Separation of *Cis* and *Trans* Isomers by Reverse Phase High Pressure Liquid Chromatography

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ABSTRACT

A reverse phase high pressure liquid chromatography procedure was devised for the analytical or preparative separation of geometric isomers of dodecenyl acetates, tetradecenyl acetates, hexadecenyl acetates, tridecadienyl acetates, and methyl oleate and elaidate. Use of μ Bondapak C-18 permitted the separation of these isomers.

INTRODUCTION

The separation of *cis* and *trans* isomers always has been a tedious process for organic chemists. A good analytical method which can be used preparatively for this separation is almost nonexistent. The use of thin layer chromatography (TLC) or open tubular chromatography with silver nitrate impregnated supports is quite time consuming, and resolution is often hampered by tailing. Resolution of geometric isomers by gas liquid chromatography (GLC) usually is limited to capillary columns (1,2) of great length which cannot be used preparatively to any great extent.

Part of the work of this Laboratory involves the isolation or synthesis of unsaturated fatty alcohol acetates. These acetates are either naturally occurring attractants or candidate attractants for Lepidopterous insects (3). It is absolutely essential that the geometric purity of these acetates be known. The literature is replete with examples of inhibition (4,5) of activity of one isomer by another or by synergism (6-9) of one isomer by another where insect attraction is involved.

When the stereochemical synthetic routes (10-14) for the production of double bonds are considered, one can see that knowledge of product geometric purity is of utmost importance. Only two reactions, reduction of alkynes with metallic sodium in liquid ammonia (15) and reduction of alkynes with iron in *n*-propanol solution (16), produce 100% geometric purity.

Adsorption chromatography is not effective in the separation of *cis* and *trans* compounds with which this Laboratory is concerned; therefore, a preliminary examination of (*Z*)- and (*E*)-7-dodecen-1-ol acetate[2] was made by high pressure liquid chromatography (HPLC) on Corasil C-18 (122 x 0.32 cm outside diameter) at 0.55 ml/min with the system methanol:water (3:1). Some resolution was achieved with this reverse phase column. A routine examination of common methyl esters was made on a μ Bondapak C-18 column (Fig. 1); resolution was excellent. The hint of resolution of geometric isomers on Corasil C-18 and the excellent resolution of methyl esters on μ Bondapak C-18 have led to the separation of *cis* and *trans* isomers on the latter column.

In this paper, the reverse phase HPLC isomeric analyses of (*Z*)- and (*E*)-5-dodecen-1-ol acetate, (*Z*)- and (*E*)-7-tetradecen-1-ol acetate, (*Z*)- and (*E*)-7-hexadecen-1-ol acetate, methyl oleate and methyl elaidate, the 4 isomers of 5,9-tridecadien-1-ol acetate, (*Z*)- and (*E*)-9-tetradecen-1-ol acetate, and (*Z*)- and (*E*)-5-tetradecen-1-ol acetate are discussed.

EXPERIMENTAL PROCEDURES

Materials

Methyl linolenate, methyl linoleate, methyl palmitate, methyl oleate, and methyl arachidate were purchased from Sigma Chemical Co., St. Louis, Mo. Methyl stearate was a Chem Service, Media, Pa., standard; methyl elaidate was a standard from the Hormel Institute (Austin, Minn.);

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[2]Use of (*Z*) and (*E*) denotes *cis* and *trans*, respectively, as set forth by the International Union of Pure and Applied Chemists' in "Tentative Rules for the Nomenclature of Organic Chemistry" (17).

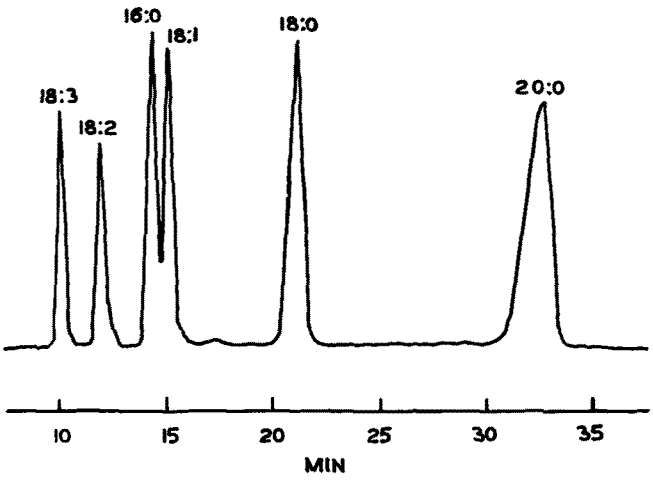

FIG. 1. Separation of methyl linolenate (18:3), methyl linoleate (18:2), methyl palmitate (16:0), methyl oleate (18:1), methyl stearate (18:0), and methyl arachidate (20:0) on μ Bondapak C-18 (30 x 0.4 cm inside diameter) at 1 ml/min methanol:water (90:10).

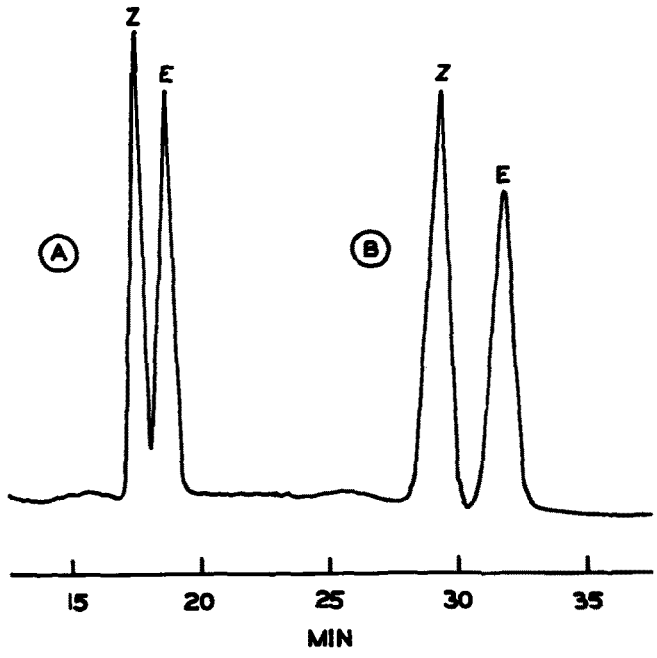

FIG. 2. Separation of A (*Z*)- and (*E*)-5-dodecen-1-ol acetate and B (*Z*)- and (*E*)-7-tetradecen-1-ol acetate on μ Bondapak C-18 (30 x 0.4 cm inside diameter) at 1 ml/min methanol:water (80:20). (*Z*) and (*E*) denote *cis* and *trans*, respectively (17).

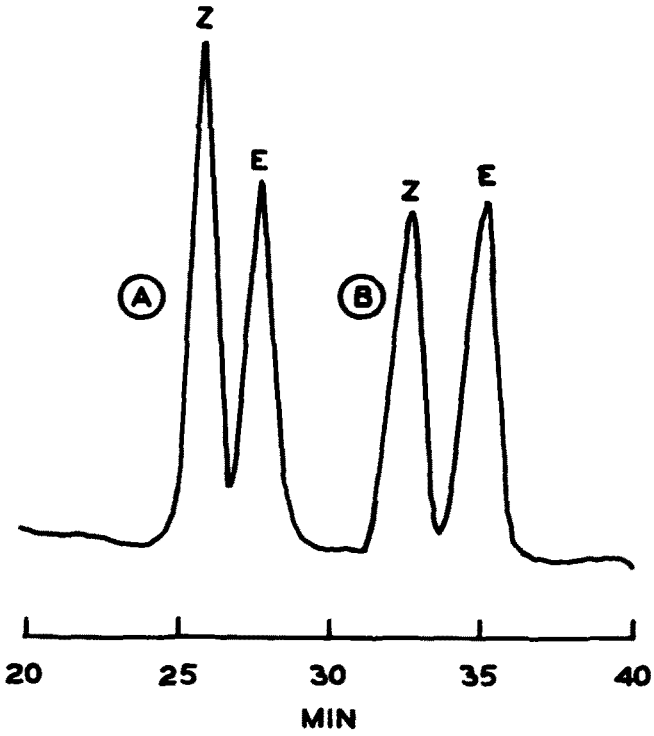

FIG. 3. Separation of A (*Z*)- and (*E*)-7-hexadecen-1-ol acetate and B methyl oleate (*Z*) and methyl elaidate (*E*) on μ Bondapak C-18 (30 x 0.4 cm inside diameter) at 1 ml/min methanol:water (85:15). (*Z*)- and (*E*) denote *cis* and *trans*, respectively (17).

(*Z*)-7-hexadecen-1-ol acetate was a Swift and Company (Oakbrook, Ill.) commercial lot; and (*E*)-7-hexadecen-1-ol acetate was prepared synthetically. The procedures for preparation of other acetates of unsaturated fatty alcohol isomers used in this study have been described previously (18-20). Acetate samples had been analyzed previously by capillary GLC (1,2) and by GLC on an additional capillary column of Silar 5CP (91 m x 0.05 cm).

HPLC

A Waters Associates (Milford, Mass.) liquid chromatograph ALC-100 equipped with an M-6000 pump was used for all analyses. A Waters Associates μ Bondapak C-18 column (30 x 0.4 cm inside diameter) was used for all analyses with concentrations of methanol:water (specified in Figs. 1-4) at 1 ml/min. The back pressure for this particular column under the specified conditions was 1000-1500 psi. Several μliters of 5% w/v solutions in methanol of each compound were injected separately and then together to reveal the separation. Detection was by a Waters Associates R-400 differential refractometer. The solvent composition in the reference cell was always the same as that used for a particular analysis.

RESULTS AND DISCUSSION

It was evident from the separation of long chain methyl esters (Fig. 1) that reverse phase chromatography on μ Bondapak C-18 could be a valuable tool for the separation of *cis* and *trans* isomers. The resolution between methyl linoleate (18:2) and methyl oleate (18:1), with one double bond difference, was enough to suggest this. One also can see the great influence which double bonds play on the polarity of a molecule, and, thus, elution occurs from most polar to least polar. The elution of methyl oleate (18:1) just after methyl palmitate (16:0) indicates that an increase in one double bond is almost equivalent to a decrease in 2 carbons of methyl stearate (18:0) on this novel small particle chemically bonded reverse phase column. The separation of methyl palmitate (16:0) from methyl oleate (18:1) doubtlessly could be improved by decreasing flow rate or increasing the percentage of water in methanol.

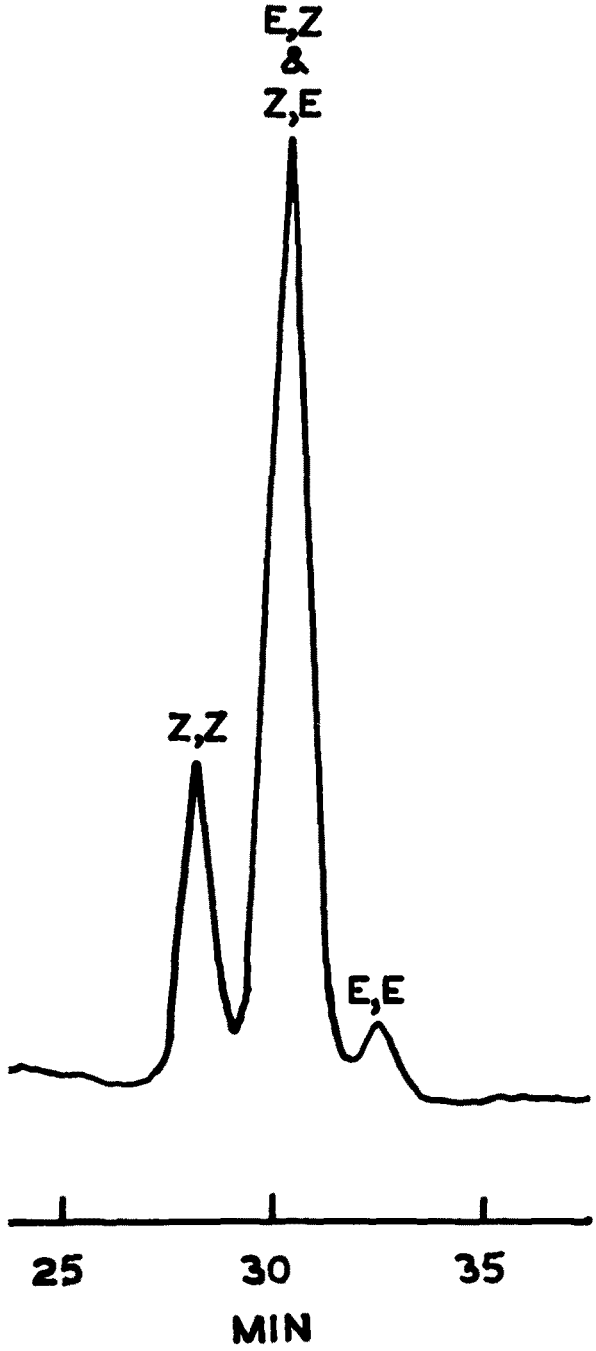

FIG. 4. Separation of the isomers of 5,9-tridecadien-1-ol acetate on μ Bondapak C-18 (30 x 0.4 cm inside diameter) at 1 ml/min methanol:water (3:1).

The resolution of (*Z*)- and (*E*)-5-dodecen-1-ol acetate and (*Z*)- and (*E*)-7-tetradecen-1-ol acetate is illustrated in Figure 2. The excellent base line resolution of the 7-tetradecen-1-ol acetates is quite evident. Analyses of (*Z*)- and (*E*)-5-tetradecen-1-ol acetate and (*Z*)- and (*E*)-9-tetradecen-1-ol acetate gave similar resolutions and retention volumes. Further resolution of the 5-dodecen-1-ol acetates could be achieved by decreasing the flow rate or increasing the percentage of water in methanol. In each of these cases, the more polar *cis* isomer elutes before the *trans* isomer.

The resolution of (*Z*)- and (*E*)-7-hexadecen-1-ol acetate and methyl oleate ([*Z*]-18:1) and methyl elaidate ([*E*]-18:1) is illustrated in Figure 3. Here, a smaller percentage of water in methanol compared to the C-12 and C-14

acetates is necessary for a reasonable retention volume. Again, the more polar *cis* isomer elutes before the *trans* isomer.

The attempted resolution of 5,9-tridecadien-1-ol acetate isomers is illustrated in Figure 4. Resolution could not be achieved for the (*E,Z*) and (*Z,E*) isomers; however, the (*Z,Z*) and (*E,E*) isomers were separated from this unresolved pair. Evidently, the difference in polarity for this pair is too subtle for the conditions used on the column. Gradient elution or recycle would be necessary if one wanted resolution of this pair.

The excellent separation of methyl oleate from methyl elaidate indicates the potential of reverse phase HPLC on μ Bondapak C-18. This small 30 cm column is competing effectively with capillary GLC in the separation of geometric isomers. There is, however, one added benefit. Although these analyses were done on tenths of mg, there is no reason why separation of geometric isomers in mg quantities could not be achieved on the same column in a preparative mode.

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