24. Noelken, M., and M. Reibstein, Arch. Biochem. Biophys. 123: 397 (1968).
25. Schellman, J.A., C. R. Trav. Lab. Carlsberg 29:230 (1955).
26. Hvidt, A., and S.O. Nielson, Adv. Prot. Chem. 21:287 (1966).
27. Oobatake, M., S. Takahashi and T. Ooi, J. Biochem. 86:65 (1979).
28. Tanford, C., J. Am. Chem. Soc. 84:4240 (1962).
29. Rowe, E.S. and C. Tanford, Biochemistry 12:4822 (1973).
30. Yutani, K., K. Osahara, and Y. Sugino, J. Mol. Biol. 144:455 (1980).
31. Pace, C.N., and K. Vanderburg, Biochemistry 18:288 (1979).
32. Schellman, J.A., and R.B. Hawkes, in Protein Folding, edited by R. Jaenicke, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, p. 331.
33. Tanford, C., Adv. Prot. Chem. 24:1 (1970).
34. Alexander, S.S., and C.N. Pace, Biochemistry 10:2738 (1971).
35. Hollecker, M., and T.E. Creighton, Biochim. Biophys. Acta 701:395 (1982).
36. Wedin, R.E., M. Delepierre, C.M. Dobson and F.M. Povlsen, Biochemistry 21:1098 (1982).
37. Kossiakoff, A.A., Nature 296:713 (1982).
38. Wagner, G., and K. Wutrich, J. Mol. Biol. 155:347 (1982).
39. Hilton, B.D., K. Trudeau and C.K. Woodward, Biochemistry 20:4697 (1981).
40. Sachs, D.H., A.N. Schechter, A. Eastlake and C.D. Anfinsen, Proc. Nat. Acad. Sci. 69:3790 (1972).
41. Privalov, P.L., P.L. Mateo, N.N. Khechinashvili, V.M. Stepanov and L.P. Revina, J. Mol. Biol. 152:445 (1981).
42. Edelhoch H., and J.C. Osborne, Jr., Adv. Prot. Chem. 30:183 (1976).
43. Schellman, J.A., M. Lindorfer, R. Hawkes and M. Grutter, Biopolymers 20:1989 (1981).
44. Grutter, M.G., R.B. Hawkes and B.W. Matthews, Nature 227: 667 (1979).
45. Yutani, K., K. Ogasahara, M. Susuki and Y. Sugino, J. Biochem. 85:915 (1979).
46. Matthews C.R., M.M. Crisanti, G.L. Gepner, G. Velicelebi and J.M. Sturtevant, Biochemistry 19:1290 (1980).
47. Knapp, J.A., and C.N. Pace, Ibid., 13:1289 (1974).
48. McLedon, G., and M. Smith, J. Biol. Chem. 253:4004 (1978).
49. Cupo, J.F., and C.N. Pace, Biochemistry, submitted for publication.
50. Wutrich, K., G. Wagner, R. Richarz and W. Braun, Biophys. J. 32:549 (1980).
51. Chlebowski, J.F., and S. Mabrey, J. Biol. Chem. 252:7042 (1977).
52. Filimonov, V.V., W. Pfeil, T.N. Tsalkova and P.L. Privalov, Biophys. Chem. 8:117 (1978).
53. Kennah, E.H., M.S. thesis, Texas A&M University (1974).
54. Pace, C.N., and T. McGrath, J. Biol. Chem. 255:3862 (1980).
55. Imoto, T., and J.A. Rupley, J. Mol. Biol. 80:657 (1973).
56. Mosbach, K., Methods Enzymol. 44:1 (1977).
57. Gurd, F.R.N., and T.M. Rothberg, Adv. Prot. Chem. 33:73 (1980).
58. McCammon, J.A., B.R. Gelin and M. Karplus, Nature 267: 585 (1977).
59. Privalov, P.L., and T.N. Tsalkova, Ibid. 280:693 (1979).
60. Edsall, J.T., Structural Chemistry and Molecular Biology, edited by A. Rich and N. Davidson, Freeman, San Francisco, 1968.
61. Tanford, C., Adv. Prot. Chem. 24:1 (1970).
62. Friend, S.H., and F.R.N. Gurd, Biochemistry 18:4612 (1979).
63. Tall, A.R., G.G. Shipley and D.M. Small, J. Biol. Chem. 251: 3749 (1976).
64. Schnarr, M., and J.C. Maurizot, Biochemistry 20:6164 (1981).
65. Mantulin, W.W., M.F. Rohde, A.M. Grotto, Jr., and H.J. Pownall, J. Biol. Chem. 255:8185 (1980).
66. Pfeil, W., Biophys. Chem. 13:181 (1981).
67. Chen, C.-H., O.H.W. Kao and D.S. Berns, Ibid. 7:81 (1977).
68. Jacobson, A.L., G. Devin and H. Braun, Biochemistry 20: 1694 (1981).
69. Contaxis, C.C., C.C. Bigelow and C.G. Zarkadas, Can. J. Biochem. 55:325 (1977).
70. Pfeil W., and P. Bendzko, Biochim. Biophys. Acta 626:73 (1980).
71. Nojima, H., A. Ikai, T. Oshima and H. Noda, J. Mol. Biol. 116:429 (1977).
72. Ahmad, F., and A. Salahuddin, Biochemistry 15:5168 (1976).
73. Holladay, L.A., R.G. Hammonds, Jr., and D. Puett, Ibid. 13: 1653 (1974).

[Received October 19, 1982]

Acetonitrile as Eluent in Silver Resin Column Chromatography

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ABSTRACT

Acetonitrile (ACN) is shown to be an effective eluent in combination with methanol (MeOH) or acetone for the rapid separation of polyunsaturated fatty acid methyl esters on a fully silver-loaded ion-exchange (XN1010) column. ACN-containing eluents thus provide on one fully siliver-loaded resin column an attractive alternative separation system to a series of partially silvered resin columns ("PARC" columns), which previously have been used for such separations. Solvent programming from 0 to 15% ACN in MeOH allows the separation of methyl oleate, linoleate, linolenate and arachidonate within 3 hr. Preparative (up to 800 mg) isocratic separations of a number of polyunsaturated fatty acid esters from natural sources (methyl linoleate from safflower esters, for example) were readily achieved.

INTRODUCTION

Macroeticular, sulfonic acid, ion-exchange resins loaded with silver ion have been useful in the chromatographic separation of unsaturated compounds, particularly geometrical isomers of unsaturated esters. The separation of geometrical isomers of methyl monoenoates was readily achieved with resins of this type using MeOH as eluent (1,2), but polyenoates were eluted only with very large volumes of MeOH or not at all. Improved resins gave practical separation of (E,E)-, (Z,E)- and (Z,Z)-nonconjugated dienoates (3). Separation of conjugated dienoates and trienoates was also possible, owing to their lower elution volumes as compared to their nonconjugated isomers (4).

The problem of elution of polyunsaturated esters with three or more nonconjugated double bonds still remained. The use of 10% 1-hexene in MeOH (5) as a step eluent was effective in eluting such esters from silver resin columns. Another approach to the problem was the development of the technique of partial silver-loading of the resin, called partial argentation resin chromatograph, or PARC (6-8). In this technique, the retention of the column for nonconjugated polyunsaturated esters was reduced by only partial loading with silver ion. The disadvantage of partial silver loading is that the retention of monoenoates and even dienoates may be so low that their resolution is poor. Thus, more than one column is required to separate ester mixtures that contain a wide range of unsaturation.

We have now found that by solvent programming with mixtures of MeOH/acetonitrile (ACN) it is possible to separate mixtures of mono- and nonconjugated di-, tri- and tetraenoates on a fully silver-ion loaded exchange resin. By selection of the proper mixture of ACN in MeOH as eluent, large-scale isocratic separations of a particular polyunsaturated ester from the esters of naturally occurring mixtures of acids may be readily achieved.

EXPERIMENTAL

Preparation of Columns

XN1010 resin (Rohm and Haas) in the sodium form (XN1010 Na) was ground, size-fractionated to yield a wet fraction (200-270 mesh) and silver-exchanged as previously described (6).

For the large-scale separations, a heavy-walled glass column (2.54 x 60 cm) was made by cutting a chromatography column (Pharmacia) and sealing a Teflon plug valve with Luer tip to the cut end. The standard expanding piston assembly furnished with the column was used to close the other end. This column (A) was slurry-packed with ca. 300 mL of the resin, which was held above the valve by a glass wool plug. A septum-sealed injector suitable for < 50 psig was used for sample introduction. The column was pumped with a piston pump (Metering Pumps, Ltd.) and column effluent was monitored with a refractometer (Waters Associates, R403). Standard reagent grade MeOH (MCB Omnisolv and Mallinckrodt ChromAR) was used as received. The column exhibited ca. 580 theoretical plates based upon the methyl oleate peak eluted with MeOH. The column void volume was 180 mL, as indicated by the methyl palmitate-stearate peak eluted with MeOH.

A small column (B) was used for the study of solvent programming with MeOH/ACN mixtures. Silver-loaded XN1010 resin formerly in the sodium form (AgXN1010Na) and < 270 mesh was air elutriated (9) to give a fraction with 15 ± 5 μm particles (measured by electron scanning microscopy). A stainless-steel column (0.45 x 60 cm) having 2 μm frits was dry-packed with the resin by increments, followed by vigorous tapping on a hard surface. The column tested at 550 theoretical plates based upon the methyl oleate peak eluted at 0.5 mL MeOH/min and contained 7 g of resin with a void volume of 5.16 mL based upon the difference of its weight when filled with resin and its weight after filling the packed column with MeOH. The small column (B) was solvent programmed by use of two pumps (Waters Associates Model 6000A) and an associated solvent programmer (Waters Associates Model 660). Column effluent in the program mode was monitored at 215 nm (Schoeffel SF770) and in the isocratic mode at 215 nm or with a refractometer (Waters Associates R401). Samples were applied by injection with a 10 μL syringe into an injector valve (Rheodyne, Model 7120) with 10 μL loop.

Tests of the Columns with ACN/MeOH Eluents

A mixture, 1, of methyl oleate (Ol), linoleate (Lo), linolenate (Ln) and arachidonate (Ar) (31.0, 22.1, 27.7 and 19.1%, respectively) was used to measure the effect on the k′ (capacity factor) for each of these esters of loading and of the percent ACN in the MeOH.

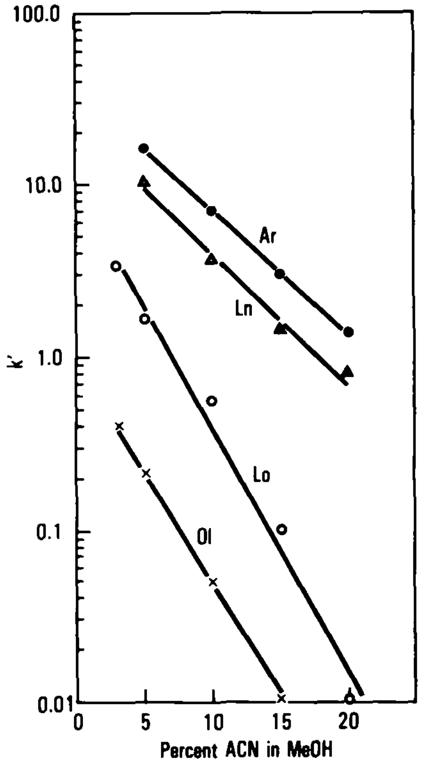

FIG. 1. Capacity factors (k′) for methyl Ol, Lo, Ln and Ar vs percent ACN in MeOH, column B, 0.5 mL/min.

The effect of the percent ACN in MeOH on the k′ for the esters of 1 was studied by injection of 5 μL of the neat mixture on column B in isocratic runs with 0, 5, 10, 15 and 20% ACN in MeOH at a flow setting of 0.5 mL/min (0.47 mL/min actual) and using one pump (Waters 6000A). Column pressure was ca. 750 lb/in.^{2}.

The effect of sample load on the k′ values of the esters of 1 was investigated by injection of decreasing volumes of a solution of 1 in 15% ACN/MeOH. A flow rate of 0.5 mL 15% ACN/MeOH per min on column B was used.

The columns were operated at room temperature (19-22 C). Tests of column A were confined to isocratic separations as illustrated in results below. Column A would flow without pump pressure and only low pressure of ca. 5 lb/in.^{2} was required for a flow rate of 7 mL/min.

RESULTS AND DISCUSSION

Figure 1 shows the effect of increasing amounts of ACN in MeOH on the k′ values (k′ being the net retention volume expressed in units of columns void volume) for methyl Ol, Lo, Ln and Ar (mixture 1). It is apparent that ACN is a powerful eluent for polyunsaturated esters from fully silver-exchanged XN1010Na. Other substances that compete for coordination with the silver ions on the resin can be expected to cause similar effects. For example, 1-hexene has already been used in step programming (5). Preliminary experiments showed water in MeOH also to be effecting in displacing unsaturated esters. However, water reduces the ability of MeOH to dissolve fatty esters, and 1-hexene is

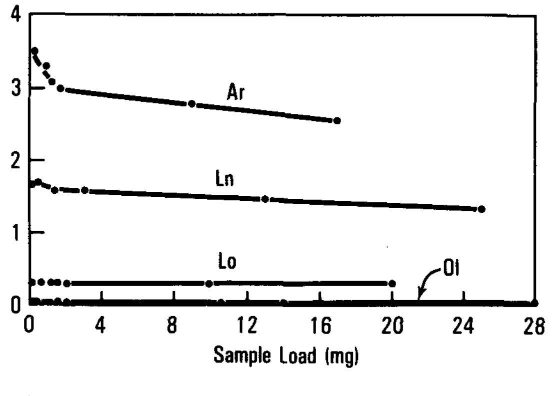

FIG. 2. k' for methyl Ol, Lo, Ln and Ar vs sample size, column B, 0.5 mL 15% ACN in MeOH/min.

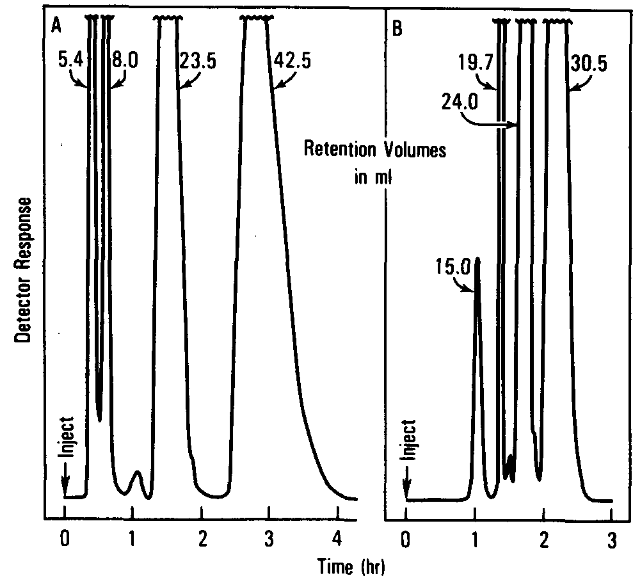

FIG. 3. Separation of methyl Ol, Lo, Ln and Ar (left to right) on column B at 0.5 mL/min; 5 μL sample of mixture 1. (A) Isocratic 10% ACN in MeOH; (B) programming 0-15% ACN in MeOH, 1/2 hr linear ramp. Retention volumes are indicated above respective peaks.

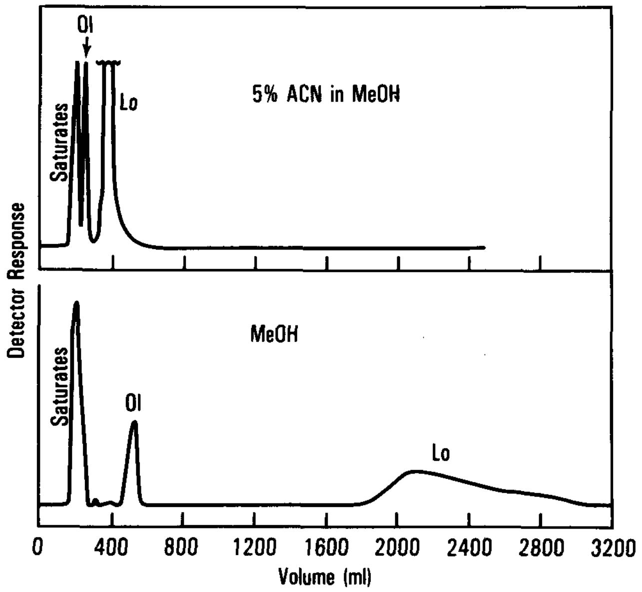

FIG. 4. Preparative separation of 800 mg methyl esters of safflower oil on column A. (A) 7.4 mL MeOH/min; (B) 6.9 mL 5% ACN in MeOH/min.

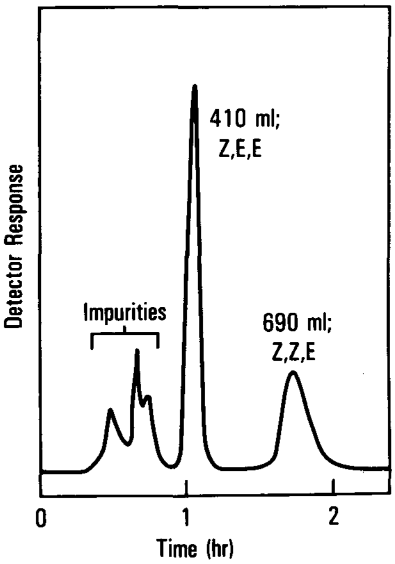

FIG. 5. Separation of synthetic mixture (100 mg) containing methyl (Z,E,E)- and (Z,Z,E)-9,12,15-octadecatrienoates on column A, 6.6 mL 5% ACN in MeOH/min.

not transparent in ultraviolet (UV) at 215 nm. Detection at 215 nm allows solvent programming because methyl esters may be detected. Thus, ACN appears particularly suitable owing to its power to elute unsaturates and its transparency at 215 nm.

The sample load on the column, as well as the percentage of ACN in MeOH, has an influence on the k'. Figure 2 shows this effect in the range of 1-20 mg for the esters of 1. The loading effect appears to be confined mainly to Ar and Ln and is most noticeable as a decrease in k' of Ar at low loading levels as the sample size is increased.

A 1/2 hr linear solvent program from 0 to 15% ACN in MeOH for the elution of 1 from column B is shown in Figure 3. Also shown is an isocratic separation of 1 on the same column and load with 10% ACN in MeOH. The four peaks were collected, and analysis by gas chromatography (GC) indicated ca. 99% purity for each.

The larger column (A) was used for isocratic separations of methyl esters of various unsaturated acid mixtures. The effect on the separation of the methyl esters of safflower oil of changing eluent from MeOH to 5% ACN in MeOH is shown in Figure 4. Figure 5 illustrates the separation of triene-geometrical isomers.

ACN is also useful in combination with solvents other than MeOH. Most of the separations utilized ACN/MeOH because of the need to use UV detection with solvent programming. Some isocratic separations were carried out using ACN/acetone and RI detection. Figure 6 shows the separation of methyl Ol, Lo, Ln and Ar with 20% ACN in acetone. ACN with other solvents may also be effective.

Column B should be reequilibrated to lower levels of ACN by passage of 5 or 6 column voids of solvent. As this amounts to ca. 30 mL and one hr of pumping, it does not represent a severe difficulty. However, with column A ca.

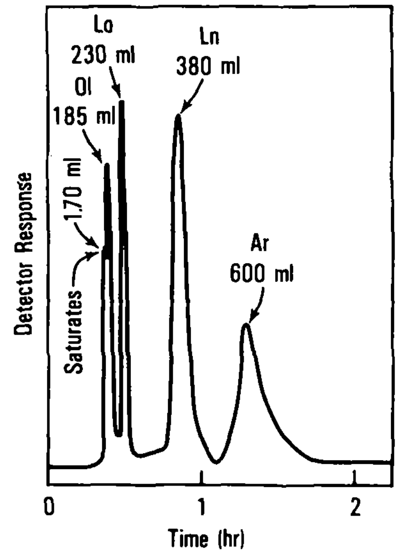

FIG. 6. Separation of: saturates, Ol, Lo, Ln and Ar on column A, 7.2 mL 20% ACN in acetone/min. Sample size 130 mg.

1.5 L of solvent would be needed for reequilibration or ca. 3.5 hr at 7 mL/min. Therefore, it is not convenient to solvent program large columns. No change in the resolution of column B was noted in several months of use. Columns should not be allowed to drain. We found it necessary to repack the large column (A) if air entered the top of the column. The resin was washed out and slurry-packed as described to restore the column.

ACN is a powerful eluent for polyunsaturated esters from fully silver-loaded ion exchange resins in column chromatography. Its use in combination with MeOH or acetone allows separation of methylene-interrupted polyunsaturated esters that are difficult to elute from silver-resin columns. By increasing the proportion of ACN in the solvent according to the degree of unsaturation of the desired component, preparative separations may be effected on one column, which previously would have required the use of a number of partial argentation resin columns (PARC columns [6-8]).

REFERENCES

1. Emken, E.A., C.R. Scholfield and H.J. Dutton, JAOCS 41:388 (1964).
2. Scholfield, C.R., and E.A. Emken, Lipids 1:235 (1966).
3. Scholfield, C.R., and T.L. Mounts, JAOCS 54:319 (1977).
4. Emken, E.A., C.R. Scholfield, V.L. Davison and E.N. Frankel, JAOCS 44:373 (1967).
5. Emken, E.A., J.C. Hartman and C.R. Turner, JAOCS 55:561 (1978).
6. Adlof, R.O., H. Rakoff and E.A. Emken, JAOCS 57:273 (1980).
7. Adlof, R.O., and E.A. Emken, JAOCS 57:276 (1980).
8. Adlof, R.O., and E.A. Emken, JAOCS 58:99 (1981).
9. Ekman, R., B.R. Johansson and U. Ravnskov, Anal. Biochem. 70:628 (1976).

[Received August 26, 1982]

Identification of Adduct Radiolysis Products from Ethyl Palmitate and Ethyl Oleate

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ABSTRACT

Radiolysis induced adduct products have been separated and identified from irradiated ethyl palmitate, ethyl α-d_2-palmitate and ethyl oleate. In the saturated compounds, adduct formation was observed mainly at the position α to the carbonyl group. The three major adduct products identified in irradiated ethyl palmitate were ethyl α-tetradecylpalmitate, ethyl α-pentadecylpalmitate and the α,α′-dimer of ethyl palmitate. Corresponding compounds were identified from the irradiated ethyl α-deuteropalmitate. Adduct radiolysis products formed in ethyl oleate were identified as the monoene and diene dimers.

INTRODUCTION

Prior studies (1-3, 5) have provided evidence for the formation of radiolysis products of higher molecular weight than that of the starting material. Recently, the adduct compounds formed from tributyrin and tripalmitin have been reported (5). This study is concerned with the identification of the adduct compounds produced by the irradiation of fatty acid esters in order to elucidate the mechanism for their formation. The compounds chosen were ethyl palmitate, ethyl α-deuteropalmitate and ethyl oleate. The selection of these compounds was based on the expectation that the separation and identification of the adduct radiolysis products could be easily performed by gas chromatography-mass spectrometry (GC-MS).

EXPERIMENTAL

The materials used in this study were purchased from Fisher Scientific Co. (Medford, MA); Applied Science Lab., Inc. (State College, PA); ICN Pharmaceuticals, Inc. (Plainview, NY); and Sigma Chemical Co. (St. Louis, MO). The α-deuterated ester was synthesized (6) from the α-deuterated palmitic acid. Purity of each compound examined by GC-