⁂Partial Argentation Resin Chromatography (PARC): III. The Effects of Sodium Ion Incorporation and Solvent on the Separation of Mixtures of Fatty Acids, of Fatty Esters, and of Triglycerides

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ABSTRACT

Partial argentation resin chromatography (PARC) was used to separate mixtures of fatty acids, of fatty esters and of triglycerides. Various eluting solvents (methanol, acetone, ether, benzene) were investigated and acetone was the preferred solvent. To prevent acid-catalyzed condensation of the acetone, the sulfonic acid protons of the XN1010 resin were replaced with sodium ions before silver ion incorporation. Atomic absorbtion spectrophotometry was used to determine the number of silver ions incorporated per volume of resin. A mixture containing stearic, elaidic and oleic acids was separated on a saturated silver resin column. A commercially available sample of linoleic acid was purified on a 53% PARC column (meaning 53% of the available sulfonic acid protons in the resin were replaced by silver ions). A 39% PARC column was used to separate a mixture of linseed acids. Methyl linoleate, linolenate and arachidonate also were isolated on a 39% PARC column. A mixture containing triolein, trilinolein and trilinolenin was separated on a 32% PARC column.

INTRODUCTION

The separation of fatty ester isomers by saturated silver resin chromatography has recently been included in a review by Scholfield (1). Silver-loaded resins also have been used to separate the geometric isomers of insect pheromones (2-4). Applications of this technique can be greatly extended if only a part of the total sulfonic acid protons of a macroreticular resin is replaced by silver ions. Partial argentation resin chromatography (PARC) has been used to separate mixtures of *trans,trans-* and *cis,trans-* and of *trans-cis-* and *cis-cis*-12,15-octadecadienoate esters (5). *cis*--Dienoic, trienoic and tetraenoic esters also have been separated on resins containing silver ions in the range of 16-54% of theoretical (meaning 16-54% of the sulfonic acid protons in the resin were replaced by silver ions) (6). Similarly, separation of polyunsaturated fatty esters have been reported with strong cation macroreticular resin columns containing varying proportions of sodium and silver ions (7).

Attempts to separate mixtures of fatty acids and of triglycerides by silver resin chromatography using methanol as elutant were unsuccessful because of sulfonic-acid-catalyzed esterification of the fatty acids and interesterification of the triglycerides (6). Acetone, while a suitable alternative, was found to slowly undergo sulfonic-acid-catalyzed condensation to form isomers of mesityl oxide (4 methyl-3-pentene-2-one) (8). These problems were minimized if the sulfonic acid protons of the resin were replaced by sodium ions before silver ion incorporation. Resins prepared in this manner will be designated 100%/Na^+ when saturated and % PARC/Na^+ when partially in the silver form.

EXPERIMENTAL PROCEDURES

Materials

XN1010 sulfonic acid resin, 16/50 mesh, was from Rohm and Haas. The mixtures used were: monoenoic esters (17% saturates, 28% elaidate, 55% oleate); dienoic esters (35% stearate, 34% oleate, 25% linoleate); tetraenoic esters (5% saturates, 10% oleate, 25% linoleate, 20% linolenate, 44% arachidonate); monoenoic acids (30% stearic, 30% elaidic, 40% oleic); linoleic acid (61%; Fisher Scientific Co.); linseed oil acids (10% saturated, 22% oleic, 15% linoleic, 53% linolenic); triglyceride (31% olein, 26% linolein, 43% linolenin); triolein (> 98%, Hormel Institute).

Methods

Grinding and sieving of the resin, preparation of PARC columns and analyses of the eluted fatty ester fractions have been described previously (5). Fatty acids were methylated with diazomethane for gas chromatographic (GC) analysis. Triglycerides were spotted on silver-impregnated thin layer chromatography (TLC) plates (Silica Gel 60F-254; 0.25 cm, E. Merck, Darmstadt, Germany), eluted with acetone and visualized with sulfuric acid and charring. Chromatographic separations were made through a 0.6 cm x 72 cm glass column containing the appropriate silver-ion form of the resin (80/100 mesh; wet-sieved).

The sodium form of the XN1010 resin was prepared by passing a 1 M solution of sodium nitrate ($NaNO_3$) in water through a column containing 30 ml of the resin until the eluent was no longer acidic. After washing the resin with water, the eluents were combined and diluted to a constant volume in a volumetric flask. A 50-ml portion was removed and titrated with 0.1 M sodium hydroxide in 90% methanol to a phenolphthalein endpoint (see PARC I, ref 5). The calculated capacity of the resin (3.1 meq/g resin) was used in adding silver to the resin to prepare PARC/Na^+ columns. The resin (in 100 ml H_2O) was placed in a 500-ml, round-bottomed flask equipped with addition funnel and mechanical stirrer. The calculated amount of $AgNO_3$ in 75 ml H_2O was added by drops over a 1/2-hr period. The slurry was stirred for 1 hr, then vacuum-filtered to remove the resin. The filtrate was collected and the volume adjusted to 250 ml. Atomic absorption spectrophotometry (Varian Techtron AA120) was used to determine the amount of silver ions remaining in the eluent. This procedure was used to prepare a series of resins saturated or partially saturated with silver ions (Table I).

RESULTS

The retention volumes of fatty acids, esters and triglycerides on resins containing various percentages of silver are summarized in Table II.

Stearic, elaidic and oleic acids were separated with acetone elution on a 100%/Na^+ column (Fig. 1). The separation of linseed oil fatty acids on a 39% PARC/Na^+ column is illustrated in Figure 2. Triolein, trilinolein and tri-

linolenin were resolved on a 32% PARC/Na^+ column (Fig. 3). A mixture containing saturated and *cis*-mono, di-, tri- and tetraenoic esters was separated on a 39% PARC/Na^+ column (Fig. 4).

A 70-mg commercial sample of linoleic acid (61%) was purified on a 65 x 0.6 cm 39% PARC/Na^+ column. The purity of the recovered linoleic acid was > 99%.

DISCUSSION

Several solvents were found to have limited applicability in silver resin chromatography (Table II). Diethyl ether is an excellent solvent for the separation of methyl oleate/methyl elaidate, but did not elute methyl linoleate. Benzene was used to separate stearic, monoenoic and dienoic esters, but did not separate the *cis/trans* monoenoic esters.

Polarity and solubility were important in the selection of a solvent. Polyenoic acids and esters were more readily eluted with methanol (smaller retention volumes). However, acetone was necessary to elute triglycerides, because of their greater solubility in this solvent. Given its wider range of applicability and lower toxicity, acetone was chosen for the bulk of our separations.

Both methanol and acetone could be used to elute fatty acids. The sulfonic acid protons of the resin had to be replaced by sodium ions to prevent sulfonic-acid-catalyzed esterification of fatty acids when methanol elution was used. Complete replacement also eliminated the formation of mesityl oxide isomers when acetone was employed. When acetone was used as the eluting solvent, preliminary saturation of the resin by sodium ions was necessary only if the resin column was infrequently used. Acetone placed in contact with 100% silver resin was found to yellow after ca. 1 week. No yellowing of the acetone was noted when 70% PARC to silver-ion-saturated columns were used every second day. Thus, both the sodium-ion-saturated and the sulfonic acid forms of the resin can be employed if use of the silver resin column is fairly frequent.

Polyunsaturated fatty acids were more rapidly eluted with methanol than with acetone. A resin containing a higher percentage of silver (with a correspondingly higher capacity) can thus be employed when methanol is used as the eluting solvent. However, when methanol was employed to elute fatty acids, a trace of fatty esters (1-2%) was found, even when 100%/Na^+ resin was used. This may have resulted from still unexchanged sulfonic acid protons on the resin.

TABLE I

Summary of Silver Resin Preparations

Prep.	Resin[a] (ml)	Capacity (meq/ml resin)	Meq silver ions added	Percent silver (theor)[b]	Percent silver actual[c]
1	28.5	1.25[d]	Saturation	100	100
2	36.2	1.1[e]	Saturation	100	100
3	20.5	1.1[e]	13.0	58	53
4	23.4	1.1[e]	10.3	40	39
5	36.2	1.1[e]	12.8	34	32

[a]80/100 mesh.
[b]Based on calculated capacity (meq/ml).
[c]Determined by atomic absorbtion spectrophotometry.
[d]Calculated by titration of eluent after sulfonic acid form of resin was saturated with 1 N $AgNO_3$ (2,3).
[e]Calculated by titration of eluent after sulfonic acid form of resin was saturated with 1 N $NaNO_3$.

TABLE II

Retention Volumes of Fatty Acids, Esters and Triglycerides

Mixtures[a]	Sample weight (mg)	Column[b]	Solvent	Flow rate (ml/min)	Retention volumes (ml) 16:0, 18:0	9t-18:1	9c-18:1	18:2[c]	18:3[d]	20:4[e]	Notes
Fatty esters											
Monoene	100	100%	Methanol	0.4	15.5	19.0	24.5				
Monoene	100	100%	Ether	1.5	16.0	24.2	44.0				f
Monoene	90	100%/Na^+	Acetone	0.4	14.0	17.1	23.0				f
Monoene	100	100%	Acetone	0.4	15.0	16.7	19.3				
Diene	100	100%	Benzene	0.4	15.0		21.5	71.0			f
Diene	100	100%	Ether	0.4	14.9		48.1	*			
Diene	100	100%	Acetone	0.4	14.8		19.6	73.1			
Diene	70	100%/Na^+	Acetone	0.4	13.8	17.8	23.9	159			g
Tetraene	30	39% PARC/Na^+	Acetone	0.2	———	14.5	———	17.6	28.3	39.6	f
Fatty acids											
Monoene	100	100%	Acetone	0.4	15.5	19.1	25.0				
Monoene	50	100%/Na^+	Acetone	0.4	17.3	34.8	67.5				f
Linoleic	50	53% PARC/Na^+	Methanol	0.3	13.6		16.5	24.2			f,h
Linoleic	50	53% PARC/Na^+	Acetone	0.3	17.2		26.5	56.4			f,h
Linseed	80	39% PARC/Na^+	Acetone	0.3	14.2		22.5	34.8	58.0		f
Triglycerides											
STD mixture	60	39% PARC/Na^+	Acetone	0.4			15.3	28.0	*		f
STD mixture	40	32% PARC/Na^+	Acetone	0.2			13.3	17.1	40.5		f
Triolein	50	100%	Acetone	0.3			48.2				
Triolein	40	100%/Na^+	Acetone	0.4			127				g

[a]See Experimental procedures for composition of mixtures.
[b]Column size 72 x 0.6 cm, 80/100 mesh resin except where noted.
[c]9c,12c-18:2.
[d]9c,12c,15c-18:3.
[e]5c,8c,11c,14c-20:4.
[f]Resolution of components greater than 95% of baseline.
[g]Last component tailed severely.
[h]65 X 0.6 cm column, 80/100 mesh resin.
*Did not elute.

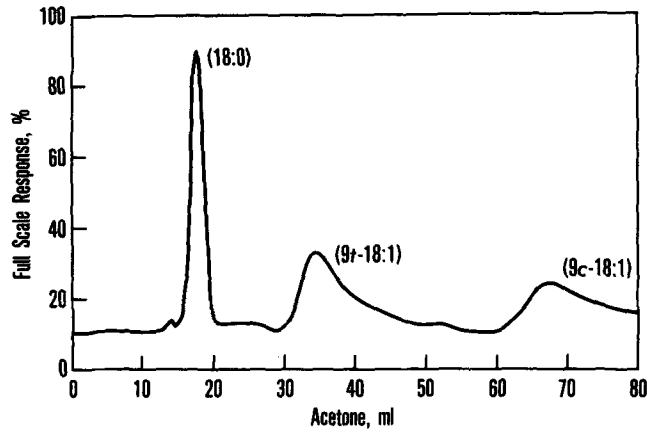

FIG. 1. Separation of the monoenoic acid standard on a 100%/Na^+ column. Flow rate: 0.4 ml acetone/min; sample size: 50 mg.

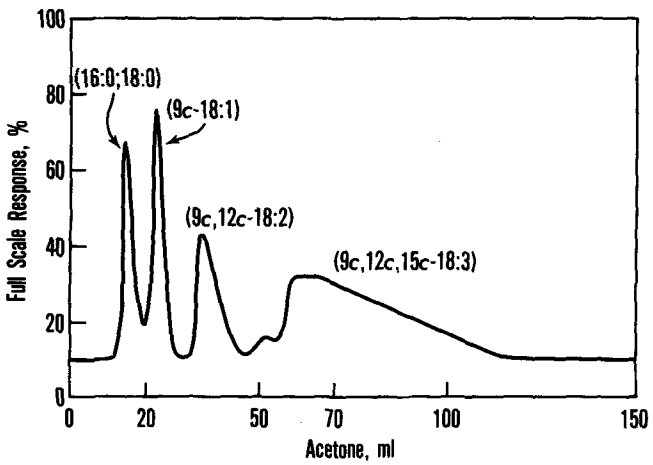

FIG. 2. Separation of linseed oil acids standard on a 39% PARC/Na^+ column. Flow rate: 0.3 ml acetone/min; sample size: 80 mg.

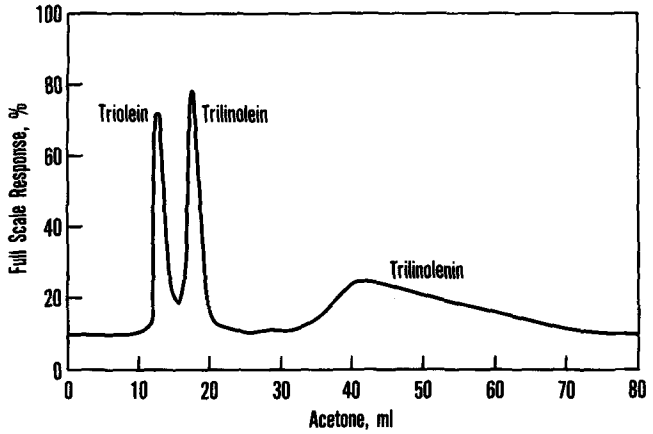

FIG. 3. Separation of the triglyceride standard on a 32% PARC/Na^+ column. Flow rate: 0.2 ml acetone/min; sample size: 40 mg.

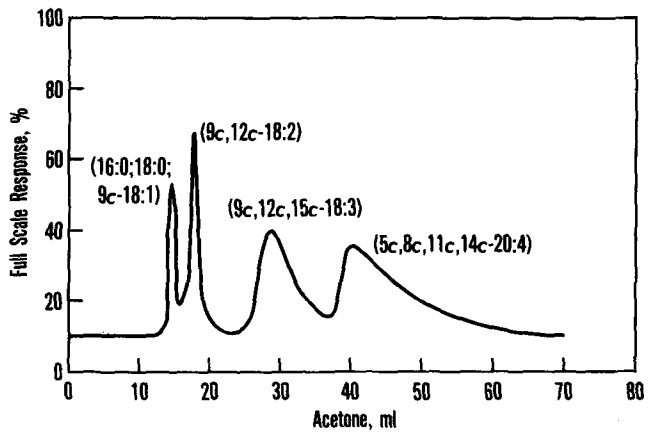

FIG. 4. Separation of the tetraenoic ester standard on a 39% PARC/Na^+ column. Flow rate: 0.3 ml acetone/min; sample size: 30 mg.

Several other observations can be made, although we cannot necessarily explain them. The retention volumes (methanol and acetone) for unsaturated fatty acids, esters and triglycerides were greatly increased on resins initially saturated with sodium ions. We have found that polyunsaturated esters are very slightly retained on sodium ion saturated resin (methanol elution). While resolution was impossible, small variations of composition were detected across the elutant peak. The resolution of monoenoic ester isomers was improved when 100%/Na^+, rather than saturated silver resin, was used (acetone elution; Table II). Thus, there must be sulfonic acid protons accessible to sodium ions, yet inaccessible to silver ions. However, methyl arachidonate was eluted with 40 ml of acetone on a 39% PARC/Na^+ column vs 300 ml of methanol on a 36% PARC column.

The use of sodium-ion-saturated silver resin chromatography and acetone elution makes it possible to separate and purify fatty acids and triglycerides and enhances PARC's utility for separation of polyunsaturated esters, such as methyl arachidonate. This extension of the PARC concept has greatly facilitated the preparation of pure samples of these important compounds.

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