenta or blue spots on a pink background when the same plate was placed in an iodine chamber for 2 min and then sprayed a second time with the fuchsin–sulfurous acid solution. Samples were also made visible by charring with sulfuric acid.

### *Gas–Liquid Chromatography*

Acetals and related compounds were analyzed in an Aerograph A-350-B chromatograph equipped with a

thermal conductivity detector, an Aerograph 200 chromatograph equipped with a flame ionization detector, and an F & M 810 chromatograph equipped with a flame ionization detector. 10-ft stainless steel columns, $^1/_4$ or $^1/_8$ inch O.D., containing 10, 13, 15, and 20% EGS on 60–80 mesh Gas-Chrom P (Applied Science Laboratories Inc., State College, Pa.) were used for chromatographic separations on a polar phase. A 6 ft column, $^1/_4$ inch O.D., containing 10% Apiezon M on 60–80 mesh Gas-Chrom P was used for chromatographic separations on a nonpolar phase. Specific operating conditions are listed with the appropriate figures and tables.

## SYNTHESIS OF ALDEHYDES AND ACETALS

### *Preparation of Fatty Acid Chlorides*

A saturated fatty acid and thionyl chloride in approximately 1:3 mole ratio were refluxed for 3 hr. Excess thionyl chloride was removed through a take-off condenser. Acid chlorides were then isolated in 80–90% yield by a Claisen distillation. Oxalyl chloride (22) was substituted for thionyl chloride in the preparation of unsaturated fatty acid chlorides. These acid chlorides were also obtained in 80–90% yield.

### *Reduction of Fatty Acid Chlorides to Aldehydes*

Acid chlorides were reduced with either commercial or freshly prepared lithium aluminum tri-*t*-butoxy hydride (15). In a typical experiment, 40 ml of diglyme was placed in a flask fitted with a pressure-equilibrated dropping funnel and stirrer. Lithium aluminum hydride, 0.05 mole, was placed in the flask and 0.15 mole of *t*-butanol was added through the dropping funnel over a period of 1 hr. This preparation of lithium aluminum tri-*t*-butoxy hydride was used immediately for the reduction reaction. 50 ml of diglyme and 0.05 mole of palmitoyl chloride were placed in a three-necked flask fitted with a pressure-equilibrated dropping funnel and stirrer. The flask was flushed with nitrogen (Hi-Pure, General Dynamics Corp.) and cooled to −70°C in a Dry Ice–acetone bath. The metal hydride was added dropwise with stirring over a period of 1 hr and the temperature maintained at −70°C. The cooling bath was removed at this time and stirring was continued for 1 hr at room temperature. Flask contents were poured over crushed ice and neutralized with hydrochloric acid. The crude aldehyde was then extracted with ether, washed several times with water to remove diglyme, and finally washed with 2% sodium carbonate.

TLC in hexane–ether–acetic acid 70:30:1 showed that the crude aldehyde (75% yield) was contaminated with fatty acid and fatty alcohol. These components were removed by eluting the aldehyde from silicic acid (Mallinckrodt, 100 mesh) with 3% ether in hexane. The purified aldehyde was obtained from the acid chloride in 55% yield.

The aldehyde was also purified by recrystallizing the 2,4-dinitrophenyl hydrazone (DNPH) derivative (8). IR analysis (Beckman IR-5) of olealdehyde DNPH synthesized from highly purified oleic acid showed no absorption at 965 $cm^{-1}$. This indicated that *cis-trans* isomerization did not occur during the reaction sequence. The free aldehyde was regenerated with α-ketoglutaric acid (23). Approximately 0.5 mmole of palmitaldehyde DNPH was mixed with 0.5 mmole of α-ketoglutaric acid and refluxed with 100 ml of 35% sulfuric acid for 1 hr. The mixture was then diluted with water and extracted with ether. The ether extract was washed several times with water, then 10% sodium carbonate, and finally water until neutral. The ether was filtered through anhydrous sodium sulfate and the aldehyde was concentrated. The purified aldehyde was obtained from the DNPH derivative in 73% yield.

### *Ozonolysis and Reduction of 1-Alkenes*

*Lindlar Catalyst.* Approximately 7.5 mmoles of 1-alkene was dissolved in 125 ml of pentane and placed in a gas-trap bottle. The solution was cooled in an ice bath. Ozone, generated in a Welsbach T-408 ozonator, was bubbled through the solution for 30 min. At the end of the reaction, the bottle was flushed with nitrogen and the solution was transferred to a hydrogenation flask together with 500 mg of Lindlar catalyst (24). Hydrogen pressure was adjusted to 5 psi and the flask was agitated for 30 min. A low temperature was maintained during hydrogenation by an ice jacket. The catalyst was removed by filtration, and the aldehyde solution was concentrated. In a typical experiment, octadecyl aldehyde was synthesized from 1-nonadecene in 70% yield. GLC showed that the aldehyde was contaminated with unreacted alkene, which could be separated from the aldehyde by elution from a Unisil column with small amounts of hexane.

*Dimethyl Sulfide.* Approximately 4 mmoles of 1-alkene was dissolved in 100 ml of absolute methanol. The solution was cooled in an ice bath and ozone was bubbled through for 90 min. The flask was flushed with nitrogen to remove excess ozone. The reaction mixture was then raised to room temperature at which time a white precipitate dissolved. 10 ml of dimethyl sulfide was added and the mixture was cooled to 0°C (16) for 2 hr. The solvent was removed under vacuum, and the product was dissolved in 200 ml of ether. The solution was transferred to a separatory funnel and washed four times with 50-ml portions of water to remove dimethyl sulfoxide. The ether was filtered through anhydrous sodium sulfate and concentrated under vacuum. TLC with xylene showed that the crude aldehyde (75% yield) contained traces of fatty

alcohol and fatty acid. The aldehyde was identified by GLC.

*Synthesis of Cyclic Acetals (Dioxolanes)*

*Aldehydes.* Approximately 100 mg of aldehyde, 250 mg of the appropriate diol, and 6–8 mg of *p*-toluenesulfonic acid were added to 100–150 ml of benzene. The mixture was refluxed in a Dean-Stark azeotrope apparatus (phase separating head) for 3 hr and the benzene–water azeotrope removed several times during this period. The acid catalyst was neutralized with solid calcium carbonate and this mixture was filtered into a separatory funnel where excess diol was removed by washing several times with 25-ml portions of ethanol–water 1:6. Each aqueous wash was reextracted with ether, and the ether extracts were combined, passed through anhydrous sodium sulfate, and concentrated under vacuum. GLC analysis showed the absence of unreacted aldehyde and a single peak corresponding to the cyclic acetal. In a typical experiment, 5 mmoles of nonyl aldehyde was refluxed with 15 mmoles of 1,3-propanediol and formed the cyclic acetal in 89% yield.

Analysis[1]: $C_{12}H_{24}O_2$; calculated: C, 71.9; H, 12.1
found with redistilled product: C, 72.5; H, 12.1

*2,4-Dinitrophenyl Hydrazones.* Approximately 100 mg of the DNPH derivative, 300–400 mg of 1,3-propanediol, and 20–30 mg of *p*-toluenesulfonic acid were added to 100–150 ml of benzene. Either 5 ml of acetone or 2 ml of acetylacetone (25) was added as an exchanger. The exchanger used to generate free aldehyde, α-ketoglutaric acid, was less effective in several experiments. The mixture was refluxed for 3 hr in a Dean-Stark azeotrope apparatus; water was removed several times during this period. The reaction products were neutralized with solid potassium carbonate and transferred to a separatory funnel with ether. The ether extract was washed twice with water and then twice with 0.5 N potassium hydroxide in methanol–water 80:20. This ether layer was washed with water until neutral, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was dissolved in hexane, decolorized with activated charcoal, filtered, and concentrated. The product, purified by elution from a Unisil column with 2% ether in hexane, was obtained in 50–60% yield. GLC analysis showed a peak corresponding to the cyclic acetal and several trace components.

*Plasmalogens.* 500 mg of bovine heart lecithin was mixed with 1 g of diol and 10–15 mg of *p*-toluenesulfonic acid in 300 ml of benzene. This mixture was refluxed for 3 hr and cyclic acetals were isolated as described under Aldehydes, above. Cyclic acetals were also separated from the phospholipid residue by elution from a Unisil column with 5% ether in hexane, or from a TLC plate after developing with xylene.

In small-scale preparations 3.2 mg of lecithin was mixed with 60 mg of diol and 3 mg of *p*-toluenesulfonic acid in 25 ml of benzene. This mixture was refluxed for 2 hr as described under Aldehydes, above. The acid catalyst was neutralized with 5 mg of solid potassium carbonate and the contents were transferred with 3 × 3 ml of hexane to a 20 ml tube fitted with a glass stopper. Water, 3 ml, was added to the tube and the contents were shaken vigorously. The upper phase was removed and the lower phase was reextracted twice with 5-ml portions of hexane. The hexane extracts were combined and dried over anhydrous sodium sulfate. The hexane was removed and the sodium sulfate residue was reextracted twice with 5-ml portions of hexane. The hexane fractions were combined, concentrated, and then analyzed for cyclic acetals by GLC.

*Synthesis of Dimethyl Acetals*

The procedures of Gray (4) and Farquhar (5) were used as described to prepare DMA. Reaction products were transferred to a separatory funnel with hexane and washed twice with small amounts of 2% potassium carbonate. The aqueous layers were reextracted with hexane and the extracts combined. This hexane fraction was washed with water until the aqueous layer was neutral to litmus paper. The hexane was then filtered through anhydrous sodium sulfate and concentrated. Yields were not estimated for DMA preparations, which generally contained unreacted aldehyde and several unidentified components (see Results).

## RESULTS

*Physical and Chemical Properties of Acetals*

*IR Spectra.* Acetals were readily distinguished from aldehydes or esters by the absence of a carbonyl absorption band and the appearance of ether absorption bands. Spectra obtained with the Perkin-Elmer Infracord 137 showed that undecyl aldehyde had a carbonyl absorption band near 1740 $cm^{-1}$ (Fig. 1*a*) while the cyclic acetal synthesized from this aldehyde and 1,3-propanediol had characteristic ether bands at 1136–1149 $cm^{-1}$, 1075 $cm^{-1}$, and 1052 $cm^{-1}$ (Fig. 1*b*). The DMA synthesized from undecyl aldehyde had absorption bands at 1179–1185 $cm^{-1}$, 1114–1117 $cm^{-1}$, 1064 $cm^{-1}$, and 1042 $cm^{-1}$ (Fig. 1*c*) which correspond to bands in the spectra for several DMA derivatives obtained by Mahadevan, Viswanathan, and Lundberg (7), and Bergmann and Pinchas (26). The DMA derivative had a distinct carbonyl band at 1740 $cm^{-1}$ (Fig. 1*c*) whereas the cyclic acetal derivative showed

[1] Microanalysis was performed by Micro-Analysis, Inc., Wilmington, Del.

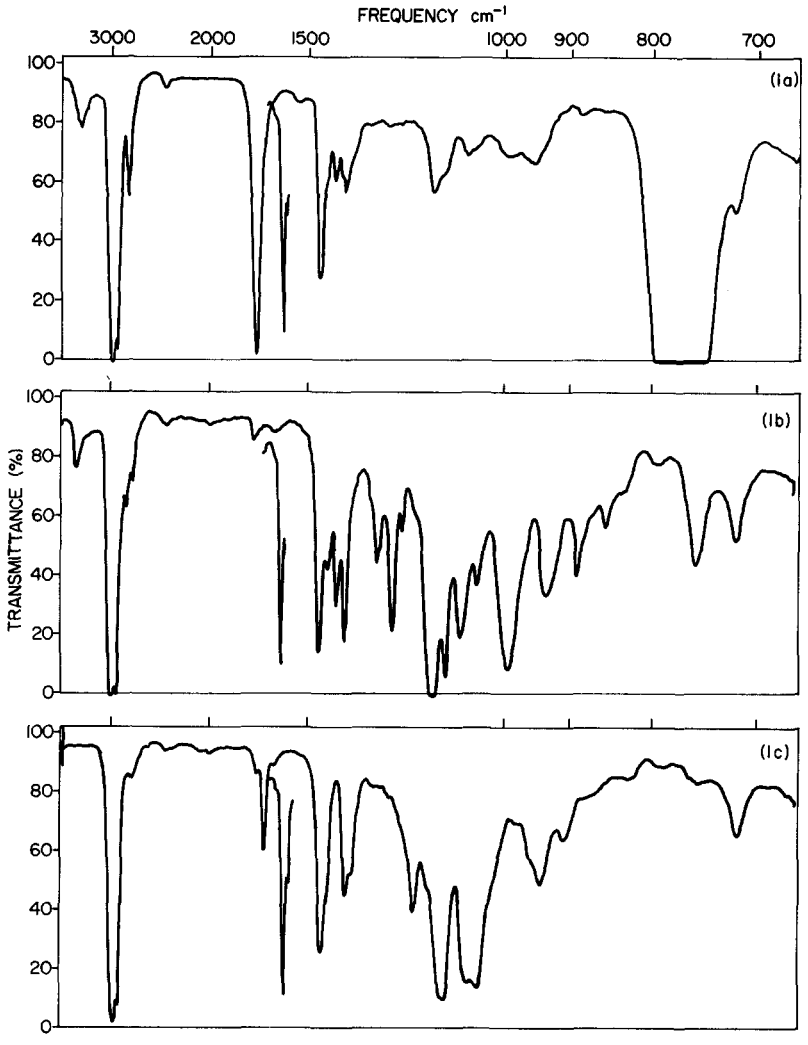


FIG. 1. IR spectra of undecyl aldehyde and acetal derivatives: 1*a*, undecyl aldehyde; 1*b*, 1,3-dioxolane; 1*c*, DMA.

a very small carbonyl absorbance (Fig. 1*b*) even though its concentration was higher than that of DMA as judged by a comparison of the % transmission of the CH stretching vibration at 2941 $cm^{-1}$ in the two spectra. A significant carbonyl band was also found in other DMA preparations synthesized during this study.

*Thin-Layer Chromatography.* Analysis by TLC of undecyl aldehyde and the DMA and cyclic acetal derivatives prepared from this aldehyde is shown in Fig. 2. The aldehyde contained two impurities which had lower $R_f$ values and did not react with fuchsin–sulfurous acid in 1 min. The DMA preparation contained two impurities with higher $R_f$ values than the DMA. The first impurity had the same $R_f$ as the aldehyde and gave the fuchsin color reaction for this compound. The second impurity turned blue after the plate was exposed to iodine and again sprayed with fuchsin–sulfurous acid. The cyclic acetal preparation contained an impurity with a low $R_f$ similar to that of an aliphatic monohydric alcohol. The impurity was not 1,3-propanediol which remained at the origin of the TLC plate. Unreacted aldehyde was not found in the cyclic acetal preparation. This observation confirms IR analyses which showed the absence of a significant carbonyl peak in cyclic acetals. Cyclic acetals prepared directly from a plasmalogen (Fig. 2) did not contain the impurity with a lower $R_f$ value.

*Saponification.* Ethanolic sodium or potassium hydroxide, 0.5 N, was prepared by dissolving 0.05 eq of base in 5 ml of water and then diluting the solution to 100 ml with absolute ethanol. Acetals were refluxed under nitrogen for 90 min with this saponification mixture. The product was transferred to a separatory funnel with ether or hexane and washed with water. The ether layer was concentrated and analyzed by GLC. Both cyclic acetals and DMA yielded the original acetal after saponification. Unreacted aldehyde was largely decomposed during this

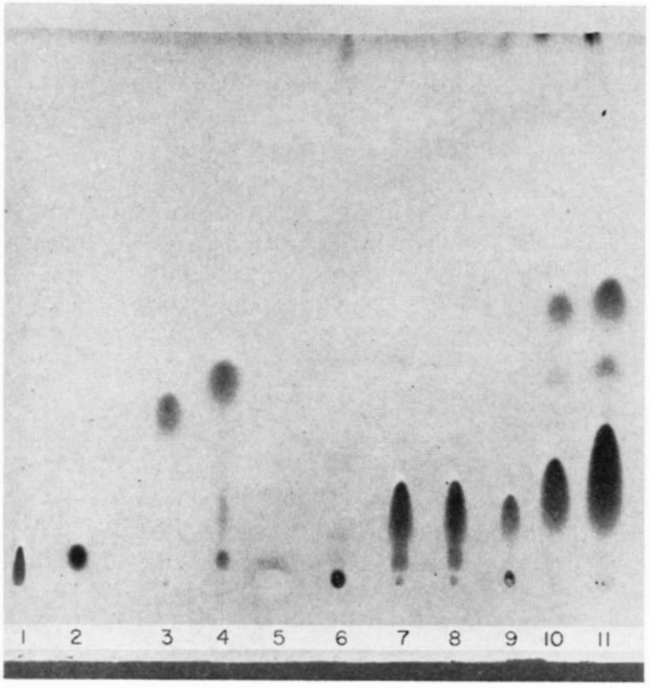


FIG. 2. TLC separation of undecyl aldehyde, reference compounds, and derivatives. 1–5 $\mu$l of a 1% solution was applied. Chromatogram was developed with xylene and made visible by charring with sulfuric acid. *1*, undecenoic acid; *2*, decyl alcohol; *3*, methyl 10-undecenoate; *4*, undecyl aldehyde; *5*, 1,3-propanediol; *6*, 1,3-propanediol refluxed with *p*-toluenesulfonic acid; *7* and *8*, 1,3-dioxolane from undecyl aldehyde; *9*, 1,3-dioxolanes from choline glycerophosphatides; *10* and *11*, DMA from undecyl aldehyde.

reaction. For example, before saponification a mixture which contained aldehyde and cyclic acetal had a 1:1 area ratio on GLC for these components (Fig. 3*a*). After saponification, GLC analysis showed that the mixture contained aldehyde and acetal in a 1:10 area ratio (Fig. 3*b*). Thus the saponification step used to remove methyl esters in the routine preparation of DMA from plasmalogens (4, 5) may also remove unreacted aldehydes if they are liberated by the acid catalyst and not converted completely to DMA.

*Catalytic Hydrogenation.* Cyclic acetals, 50 mg, were dissolved in hexane–ethanol 5:1 containing one drop of pyridine and hydrogenated with 20 mg of a platinum oxide catalyst. The flask contents were under 20–25 psi hydrogen and were shaken at room temperature for 3 hr. The product was filtered and concentrated prior to GLC analysis. This procedure completely hydrogenated the cyclic acetals as judged by the disappearance of the 18:1 component in both standard acetal mixtures and the acetals synthesized from plasmalogens (see Table 2 below).

*Reduction with Lithium Aluminum Hydride.* Cyclic acetals were refluxed with excess lithium aluminum hydride in ether or diglyme. Unreacted metal hydride was destroyed with moist ether. Water was added and the cyclic acetals in the ether layer were removed and dried over anhydrous sodium sulfate. GLC analyses showed that the original cyclic acetal mixture was recovered unchanged in the reaction product.

*Acid Hydrolysis.* A cyclic acetal was refluxed with 3 N HCl for 3 hr. The product was extracted with ether and washed with water until neutral. The ether phase was filtered through anhydrous sodium sulfate and concentrated. GLC analysis showed that the 1,3-dioxolane derivative of hexadecyl aldehyde was reconverted quantitatively to the aldehyde. The cyclic acetal was not hydrolyzed by 90% acetic acid or 90% acetic acid containing mercuric chloride (5) even though the DMA derivative was readily hydrolyzed by these reagents.

### *Gas–Liquid Chromatography*

*Relative Retention Volume.* Typical separations of 1,2- and 1,3-cyclic acetals by GLC on an EGS column are shown in Figs. 4 and 5. Peaks were symmetrical when the flame ionization detector was used. Unsymmetrical peaks were found when the thermal conductivity detector was used with a larger sample. Cyclic acetals were not decomposed on EGS columns. Mixtures which contained 1,3-dioxolane derivatives and methyl stearate had similar area ratios before and after chromatography on 10% EGS in a preparative GLC instrument (Aerograph A-700). The acetal/ester ratios were 1.42 before and 1.59 after preparative GLC for a 2-hexadecyl-1,3-dioxolane–methyl stearate mixture, and 1.19 and 1.26, respectively, for a 2-octadecyl-1,3-dioxolane–methyl stearate mixture.

Retention volume data obtained with Apiezon M (Fig. 6) and EGS (Fig. 7) columns demonstrate that $\log_{10} V'_R$ is a function of carbon number for 1,2- and 1,3-cyclic acetals, and the related aldehydes, alcohols, methyl esters, and alcohol acetates. DMA derivatives were not eluted from Apiezon M columns. This observation is in agreement with earlier studies (10). Since aldehydes and DMA derivatives had very similar retention volumes on EGS columns, GLC could not be used to estimate the conversion of an aldehyde to its DMA derivative. Cyclic acetals had much greater retention volumes than the other derivatives (Table 1) and were readily separated from the parent aldehydes by GLC.

*Analysis of Plasmalogens.* The aldehydogenic moieties of choline and ethanolamine glycerophosphatides obtained from bovine heart lipids were converted to 1,3-cyclic acetals and estimated by GLC (Table 2). Peak areas were measured by triangulation. The data are similar to data reported by Gray (27), who liberated aldehydes from plasmalogens with 90% acetic acid, separated them on a silicic acid column, and then converted them to DMA. Analyses before and after hydrogenation (Table 2) showed that the aldehydes were largely saturated. Retention volume data suggested that two-branched

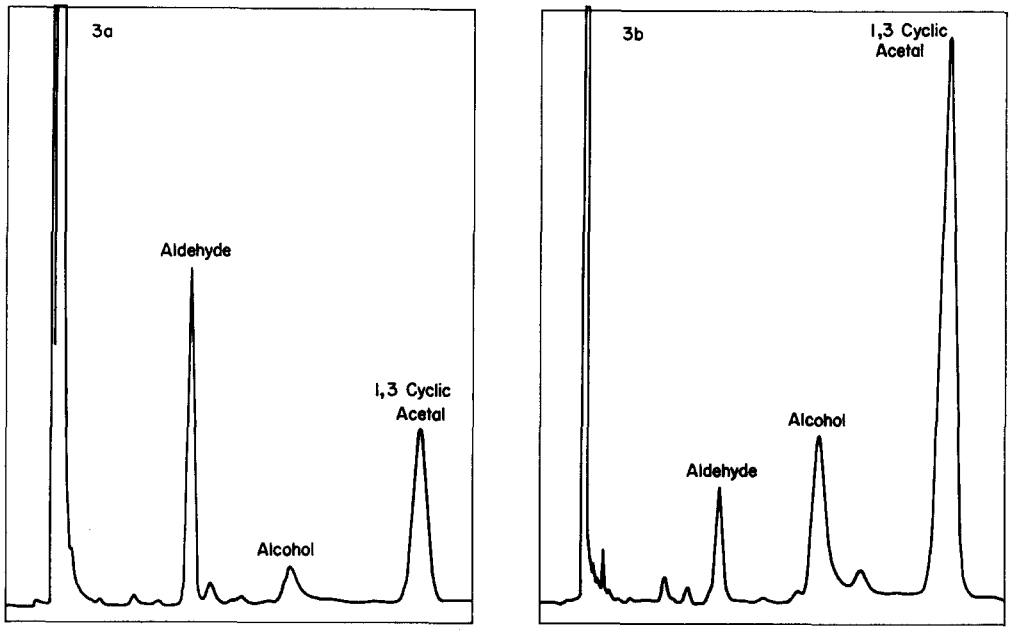


FIG. 3. GLC separation of hexadecyl aldehyde, hexadecyl alcohol, and 2-hexadecyl-1,3-dioxolane. The column contained 20% EGS on Gas-Chrom P and the temperature was 180 °C. 3*a*, before saponification; 3*b*, after saponification.

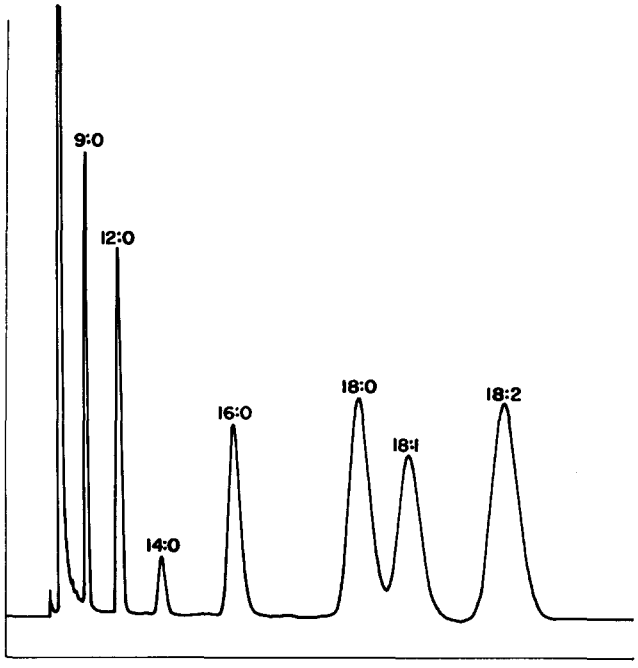


FIG. 4. GLC separation of the cyclic acetals prepared from ethylene glycol and 9:0, 12:0, 14:0, 16:0, 18:0, 18:1, and 18:2 aldehydes. The 10 ft, 1/4 inch column contained 20% EGS on Gas-Chrom P and the temperature was 180 °C. An F & M chromatograph model 810 was used.

chain analogues and the straight chain analogue were present for compounds with carbon numbers of 15 through 18. Gray (27) reported 5.3% more 16:0 and 6.5% less 18:0 in choline glycerophosphatides. He also found 3.4% less 16:0 and 4.8% less 18:0 in ethanolamine glycerophosphatides. The 14:1, 16:1, and 18:2 components reported by Gray were not identified in this study; however, small differences in composition were found after hydrogenation and these differences may indicate that several peaks included unsaturated components.

## DISCUSSION

Unsaturated aliphatic aldehydes have been prepared by a modified Grundmann synthesis (28), an acyloin condensation reaction (29), and the controlled oxidation of tosylates and mesylates by dimethyl sulfoxide (30). The reduction of acid chlorides with lithium aluminum tri-*t*-butoxy hydride is an alternative synthetic method. The reaction is rapid and a crude product is obtained in good yield. Fatty acid and fatty alcohol contaminants are readily separated by silicic acid chromatography or the preparation of the 2,4-dinitrophenyl hydrazone derivatives. IR analysis demonstrates that *cis-trans* isomerization does not occur.

Saturated odd and even numbered aliphatic aldehydes are readily prepared by the ozonolysis–reduction of 1-alkenes. The aldehydes are prepared in good yield from ozonides either by catalytic hydrogenation or reduction with dimethyl sulfide. However, hydrogenation requires the preparation of a Lindlar catalyst. The ozonolysis–reduction procedures are limited by the availability of highly purified 1-alkenes.

The cyclic acetals are interesting and important new derivatives for the analysis of fatty aldehydes. These com-

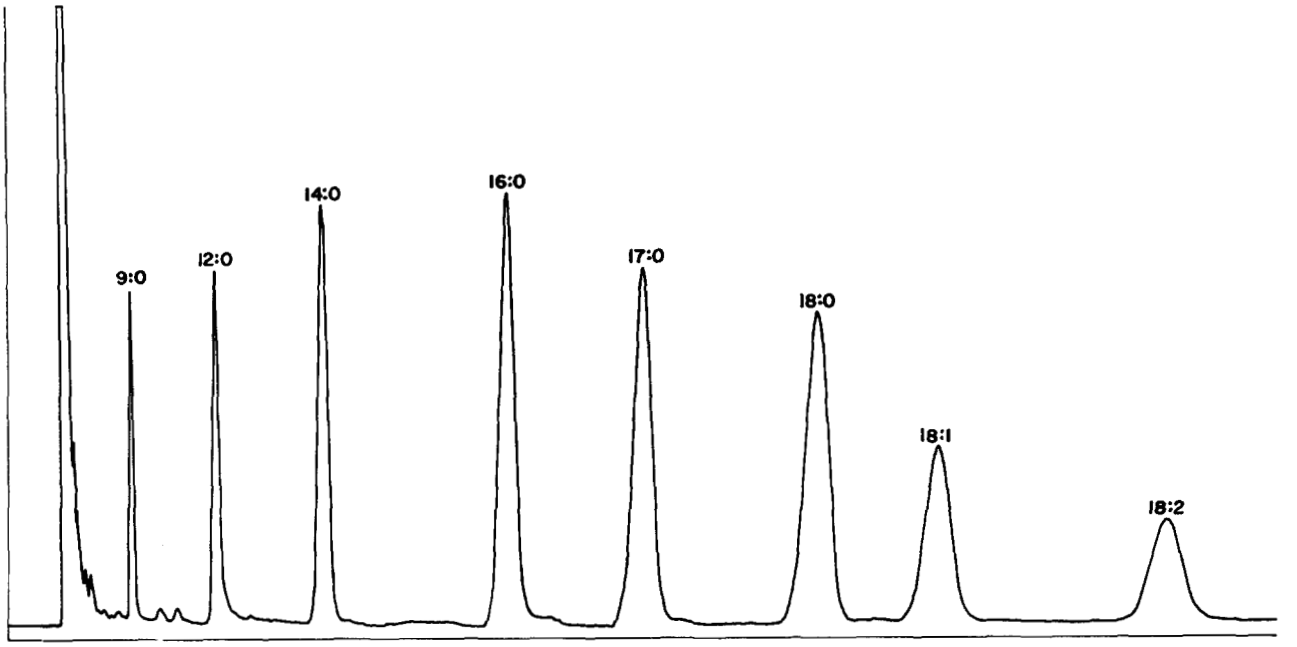


FIG. 5. GLC separation of the cyclic acetals prepared from 1,3-propanediol and 9:0, 12:0, 14:0, 16:0, 17:0, 18:0, 18:1, and 18:2 aldehydes. The 10 ft, 1/8 inch column contained 15% EGS on Gas-Chrom P and the temperature was 180°C. An Aerograph A-200 was used.

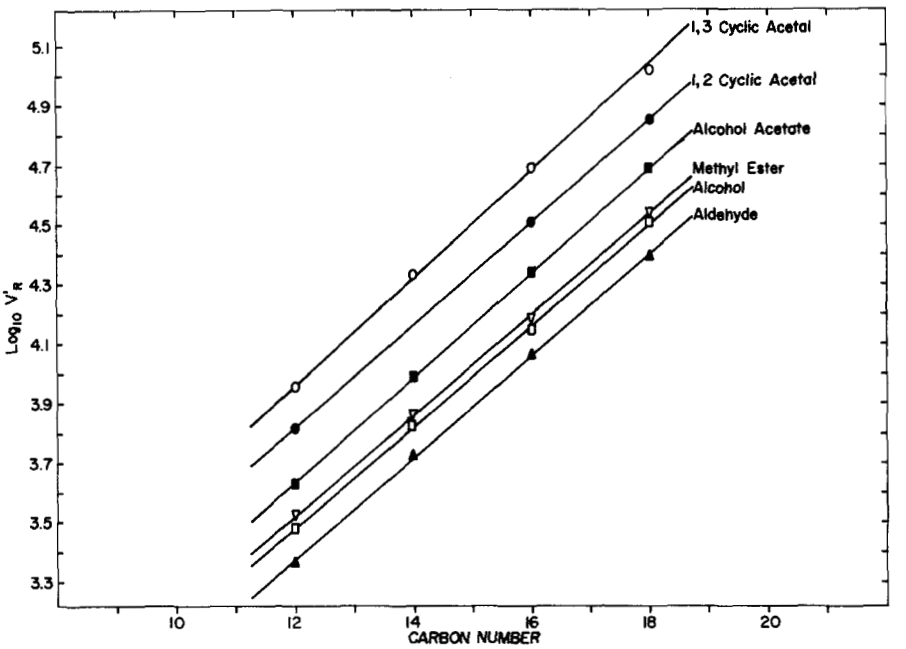


FIG. 6. The relationship between $\log_{10} V'_R$ and carbon number for aldehydes and their derivatives separated on a nonpolar column. The 6 ft, 1/4 inch column contained 10% Apiezon M on 60–80 mesh Gas-Chrom P and the temperature was 215°C. An Aerograph A-350-B was used.

pounds have large retention volumes. For example, the 1,2-cyclic acetals have twice the retention volume of the analogous DMA derivatives (Table 1). Relative volatility data (Fig. 8) show that polarity increases in the order: DMA < alcohol acetate < methyl ester < 1,2-cyclic acetal < 1,3-cyclic acetal < aldehyde < alcohol. Decreased volatility is probably related to the increased dipole moment which is found with cyclic derivatives (31). Thus dimethyl carbonate, molecular weight 90 and dipole moment 1.06 $\mu$ (32), boils at 90°C whereas ethylene carbonate, molecular weight 88 and dipole moment 4.87 $\mu$ (33), boils at 248°C. Since retention volumes are

TABLE 1 RELATIVE RETENTION VOLUMES FOR FATTY ALDEHYDES AND THEIR DERIVATIVES SEPARATED ON A POLAR STATIONARY PHASE*

| | 12:0 | 14:0 | 15:0 | 16:0 | 17:0 | 18:0 | 19:0 | 18:1 | 18:2 |
|---|---|---|---|---|---|---|---|---|---|
| Aldehyde | 0.14 | 0.27 | 0.34 | 0.46 | 0.61 | 0.81 | 1.08 | 0.95 | 1.18 |
| DMA | 0.15 | 0.24 | 0.43 | 0.46 | 0.62 | 0.84 | 1.13 | — | — |
| Methyl ester | 0.19 | 0.32 | 0.42 | 0.57 | 0.75 | 1.0 | 1.32 | 1.11 | 1.38 |
| Alcohol acetate | 0.22 | 0.37 | — | 0.70 | — | 1.15 | — | — | — |
| Alcohol | 0.26 | 0.45 | — | 0.81 | — | 1.45 | — | — | — |
| 1,2-Cyclic acetal | 0.30 | — | 0.72 | 0.95 | 1.25 | 1.68 | 2.22 | 1.89 | 2.36 |
| 1,3-Cyclic acetal | 0.43 | 0.73 | 0.97 | 1.32 | 1.71 | 2.27 | 3.07 | 2.62 | 3.15 |

* The 10 ft stainless steel column, 1/4 inch O.D., contained 10% EGS on 60–80 mesh Gas-Chrom P. Temperature was 192°C. Helium was the carrier gas and the flow rate was 98.3 ml/min. The adjusted retention volume of methyl stearate was 649 ml. A thermal conductivity detector was used.

TABLE 2 ALDEHYDE COMPOSITION OF BOVINE HEART PLASMALOGENS ANALYZED AS 1,3-CYCLIC ACETALS*

| | $r_{18}$† | | Choline Glycerophosphatides‡ | | Ethanolamine Glycerophosphatides§ | |
|---|---|---|---|---|---|---|
| Aldehyde | Standard | Sample | Total | Hydrogenated | Total | Hydrogenated |
| | | | | *% of total* | | |
| (br 12:0)‖ | | 0.38 | 0.7 | 0.8 | 0.4 | 0.8 |
| 12:0 | 0.43 | 0.44 | 2.4 | 3.1 | — | — |
| (br 13:0) | | 0.52 | 0.3 | 0.5 | — | — |
| 13:0 | 0.58 | 0.62 | 0.4 | 1.5 | tr. | 0.8 |
| 14:0 | 0.73 | 0.75 | 0.4 | 0.7 | 0.4 | 0.6 |
| (br 15:0) | | 0.79 | 2.0 | 1.7 | tr. | 1.1 |
| (br 15:0) | | 0.95 | 1.4 | 1.0 | 0.2 | 0.3 |
| 15:0 | 0.97 | 1.00 | 0.9 | 1.5 | 0.6 | 0.9 |
| (br 16:0) | | 1.05 | 1.8 | 2.1 | 1.4 | 1.6 |
| (br 16:0) | | 1.19 | 2.0 | 1.4 | 0.4 | 0.5 |
| 16:0 | 1.32 | 1.32 | 62.2 (62.0–62.5)¶ | 61.5 (60.5–62.2) | 36.7 | 34.7 |
| (br 17:0) | | 1.55 | 2.4 | 2.9 | 1.2 | 0.9 |
| (br 17:0) | | 1.64 | 3.5 | 2.5 | 1.3 | 1.8 |
| 17:0 | 1.71 | 1.75 | 1.7 | 1.5 | 1.7 | 2.6 |
| (br 18:0) | | 1.98 | tr. | tr. | — | — |
| (br 18:0) | | 2.09 | tr. | tr. | — | — |
| 18:0 | 2.27 | 2.27 | 15.5 (15.1–15.8) | 18.6 (18.3–18.8) | 47.4 | 55.4 |
| (br 19:0) | | 2.43 | tr. | tr. | — | — |
| 18:1 | 2.62 | 2.63 | 2.8 | 0 | 8.4 | 0 |

Aldehydes denoted by chain length: no. of double bonds; br, branched.
* The 10 ft stainless steel column, 1/8 in O.D., contained 15% EGS on 60–80 mesh Gas-Chrom P. Temperature was 185°C. A flame ionization detector was used.
† Retention volume relative to 18:0 methyl ester.
‡ Choline glycerophosphatides were purchased from Sylvana Chemical Co.
§ Ethanolamine glycerophosphatides were isolated as described.
‖ Parenthesis indicates a tentative peak assignment from $r_{18}$ data.
¶ Range obtained in analysis of three tracings.

similar, it is not possible to measure the conversion of aldehydes to DMA by GLC. This difficulty is overcome by the large retention volumes of cyclic acetals.

The quantitative conversion of free aldehydes to cyclic acetals is demonstrated by the absence of an aldehyde peak on GLC, of an aldehyde spot on TLC, and of a significant carbonyl band in the IR spectrum of the reaction product. When aldehydes are converted to their DMA derivatives by refluxing with anhydrous methanolic HCl, TLC plates and IR spectra both show that the product contains unreacted aldehyde. A recent study has shown that the conversion of aldehydes to DMA is quantitative when HCl is replaced by 100% sulfuric acid and the reaction is controlled at a low temperature (7). Cyclic acetals can be synthesized directly from 2,4-dinitrophenyl hydrazones when an exchanger such as acetone or acetylacetone is included in the reaction mixture. The yields in the reaction are only 50–60%; however, the method is suitable for the preparation of pure reference compounds.

Cyclic acetals which are prepared from plasmalogens do not show a free aldehyde spot on TLC. The saponification and extraction steps necessary for the removal of methyl esters and the mild acid hydrolysis and extraction

steps used for the isolation of free aldehydes are not required when cyclic acetal derivatives are used for GLC analysis. Saponification destroys free aldehydes (Fig. 3) and may, therefore, remove the aldehydes that are present in a DMA–methyl ester reaction product. If conversion to the DMA derivative is judged by the amount of aldehyde remaining in the sample, the reaction product should be examined before saponification and extraction.

The stability of DMA derivatives has been a significant problem in their analysis. Recent studies have shown conclusively that alk-1-enyl methyl ethers are formed during GLC. These derivatives may also represent one of the

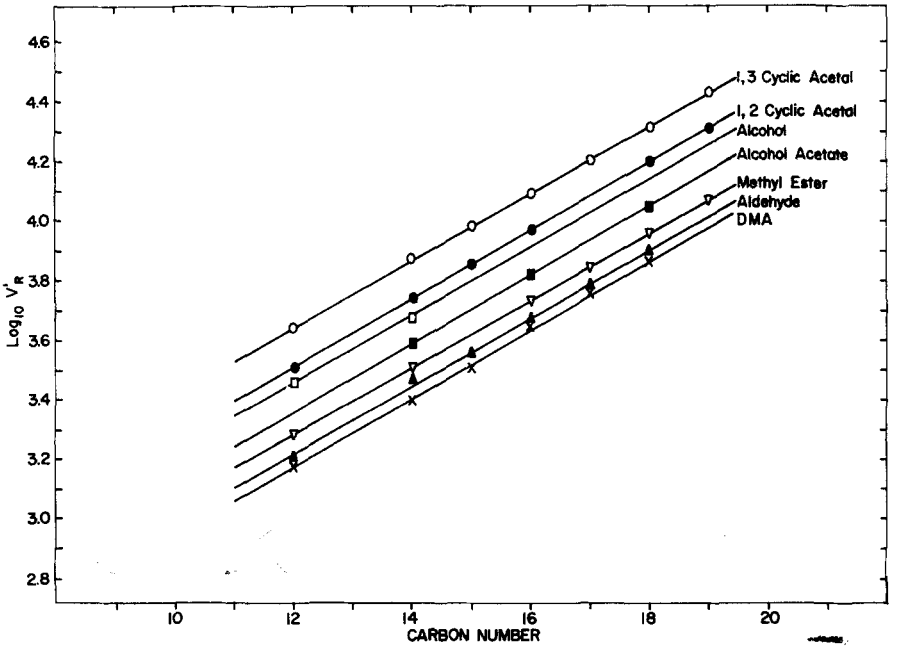


FIG. 7. The relationship between $\log_{10} V'_R$ and carbon number for aldehydes and their derivatives separated on a polar (EGS) column. Operating conditions are described in Table 1.

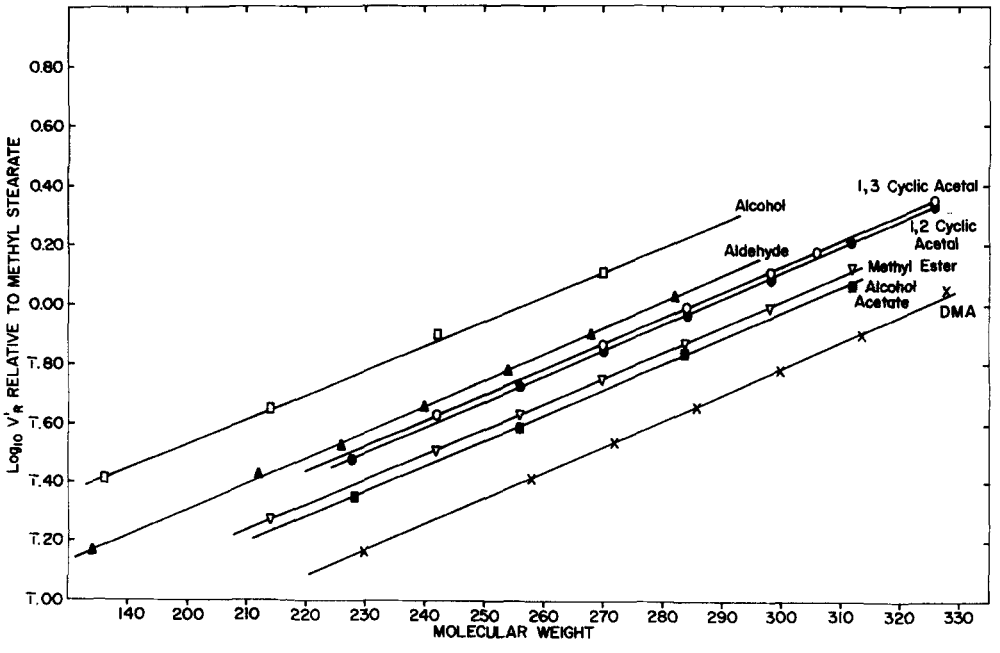


FIG. 8. The relative volatility of aldehydes and their derivatives expressed as the relationship between relative retention volume ($\log_{10} V'_R$ relative to methyl stearate) and molecular weight. Operating conditions are described in Table 1.

several compounds separated from the DMA reaction product by TLC. Cyclic acetals are stable compounds which do not appear to decompose during GLC. They have been stored for as long as 1 yr without measurable decomposition to the free aldehyde. In fact, cyclic acetals are not hydrolyzed appreciably by 90% acetic acid, a reagent which readily hydrolyzes DMA.

A number of other diols are available for the preparation of cyclic acetal derivatives. Fischer and Smith (13) have synthesized stable cyclic acetals with 2-methyl-2,4-pentanediol in aqueous solution. When this diol is used to form cyclic acetals with fatty aldehydes, two peaks are obtained on GLC analysis. These peaks may represent two conformations with different dipole moments such as the 2-alkyl-5-*t*-butyl-1,3-dioxanes which were recently described by Eliel and Knoeber (34). Cyclic acetals suitable for GLC analysis have been synthesized from 2,2-dimethyl-1,3-propanediol and 2,2-diethyl-1,3-propanediol. The separations achieved on GLC with these derivatives are similar to the separations obtained with 1,3-propanediol and 1,2-ethylene glycol. They were not investigated further.

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