Partial Argentation Resin Chromatography (PARC): II. Separation of Saturated and Mono-, Di-, Tri- and Tetraenoic Fatty Esters

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ABSTRACT

Partial argentation resin chromatography (PARC) was used to separate a mixture of saturated and monoenoic, dienoic, trienoic and tetraenoic fatty esters. A series of columns containing XN1010 sulfonic acid resin silvered in the range of 17-91% of theoretical (meaning 17-91% of the sulfonic acid protons were replaced by silver ions) were prepared and were used to correlate the percentage silver with the separation and the retention times of unsaturated fatty esters. *cis*-Trienoic and tetraenoic fatty esters were eluted with methanol on PARC columns containing 36 and 17% silver. Partial silvering of the resin improved peak shapes while sample elution time and elution volume were reduced. Application of PARC to the separation of mixtures of fatty acids and mixtures of triglycerides was investigated and found to be not feasible with the present system.

INTRODUCTION

Saturated silver resin chromatography has been reported to give excellent separation of saturated and monoenoic fatty esters (1-4), but the more highly unsaturated esters were found to be difficult or impossible to remove from the column by methanol (CH_3OH) elution alone (5,6). Elution of larger quantities of methyl linoleate (>20 g) from a fully saturated silver resin column required huge volumes of methanol and times approaching 1 week. A displacing agent such as 1-hexene could be used elute polyunsaturated esters, but no separation of components could then be achieved (6).

The application of partial argentation resin chromatography (PARC) has been reported for the separation of methyl 12,15-octadecadienoate isomers (7). This paper describes the extension of the PARC concept to the separation of saturated and *cis*-mono-, di-, tri- and tetraenoic fatty esters.

EXPERIMENTAL PROCEDURES

Materials

XN1010 sulfonic acid resin, 16/50 mesh, was from Rohm and Haas. The standard mixtures used were: Dienoic ester (20% saturates, 20% oleate and 60% linoleate); tri- and tetraenoic esters (5% saturates, 10% oleate, 20% linoleate, 20% linolenate and 44% arachidonate); and safflower oil methyl esters (11% saturates, 13% oleate and 75% linoleate). Saturates contain both methyl palmitate and methyl stearate. Methyl arachidonate was obtained from Hoffmann-La Roche, Inc., Nutley, NJ.

Methods

The grinding and sieving of the resins, preparations of PARC columns, silver percentage determinations (Table I) and analyses of the various eluted fractions have been described previously (7). Thin layer chromatography (TLC) (Silica Gel 60F-254; 0.25 cm; E. Merck, Darmstadt, Germany) was used to determine the effect of silvered resin on fatty acids and triglycerides. Samples were developed using a 50:50 mixture of petroleum ether (P.E.) and diethyl ether (Et_2O) and visualized with sulfuric acid and charring. Di- and tetraenoic standard mixtures (100-mg) were separated on an 85 × 0.6 cm glass column packed with 20-ml samples of 17-54% silvered (meaning 17-54% of the resin sulfonic acid protons were replaced by silver ions) resins. Safflower oil methyl esters (∼20 g) were separated by a 4 × 53 cm glass column containing 700 ml of 40/80 mesh, 91% silvered resin.

RESULTS

Figures 1-3 illustrate the separation of 100-mg samples of fatty esters on 54, 36 and 17% PARC (silvered) columns.

TABLE I

Summary of Silver Resin Preparations

Prep. no.[a]	Resin (ml)	Resin size (mesh)	Total volume[b] (ml)	$AgNO_3$ soln. (N)	Percent silver (theor.)	Percent silver[c]	meq $AgNO_3$/g resin
1	27.8	40/ 80	400	0.55	60	54	1.9
2	26.3	80/100	400	0.040	39	36	1.3
3	23.2	80/100	400	0.016	18	17	0.6
4	36.0	16/ 50	Saturation[d]	0.16	100	100	3.3
5	820	40/ 80	1640	0.645	100	70[e]	2.5
			1640	0.645	100	91[f]	3.3

[a] Runs 1-4 are one-batch silverings; run 5 is one batch silvered twice.

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

[c] Determined by titration.

[d] Unground resin; washed with 0.16 N $AgNO_3$ until saturated.

[e] Total percent silver on resin after first silvering.

[f] Total percent silver on resin after second silvering.

The separation of safflower oil methyl esters is illustrated in Figure 4. About 14 g of 99+% pure methyl linoleate was isolated from each 20-g sample. A 25-ml sample of unground (16/50 mesh), 100% silvered resin was also prepared to determine if the resin could be used as received. Separations were found to be poor (Fig. 5). Although saturated and monoenoic esters could be partially separated, the dienoic esters began to elute from the column as part of the monoenoic ester peak.

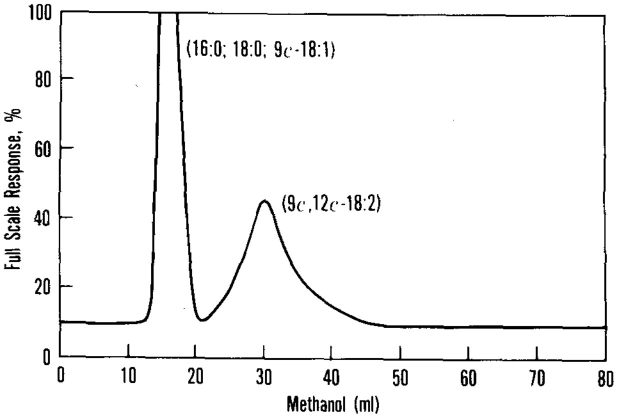

FIG. 1. Separation of the dienoic ester standard on an 85 × 0.6 cm 54% PARC column. Flow rate: 0.55 ml CH_3OH/min; 40/80 mesh; sample size: 100 mg.

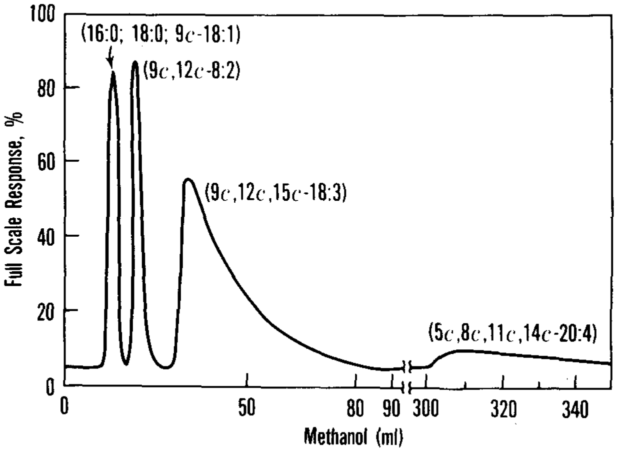

FIG. 2. Separation of the tetraenoic ester standard on an 85 × 0.6 cm 36% PARC column. Flow rate: 0.40 ml CH_3OH/min; 80/100 mesh; sample size: 100 mg.

The feasibility of separating mixtures of fatty acids or triglycerides using PARC was also investigated. Small samples of oleic acid (50 mg) were placed in vials containing ca. 10 ml of methanol and either 5 ml of 100% silvered or 50% silvered resin. The vials were left at room temperature overnight and the supernatant was tested by thin layer chromatography (TLC) for the presence of methyl oleate. A significant amount of methyl oleate (20-30%) was found, thus indicating esterification had occurred. Similar tests using triolein also produced methyl oleate (<5% for 100% silvered; 15-20% for 50% silvered). Thus, both esterification and transesterification occurred, even when silver-saturated resin was used. Apparently these reactions were catalyzed by traces of free sulfonic acid groups remaining on even the 100% silvered resin.

DISCUSSION

The applications of silver resin chromatography have been expanded by the PARC concept. Polyenoic esters can now be separated by control of the percentage of silver on the resin. The effect of partial silvering is illustrated in Figures 1-3. Dienoic esters can be separated from monoenoic and saturated esters using a 54% PARC column. A 36% PARC column can be used to isolate both dienoic and trienoic esters and tetraenoic esters can be eluted from a 16% PARC column. The poor separation of trienoic and tetraenoic esters on the 16% PARC column may indicate the limit to which the percentage of silvering of the resin can be lowered and usable separation still be achieved. However, a 36% PARC column could be used to selectively separate all *cis*-dienoic, trienoic and tetraenoic fatty esters if the tetraenoic fatty esters were eluted with 1-hexene. These separations are currently impractical using a saturated silver resin column (5,6).

Since flow rate,percentage of silver and column length can be varied, the parameters used in this paper provide only general guidelines for the preparation of suitable columns. Optimization of the parameters for specific separations was not investigated. Consequently, the use of high flow rates with columns containing the highest possible percentage of silver (where the sample can still be eluted) was the best strategy for preparative separation of polyenoic fatty esters.

Elution volume for saturated fatty esters was essentially constant despite large variations in the percentage of silver. Only with the unground resin did the saturates begin to elute earlier (Fig. 5), perhaps because of channeling. Thus

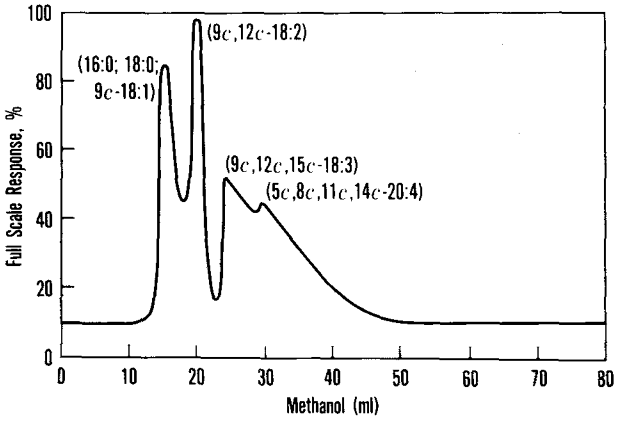

FIG. 3. Separation of tetraenoic ester standard on an 85 × 0.6 cm 17% PARC column. Flow rate: 0.19 ml CH_3OH/min; 80/100 mesh; sample size: 100 mg.

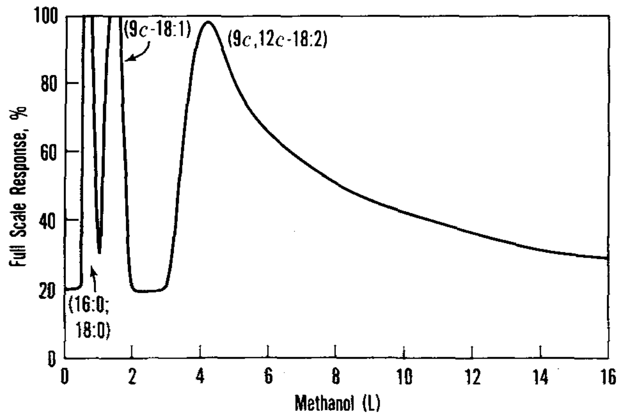

FIG. 4. Separation of safflower oil methyl esters on a 53 × 4 cm 91% PARC column. Flow rate: 10 ml CH_3OH/min for 4 liters, then 12 ml/min; 40/80 mesh; sample size: 20 g.

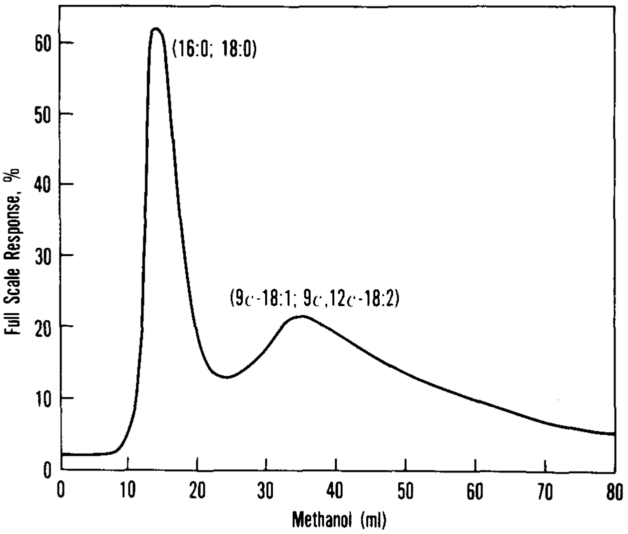

FIG. 5. Separation of the dienoic ester standard on an 85 × 0.6 cm 100% silvered column. Flow rate: 0.27 ml CH_3OH/min; 16/50 mesh; sample size: 100 mg.

grinding and sieving of the resin are necessary for good sample resolution. A column packed with 40/80 mesh resin is a good choice. With this mesh size, high flow rates (at low back pressure) and capacities can be maintained while channeling is kept to a minimum.

The advantages of PARC over other separation techniques are numerous. Unlike previously employed resins such as XN1005, the XN1010 resin is commercially available. Older XN1005 saturated silver resin columns have lasted for more than 10 years, thus demonstrating their durability. In general, the columns can be reused indefinitely. The silver incorporated onto the resin can be easily recovered if necessary by regenerating the resin with nitric acid. Polyunsaturated esters or tightly bound impurities were previously shown to be displaced using a 10-20% solution of 1-hexene in methanol (6). The capacities of the silver resin columns are high compared to silver-silica gel columns, since a 4 × 53 cm, 91% PARC column can easily separate 20-g samples of safflower oil methyl esters, and samples twice as large can be used if only the dienoic esters are to be isolated. The PARC concept allows a column to be tailored to the specific separation needed.

Although this method is not applicable to fatty acids and triglycerides, preparative separation of large quantities of saturated and *cis*-mono-, di-, tri- and tetraunsaturated fatty esters is now feasible. The PARC concept should be a useful technique for those laboratories requiring large, highly pure samples of these important compounds.

Since this work was completed and reviewed by *JAOCS*, the authors have become aware of a patent issued in February 1980 to the Procter and Gamble Company which described some of the concepts of partial argentation resin chromatography used in this paper (8).

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