Silver Resin Chromatographic Separation of Methyl *cis*- and *trans*- Mono- and Dihydroxy Fatty Esters

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ABSTRACT

Column chromatography on silver ion-saturated Amberlyst XN 1010 cation exchange resin gave very good separation of a mixture of methyl 12-hydroxy-*cis*- and *trans*-9-octadecenoates and of methyl *threo*-12,13-dihydroxy-*cis*- and *trans*-9-octadecenoates. Comparison of the retention volumes of nonhydroxy, monohydroxy, and dihydroxy saturated and monoenoic methyl esters and of dienoic methyl esters shows that the hydroxy group interacts with the column packing to slow passage of the compound through the column, although the effect of a hydroxy group is less than that of a *trans* double bond. The effects of the hydroxy groups are additive; the ratio of retention volumes of dihydroxy ester to monohydroxy ester is slightly larger that that of monohydroxy ester to nonhydroxy ester. The retention volume of a *cis* monoenoic ester is equal to that of a hydroxy *trans* monoenoic ester and that of a hydroxy *cis* monoenoic ester is equal to that of a dihydroxy *trans* monoenoic ester.

INTRODUCTION

In 1964, Emken, Scholfield, and Dutton (1) reported on the chromatographic separation of *cis* and *trans* fatty esters on a silver ion-saturated macroreticular cation exchange resin. Since that time, several papers (2-6) have appeared describing further applications of this system or better separations on new resins. The most recent paper (6) deals with the separation on silver-saturated Amberlyst XN 1010 of methyl octadecadienoates, octadecatrienoates, and octadecynoates. We now have extended the use of this system to the separation of geometrically isomeric methyl mono- and dihydroxy fatty esters and have analyzed the effects of these substituents on retention volume.

EXPERIMENTAL PROCEDURES

Materials

Methyl octadecanoate, methyl *cis*-9-octadecenoate, methyl *trans*-9-octadecenoate, and methyl *cis*-9,*cis*-12-octadecadienoate were purchased from Nu-Chek-Prep. Methyl 12-hydroxyoctadecanoate, methyl 12-hydroxy-*cis*- and *trans*-9-octadecenoates, methyl *threo*-9,10-dihydroxyoctadecanoate and methyl *cis*-9,*trans*-12-, and methyl *trans*-9,*trans*-12-octadecadienoates were available from other workers in these laboratories (7-10).

Methyl *threo*-12,13-dihydroxy-*cis*-9-octadecenoate

This dihydroxy ester was obtained from *Vernonia anthelmintica* seed oil by a modification of the procedure of Gunstone (11). After acetolysis and saponification of the *Vernonia* oil, the dihydroxy-*cis*-9-octadecenoic acid was separated from the nonhydroxy acids by partition between acetonitrile (AN) and petroleum ether (PE). The mixed *Vernonia* acids (200 g) were dissolved in AN (500 ml) and extracted with PE (2 x 500 ml and 2 x 300 ml). The combined PE fractions were back-extracted with AN (2 x 200 ml). On removal of the solvents on a rotary evaporator, the AN layer yielded 154 g of oil (fraction 1A), the PE layer 21 g of oil (fraction 2A), and the AN extract of the PE layer 9 g (fraction 3A). Each of these fractions was converted separately to methyl esters (fractions 1E, 2E, and 3E, respectively) with methanol and sulfuric acid. Each ester fraction was treated with acetone and boron trifluoride-methanol to form the dioxolane (12) of the dihydroxy ester and analyzed by gas liquid chromatography. The chromatogram of fraction 2E showed all the *Vernonia* esters except the dihydroxy ester, that of fraction 3E showed all the *Vernonia* esters (about 50% was the dihydroxy ester), while that of fraction 1E showed about 95% dihydroxy ester with the principal contaminant being methyl linoleate. The dihydroxy ester can be purified further by crystallization from PE solution at -30 C or by silica gel chromatography. Thus, fraction 1E (5 g) was placed on a column (22 mm ID) of silica gel (40 g) in PE. Elution with PE:ethyl ether (EE) = 90:10 gave 1.9 g of material which contained all the *Vernonia* esters including the dihydroxy ester. Further elution with PE:EE = 50:50 gave 3.1 g of oil which contained only the dihydroxy ester. ^{13}C-NMR chemical shifts for the *cis* isomer, measured from TMS, are: C-14, δ33.7; C-13, δ73.9; C-12, δ74.0; C-11, δ31.8; C-10, δ133.0; C-9, δ125.1; C-8, δ27.4.

Methyl *threo*-12,13-dihydroxy-*trans*-9-octadecenoate

Pure methyl *threo*-12,13-dihydroxy-*cis*-9-octadecenoate (2.54 g) was isomerized by the method of Litchfield et al. (13) to yield a yellow liquid (1.94 g) which was separated from nitrogenous by-products on a silica gel column as described for the purification of the *cis* isomer. This product was separated on the Ag resin column to yield 1.29 g of the *trans* isomer and 0.33 g of the *cis* isomer (i.e., 80% *trans*). ^{13}C-NMR chemical shifts for the *trans* isomer, measured from TMS, are: C-14, δ33.7; C-13, δ73.8; C-12, δ74.0; C-11, δ37.2; C-10, δ133.9; C-9, δ126.1; C-8 δ32.7.

Methods

Gas liquid chromatographic analyses were conducted on F&M Model 720 gas chromatograph equipped with a thermal conductivity detector. The column (10 ft x ¼ in.) was packed with 15% EGSS-X on 100/120 mesh Gas Chrom P and operated at 200 C with a helium flow rate of 65 ml/min at 70 psi. ^{13}C-NMR spectroscopy was conducted on a Bruker WH-90 Fourier Transform NMR spectrometer operating at 22.63 MHz.

The procedure for saturating the resin with silver and preparing the silver resin column has been described (1). Methanol was used as the eluant. Effluent from the column (2 cm x 100 cm) was monitored by a Waters Associates Differential Refractometer, and changes in refractive index were plotted on a strip chart recorder. Retention volumes were measured from the time the sample was placed on the column.

RESULTS AND DISCUSSION

The separation of a 245-mg mixture of methyl 12-hydroxy-*cis*-9-octadecenoate and of methyl 12-hydroxy-*trans*-9-octadecenoate on a 2 cm x 100 cm XN 1010-Ag resin column is shown in Figure 1a. Almost baseline separa-

tion is achieved, and the entire sample is eluted from the column in less than 8 hr.

Figure 1b illustrates the separation of a 340-mg mixture of methyl *threo*-12,13-dihydroxy-*cis*-9-octadecenoate and methyl *threo*-12,13-dihydroxy-*trans*-9-octadecenoate on this same Ag resin column. Here, again, almost baseline separation is achieved and the entire sample is through the column in about 15 hr.

Retention volumes were determined for pure samples of methyl octadecanoate, methyl *cis*-9-octadecenoate, methyl *trans*-9-octadecenoate, methyl 12-hydroxyoctadecanoate, methyl *threo*-9,10-dihydroxyoctadecanoate, and the methyl *cis,cis*, methyl *cis,trans*, and methyl *trans,trans*-octadecadienoates and are recorded in Table I together with the values from Figure 1.

It is apparent from the figure and the table that the hydroxy group interacts with the column packing to slow substantially the passage of the unsaturated molecules through the column. For example, the retention volume of methyl *trans*-9-octadecenoate is 183 ml, that of methyl 12-hydroxy-*trans*-9-octadecenoate is 253 ml, and that of methyl *threo*-12,13-dihydroxy-*trans*-9-octadecenoate is 389 ml. This retarding effect is detectable even with the saturated mono- and dihydroxy fatty esters, but, in this case, the retardation is not sufficient to permit separation of a mixture of methyl octadecanoate, methyl 12-hydroxyoctadecanoate, and methyl *threo*-9,10-dihydroxyoctadecanoate. This retardation of compounds containing hydroxy groups is consistent with the observation of Scholfield and Mounts (5) that radioactive methanol is retarded on the column relative to methyl octadecanoate.

Examination of Table I reveals that the effect of the hydroxy groups on increasing the retention volumes of the fatty esters increases as the retention volume increases. Thus, for the mono- and dihydroxy compounds the dihydroxy-to-monohydroxy ratio is 1.09 for the saturated compounds, 1.53 for the *trans* monoenes, and 1.81 for the *cis* monoenes. Similar results are noted in the monohydroxy-to-nonhydroxy and the dihydroxy-to-nonhydroxy ratios.

Further examination of this table reveals that the retarding effects of the hydroxy groups are additive providing the configuration about the double bond is the same. Thus, in the saturated compounds, the *trans* monoenes, and the *cis* monoenes, the dihydroxy-to-monohydroxy ratio is only slightly larger than the monohydroxy-to-nonhydroxy ratio.

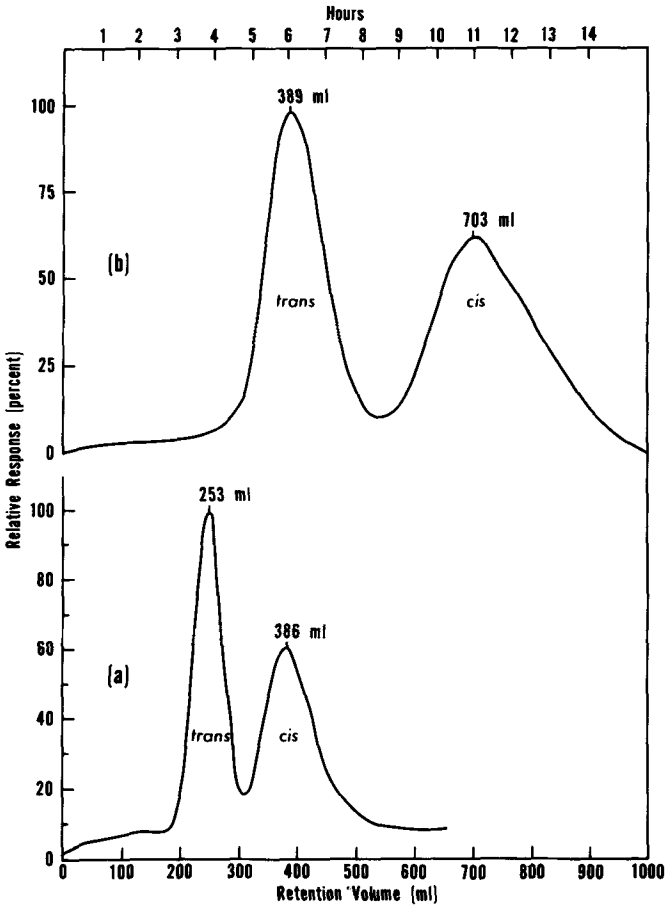

FIG. 1. Separation on 40-80 mesh XN 1010-Ag resin column (100 cm x 2 cm) with a flow rate of 1.1 ml/min methanol of (a) a mixture of methyl 12-hydroxy-*trans*-9-octadecenoate (129 mg) and methyl 12-hydroxy-*cis*-9-octadecenoate (116 mg) and (b) a mixture of methyl *threo*-12,13-dihydroxy-*trans*-9-octadecenoate (175 mg) and methyl *threo*-12,13-dihydroxy-*cis*-9-octadecenoate (165 mg).

This is in keeping with the observation that the ratios increase as the retention volume increases. By contrast, in the diene series, the *cis,cis*-to-*cis,trans* ratio (1.73) is equal to the *cis,trans*-to-*trans,trans* ratio (1.75) even though the

TABLE I

Retention Volumes of Fatty Esters on XN 1010-Ag Resin Column[a]

Compound		Retention volume (ml)[c]	Ratios		
Type[b]	Name		M/N	D/M	D/N
N	Methyl octadecanoate	146			
M	Methyl 12-hydroxyoctadecanoate	151			
D	methyl *threo*-9,10-dihydroxyoctadecanoate	165			
			1.03	1.09	1.13
N	Methyl *trans*-9-octadecenoate	183			
M	Methyl 12-hydroxy-*trans*-9-octadecenoate	253			
D	Methyl *threo*-12,13-dihydroxy-*trans*-9-octadecenoate	389			
			1.38	1.53	2.13
N	Methyl *cis*-9-octadecenoate	258			
M	Methyl 12-hydroxy-*cis*-9-octadecenoate	386			
D	Methyl *threo*-12,13-dihydroxy-*cis*-9-octadecenoate	703			
			1.50	1.82	2.72
	Methyl *trans*-9-,*trans*-12-octadecadienoate	299			
	Methyl *cis*-9-,*trans*-12-octadecadienoate	524			
	Methyl *cis*-9-,*cis*-12-octadecadienoate	907			
			1.75[d]	1.73[e]	3.03[f]

[a] 2 cm x 100 cm.
[b] N = nonhydroxy; M = monohydroxy; D = dihydroxy.
[c] Measured from time sample is placed on column.
[d] *cis,trans/trans,trans* Ratio.
[e] *cis,cis/cis,trans* Ratio.
[f] *cis,cis/trans,trans* Ratio.

cis,cis isomer elutes much later than the others.

It is also interesting to note that the 12-hydroxy-*trans*-9-octadecenoate has the same retention volume (253 ml) as the *cis*-9-octadecenoate and that the 12,13-dihydroxy-*trans*-9-octadecenoate has the same retention volume (386 ml) as the 12-hydroxy-*cis*-9-octadecenoate. Thus, a hydroxy *trans* is equivalent to a *cis* and a dihydroxy *trans* is equivalent to a hydroxy *cis* in its retention by the column. The *trans*-9,*trans*-12-octadecadienoate falls between these pairs of compounds.

From these data, it is possible to predict which mixtures of these compounds would be separable by silver resin chromatography. Mixtures not separable by this technique, for example, methyl *threo*-9,10-dihydroxyoctadecanoate and methyl *cis*- and *trans*-9-octadecenoates, could be separated by other techniques (14) (e.g., silica gel chromatography) before geometric isomer separation on the silver resin column.

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