J. Lipid Research, January, 1962
Volume 3, Number 1

Identification and gas-liquid chromatographic behavior of plasmalogen aldehydes and their acetal, alcohol, and acetylated alcohol derivatives*†

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[Received for publication July 10, 1961]

SUMMARY

The aldehydogenic chains of human erythrocyte plasmalogens were isolated as a group and converted to their aldehyde, alcohol, acetylated alcohol, fatty acid methyl ester, and dimethylacetal derivatives; similar derivatives were prepared from reference fatty acids. Gas-liquid chromatographic (GLC) constants of these reference and unknown compounds were determined under a variety of chromatographic conditions. These data permit the identification of 19 aldehydogenic compounds in erythrocyte plasmalogens, ranging in chain length from 14 to 19 carbons and in unsaturation from 0 to 2 double bonds. With this information, aldehydogenic chains as dimethylacetals now can be identified even when present with methyl esters of fatty acids. For purposes of studying selectivity of the GLC solvents for the various substituents listed above, it was profitable to relate the chromatographic constants of the various derivatives to those of a series of *n*-alkanes. In studying the relative volatility per unit mass of the various derivatives, it was shown that the order of polarity is alkane < acetal < acetate < methyl ester < aldehyde.

Plasmalogens are a special type of phosphoglyceride from which fatty aldehydes are released by acid hydrolysis. First detected in 1924 by Feulgen and Voit (1), their structure has recently been clarified by Rapport and co-workers (2, 3) and by Debuch (4). They contain one long-chain aldehydogenic chain linked as an α,β unsaturated ether in place of one acyl group of the classical diacyl phosphoglyceride.

A complete description of structural variations of aldehydogenic chains was not possible prior to the introduction of gas-liquid chromatography (GLC).[1]

In order to identify these unknown chains by their GLC constants, it is necessary to convert them without alteration to a class of stable compounds that are suitable for GLC and for which compounds are available for comparison. Long-chain primary alcohols were chosen for this purpose and were obtained by reduction of reference fatty acids. Fatty alcohols obtained by reduction of unknown aldehydes were compared with reference alcohols by GLC on two solvents and at different temperatures. A majority of the aldehydes could be structurally identified in this way. Confirmation was obtained by similar comparisons of the acetylated alcohols. Prior to the completion of this work, Gray (5) reported the identification of tissue aldehydes by GLC comparison of dimethylacetals. More recently, Schogt and co-workers reported identification of aldehydes by comparison of methylated fatty acids obtained after oxidation (6).

In this paper, studies are presented that identify 19 aldehydes derived from human erythrocyte plasmalogens. This identification permits subsequent measurements of these components as dimethylacetals, a derivative more conveniently prepared but for which reference compounds are less readily accessible.

These studies also report the relative retention factors and the temperature dependency of fatty alcohols, acetylated fatty alcohols, fatty aldehydes, and fatty aldehyde dimethylacetals.

A method of plotting relative volatility[2] against mass

* Supported in part by Grant H-2539, National Institutes of Health, Bethesda, Maryland.

† Some of these findings were presented at the meetings of the Federated Societies, Atlantic City, New Jersey, April, 1961 (*Federation Proc.* **20**: 279, 1961).

[1] Abbreviations to be used are: GLC = gas-liquid chromatography; ME = methyl ester of a fatty acid; ald = long-chain aliphatic aldehyde; DMA = dimethylacetal of a long-chain aliphatic aldehyde; alc = long-chain aliphatic primary alcohol; EGA = ethylene glycol adipate polyester.

[2] Relative volatility = relative retention factor = ratio of partition coefficients of solutes in question.

is also presented, and data are derived from these ratios that are relevant to the nature of the forces of interaction of the alkyl chains and of the functional groups of these compounds and the solvents used in GLC.

METHODS

The results reported herein were obtained from studies of human erythrocytes obtained from normal donors. Reference compounds used are listed in Table 1. A shorthand system will be employed in naming compounds: for example, a diunsaturated aliphatic fatty acid such as linoleic acid = *cis*, *cis* 18:$2^{9,12}$ acid; a branched, saturated 17-carbon aldehyde dimethylacetal with a terminal isopropyl group = br 17:0 iso DMA.

Solvents were redistilled. Ethyl ether was peroxide-free, and petroleum ether was the fraction with boiling point 30° to 60°. Solvent mixtures were made on a v/v basis.

TABLE 1. IDENTITY, STRUCTURE, AND SOURCE OF REFERENCE COMPOUNDS*

Reference Compound	Shorthand Designation	Source
Tetradecanoic (myristic)	14:0 acid	Hormel Inst., Austin, Minn.
13-Methyltetradecanoic	br 15:0 ante-iso acid	Dr. H. Sobotka, New York, N.Y., and Dr. A. W. Weitkamp, Whiting, Ind.
Pentadecanoic	15:0 acid	
13-Dimethyltetradecanoic	br 16:0 neo acid	
15-Methylpentadecanoic	br 16:0 iso acid	
cis-Hexadeca-9-enoic (palmitoleic)	*cis* 16:1^{9} acid	menhaden body oil (see ref. 10)
Hexadecanoic (palmitic)	16:0 acid	Hormel Inst., Austin, Minn.
15-Methylhexadecanoic	br 17:0 ante-iso acid	Dr. H. Sobotka, New York, N.Y., and Dr. A. W. Weitkamp, Whiting, Ind.
Heptadecanoic	17:0 acid	
15-Dimethylhexadecanoic	br 18:0 neo acid	
17-Methylheptadecanoic	br 18:0 iso acid	
cis,*cis*-Octadeca-9,12-enoic (linoleic)	*cis*, *cis* 18:$2^{9,12}$ acid	Hormel Inst., Austin, Minn.
cis-Octadeca-9-enoic (oleic)	*cis* 18:1^{9} acid	Hormel Inst., Austin, Minn.
trans-Octadeca-9-enoic (elaidic)	*trans* 18:1^{9} acid	Dr. F. Mattson, Cincinnati, Ohio
cis-Octadeca-6-enoic (petrosolinic)	*cis* 18:1^{6} acid	K and K Labs., Long Island City, N.Y.
trans-Octadeca-6-enoic (petroselaidic)	*trans* 18:1^{6} acid	Dr. F. Mattson, Cincinnati, Ohio
trans-Octadeca-11-enoic (vaccenic)	*trans* 18:1^{11} acid	K and K Labs, Long Island City, N.Y.
cis-Octadeca-12-enoic	*cis* 18:1^{12} acid	Dr. A. Fulco, Los Angeles, Calif.
Octadecanoic (stearic)	18:0 acid	Hormel Inst., Austin, Minn.
17-Methyloctadecanoic	br 19:0 ante-iso acid	Dr. H. Sobotka, New York, N.Y., and Dr. A. W. Weitkamp, Whiting, Ind.
Nonadecanoic	19:0 acid	
Nonanal	9:0 ald	Dr. E. Horning, Bethesda, Md.
Undecanal	11:0 ald	
Tetradecanal (myristaldehyde)	14:0 ald	
Hexadecanal (palmitaldehyde)	16:0 ald	
Octadecanal (stearaldehyde)	18:0 ald	
Palmitaldehyde dimethylacetal	16:0 DMA	Dr. G. Schmidt, Boston, Mass.

* The shorthand designation follows principles outlined previously (10). In addition, abbreviations indicate the class of compound: ald = long-chain aliphatic aldehyde; DMA = dimethyl acetal of aldehyde; acid = fatty acid.

A. Preparation and Isolation of the Aldehydes as Dimethylacetals. Various chloroform-methanol eluates from silicic acid chromatography of total lipids of human red cells served as sources of plasmalogens. Techniques of recovery and the nature of plasmalogen fractions will be reported in a separate communication.[3] Methods similar to those previously described (7) were employed to convert the fatty acids of the phosphoglycerides to methyl esters and the aldehydogenic chains of phosphoglyceride plasmalogens to their dimethylacetals. The lipids were methylated in 5% HCl in anhydrous methanol in sealed ampules at 90° for 2 hours. Prior to sealing, the ampules were flushed with nitrogen, then sealed after cooling in dry ice and acetone.

Conversion of nonanal, undecanal, tetradecanal, hexadecanal, and octadecanal to dimethylacetals was complete (over 90% of theoretical weight) under these conditions. Over 90% of theoretical weight of methyl esters was recovered from egg lecithin and egg phosphatidyl ethanolamine prepared by silicic acid chro-

[3] J. W. Farquhar. In preparation.

matography (8). The methyl esters and dimethylacetals were recovered by adding one-third volume of water and extracting 5 times with equal volumes of petroleum ether. The petroleum ether extracts were washed with an equal volume of ethanol-water, 1:3 (v/v).

Dimethylacetals and methyl esters were then separated as follows. The methyl esters were converted to their sodium salts by saponification with 0.5 N NaOH in 90% methanol at 85° under reflux for 2 hours. Alternatively, samples were saponified overnight at 37° under nitrogen in glass-stoppered tubes. The saponification mixture was extracted 3 times with equal volumes of petroleum ether, and the combined upper phases were washed with the alkaline lower phase (water-ethanol-3 N NaOH, 4:1:0.1 [v/v]). The unsaponifiable dimethylacetals were recovered in the petroleum ether phase, the soaps in the aqueous phase. In experiments with reference compounds (methyl laurate, methyl palmitate, methyl linoleate, palmitaldehyde dimethylacetal), complete saponification of methyl esters (over 90% of theoretical weight) and quantitative recovery of dimethylacetals (over 90% of weight) was obtained. Traces of other nonsaponifiable material in dimethylacetal fractions were removed by sublimation (9).

B. Preparation of Derivatives of Dimethylacetals and Reference Fatty Acids. In order to identify aldehydogenic chains by GLC, it was found useful to convert dimethylacetals and reference fatty acids to other compounds: 1. Unsaturated dimethylacetals could be converted to the saturated derivatives by hydrogenation; 2. Dimethylacetals and reference fatty acids were reduced to alcohols with lithium aluminum hydride; 3. Alcohols were acetylated with acetic anhydride; and 4. Saturated dimethylacetals were converted to methyl esters by methylation (9) after hydrolysis and oxidation with silver nitrate. Methods 2 and 3 had greater utility than 1 and 4 since original unsaturation was preserved. The detailed techniques follow:

Hydrogenation. Dimethylacetals were hydrogenated as outlined previously (10).

Reduction to Alcohols. Fatty acids can be reduced directly, but dimethylacetals must first be hydrolyzed. Dimethylacetals were dissolved in 90% acetic acid 1:30 (w/v), 1 drop of a saturated solution of mercuric chloride was added, and the mixture was heated for 8 to 24 hours at 37° in a sealed ampule under nitrogen. Aldehydes were recovered as follows: An equal volume of water was added, the solution was neutralized with 3 N NaOH, then extracted 4 times with equal volumes of petroleum ether. The extracts were washed once with the alkaline lower phase (water-ethanol-3 N NaOH, 4:1:0.1), then dried over sodium sulfate. If necessary, the aldehydes may be stored for brief periods prior to reduction; however, nonvolatile polymers and partly oxidized products may form unless the samples are kept under nitrogen at −20°.

Aldehydes and fatty acids were reduced to the alcohols with lithium aluminum hydride (11). The amount of reducing agent is not critical if in excess of 1.5 moles per mole of substrate. Ten milliliters of a 3.0% anhydrous ethereal solution of lithium aluminum hydride was added dropwise at −20°, and kept at −20° during a 2-hour period with occasional stirring, to a sample of from 3 to 30 mg of aldehydes or fatty acids dissolved in 5 ml of anhydrous ethyl ether. (It is important that water be excluded prior to cooling because of the violent reaction of the lithium reagent with traces of water.) Then the reaction was stopped by dropwise addition of 5 ml of water to the reactants maintained at −20°. Five milliliters of ethanol was added, the supernatant ether was removed, the lower phase extracted 5 times with petroleum ether, and the combined extracts washed once with fresh lower phase (water-ethanol, 4:1). Recovery of fatty alcohols averaged 82%.

Acetylation of Alcohols. The fatty alcohols (1 to 10 mg) were dissolved in 9 ml of acetic anhydride-pyridine, 3:6 (v/v) in a glass-stoppered tube and kept at 37° with occasional shaking for 15 minutes. After addition of 5 ml of water, the acetates were recovered in three extractions with 5-ml portions of petroleum ether.

Oxidation of Dimethylacetals to Fatty Acids. This was performed after hydrolysis of dimethylacetals as described in Method 2. The aldehydes were then oxidized with silver oxide (12), acids were methylated with HCl-MeOH (9), and the methyl esters were identified by GLC. Since unsaturated aldehydes are decomposed by this oxidation method, this procedure was useful only in identification of saturated dimethylacetals.

C. Identification and Quantification of Aldehydes and Their Derivatives by GLC. The preparations of columns and other GLC techniques have been described previously (10), except for an improved procedure of adipate polyester synthesis and purification that minimizes bleeding, prolongs column life, and may prevent certain reactions during GLC with the dimethylacetal alcohol compounds. In this procedure, the acid catalyst (para-toluene sulfonic acid) was omitted, and the lower molecular weight polymers were selectively removed from the mixture and discarded.[4]

[4] The details of this useful method of polyester preparation were kindly furnished by Drs. J. N. R. Ridyard and D. J. Hanahan.

Chromatographic constants of dimethylacetals from red cell plasmalogens were compared to those of dimethylacetals prepared from saturated reference aldehydes and to similar data on known methyl esters. Comparisons on two solvents and at various temperatures permitted the positive identification of a few saturated dimethylacetals. However, the structures of other dimethylacetals were elucidated by GLC study of derivatives, the preparation of which has been described above. Tables of relative retention factors for dimethylacetals, methyl esters, long-chain aldehydes, alcohols, and acetylated alcohols were constructed from GLC runs made at two or more temperatures and on polar and nonpolar solvents. On the basis of this total experience, it was possible to identify all the aldehydogenic units of human RBC plasmalogens by GLC of the mixed dimethylacetals.

RESULTS

A. Identification of Aldehydogenic Chains of Human Red Cell Plasmalogens. Nineteen major components were noted when the dimethylacetals obtained from red cell plasmalogens were chromatographed at 197° on Apiezon-M (Fig. 1). The identification of these compounds by GLC proceeded in various steps, and the data obtained by one method often confirmed structures suggested by another. The final assignment of structure (Fig. 1, Table 5) to these dimethylacetals was based on the accumulated evidence.

Identification of 16:0 DMA (No. 8, Fig. 1). The initial identification of peak No. 8 as 16:0 DMA by comparison of relative retention factors of a reference sample[5] indicated that a number of unidentified peaks observed on chromatograms of the products of methylation of red blood cell lipids might represent dimethylacetals rather than methyl esters. To establish the presence of acetals, the methylated mixture was saponified and the nonsaponifiable products were chromatographed on Apiezon-M at 197° (Fig. 1).

Identification of Peaks No. 1, 8, and 17. These peaks (Fig. 1) were chromatographically indistinguishable from reference 14:0 DMA, 16:0 DMA, and 18:0 DMA, respectively. The GLC constants of these reference dimethylacetals and their precursor aldehydes are listed in Table 2.

Identification of Peaks No. 7, 9, 10, 14, 15, and 16 as Unsaturated Dimethylacetals. Since these peaks disappeared after hydrogenation, it was evident that these 6 compounds were unsaturated.

[5] This sample had been furnished by Dr. Gerhardt Schmidt to test the suitability of GLC as a tool for the study of this class of compounds.

Identification of Chain Length of Peaks No. 15 and 16. After hydrogenation of a 3-mg quantity of peaks 15 and 16, 18:0 DMA was found to be the sole product, indicating that peaks 15 and 16 were unsaturated C_{18} aldehydes.

Identification of Peaks No. 3, 4, 7, 12, 13, 14, 15, 16, and 19. Table 3 lists relative retention data (related to 18:0 alcohol) of reference alcohols and of alcohols prepared from the mixed red cell dimethylacetals. Data are given only for runs on Apiezon-M; however, similar comparisons were obtained on EGA columns. Peaks No. 4, 13, and 19 were identified as odd-numbered, saturated straight-chain alcohols (15:0 alc, 17:0 alc, 19:0 alc). Peaks No. 3 and 12 were identified as branched, saturated iso or ante-iso alcohols of C_{15} and C_{17} chain length, and peaks No. 7, 14, and 15 as *cis* hexadeca-9-enol (*cis* $16:1^{9}$ alc); *cis, cis*-octadeca-9-12-dienol (*cis, cis* $18:2^{9,12}$ alc); and *cis*-octadeca-9-enol (*cis* $18:1^{9}$ alc), respectively. The identity of peak No. 16 was not completely clarified: in alcohol form it corresponded most closely to *cis* $18:1^{12}$ alc. The precise double-bond structure of the isomers of octadecenol (peaks No. 14 and 15) is still under study.

Identification of Peaks No. 2, 5, 6, 11, and 18. After conversion of dimethylacetals to alcohols, peaks No. 2, 5, 6, 11, and 18 did not match any of the reference alcohols. Their retention characteristics before and after hydrogenation suggested branched dimethylacetals of structure other than iso or ante-iso forms. They may be more highly branched, since they eluted earlier than these 2 reference alcohols.

Identification of Peaks No. 9 and 10. Their elution position in Figure 1 and disappearance after hydrogenation suggests branched C_{17} unsaturated aldehydes; they have not been further defined.

Confirmatory Studies on Acetylated Alcohols. Chromatography of red cell dimethylacetals and reference compounds after conversion to alcohols and then to acetates confirmed the identities suggested by previously described procedures. This confirmation was particularly valuable for the unsaturated peaks No. 7, 14, 15, and 16. Table 4 lists the GLC constants of some reference acetylated alcohols.

Confirmatory Studies on Acids Formed from Dimethylacetals. Conversion of saturated dimethylacetals to aldehydes and then to acids with silver oxide was quantitative (over 90% of theoretical weight); unsaturated dimethylacetals are destroyed by treatment with silver oxide. Chromatographic behavior of the methyl esters of the saturated acids was compared to reference methyl esters on both stationary phases. The results confirmed the identities of the saturated dimethylacetals described above. Chromatographic

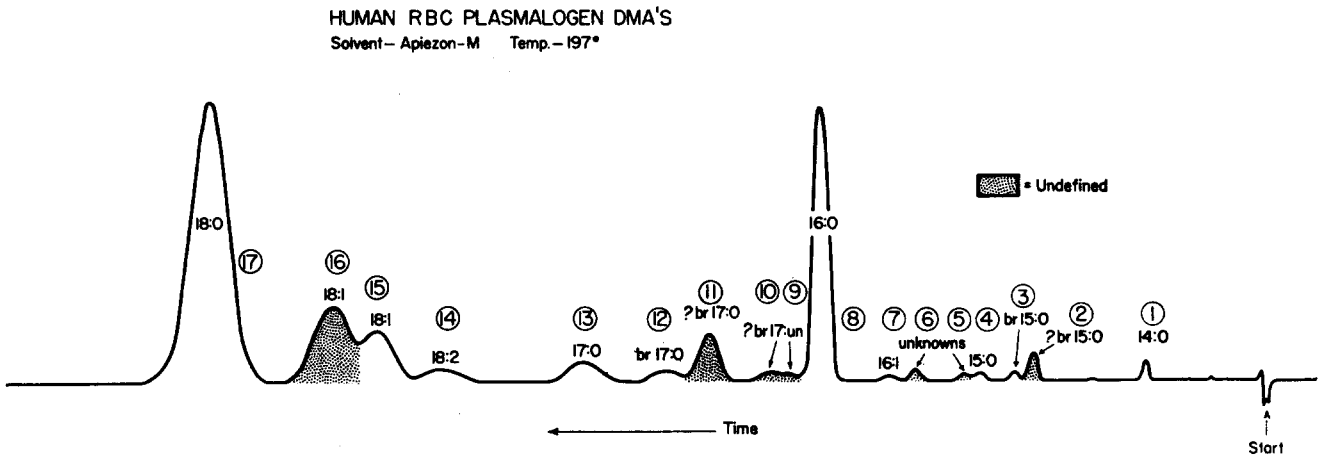

FIG. 1. Chromatogram of human red cell plasmalogen aldehydes (as DMA's). Solvent = Apiezon-M. Temperature = 197°. Retention factors relative to 18:0 DMA are listed in parentheses after each component: 1. 14:0 (0.180); 2. sat. unknown (0.224); 3. br 15:0 (iso or ante-iso) (0.239); 4. 15:0 (0.272); 5. sat. unknown (0.288); 6. sat. unknown (0.335); 7. *cis* 16:1^{9} (0.361); 8. 16:0 (0.424); 9. unsat. unknown (0.455); 10. unsat. unknown (0.471); 11. br 17:0, type unknown (0.530); 12. br 17:0 (iso or ante-iso) (0.567); 13. 17:0 (0.646); 14. *cis, cis* 18:2^{9,12} (0.782); 15. *cis* 18:1^{9} (0.847); 16. 18:1 (structure undefined) (0.887); 17. 18:0 (1.00); 18. br 19:0 (type unknown) (1.22); 19. 19:0 (1.54). Numbers 18 and 19 not shown.

constants of methyl esters have been reported (10).

B. GLC Characteristics of Derivatives of Aldehydogenic Chains.

Apiezon-M Columns. Saturated, branched, and unsaturated aldehydes in the form of alcohols, methyl esters, acetates, or dimethylacetals are successfully chromatographed on the nonpolar stationary phase Apiezon-M, and the major members of each class are easily resolved at 197° (Fig. 2). As recommended by Ambrose, Keulemans, and Purnell (13), tables of relative retention factors of these compounds are listed at different temperatures (Tables 2, 3, 4, and 5), and in each table the specific retention volume of one reference compound (18:0 ME) is included. In addition, temperature effects are demonstrated in plots of r against the reciprocal of absolute temperature (Fig. 3). The absence of intersection or superposition of lines indicates that the common members of the classes of dimethylacetals, alcohols, and methyl esters may be resolved at all temperatures between 173.5° and 197°. Despite lesser resolution, the more rapid elution at 197° would dictate the choice of this temperature in GLC analyses of alcohols and methyl esters.

In order to indicate the selectivity of this stationary phase more effectively than is possible with customary methods of plotting data, the relative retention factors of the various classes of derivatives and of n-alkanes have been plotted against their molecular weight (Fig. 4). This plot shows the expected linearity of log r against molecular weight, and the slopes of the lines for each

TABLE 2. RELATIVE RETENTION FACTORS (r) OF REFERENCE ALDEHYDES AND THEIR DMA DERIVATIVES*

Shorthand Designation	Stationary Phase: EGA 154°	EGA 173°	EGA 184.8°	EGA 197°	EGA 204°	Apiezon-M 173.5°	Apiezon-M 184.8°	Apiezon-M 197°
11:0 ald	0.050	0.068	0.083	0.110				
11:0 DMA	0.059	0.076	0.083	0.110				
14:0 ald	0.160	0.194	0.216	0.258	0.268			
14:0 DMA	0.183	0.218	0.230	0.262	0.266	0.188	0.207	0.228
16:0 ald	0.347	0.392	0.425	0.459	0.477			
16:0 DMA	0.403	0.432	0.449	0.476	0.480	0.493	0.518	0.546
18:0 ald	0.755	0.792	0.816	0.836	0.868			
18:0 DMA	0.879	0.872	0.867	0.874	0.875	1.28	1.28	1.28
18:0 ME	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vg 18:0 ME, ml/g			619			7,840	4,940	2,430

* All r's expressed relative to 18:0 ME. Specific retention volumes (Vg) of 18:0 ME are listed.

TABLE 3. IDENTIFICATION OF HUMAN ERYTHROCYTE PLASMALOGEN ALDEHYDES (AS ALCOHOLS) BY COMPARISON OF GLC BEHAVIOR WITH REFERENCE FATTY ACIDS (AS ALCOHOLS)*

Fatty Alcohols	Shorthand Designation	Relative Retention Factors of Alcohols on Apiezon-M			
		Temp.	$r\left(\frac{\text{unknown RBC alcohol}}{\text{18:0 alcohol}}\right)$	Temp.	$r\left(\frac{\text{reference alcohol}}{\text{18:0 alcohol}}\right)$
Tetradecanol	14:0 alc	197 185	0.178 0.156	197 185	0.179 0.157
13-Methyl-tetradecanol	15:0 ante-iso alc	197 185	0.242 0.216	197 185	0.230 0.216
Pentadecanol	15:0 alc	197 185	0.276 0.249	197 185	0.276 0.249
cis-Hexadeca-9-enol	*cis* 16:1^{9} alc	197 185	0.372 0.342	197 185	0.378 0.343
Hexadecanol	16:0 alc	197 185	0.424 0.397	197 185	0.424 0.397
15-Methyl-hexadecanol	17:0 ante-iso alc	197 185	0.572 0.545	197 185	0.574 0.545
Heptadecanol	17:0 alc	197 185	0.650 0.630	197 185	0.650 0.630
cis, cis-Octadeca-9,12-enol	*cis, cis* 18:$2^{9,12}$ alc	197 185	0.815 0.786	197 185	0.816 0.786
cis-Octadeca-9-enol	*cis* 18:1^{9} alc	197 185 155	0.864 0.845 0.806	197 185 155	0.862 0.841 0.804
Octadecenol (unknown isomer)	18:1 alc	197 185 155	0.895 0.882 0.857		
cis-Octadeca-6-enol	*cis* 18:1^{6} alc			185	0.853
trans-Octadeca-6-enol	*trans* 18:1^{6} alc			185	0.874
trans-Octadeca-11-enol	*trans* 18:1^{11} alc			185 155	0.894 0.870
cis-Octadeca-12-enol	*cis* 18:1^{12} alc			185 155	0.876 0.850
trans-Octadeca-9-enol	*trans* 18:1^{9} alc			185 155	0.875 0.848
Octadecanol	18:0 alc		1.00		1.00
Nonodecanol	19:0 alc	197 185	1.53 1.58	197 185	1.53 1.59

* All r's expressed relative to 18:0 alc.

class are very similar. Differences in retention per unit mass are readily derived by construction of a vertical line through an arbitrary molecular weight. The relative retention factors of members of each class of compound, matched in molecular weight, may be obtained from the intersects with the vertical line. Figure 4 shows very similar retention times per unit mass for all derivatives except the alcohols, which are

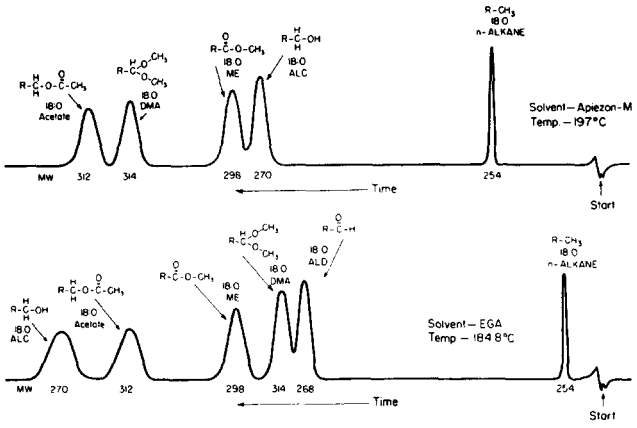

FIG. 2. Chromatographic patterns of different compounds on two stationary phases. Variations in elution behavior of classes (acetates, DMA, etc.) on the two types of solvents are evident. Molecular weight of each compound is listed.

retained 130% longer than saturated straight-chain methyl esters. Acetates and straight-chain methyl esters are nearly superimposable, whereas the elution of dimethylacetals nearly matches that of the neo form of branched methyl esters.

Aldehydes are not eluted from Apiezon columns at temperatures tested (173° to 212°).

EGA Columns. The aldehydes, methyl esters, acetates, dimethylacetals, and alcohols were successfully chromatographed on the polar solvent EGA. The alcohols did not chromatograph on EGA synthesized in the manner previously described (10) until these columns had been thoroughly conditioned by at least a week of continuous use. Figure 2 demonstrates that the major members of each class were well resolved on these polyester columns, but their order of elution was different from that on the nonpolar stationary phase, Apiezon-M. Chromatographic constants (Tables 2, 3, 4, and 5), temperature effects (Fig. 5), and retention times per unit mass (Fig. 6) are presented for comparison with the Apiezon data. Figure 5 reveals that temperatures between 154° and 204° allow resolution of all classes except aldehydes and dimethylacetals, which overlap at the higher temperatures. Figure 6 demonstrates that, on a unit mass basis, alcohols and aldehydes are both considerably less volatile than saturated straight-chain methyl esters. They are retained 280% and 66% longer, respectively, at 184.8°. As on Apiezon, the saturated straight-chain acetates and methyl esters elute at almost the same speed. The dimethylacetals are considerably less volatile on EGA columns than the neo methyl esters, whereas they eluted at similar rates on Apiezon columns.

DISCUSSION

Identification of Aldehydogenic Chains of Plasmalogens. Gas-liquid chromatography techniques have revealed complex structural variations in bound aldehydes in various tissues; however, the instability of free aldehydes and lack of reference compounds are factors that have led other workers in this field to seek stable derivatives for which reference compounds are available. Gray (5) identified tissue aldehydes by comparing their dimethylacetals with reference compounds produced by reduction and methylation of fatty acids. In contrast, Schogt and co-workers (6) identified aldehydes after mild oxidation and methylation to fatty acid methyl esters. The main method employed in this study for identification was comparison of the retention time of alcohols after reduction of unknown aldehydes and reference fatty acids. Identifications of branched and unsaturated aldehydes were strengthened by using a larger number of reference compounds than were used by Gray and by comparing the unknown and reference alcohols after acetylation. With the information thus available, it is now possible to identify with assurance many of the peaks obtained by routine GLC analyses of dimethylacetals. Methylation of lipids containing mixtures of fatty acids and aldehydes will allow identification of methyl esters and dimethylacetals as separate peaks on a single chromatogram. In certain cases overlap will occur; these may be chromatographed separately after saponification and remethylation of the fatty acids. A few areas of uncertainty remain to be explored: double bond positions in certain aldehydes must be established by degradative

TABLE 4. RELATIVE RETENTION FACTORS (r) OF REFERENCE ACETYLATED FATTY ALCOHOLS ON APIEZON-M AND EGA*

Acetate Shorthand Designation	Stationary Phase	
	EGA 184.8°	Apiezon-M 197.0°
br 14:0 iso	0.303	0.216
14:0	0.348	0.248
br 15:0 iso	0.445	0.332
15:0	0.481	0.382
br 16:0 iso	0.580	0.515
cis 16:1[9]		0.498
16:0	0.666	0.591
br 17:0 ante-iso	0.851	0.792
17:0	0.920	0.906
br 18:0 neo	0.928	0.955
cis, cis 18:2[9,12]	1.71	1.11
cis 18:1[9]	1.41	1.19
18:0	1.28	1.39

* All r's expressed relative to 18:0 ME. Specific retention volumes (Vg) of 18:0 ME at these temperatures are given in Table 2.

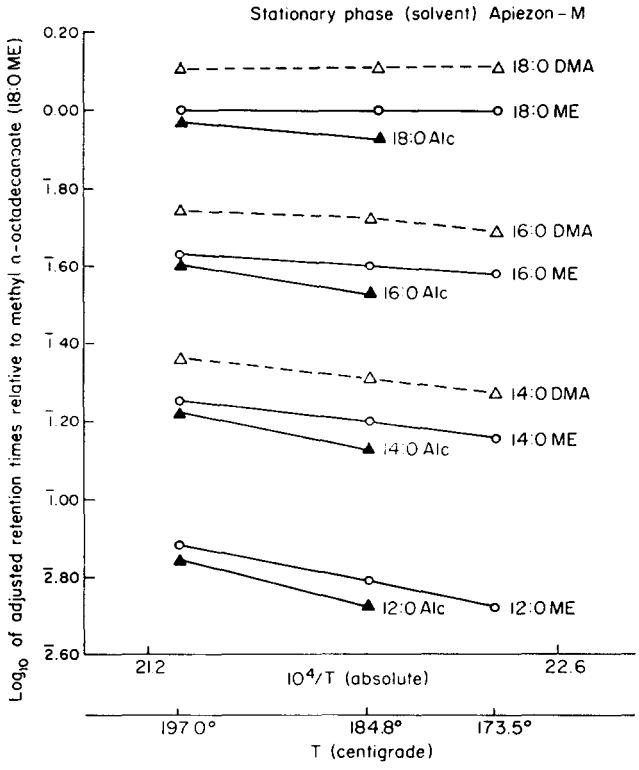

FIG. 3. Plot of $\log_{10} r$ (compound/18:0 ME) of certain saturated alcohols, ME, and DMA vs. $10^4/T$ (absolute) to demonstrate effect of temperature on solvent efficiency. Solvent = Apiezon-M.

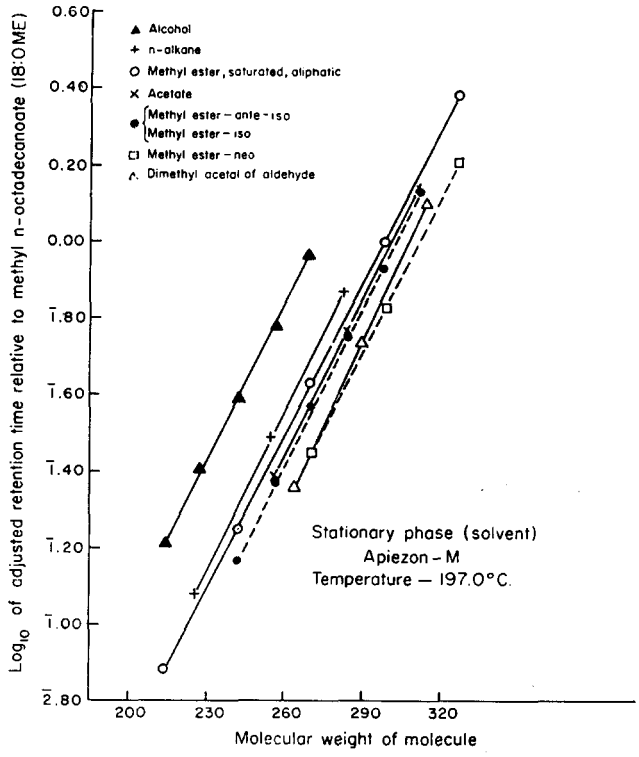

FIG. 4. Plot of $\log_{10} r$ (compound/18:0 ME) of certain saturated alcohols, ME, acetates, and DMA vs. molecular weight of compound to demonstrate solvent selectivity. Solvent = Apiezon-M. Temperature = 197.0°.

methods, and the location of branch points in branched aldehydes will require further studies (the recent work of Schogt and co-workers (14) outlines some useful techniques).

One difference between our results and those of Gray (5) deserves comment. The dimethylacetals were found to be stable when chromatographed on EGA, whereas Gray presented evidence for hydrolysis on such columns. Since the EGA used in these studies was synthesized without catalyst, Gray's results possibly are due to the residual acid catalyst in his EGA.

It seems likely that biochemical studies of aldehydes will be stimulated by the availability of these simple methods for their identification and quantification. For example, comparisons of fatty acid and fatty aldehyde compositions under the influence of alterations of dietary fat have indicated certain common pathways of biosynthesis, which will be discussed in another publication.[6] Comparison of the types of aldehydes present in the various phospholipids of the red blood cell with those reported in other tissues by Gray (5), Leupold (15), and Klenk *et al.* (16) also will be discussed separately.[7]

GLC Characteristics of Aldehydes, Acetals, Alcohols, Acetylated Alcohols, and n-Alkanes. Chromatography of all these compounds is feasible on at least one of the two stationary phases employed, although alcohols may not emerge from unconditioned columns containing EGA (prepared with acid catalyst) and aldehydes are not recoverable from Apiezon-M columns. Some limited data exist on the relative retention factors of dimethylacetals (5); however, the GLC constants of an extensive series of fatty alcohols and their acetates have not been previously described. In view of the occurrence of fatty alcohols as major components of the lipids of certain animals (17), these data should aid in extending knowledge of the structure and metabolism of these alcohols.

Dimethylacetals, alcohols, and acetates are very stable and may be stored for months in petroleum ether at −18° without apparent alteration in composition of mixtures of saturated and unsaturated products. Confirmation of the well-known instability of aldehydes

[6] See footnote 3.

[7] See footnote 3.

TABLE 5. TABLE OF RELATIVE RETENTION FACTORS (r) OF THE HUMAN ERYTHROCYTE PLASMALOGEN DMA'S (NUMBERED 1 THROUGH 19) OF FIGURE 1*

DMA Shorthand Designation	Stationary Phase EGA 184.8°	Apiezon-M 197°
1. 14.0	0.266	0.180
2. Sat. unknown		0.224
3. br 15:0 (iso or ante-iso)		0.239
4. 15:0	0.372	0.272
5. Sat. unknown		0.288
6. Sat. unknown		0.335
7. *cis* 16:1[9]		0.361
8. 16:0	0.518	0.424
9. Unsat. unknown		0.455
10. Unsat. unknown		0.471
11. br 17:0 (type unknown)		0.530
12. br 17:0 (iso or ante-iso)		0.567
13. 17:0	0.724	0.646
14. *cis, cis* 18:2[9,12]	1.32	0.782
15. *cis* 18:1[9]	1.10	0.847
16. 18:1 (type unknown)	1.11	0.887
17. 18:0	1.00	1.00
18. br 19:0 (type unknown)		1.22
19. 19:0	1.39	1.54

* All r's expressed relative to 18:0 DMA.

was obtained; their storage either as bisulfite addition products or as dimethylacetals is recommended. The lack of success of chromatography of aldehydes on the nonpolar stationary phase is difficult to explain. They may either oxidize or polymerize by aldol condensation at the high temperature of the column; however, it is not clear why this should not also occur on the polar stationary phase EGA.

The failure of alcohols to chromatograph when applied to the usual type of EGA column is possibly due to acid catalyzed interchange of the alcohol with the glycol portion of the polyester or to esterification with carboxyl groups of residual adipic acid. It is interesting that other compounds containing hydroxyl groups (for example, the methyl ester of 12-hydroxy-octadecanoic acid), like the primary alcohols, may be irreversibly retained on the older type of EGA column. However, this compound does appear on conditioned EGA columns at a retention time 7.98 times that of 18:0 ME at 184.8°.[8] Acetylation of the hydroxyl group of 12-hydroxy-octadecanoate decreases the retention time (to 5.70 times 18:0 ME), prevents tailing, and therefore facilitates GLC of this hydroxy acid.[9] It is likely that the acetylation reaction will be useful as a method of producing more volatile derivatives of hydroxy compounds suitable for chromatography on the usual polyester phases.

[8] Unpublished data.
[9] See footnote 8.

Comparison of relative retention factors of unknown compounds before and after acetylation may be useful for identification of compounds containing hydroxyl groups since their relative retention factors change in a specific fashion for each compound. Also, acetylation may facilitate chromatography of a wide variety of other compounds that have hydroxyl groups in their molecules by permitting analysis on polyester stationary phases.

Selectivity of Apiezon-M and EGA Solvents for the Various Classes of Compounds Studied. In the course of our work, GLC constants of n-alkanes and of the various classes of fatty compounds (methyl esters, alcohols, alcohol acetates, aldehydes, dimethylacetals) were obtained; in order to determine the influence of the functional groups of these compounds on their GLC behavior, their relative volatility is compared with that of n-alkanes (Figs. 4 and 6). Useful thermodynamic data, including partition and activity coefficients, may be derived from these constants (18) and calculations may be made of the molecular weight of unknowns or of the degree of dimerization of associated molecules. In the practice of GLC, however, they serve also to facilitate the identification of unknown compounds by clarifying the different selectivity of each solvent for the various classes. These differences are evident on inspection of their r values (Tables 2, 3, and 4) and of Figures 4 and 6. Figure 4 shows that on nonpolar Apiezon the volatility relative to mass of all classes of compounds is quite similar except for alcohols. The compounds may be arranged in ascending order of retention (or decreasing volatility) on Apiezon as follows: neo methyl esters, dimethylacetals, iso and ante-iso methyl esters, acetates, methyl esters, n-alkanes, and alcohols. The similarity in volatility per mass of all the straight-chain compounds except the alcohols, as well as the decreased retention of the branched molecules, is consistent with the hypothesis that London dispersion forces predominate in the forces of interaction of solute and this nonpolar solvent. Since dimethylacetals are quite close in elution behavior to the branched methyl esters, it is reasonable to consider the acetal group analogous to the branching of alkyl groups. The much greater retention of alcoho's is most likely explained by their tendency to associate by hydrogen bonding, resulting in decreased volatility and tailing—a phenomenon previously described in GLC of fatty acids (19).

Figure 6 portrays a greater difference in elution behavior between classes. The n-alkanes elute considerably earlier than any of the other compounds on EGA. The rapid elution of n-alkanes on EGA indicates that all the functional groups (aldehyde, methyl

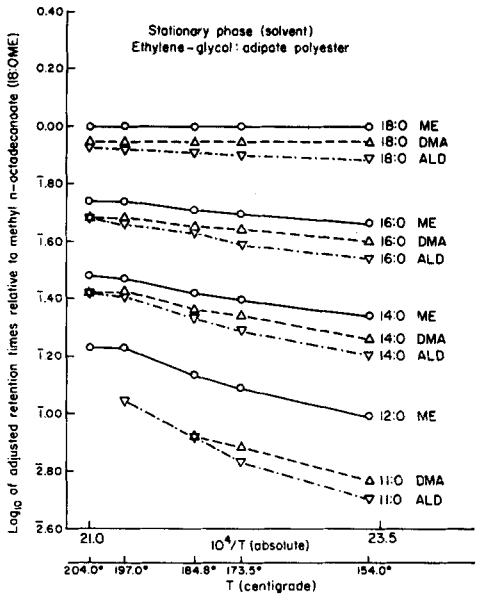

FIG. 5. Plot of $\log_{10} r$ (compound/18:0 ME) of certain saturated aldehydes, ME, and DMA vs. $10^4/T$ (absolute) to demonstrate effect of temperature on solvent efficiency. Solvent = EGA.

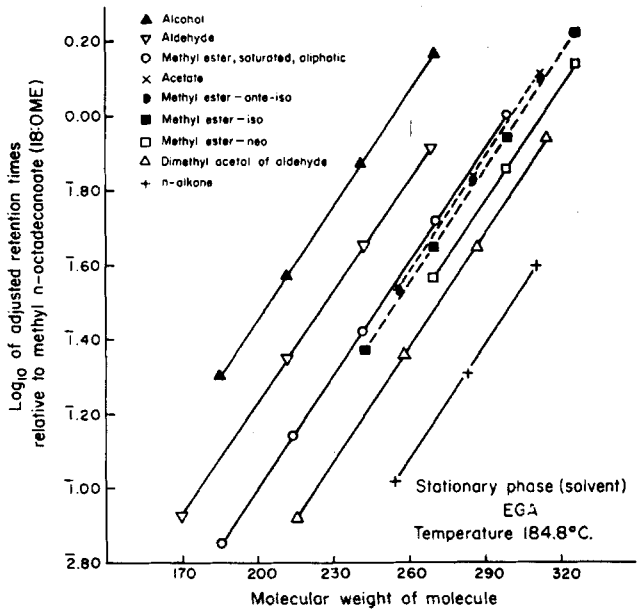

FIG. 6. Plot of $\log_{10} r$ (compound/18:0 ME) of certain saturated aldehydes, ME, acetates, and DMA vs. molecular weight of compound to demonstrate solvent selectivity. Solvent = EGA. Temperature = 184.8°.

ester, acetate, alcohol, and acetal) considerably augment the retention of these molecules. Although the differences in relative volatility are most likely due to variations in the amount of total polarity, these data do not allow one to differentiate the contribution of the interaction due to permanent dipoles (Keesom forces) from that due to induced dipoles (Debye forces). The compounds may be arranged in order of increasing retention on EGA as follows: *n*-alkanes, dimethylacetals, neo methyl esters, iso methyl esters, ante-iso methyl esters, acetates, saturated aliphatic methyl esters, aldehydes, and, lastly, the alcohols. It is probable that at least some of the decreased volatility of the alcohols on EGA is independent of the solvent and is due to their tendency to associate by hydrogen bonding, as discussed in reference to their long retention on Apiezon-M. Since the separation of compounds in GLC is largely dependent on the nature of the intermolecular forces of solute and solvent, the data reported here may help to increase our understanding of these forces.

The author is indebted to Miss Eileen McMullen, Mrs. Nancy Dean, and Miss Mary Nahon for excellent technical assistance; to Drs. Gerhardt Schmidt, Robert Franzl, George Popják, Te Piao King, and David Mauzerall for invaluable guidance in methodology; and especially to Dr. E. H. Ahrens, Jr., for his wise counsel during the course of this work and for his valuable assistance in the preparation of the manuscript.

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