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Note

Silicic acid column chromatography of phosphonolipids

III. Separation of 1-O-alkyl-2-acetyl-*sn*-glyceryl-3-phosphonocholine from *trans*-D-*erythro*-ceramide-N,N,N-trimethylaminoethyl phosphonate

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In a previous paper was reported the separation of 1-O-alkyl-2-acetyl-*sn*-glyceryl-3-phosphonocholine (derived from chimyl alcohol) (phosphono-AGEPC) from phosphoryl-AGEPC, other related phospholipids and partially from *trans*-D-*erythro*-ceramide-N,N,N-trimethylaminoethyl phosphonate (phosphono-sphingomyelin)[1].

In this paper the above partial separation has been studied further and use has been made of silicic acid column chromatography for their complete and efficient separation. The collected fractions were analysed by thin-layer chromatography (TLC) and infrared (IR) spectroscopy for confirmation of identification. By this means it is possible to separate completely and effectively two completely different phosphonolipids possessing similar chromatographic properties.

EXPERIMENTAL

Instrumentation

IR spectra were recorded on a Perkin-Elmer double-beam spectrophotometer. A glass column (35 × 1.6 cm I.D.) was employed for the separation.

Reagents

Solvents for column chromatography and TLC were of analytical-reagent grade (Merck, Darmstadt, G.F.R.) and were distilled before use. TLC was conducted on 20 × 20 cm plates with a 0.25 mm thick layer of silica gel G for 60 F_{254} (Merck) and detection was effected with molybdenum blue, iodine vapour, UV irradiation and ninhydrin spray reagent.

Standards

Both phosphono-AGEPC[2] and phosphono-sphingomyelin[3,4] were synthetic products.

Procedure

The chromatographic column, which was fitted at the bottom with a glass-

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wool plug, was loaded with a slurry of 8.0 g of regenerated silicic acid (Sigma, St. Louis, MO, U.S.A.) in 50 ml of chloroform to a column height of 8.5 cm and a total column volume of 20.0 ml. The column was washed with two column volumes of chloroform and the flow-rate maintained in the elution was 1.4–1.8 ml/min. The volume of the eluate collected by the fraction collector was about 5.0 ml. When the total weight was desired, a total of 15–25 ml of the eluate was obtained by pooling of fractions. Evaporation of the solvents was accomplished under vacuum and at a bath temperature of 35°C or under nitrogen. Column elution was effected with mixtures of methanol in chloroform as indicated in Table I.

TABLE I

ELUTION OF THE CHROMATOGRAPHIC COLUMN (35 × 1.6 cm I.D.) PACKED WITH REGENERATED SILICIC ACID

Flow-rate, 1.4–1.8 ml/min. Fractions of approximately 3.0 ml were collected.

Methanol in chloroform (%)	*Column volumes*	*Total volume of solvent (ml)*	*Fractions collected*
0	1.5	30	1–7
5	2.8	55	8–25

IR spectra of the various pilot fractions were run as chloroform solutions or potassium bromide discs.

Thin-layer chromatograms were run on silica gel G F_{254} plates and also on plates coated in this laboratory to a thickness of 0.30 mm. Development of the chromatograms was carried out in two chambers of dimensions 8 × 20.5 cm (Desaga, Heidelberg, G.F.R.) and the run normally took about 45 min. The plates were developed in chloroform–methanol–water (65:25:4) (system A) and methanol–water (2:1) (system B). Standards were also spotted on the plates to assist in the detection of developed spots. Detection was effected with molybdenum blue, iodine vapour, ninhydrin spray and under UV irradiation.

RESULTS

Column elution was effected with methanol–chloroform and chloroform (as indicated in Fig. 1). Fractions were identified by TLC and IR spectroscopy (Table II). The nature of the fractionation pattern of the phosphonolipids under examination is shown in Fig. 1. With the solvents used, 99.95% of the lipids applied could be recovered.

DISCUSSION

The unusual chromatographic behaviour of both phosphono-AGEPC and phosphono-sphingomyelin, in that both are eluted together from a silicic acid column in an early fraction (cardiolipin fraction) with 5% methanol in chloroform, has already been noted[1]. Because of the importance of phosphono-AGEPC as a biologically active agent[1], the need arose to attempt to separate phosphono-AGEPC from phosphono-sphingomyelin. The former compound has R_F values of 0.78 and 0.64 in

TABLE II

COMPOSITION OF FRACTIONS OBTAINED FROM CHROMATOGRAPHY OF PHOSPHONOLIPIDS ON THE SILICIC ACID COLUMN

9.0 mg of phosphonolipids were applied to the column. Total recovery was 8.996 mg (99.95%).

Solvent	*Fractions collected*	*TLC R_F values*		*Component identified from IR spectral data*
		System A	*System B*	
Chloroform	3–5	0.78	0.64	Phospho-AGEPC
5% methanol in chloform	9–13	0.96	0.91	Phosphono-sphingomyelin

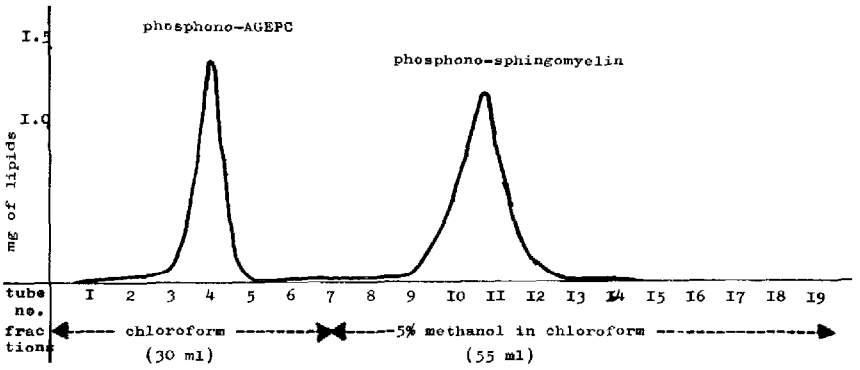

Fig. 1. Chromatography of phosphonolipids on a silicic acid column using chloroform and 5% methanol in chloroform. The composition of the phosphonolipids was phosphono-AGEPC 5.0 mg and phosphono-sphingomyelin 4.0 mg. The lipids were applied to the column in 3.0 ml of chloroform as solvent.

TLC systems A and B, respectively, whereas phosphonosphingomyelin has R_F values of 0.96 and 0.91, respectively. The compounds are also separated completely and effectively on a silicic acid column, being eluted early in the process with chloroform and 5% methanol in chloroform.

Hence phosphono-AGEPC and phosphono-sphingomyelin can be effectively separated by TLC and/or silicic acid column chromatography. From the results, it is also evident that phosphono-AGEPC is less polar than phosphono-sphingomyelin.

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REFERENCES

1 M. C. Moschidis, *J. Chromatogr.*, 259 (1983) 356.
2 M. C. Moschidis, C. A. Demopoulos and L. G. Kritikou, *Chem. Phys. Lipids*, (1982) in press.
3 M. C. Moschidis, *Ph.D. Thesis*, University of Athens, Athens, 1977.
4 V. M. Kapoulas and M. C. Moschidis, *Chem. Phys. Lipids*, 28 (1981) 357.