Separation of Conjugated Methyl Octadecadienoate and Trienoate Geometric Isomers by Silver-Resin Column and Preparative Gas-Liquid Chromatography[1]

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Abstract

Silver-resin column chromatography, preceded by preparative gas-liquid chromatography to remove unconjugated esters, has been used to separate conjugated methyl octadecadienoates into *cis,cis-*, *cis,trans-*, and *trans,trans*-isomers, also methyl eleostearate into α- and β-isomers in 91 to 100% purity.

Introduction

VARIOUS PROCEDURES, based upon the formation of silver ion-olefin complexes, have been developed to separate geometric isomers of unsaturated fatty esters. These methods all depend on the difference in stability between the silver-*cis* olefin complex and the silver-*trans* olefin complex (10). In on such procedure—argentation countercurrent distribution (CCD)—a solvent system consisting of hexane and methanolic silver nitrate is employed to fractionate various mixtures of geometric and positional isomers (2,15). Also silver nitrate-impregnated silicic acid has been used both in column (17) and thin-layer chromatography (12) to separate geometric fatty ester isomers.

In a previous publication (6) column chromatography was described with a silver-containing, cation-exchange resin to separate *cis* from *trans* octadecenoic esters and mono-*trans* from di-*trans* octadecadienoates. The unconjugated *cis,cis* dienes are held very strongly on the silver-resin column. In this paper is described the application of the silver-resin column to the isolation of pure geometric isomers from alkali-conjugated methyl linoleate, dehydrated ricinoleate, and α- and β-eleostearate. Also described is a preparative gas-liquid chromatographic (GLC) procedure to separate conjugated and unconjugated dienes. This procedure, which was used previously on conjugated and unconjugated dienes from methyl linoleate hydrogenated with $Fe(CO)_5$ (7), was necessary because the silver-resin column did not separate *trans* unconjugated dienes from conjugated dienes.

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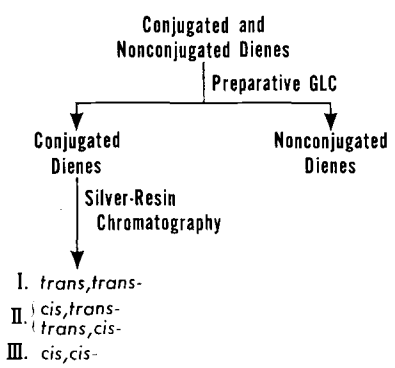

FIG. 1. Scheme for separation of conjugated dienes by preparative gas-liquid chromatography and silver-resin column chromatography.

Materials and Methods

Materials

Partially alkali-isomerized linoleate was prepared, according to the procedure of Kass and Burr (9), by refluxing methyl linoleate with 20% KOH. Methyl esters prepared from the conjugated acids contained 47% nonconjugated and 53% conjugated diene by GLC analysis.

A mixture of *trans,trans-*, *cis,trans-*, and *cis,cis*-conjugated octadecadienoates was prepared by dehydrating methyl ricinoleate. Ricinoleate was distilled in the presence of 10% by weight of $KHSO_4$ through a Vigreux column under 0.07-mm of pressure at 110C. The distilled esters were estimated by GLC analysis to contain 44.7% conjugated diene, of which approximately 35% was *trans,trans*, 45% *cis,trans*, and 20% *cis,cis*.

Pure α- and β-eleostearate isomers were prepared by crystallization from tung fatty acids by the procedure of Mod et al. (11). UV spectral analysis of the methyl α-eleostearate gave an absorptivity at 271 mμ of 175.3 and of methyl β-eleostearate, an absorptivity at 268 mμ of 207.

Preparative GLC

An Aerograph preparative gas-liquid chromatograph model A-700 (5) was used with either a 20-ft × 3/8-in. coiled aluminum column packed with 30% SE-30 on 60/80 mesh Chromosorb P or a 10-ft × 3/8-in. aluminum column packed with 30% DEGS on 60/80 Chromosorb W. Operating conditions for the SE-30 column were: column temperature, 210C; injection temperature, 240C; detector temperature, 243C; collector temperature, 212C; flow rate, 300 ml/min of helium gas. Average retention time for the conjugated dienes was 2.5 hr. With the DEGS column, operating temperatures were approximately 15C lower than those used for the 30% SE-30 column. Flow rates of 100 to 150 ml/min and sample sizes of 35 to 50 μl were used. The average retention time for the conjugated dienes was 1.25 hr. With the DEGS column, bleed material is found in the separated fractions. This material, together with any oxidation products present, was easily removed from the fractions by chromatographing on a column (12 × 16.5 mm) packed with 15 g of silicic acid (3).

TABLE I
Recovery of Pure (100%) Fatty Esters by Preparative GLC[a]

Methyl esters	Recovery, %
1. Oleate	90.8
2. Linoleate	92.0
3. Linolenate	90.3
4. Mixture: 1, 2, and 3	94.0

[a] Column = DEGS (see text for conditions).

TABLE II
Effect of Preparative GLC on Double Bond Positions Methyl *trans,trans*-9,11-Octadecadienoate from Dehydrated Methyl Ricinelaidate[a]

Analyses		Before GLC	After GLC
IR, $a_{10.2\,\mu}$		1.02	1.10
UV, $a_{231\,m\mu}$		86.3	104.0
Double bond position:			
	7	1.7	2.7
	8	4.8	5.5
	9	41.7	37.8
	10	1.0	6.6
	11	47.3	43.0
	12	3.5	4.4

[a] Columns = SE-30 (see text for conditions).

TABLE III
Analyses of Conjugated Diene Fractions from Alkali-Isomerized Methyl Linoleate

Analysis	Fraction II		Fraction III	
	Exp.	Lit.[a]	Exp.	Lit.[a]
Infrared	$a_{10.1\,\mu} = 1.30$	1.41[b]	$\frac{a_{10.18\,\mu}}{a_{10.54\,\mu}} = 1.23$	1.198[b]
Ultraviolet	$a_{232\,m\mu} = 99.5$	112.7[b]	$a_{233\,m\mu} = 88.2$	90.4[b]
GLC, %:				
trans,trans-	96		0	
cis,trans-	4		100	

[a] Literature values are for the pure isomers.
[b] Chipault and Hawkins (4).

Approximately 200 ml of hexane were used to elute the conjugated dienes from the column.

GLC samples were collected in glass vials supplied with the Autoprep A-700. Because the samples formed aerosols in the collection gas, it was necessary to pack these vials with glass wool and to use tightly fitting rubber caps for good recoveries. The glass wool had to be packed evenly and sufficiently tight to offer some resistance to gas flow. If the glass wool packing was too tight, the aerosol escaped around the seal between the collector outlet and the collector vial. Repetitive manual injections of 35 to 50 μl were made until the desired amount of sample was collected.

Silver-Resin Chromatography

The technique which was followed for the preparation of the silver-treated resin, packing of the column, and sample injection, also for monitoring the sample was the same as described previously (6). Only the diameter of the column, sample size, and solvent flow-rate were varied. A larger diameter column (21 mm) with a maximum capacity of 1.5 g of sample was used. With the larger diameter column, optimum separations were possible with a higher flow-rate of 2 ml/min. With alkali-isomerized methyl linoleate the nonconjugated diene, being nearly all *cis,cis*, could also be separated from the conjugated diene with a short silver-resin column (50 × 1.8 cm) instead of preparative GLC.

The methodology relating to analytical GLC, IR, UV, and $KMnO_4$-KIO_4 cleavage analyses was the same as reported earlier (8).

Results and Discussion

The fractionation scheme adopted in this work is shown in Figure 1. Preparative GLC provided samples containing 95 to 98% pure conjugated fatty ester geometric isomers. Samples were then fractionated by silver-resin column chromatography into the different conjugated methyl octadecadienoate geometric isomers. With alkali-isomerized methyl linoleate the unconjugated *cis,cis* diene was removed by the short silver-resin column instead of preparative GLC.

The recovery and purity of fatty methyl esters obtained by preparative GLC were checked by chromatographing known samples. Table I shows the recoveries from pure samples of methyl oleate, linoleate, and linolenate, also a mixture of the three collected by preparative GLC. Total recovery averaged 90% by weight, based on collection of completely separated peaks.

To check whether isomerization of unsaturated fatty esters occurred during preparative GLC, double bond distribution was determined by $KMnO_4$-KIO_4 cleavage. Table II shows the analysis of methyl *trans,trans*-9,11-octadecadienoate (14) before and after preparative GLC. A 5% increase in bond migration is indicated, which is probably within the experimental error of the technique and is not considered significant. IR and UV absorptivities given in Table II were higher after preparative GLC probably because small amounts of oxidative or polymeric impurities were removed. These experiments showed that good recovery of esters could be achieved with little alteration of the rather labile conjugated esters.

Figure 2 gives the silver-resin column separation of the conjugated diene fraction from alkali-isomerized methyl linoleate. The starting material for this separation can be obtained by either preparative GLC or by silver-resin chromatography through a short column. Analytical GLC showed it to contain 90% mono-*trans* (*cis,trans* and *trans,cis*) esters, 5% *trans,trans*- and 5% *cis,cis*-conjugated diene. Two definite peaks were eluted from the silver-resin column; the larger peak also had a shoulder. The eluate was combined in three fractions as indicated in Figure 2.

Fraction I appeared to be polymeric oxidation products. The IR absorption curve had a hydroxyl band at 3300 cm^{-1}. There was no conjugated diene absorption at 230–235 mμ. Although GLC gave a few small peaks with short retention times, the majority of the sample was not eluted from the GLC column. Table III includes the analyses of fractions II and III. The alkali-isomerized sample apparently contained mostly mono-*trans*-conjugated diene, as shown by IR, UV, and GLC analysis of the separated fractions. Both GLC and UV analyses indicated that fraction II has 96% *trans,trans*-conjugated diene. No *cis,cis*-conjugated diene could be detected by GLC analysis. These results agree with data from argentation CCD (15) which show that alkali-isomerized

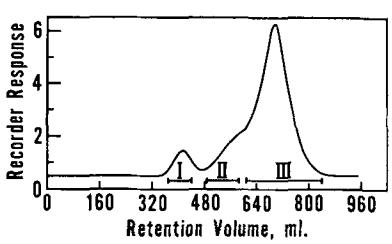

FIG. 2. Refractometric curve for separation of alkali-conjugated methyl linoleate.

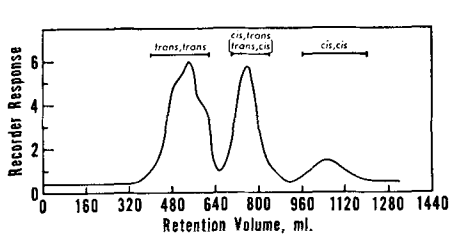

FIG. 3. Refractometric curve for separation of dehydrated methyl ricinoleate.

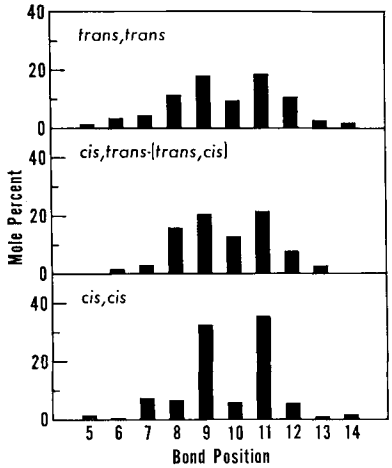

FIG. 4. Double bond distribution of conjugated octadecadienoate fractions from Figure 3.

methyl linoleate is mostly mono-*trans*-conjugated diene and has only small amounts of *trans,trans*- and *cis,cis*-conjugated dienes. Fraction III was pure mono-*trans*-conjugated diene.

Figure 3 gives the silver-resin column separation of the conjugated esters prepared by dehydration of methyl ricinoleate. Unconjugated esters were previously removed by preparative GLC. The complex shape of the *trans,trans*-conjugated diene peak was caused by contamination from both oxidation products and bleed-off GLC column material. Because of these impurities, no quantitative measurements can be based on the areas under the curve. For preparative purposes, both of these impurities were readily removed by silicic acid chromatography. Analysis of the conjugated methyl octadecadienoate fractions are given in Table IV. These experimental absorptivities are in general agreement with published values. For the *cis,trans* fraction, the low value of the ratio of absorptivities at 10.18 μ and 10.54 μ indicates the absence of *trans,trans* conjugation in agreement with GLC data.

Figure 4 shows the $KMnO_4$-KIO_4 cleavage analysis of the *trans,trans*-, *cis,trans*-, and *cis,cis*-conjugated octadecadienoate isomers from dehydrated methyl ricinoleate. Double bond migration was indicated in all three samples. The greatest amount of bond migration occurred in the *trans,trans* fraction with only 40% of the double bonds in the original 9,11-positions. The *cis,cis* fraction contained approximately 70% of the double bonds in the 9,11-positions. These results are in agreement with unpublished data of this laboratory on samples prepared by argentation CCD of dehydrated methyl ricinoleate.

Figure 5 illustrates the separation of α- and β-

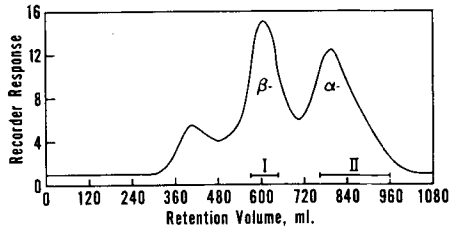

FIG. 5. Refractometric curve for separation of methyl α- and β-eleostearate.

eleostearate from a mixture. UV analysis of fraction I gave an absorptivity at 268 mμ of 205 and of fraction II, 179.5 at 271 mμ. Paschke and Wheeler (13) reported absorptivity at 271.5 mμ of 178.3 and at 269 mμ of 201.0 for α- and β-eleostearic acid respectively, corresponding to 169.7 and 191.4 for the methyl esters. Therefore fractions I and II are essentially pure methyl β- and α-eleostearate.

These results demonstrate that silver-resin column chromatography is applicable to the fractionation and separation of pure conjugated octadecadienoate and octadecatrienoate geometric isomers. The technique is suitable for small preparative separations. It is more rapid, requires less elaborate equipment, and is suitable for smaller samples than argentation CCD. Also the silver resin seems to add greater selectivity since the separation of conjugated diene isomers is more complete than those reported by argentation CCD. It is necessary to remove unconjugated esters. This was done by preparative GLC. Alternative methods include fractional distillation (16) or CCD (15). The conjugated diene isomers from this study are comparable in purity with those produced by fractional crystallization techniques (1).

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TABLE IV
Analyses of Conjugated Octadecadienoate Fractions from Dehydrated Methyl Ricinoleate

Analysis	Fraction					
	tran,trans		*cis,trans*		*cis,cis*	
	Exp.	Lit.[a]	Exp.	Lit.[a]	Exp.	Lit.[a]
Infrared	$a_{10.1\ \mu} = 1.16$	1.41[b]	$\frac{a_{10.18\ \mu}}{a_{10.54\ \mu}} = 1.160$	1.198[b]		
Ultraviolet	$a_{231.5\ m\mu} = 99.0$	112.7[b]	$a_{233\ m\mu} = 86.9$	90.4[b]	$a_{235.5\ m\mu} = 83.0$	90.8[c]
GLC, %:						
Conj. diene:						
trans,trans	91.4		0.0		0.0	
cis,trans	7.0		99.5		4.5	
cis,cis	0.0		0.0		94.0	
Nonconj. diene	1.6		0.5		1.5	

[a] Literature values are for the pure isomers.
[b] Chipault and Hawkins (4).
[c] Body and Shorland (1).