Chromatographic Separation of *cis* and *trans* Fatty Esters by Argentation with a Macroreticular Exchange Resin[1]

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Abstract

Methyl oleate and methyl elaidate, as well as other monoene *cis* and *trans* isomers of fatty esters, can be separated quickly and conveniently by a preparative chromatographic procedure in which a silver-saturated ion-exchange resin is used. Separations are based on differences in stabilities of the silver-olefin complexes. Recoveries of better than 95% were made, and pure *trans* and *cis* monoene fractions were collected. This method can be used to separate saturates from *cis* and *trans* monoenes. The *cis,trans, cis,cis,* and *trans,trans*-9-12-octadecadienoates were separated. While *cis,trans* and *trans,trans* dienes were eluted separately, the *cis,cis* diene isomer remained on the column.

Introduction

In 1943, Lucas, Moore, and Pressman (6) showed that silver ions would form weak complexes with olefins. Dutton, Scholfield, and Jones (3,10) reported that separation of *cis* and *trans* isomers of methyl linoleate and methyl oleate was possible by using countercurrent distribution (CCD) with a silver-nitrate solvent system. According to Helfferich (4), organic compounds can be separated by "ligand exchange" using an ion exchanger containing a complexing metal ion as the solid absorbent, while de Vries (11) separated methyl oleate and methyl elaidate using a silver nitrate-impregnated silicic acid column. Since this work was completed, C. F. Wurster, Jr. and co-workers (12) reported the use of a silver-containing cation exchange resin with column chromatography for the separation of oleic, linoleic and linolenic methyl esters but not for geometric isomers.

A rapid and effective method for separating approximately 0.5-g samples of *cis* and *trans* monoene methyl esters has frequently been needed at this laboratory. CCD is an excellent method for separating 30–40 g samples but it is impractical for only 500–600 mg. Four days are required to separate the geometric monoene isomers and also some difficulties are involved in keeping the apparatus clean while handling silver nitrate-methanol solutions. The silver nitrate-silicic acid column is not suitable for automatic monitoring of eluate with a differential refractometer because the solvent composition usually must be changed during each run.

An alternative procedure, therefore, was investigated which resulted in the establishment of an effective chromatographic procedure for separating *cis* and *trans* monoenes. It consisted in using a silver-saturated macroreticular cation exchange resin for the column packing and a recording refractometer for monitoring the chromatographic peaks. No solvent changes are necessary and repeated use with monoenes does not affect the degree of separation.

Materials and Methods

The methyl oleate and methyl elaidate mixture used in the experiments was derived from oleic acid, which in turn was obtained by an acid soap crystallization method from olive fatty acids (9). Nitrous oxide isomerization gave a mixture containing 76% elaidic acid, 21% oleic acid and 3% saturates. The methyl esters were made and pure oleate was added to the mixture to give a 50:50 oleate and elaidate mixture used in the experiments. Analysis by GLC showed a slight impurity of 2–3% saturates in the final mixture.

The column packing consisted of a silver-saturated cation exchange resin which was very porous and had a large surface area of approx 122 m^2/g. The resin (Amberlyst XN1005) (13) was obtained through the courtesy of Rohm and Haas but is not commercially available. It was received in the dry state and was prepared for use by first placing it in a large diam column, washing with methanol, allowing it to stand overnight in methanol, and then washing with distilled water. Next, the resin was saturated with silver ions by passing a 0.2 *N* silver nitrate solution through the column until ionic silver was detected in the eluate. (One liter 0.2 *N* silver nitrate solution will saturate approx 60 g dry resin.) The treated resin was then washed with distilled water until neutral. Finally, after being washed again with 3–4 bed volumes of methanol, it was ready for use.

The prepared resin was packed in a glass column approx 200 cm long x 13 mm diam. A Waters recording differential refractometer (Model 2143) with a strip chart recorder was used to monitor the eluted solution (2). Full-scale deflection on the recorder was equal to 2.5 x 10^{-3} refractive index units. A Beckman metering pump (0–2 ml/min capacity) was used to pump the eluting solvent.

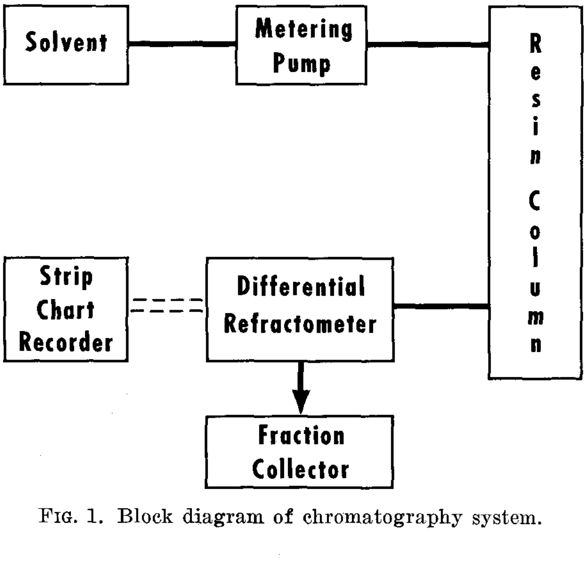

FIG. 1. Block diagram of chromatography system.

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TABLE I

Separation of Methyl Oleate and Methyl Elaidate

Experiment	Flow rate ml/min	Sample size mg	Fractions recovered					
			trans [a]		Intermediate		*cis*	
			mg	% *trans*	mg	% *trans*	mg	% *trans*
1	0.5	488	229	90	25	21	182	0.0
2	1.0	432	225	96	0.0	0.0	188	0.0
3	1.5	676	202	85	224	66	222	0.0
4	2.0	459	60	92	206	59	181	0.0

[a] The *trans* fractions contained 4–6% methyl stearate as an impurity.

The apparatus was assembled as shown in Figure 1. A sample of 100–500 mg of the 50:50 mixture of methyl oleate and methyl elaidate was injected from a weighed hypodermic syringe into the top of the column packing. The sample was then allowed to flow completely into the resin packing until it was no longer visible before being eluted with methanol. The eluted solution was monitored by the refractometer and the change in refractive index (ΔN) was recorded against time by the strip chart recorder. The collection of eluate fractions was based upon information from the recorder chart. In most experiments the flow rates of the eluting solvent were between 0.5 and 1.5 ml/min, and retention time of the sample was between 3 and 4 hr.

After the various fractions were collected in tared flasks, the solvent was evaporated on a rotary evaporator equipped with a water aspirator. Fractions were analyzed by IR spectroscopy for the percentage of isolated *trans;* methyl elaidate served as a standard.

The silver resin column proved very stable and can be used repeatedly. Although the column does slowly darken with exposure to light, this does not seem to affect the degree of separation. Our present column has been used for over 40 separations and still retains its original resolving power.

Flow rate and sample size were varied to find conditions which gave optimum separation. Even though the degree of separation started to suffer with sample sizes of 400 mg or more, good results could still be obtained with 500–600 mg samples. In varying the flow rate between 0.5 ml/min and 2 ml, those up to 1 ml/min gave the best separations, although a flow rate of 1.5 ml/min was satisfactory. A 1 ml/min flow rate and approximately a 300–400 mg sample were the best conditions for obtaining max separation in the least amount of time (Table I).

The complexing ability of the silver ion apparently depends on the large surface area of the new macroreticular resins (5). Preliminary experiments with common cation exchange resins showed very little separation of the methyl oleate and methyl elaidate mixture. Even with the commercially available macroreticular resin, Amberlyst 15, methyl oleate and elaidate were eluted as one broad band with partial separation, as shown in the top curve of Figure 2. Fractions A, B, and C were collected across the peak and analyzed for per cent *trans*. Good separation was obtained only with Amberlyst XN1005, an experimental resin with ca. three times the surface area of Amberlyst 15. Table II compares the physical properties of these two resins (1,8).

TABLE II

Physical Properties of Ion Exchange Resins

Property	Conventional resin	Amberlyst 15	Amberlyst XN1005
Surface area, m^2/g dry resin............	<0.1	42.5	122
Exchange capacity, meq/g dry resin...	≈ 4.5	4.3	3.5
Particle size, %			
20–40 mesh..................................	....	91	....
40–60 mesh..................................	....	......	70
60–100 mesh................................	....	......	20

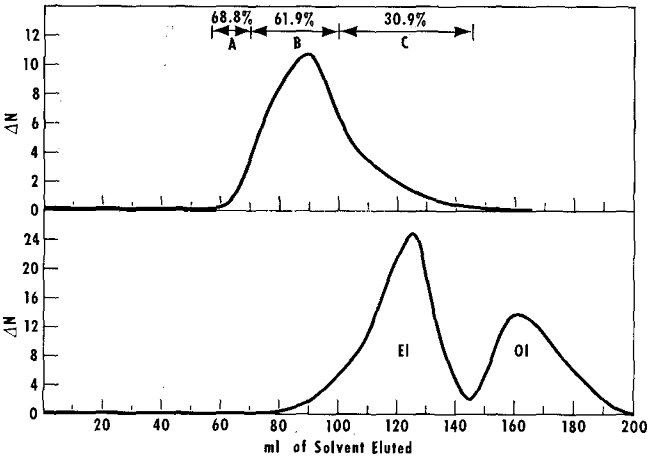

FIG. 2. Comparison of the separation of methyl elaidate (El) and methyl oleate (Ol) on 200-cm Amberlyst 15-Ag (top) and Amberlyst XN1005-Ag (bottom) resin columns.

As seen from Figure 2, the 50:50 mixture of methyl oleate and methyl elaidate can be separated into two relatively pure fractions when optimum conditions are used. The methyl elaidate elutes from the columns first because of the lesser complexing ability of the *trans* double bond. Usually no attempt was made to separate the saturates from the methyl elaidate fraction. Since the methyl elaidate fraction contains 2–3% saturates, a 96–97% *trans* value would indicate that the methyl elaidate fraction was free from methyl oleate impurity. The total recovery was better than 95% for most runs.

A 269-mg sample of a synthetic mixture containing 25% stearate, 57% elaidate, and 18% oleate was separated under optimum conditions. The separation yielded 71% of the starting material as pure fractions and 29% as impure intermediate fractions (Fig. 3).

Figure 4 shows the separation of a 307-mg sample consisting mainly of saturates, 9,12, and 15-*cis,* and 9,12, and 15-*trans* positional monoene isomers. Although the refractometer curve does not show any actual separation of the *trans* or *cis* positional monoene isomers, some fractionation did occur. The *trans* and *cis* monoene peaks were both split in half and the resulting fractions were then analyzed by capillary GLC. Analysis showed that the 9-*cis* and 12-*cis* monoene isomers were only slightly separated from each other; whereas the 15-*cis* monoene was almost absent from the first *cis* monoene fraction. However, this fractionation does not bother the separation of a *cis* and *trans* monoene mixture because the 15-*cis*

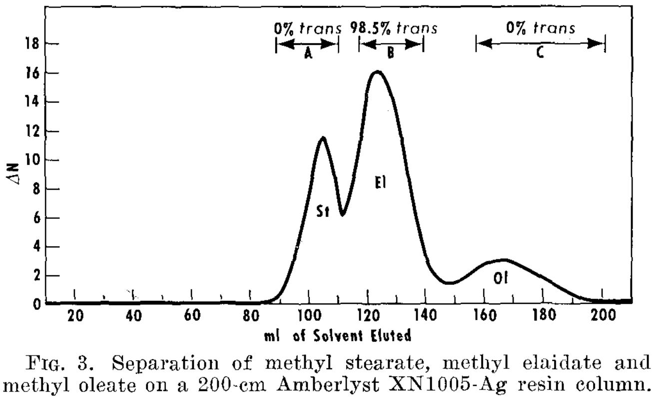

FIG. 3. Separation of methyl stearate, methyl elaidate and methyl oleate on a 200-cm Amberlyst XN1005-Ag resin column.

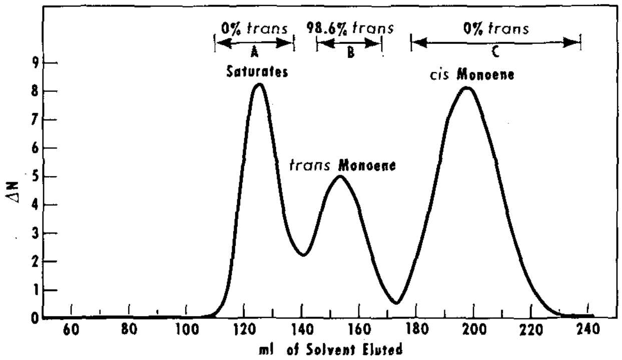

FIG. 4. Separation of saturates, *trans* monoenes and *cis* monoenes obtained from hydrogenated methyl linolenate, on a 225-cm Amberlyst XN1005-Ag resin column.

monoene is the last component to be eluted from the column and may be later combined with the other *cis* monoene fractions. The 9,12- and 15-*trans* positional monoene isomers did not present any problems as there was very little fractionation between these isomers.

Trial runs were made to determine if the diene and triene isomers were separable. The *cis,cis* diene and the *cis,cis,cis* triene isomer were not eluted from the column, but the nonconjugated *trans,trans* and *trans, cis* dienes and the conjugated geometric diene isomers were eluted. Poor separation was obtained between the *trans,trans* diene and the conjugated dienes. A 50:50 mixture of mono-*trans* (*cis,trans, trans, cis*) and *trans,trans* dienes was prepared. In Figure 5, excellent separation of these isomers was obtained from a 343-mg sample of the 50:50 mixture. At present, no attempt has been made to separate the various triene geometric isomers.

To confirm the very strong absorption of the *cis,cis* dienes, methanol was pumped through a shorter column for a comparatively long time. A 45 cm x 13 mm column was packed and a sample containing *cis, trans* and *cis,cis* dienes was run. The *cis,trans* diene was eluted from the column after 3 hr. The *cis,cis* diene was never eluted even after pumping methanol through the column for 5 hr at a rate of 1 ml/min. Apparently the *cis,cis* diene forms a very stable complex with the silver ions and does not move through the column at any appreciable rate. When 5% acetic acid/methanol was pumped through the columns, a slow bleed off of the *cis,cis* diene occurred but a good recovery could still not be made. Using higher concn of acetic acid in the methanol caused the silver to be stripped off the resin, but the elution rate of the *cis,cis* diene was not affected. Some of the other solvents tried, in which methyl esters are more soluble, were diethyl ether, chloroform, hexane and benzene. However, none of these showed any signs of being able to elute either the complexed methyl linoleate or linolenate. An explanation has not yet been postulated that will explain this behavior of the *cis,cis* diene. Using a different resin which was capable of separating oleate, linoleate and linolenate, Wurster (12) was able to elute linoleate with methanol but used displacement by a butene-1 solution to elute linolenate.

The use of such a low mol wt olefin may also prove useful for eluting methyl linoleate and methyl linolenate from the silver-XN1005 resin columns.

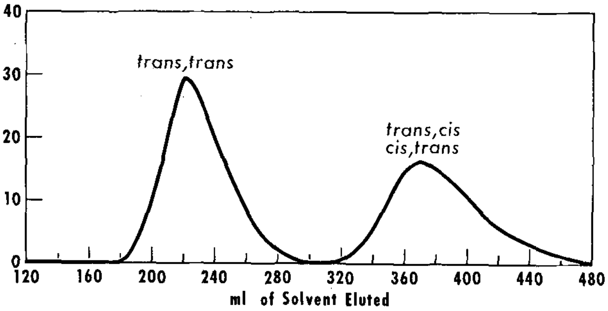

FIG. 5. Separation of a *cis,trans,trans,cis*, and *trans, trans*-9,12-octadecadienoate mixture on a 225-cm Amberlyst XN1005-Ag resin column.

Results and Discussion

According to Muhs and Weiss (7), the bonding of the silver ion with the olefinic double bond involves:

a) A σ bond, which is formed by the overlap of a filled "π" orbital with an empty "s" orbital of the silver ion.

b) A "π" bond fromed by the overlap of the empty antibonding "π" orbital with the filled *d*-orbital of the silver ion.

The greater stability of the silver-*cis* olefinic complex can be explained in terms of strain relief. This theory holds that in the formation of a silver-olefinic complex, the 5s electron shell of the silver ion is capable of twisting the *p*-orbitals of the *cis* configuration out of their plane. Thus, strain between the *p*-orbitals is relieved and an energy gain occurs. Since the *trans* configuration originally had a lesser amount of strain, the energy gain that occurs when a *cis*-silver complex is formed is greater than the energy gain that occurs during a *trans*-silver complex. Therefore, thermodynamically the silver-*cis* complex should be, and is, the more stable of the two complexes.

A silver-saturated resin column can be used to separate a *cis* and *trans* mixture of monoenes into pure fractions of *cis* monoene and *trans* monoene. An intermediate fraction, consisting of less than 10% of the total sample, is usually collected. Mixtures of methyl oleate and methyl elaidate can be almost completely separated with only a small percentage of the sample being collected as an intermediate fraction. A fairly good separation of saturates, methyl oleate, and methyl elaidate can be accomplished by judiciously collecting the fractions to obtain the max amt of pure sample. The *cis,cis, trans, cis,* and *trans, trans* dienes can be separated, although the *cis,cis* diene is not recovered. Thus, the silver-saturated exchange resin column should provide a convenient and useful tool to separate monoene, and some diene, *cis* and *trans* fatty methyl esters.

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