Partial Argentation Resin Chromatography (PARC): I. Effect of Percent Silver on Elution and Separation of Methyl Octadecadienoate Isomers

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ABSTRACT

Partial argentation resin chromatography (PARC) for the separation of octadecadienoate ester isomers was investigated. In comparison to saturated silver resin chromatography, the time necessary to elute methyl *cis,cis*-octadecadienaotes was dramatically shortened when columns containing sulfonic acid ion exchange resin silvered in the range of 60-90% of theoretical (meaning 60-90% of the sulfonic acid protons in the resin were replaced by silver ions) were used. Methods for preparation and silvering of the resin are discussed. The XN1010 resin (Rohm and Haas) was analyzed for total sulfonic acid groups and the amount of silver that can be incorporated by one or 2 treatments with silver nitrate was determined. A series of partially silvered resin columns was prepared and samples of methyl linoleate were eluted to study the effect of the percentage silvering on elution volumes and peak shapes. Twenty-gram samples of mixtures of *cis,trans-* and *trans,trans-* and of *trans,cis-* and *cis,cis*-methyl 12,15-octadecadienoates were separated on a 91% PARC (91% silvered) column.

INTRODUCTION

Separation of geometric isomers of unsaturated fatty methyl esters using counter-current distribution, silver nitrate impregnated silica gel, thin layer chromatography (TLC) and silver resin chromatography has been reviewed recently by Scholfield (1).

The separation of monoenoic and dienoic fatty ester isomers using silver saturated, macroreticular cation exchange resins (silver resin chromatography) has been reported (2-7). Although the separation of 10- to 20-g samples of monoenoic ester isomers by this technique was easily accomplished, the *cis,cis*-dienoic and *cis,cis,cis*-trienoic esters required long elution times and large volumes of methanol and resulted in poor peak shapes. These all-*cis* polyunsaturated fatty esters could be displaced from the silver resin column and their elution times thus shortened by the addition of 1-hexene to the eluting solvent (6).

This paper reports the effect of partial silvering of the XN1010 ion exchange resin (where only a portion of the total sulfonic acid protons is replaced by silver ions) on the retention times of methyl linoleate and the application of partial argentation resin chromatography (PARC) for separation of mixtures of *trans,trans-* and *cis,trans-* and of *trans,cis-* and *cis,cis*-octadecadienoate esters. This technique of replacing only a portion of the sulfonic acid protons on a cationic exchange resin with silver ions will be referred to as partial argentation resin chromatography (PARC).

EXPERIMENTAL PROCEDURES

Materials

Amberlyst XN1010 ion exchange resin is a sulfonated, cross-linked, polystyrene-type resin, which is supplied in the hydrogen form. It is hard, dry, spherical and gray-black in appearance. The resin (16- to 50-mesh particles [U.S. Standard Sieve]) has a skeletal density of 1.35 g/ml, a bulk density of 5.45 kg/m^3 and a surface area of 500 sq/g. The mean pore diameter is 50 Å units and the resin has a cation exchange capacity of 3.1 milliequivalents (meq)/g (8).

Methyl linoleate was separated by PARC from transesterified safflower oil (PVO International, San Francisco, CA). The isomeric methyl 12,15-octadecadienoates-9,10-d_2 were synthesized as previously reported (9).

Methods

Chromatographic separations, unless specifically noted, were made through an 85 × 0.6-cm glass column packed with ca. 20 ml of the appropriately silvered resin. A Milton Roy "mini-pump" was used to meter methanol to the column and the effluent was monitored by a Waters Associates Differential Refractometer (Model R403). Fractions were analyzed with an F & M 810 gas chromatograph equipped with a flame ionization detector. A 12-ft 1/4-in. stainless steel column containing 20% OV 275 on Chromosorb WAW, 100/120 was used. The XN1010 resin was ground in a Model 900 Burr Mill set at an opening of 0.06 in. (Labconco Corp., Kansas City, MO).

Preparation of Partially Silvered Resin

The ground resin was soaked in methanol (CH_3OH) for 1 hr (Caution: Heat is generated and the resin swells ca. 30%). The resin was suspended in CH_3OH 5 more times, with the solvent being rapidly decanted each time to remove the super-fines. The CH_3OH-treated resin was then washed with water (ca. 10% swelling by vol) and the addition/decantation process again repeated 5 times. The resin was allowed to settle for 15 min after each wash and was then stored overnight under the final water wash.

USA standard sieves (8 in.; Newark Wire Cloth Co., Newark, NJ) were used to separate the resin particles into various mesh sizes. About 300-ml portions of the resin were placed on the appropriate sieve and flushed with a vigorous stream of distilled water. A plastic bucket was used to collect the resin particles that passed through the sieve. After allowing the resin in the bucket to settle for 3 min, the water and super-fine resin particles (>400 mesh) were discarded. The resin remaining in the bucket was transferred to a finer sieve and the process was repeated. By this procedure, a range of mesh sizes (on 40, 40/80, 80/100, 100/120, 120/140 and 140/400) were collected. Only 40/80 and 100/120 mesh sizes were used in our study. The volume of the wet resin was measured with a graduated cylinder.

Resin containing different amounts of silver was prepared as follows: (a) for resin saturated with silver ions, a 0.2 M solution of silver nitrate ($AgNO_3$) in water was passed through a column containing the resin until the eluent was no longer acidic. The $AgNO_3$ solution and the resin were allowed to equilibrate for 1 hr, and the excess $AgNO_3$ was removed by washing with distilled water.

Gradually increasing amounts of CH_3OH in water (i.e., 25, 50, 75 and 100%) were then passed through the column. Resins prepared in this manner were assumed to be saturated (100%) with silver ions. (b) Resins not fully saturated with silver ions were prepared by a batch process. About 25 ml of the resin (in water) was transferred with 200 ml of water to a 3-necked, 500-ml round-bottomed flask equipped with an additional funnel and mechanical stirrer. The calculated amount of $AgNO_3$ (based on meq sulfonic acid/g resin) in 200 ml of water was added by drops over a 20-min period. The slurry was stirred for 1 hr and the resin was separated by vacuum filtration. After being washed with water, the resin was transferred directly into CH_3OH.

The incorporation of silver ion is described by the equation:

$$R\text{-}SO_3H + AgNO_3 \rightleftharpoons R\text{-}SO_3Ag + HNO_3, \quad \text{where } R = \text{resin} \qquad [1]$$

The amount of silver incorporated on the resin could thus be determined by titration of the HNO_3 produced according to the following procedure. The aqueous eluent or filtrate was collected and diluted to a constant volume in a volumetric flask and a 50-ml portion was removed. Saturated sodium chloride was added to precipitate the excess silver ion (let stand for 15 min), and the mixture was titrated with 0.1 M sodium hydroxide in 90% CH_3OH to a phenolphthalein endpoint.

The HNO_3-containing eluents from the preparation of silver-saturated columns were titrated to determine the cation exchange capacity in meq/vol resin. The capacity of the resin in meq/ml varied little with mesh size (i.e., 40/80 at 3.6 meq/g vs 100/120 at 3.7 meq/g). The volume of the resin was measured after storing the resin in a graduated cylinder overnight. The resin was then removed by vacuum filtration, dried overnight in a vacuum oven (110 C/70 mm Hg) and weighed to determine the density in g/ml.

RESULTS

A series of partially silvered resins (54, 61, 72 and 91%) was prepared using procedure b. The theoretical percentage of silvered vs actual percentage silvered is plotted in Figure 1. From these data, the maximal percentage of silver ion that can be incorporated by a single $AgNO_3$ treatment is ca. 85%.

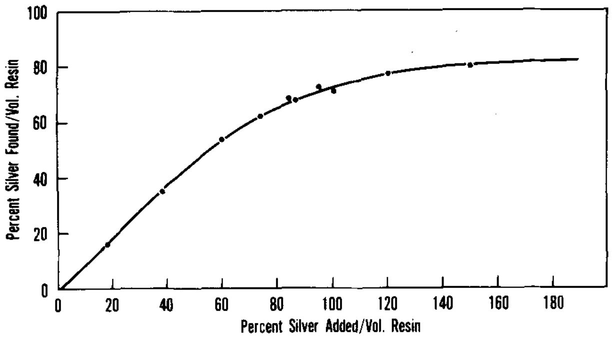

FIG. 1. Comparison of the theoretical amount of silver added by procedure b to the actual amount of silver retained on the resin (determined by titration of eluent).

Two successive batch treatments were employed when more than 75% silvering of the resin was desired. When the theoretical amount of $AgNO_3$ (equivalent to the number of meq sulfonic acid on the unsilvered resin) was used in both treatments, the same percentage of silver ion was incorporated each time. If $AgNO_3$ equivalent to the number of meq of resin unsilvered after the first treatment was used for the second silvering, the percentage of silver incorporated on the second batch was less than on the first batch. For example, to prepare a 91% silvered sample of resin, the resin was treated twice, each treatment using 100% of the theoretical quantity of silver ions (see run no. 8, Table I). Between treatments, the resin was vacuum-filtered and washed with water until neutral.

A single-treatment process was used to prepare a series of PARC columns containing 54-72% silver. A 100-mg sample of methyl linoleate was eluted with CH_3OH from each column to determine the effect of the percentage silver on peak shape and elution volume (Fig. 2). A preparative size (4 x 53 cm, 700 ml resin, 40/80 mesh) 91% PARC column was also prepared by treating the resin twice with $AgNO_3$. This column was used to separate 15- to 20-g samples of methyl 12,15-octadecadienoate-9,10-d_2 geometrical isomers as shown in Figures 3 and 4.

Table I summarizes the preparation of several partially silvered resins. Preparations 1 and 2 were saturation tests to determine the number of meq of sulfonic acid/g of resin. This number (3.6 or 3.7) was higher than the cation ex-

TABLE I

Summary of Silver.Resin Preparations

Prep. no.[a]	Resin[b] volume (ml)	Resin size (mesh)	Total volume (ml)[c]	$AgNO_3$ soln (N)	Percent silver (theor.)	Percent silver[d]	meq $AgNO_3$/g resin
1	25.1	100/120	Saturation	0.2	–	100[e]	3.7
2	28.5	40/ 80	Saturation	0.2	–	100[e]	3.6
3	27.8	40/ 80	400	0.55	60	54	1.9
4	44.6	100/120	400	0.133	73	61	2.3
5	21.3	100/120	400	0.063	99	72	2.7
6	10.0	40/ 80	30	0.436	100	71	2.6
7	9.95	40/ 80	20	0.633	100	71	2.6
8	820	40/ 80	1640	0.645	100	70[f]	2.5
	820	40/ 80	1640	0.645	100	91[f]	3.3

[a] Preparations 1 and 2 were made by method a; preparations 3-7 were 1-batch silverings; preparation 8 was a 2-batch silvering.

[b] Can be converted to g at 3.02 ml/g (40/80 mesh) and 2.83 ml/g (100/120 mesh).

[c] Milliliters of water after addition of $AgNO_3$ solution.

[d] Determined by titration.

[e] Assuming 100% silver ion incorporation.

[f] Total percentage of silver on resin after first silvering.

[g] Total percentage of silver on resin after second silvering.

change capacity of 3.1 given in the preliminary data sheet for Amberlyst XN1010 resin. Comparison of preparations 5, 6 and 7 indicates that changing the normality of the $AgNO_3$ solution in the range of 0.05-0.60 has little effect on the final percentage of silver on the resin.

DISCUSSION

Development of PARC greatly extends the application of silver resin chromatography. By varying the percentage silver on the resin, problems resulting from sample tailing and large solvent elution volumes can be minimized while an acceptable degree of separation is maintained. This concept was illustrated in the separation of the various geometrical isomers of methyl 12,15-octadecadienoate. A 91% PARC column gave almost baseline separation of these isomers. The peak shape and elution volume for the 12*c*,-15*c*-18:2 isomer is greatly improved over that previously reported for the 9*c*,12*c*-18:2 isomer.

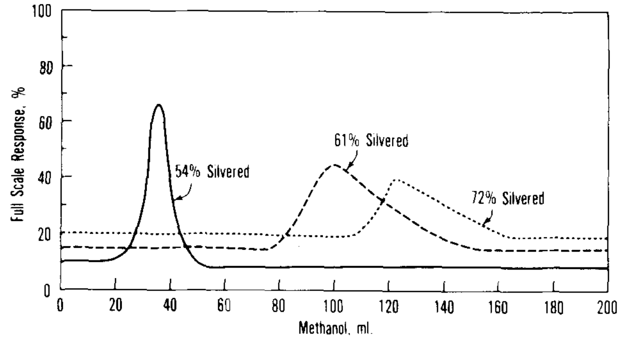

FIG. 2. Plot of the retention volume of methyl linoleate as a function of percentage silver on resin. Flow rate-0.7 ml/min CH_3OH; mesh size-40/80 or 100/120; sample size-100 mg; glass column-0.6 X 85.0 cm.

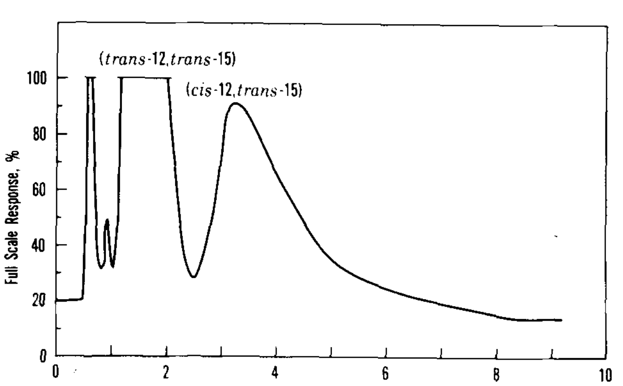

FIG. 3. Separation of 66% *trans*-12,*trans*-15- and 34% *cis*-12,*trans*-15-methyl octadecadienoate. 91%, 40/80 mesh PARC column; flow rate-10 ml/min CH_3OH; sample size-20 g.

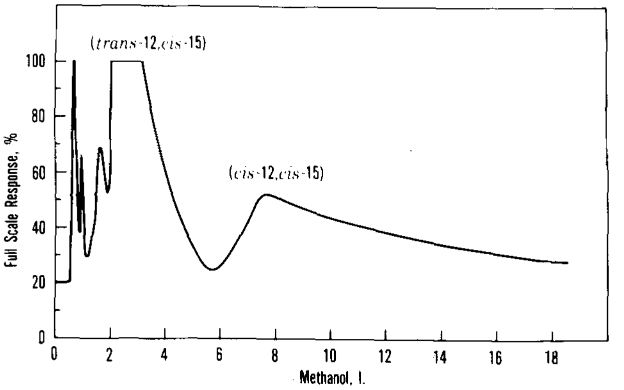

FIG. 4. Separation of 61% *trans*-12,*cis*-15- and 31% *cis*-12,*cis*-15-methyl octadecadienoate. 91%, 40/80 mesh PARC column; flow rate-10 ml/min CH_3OH; sample size-20 g.

The 91% PARC columns have been reused many times without repacking or resilvering with no deterioration in their separation capabilities. The only precautions are that the columns cannot be allowed to run dry and that large amounts of impurities cannot be present in the samples which will react with the silver ions. Compounds containing halides, e.g., can cause problems. As previously noted, a terminal olefin such as 1-hexene can be added to the methanol in order to displace highly unsaturated compounds such as polymers from the column (6). This procedure effectively cleans the column of strongly retained compounds and can be used to clean the column in order to prevent cross contamination from previous samples.

Advantages of sieving the resin wet rather than dry are that less time is required and problems with dust are eliminated. When dry sieving was used, the resin had to be sieved again after the addition of CH_3OH and H_2O because of expansion and fracturing of the resin particles and the consequent production of fines. Fines in the final mesh cut were removed to obtain the maximal flow rate through the column. A column packed with 40/80 or 80/100 mesh resin produced high flow rates at low pressure, good column capacities and adequate separation capabilities for preparative work (Figs. 3 and 4).

Use of wet resins to prepare PARC columns makes the silvering procedure somewhat empirical. The resin was kept overnight in a graduated cylinder before the volume was measured and the weight of resin/ml resin was calculated. Longer or shorter settling times would undoubtedly have changed the resin volume and affected the results. Given the errors inherent in determining the weight of the starting resin, the final percentage of silver on the resin is only relative. Despite this limitation, the method is very reproducible, and results vary by only 3-4% in the final percentage of silver on the resin.

The data in this paper explain the variations in retention of unsaturated compounds on various "saturated" silver resin columns previously prepared in our laboratory (2-8). Some of the resin columns were prepared by saturation with $AgNO_3$ (method a) and others by 3 treatments with 0.2 M $AgNO_3$ (similar to method b). Procedure b would result in silver concentrations of only 96-98% and a subsequent decrease in elution times.

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