Technical

Separation of Saturated, Unsaturated, and Acetylenic Fatty Acid Isomers by Silver Resin Chromatography

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ABSTRACT

A column packed with silver-saturated ion exchange resin (Amberlyst XN 1010) was found to have lipid separation capabilities superior to Amberlyst XN 1005 and similar to Amberlite XE 284. The separation of unsaturated fatty methyl ester isomers by silver resin chromatography using methanol as the eluting solvent has been extended to mixtures containing polyunsaturate and acetylenic fatty esters. Separations are possible on the basis of both total number of double bonds and the geometric configuration. Mixtures containing saturates, elaidate, oleate, linoleate, and linolenate can be separated, but 10% 1-hexene must be added to the methanol to elute the linolenate. Mixtures containing *trans,trans*-;*trans,cis*-; and *cis,cis*-octadecadienoate isomers have also been separated, and partial resolution of *cis*-9,*cis*-12- and *cis*-12,*cis*-15-octadecadienoate isomers was obtained. Sterolate, a monounsaturated acetylenic fatty ester was eluted at the same time as oleate. Crepenynate (*cis*-9-octadecen-12-ynoate) can be separated from linoleate but not from *cis,trans*-octadecadienoate.

INTRODUCTION

A new silver ion saturated cation exchange resin has recently been described which gives improved separation of *cis* and *trans* fatty methyl esters (1). This new silver-saturated resin (Amberlite XE 284) gave better separations than the previously described silver-saturated Amberlyst XN 1005 resin (2,3,4) and had the additional capability of separating octadecadienoate geometric isomers.

Our investigation of a similar macroreticular resin from Rohm & Haas Co. (Amberlyst XN 1010) has demonstrated that it has properties very similar to Amberlite XE 284. In addition, the separation capabilities of silver-resin chromatography using XN 1010-Ag have been extended to various mixtures containing methyl palmitate, stearate, and crepenynate. The partial separation of *cis*-9-,*cis*-12- and *cis*-12,*cis*-15-octadecadienoate isomers was also noted.

EXPERIMENTAL PROCEDURES

Methyl stearate, methyl oleate, methyl elaidate, and methyl linoleate used in the mixtures were purchased from Nu Chek Prep Inc., Elysian, MN. The preparation of methyl stearolate (5); methyl crepenynate (6); methyl *trans*-9,*trans*-12-octadecadienoate (7); methyl *cis*-9,*trans*-12-octadecadienoate (8); methyl *cis*-12,*cis*-15-octadecadienoate and *cis*-9,*cis*-12-octadecadienoate mixture (9); safflower oil methyl esters and linseed oil methyl esters (10) were by previously described procedures.

The mixture and fractions containing *cis*-9,*cis*-12- and *cis*-12,*cis*-15-octadecadienoate were analyzed for double bond position by cleaving the double bond with ozone and reducing the ozonide with $NaBH_4$. The alkyl alcohol and alcohol-ester fragments were determined by gas chromatography (11).

Experimental procedures used for saturating the resin with silver ion, eluting the sample, and monitoring the eluant were similar to those previously described (1,2). The only major difference was the mesh size of the resin par-

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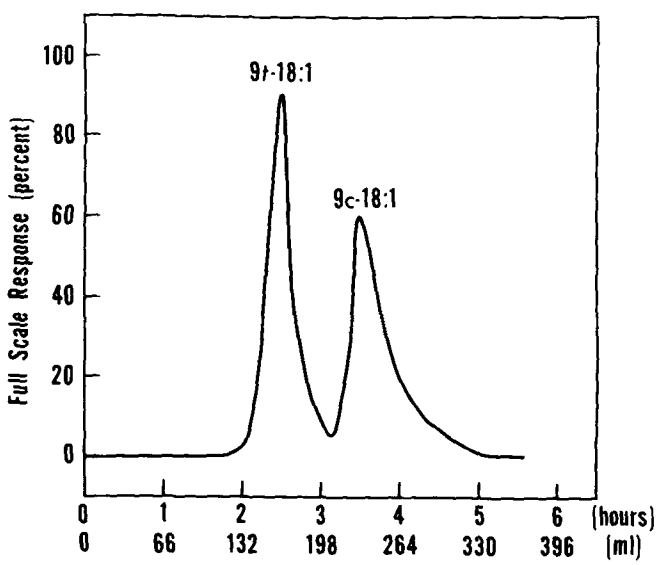

FIG. 1. Mixture of 9t-18:1 (376 mg) and 9c-18:1 (264 mg) separated on 40-80 mesh XN 1010-Ag resin column (110 cm x 2.0). Flow rate: 1.1 ml/min methanol.

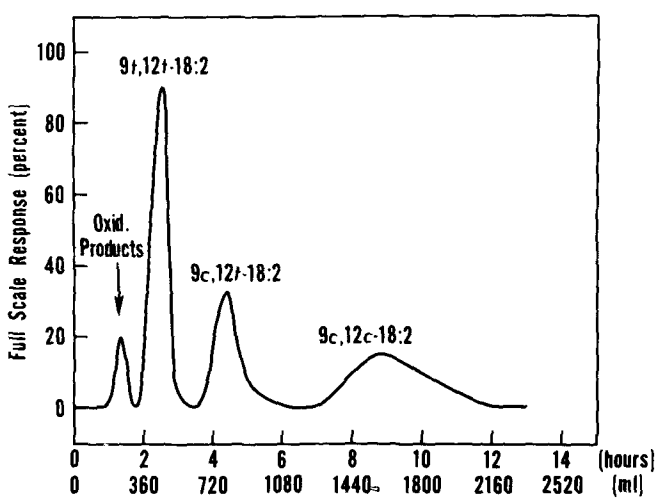

FIG. 2. Mixture of 9t,12t-18:2 (677 mg); 9c,12t-18:2 (390 mg); 9c,12c-18:2 (450 mg)separated on 40-80 mesh XN 1010-Ag resin column (60 cm x 3.0 cm). Flow rate: 3 ml/min methanol.

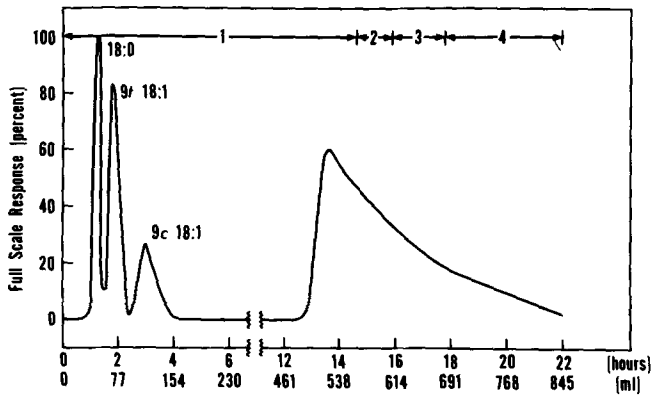

FIG. 3. Mixture (200 mg) of 9t-18:1; 9c-18:1; 9c,12c-18:1; 12c,15c-18:2 separated on 80-100 mesh XN 1010-Ag resin column (150 cm x 1.1 cm). Flow rate: 0.64 ml/min methanol. Numbers 1 to 4 indicate fractions collected and analyzed in Table I. Fractions 3 and 4 contain 9c,12c-18:2 and 12c,15c-18:2.

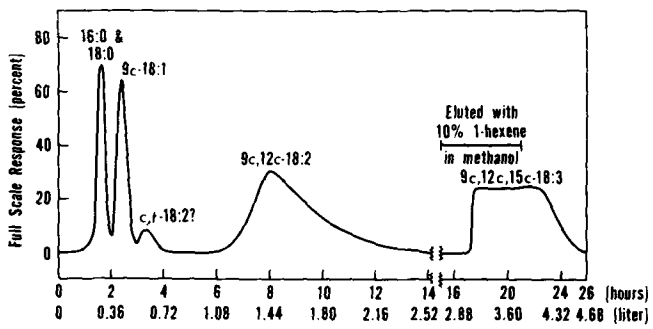

FIG. 4. Mixture of safflower oil methyl esters (1.3 g) and linseed oil methyl esters (1.3 g) separated on 40-80 mesh XN 1010-Ag resin column (60 cm x 3.0 cm). Flow rate: 3.0 ml/min methanol. Linolenate eluted with 1000 ml of 10% 1-hexene in methanol.

ticles. In this work, resin particles in the 40-80 mesh (dry) and 80-100 mesh (dry) range were used in place of the 100-120 mesh particles reported earlier (1). The resin particles were obtained by grinding the original resin in a Labconco, Model 900 Curr mill (Labconco, Corp., Kansas City, MO) set at an opening of 0.06 in. and then screening the ground resin through standard mesh sieves. The use of larger resin particles results in less efficient columns, but lower back pressures are obtained which facilitate high flow rates. A significant improvement in flow rate with the 80-100 mesh (dry) resin was obtained by resieving the resin after it had been swelled with CH_3OH and complexed with silver ion. The mesh size of the CH_3OH swelled 80-100 mesh (dry) resin was 40% 60-80 mesh and 60% 80-100 mesh. A flow rate of 0.5 ml/min methanol using 20 psi head pressure could be obtained with a 150 cm x 1.1 cm XN 1010-Ag resin column packed with resieved 80-100 mesh dry resin. Over 200 psi head pressure was required to

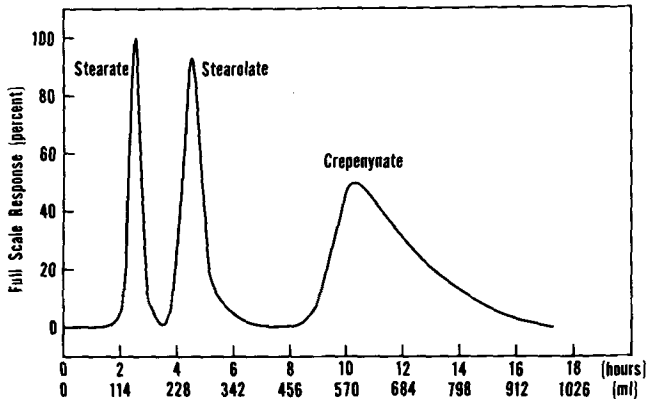

FIG. 5. Mixture of methyl stearate (100 mg); methyl stearolate (200 mg); methyl crepenynate (350 mg) separated on 40-80 mesh XN 1010-Ag resin column (110 cm x 2 cm). Flow rate: 0.95 ml/min methanol.

obtain this flow rate when the column was packed with 80-100 mesh (dry) resin which had not been resieved.

RESULTS AND DISCUSSION

Figure 1 shows the separation of a 640 mg mixture of 9t-18:1 and 9c-18:1 by a 110 cm x 2.0 cm XN 1010-Ag resin column. Almost base line separation is achieved in a total time of 5 hr compared to 2.0 hr with the smaller mesh XE 284-Ag resin column and to 7 hr with the XN 1005-Ag resin column. Resin particle size, sample size, column length, and flow rate are all factors which influence total retention times and completeness of separation.

Figure 2 illustrates the separation of 1.5 g t,t-18:1; c,t-18:2; c,c-18:2 mixture on a 60 cm x 3.0 cm XN 1010-Ag resin column. Total retention time for c,c-18:2 was 13 hr compared to 4.5 hr for the XE 284-Ag resin column. It should be noted that elution of c,c-18:2 could never be achieved on a practical time scale with the older XN 1005-Ag resin columns.

The possibility of expanding the scope of silver-resin column separation to positional isomers as well as geometric isomers was explored, and the results are shown in Figure 3. The curve in Figure 3 shows the separation of a 9c,12c-; 12c,15c-18:2 mixture by a 150 cm x 1.0 column packed with 80-100 mesh XN 1010-Ag resin.

Table I lists percentage of 9c,12c- and 12c,15c-18:2 in fraction taken across the curve in Figure 3. Although no clear separation was achieved, the fractions taken across the peak were enriched as shown in Table I. These data indicate that Ag-resin chromatography may have the potential for separating positional isomers by using highly efficient columns and high-pressure liquid chromatography techniques.

The separation of a safflower oil-linseed oil methyl ester mixture is given in Figure 4.

The 18:2 was easily separated from 18:3 by elution of

TABLE I

Silver-Resin Column Separation of 9c, 12c-18:2 and 12c, 15c-18:2[a]

Fraction[b]	Wt (mg)	9c,12c-18:2 (%)	12c,15c-18:2 (%)
2	30	100	0
3	33	90.9	9.1
4	68	40.7	59.3
Mixture		75.5	24.5

[a]18:2 Positional isomers separated on a 80-100 mesh XN 1010-Ag resin column (150 cm x 1.1 cm). Flow rate: 0.64 ml/min methanol.

[b]Fraction numbers are indicated in Figure 3.

the 18:2 with 100% methanol. The 18:3 was not removed from the XN 1010 resin column with methanol but was completely displaced from the column by eluting with 10% 1-hexene in methanol. 1-Hexene forms a more stable complex with the silver ion and effectively displaces the 18:3 (12,13). Unlike the 18:3, the 1-hexene is readily removed from the silver-resin column by elution with 100% methanol which regenerates the column for future use. A probably explanation for the difficulty in eluting 18:3 is that one or more of its double bonds are always complexed to a silver ion which prevents 18:3 from moving through the column. A similar difficulty with the older XN 1005-Ag resin columns was observed for linoleate which was not eluted with methanol. The linoleate can be eluted from the XN 1010-Ag resin columns apparently because of an increase in the spacing between the Ag-resin groups which prevent one of the linoleate double bonds from always being complexed with the silver ion. The addition of a terminal olefin, which forms a stronger silver-olefin complex than a *cis* internal olefin, effectively competes for the silver ion sites and displaces the 18:3. However, since the terminal olefin has only one double bond per molecule it can in turn be easily eluted form the column with 100% methanol. The 60 cm x 3.0 cm column used in Figure 4 required ~1000 ml of a 10% 1-hexene in methanol solution to completely clear the column of methyl linolenate. A 25% 1-hexene in methanol solution worked equally as well, and less than 400 ml of solvent was required to remove the linolenate. The resin column is regenerated by eluting with 800 ml of methanol in order to remove the 1-hexene.

The development of this simple technique for regeneration of Ag-resin columns which have been used in the samples containing more than two *cis* double bonds greatly expands the utility of the method.

Figure 5 shows the separation of acetylenic fatty esters. A mixture (650 mg) of stearate, stearolate, and crepenynate was separated on a 110 cm x 2.0 cm silver-resin column. The acetylenic bond apparently forms a silver complex that has a dissociation constant similar to the silver-*cis*-olefin complex. A mixture of stearoleate and oleate could not be resolved into two components. Mixtures of crepenynate (*cis*-9-octadecen-12-ynoate) and linoleate could be resolved, which indicates that the silver-*cis*-olefin and silver-acetylene complexes have similar but not exactly equal dissociation constants. Mixtures of crepenynate and *cis,trans*-18:2 were not separated. Apparently only one pair of pi electrons in the acetylenic bond is involved in the silver complex at any on time, and the presence of a second pair of pi electrons does not drastically change the dissociation constant of the silver-pi electron complex. For monounsaturated fatty esters, one *cis* double bond is thus about equivalent to one acetylenic bond. However, for diunsaturated fatty esters, two *cis* bonds result in a stronger silver complex than does a combination of one acetylenic and one olefin bond. These data suggest that the bent configuration of *cis,cis*-18:2 may allow both double bonds to interact with the same silver ion and therefore form a stronger complex than is possible with the straighter configuration of crepenynate.

The extension of silver resin chromatography to the separation of mixtures containing saturates, monoenes, dienes, trienes, and acetylenes or alkynes greatly expanded the usefulness of silver-resin column separations. The demonstration of 1-hexene as a useful eluting solvent for tightly bound compounds should also be valuable if small mesh size silver-resin columns are used in high-pressure liquid chromatography applications. The technique has been developed for separation of unsaturated fatty esters, but the method should be applicable for separation of many *cis* and *trans* unsaturated organic compounds.

Amberlite XE 284-Ag resin columns were used by Scholfield (1) to accomplish separations similar to those reported in this paper for Amberlyst XN 1010-Ag resin columns. The procedures for use of the XN 1010-Ag resin were developed because XE 284 resin was no longer available. Retention time for 18:0 on a 65 cm x 1.0 cm XE 284-Ag column (1) was 0.7 hr compared to 1.6 hr for the 60 cm x 3.0 cm XN 1010-Ag column (Fig. 4). Since 18:0 has no double bonds to interact with the silver ion, these retention times reflect the approximate void volumes of the columns which are calculated to be 42 ml for the 65 cm x 1 cm XE 284-Ag column and 290 ml for the 60 cm x 3.0 cm XN 1010-Ag column. The flow rate used with the XN 1010-Ag column (Fig. 4) was obviously not large enough to compensate for the large differences between the void volumes of the two columns which is caused by differences in their diameters. Thus the longer retention times observed with the XN 1010-Ag columns compared to the XE 284-Ag columns are the result of differences in column void volume and flow rate. XE 284-Ag and XN 1010-Ag columns would be expected to give similar retention times under identical conditions for both 18:0 and unsaturated fatty esters. However, if comparable flow rates were used, the separations with the 40-80 mesh XN 1010-Ag column would not be expected to be as good as with the 100-200 mesh XE 284-Ag column since a more efficient column can be packed with the smaller XE 284-Ag resin particles.

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