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Note

Silicic acid column chromatography of phosphonolipids

IV. Separation of phosphonolecithin and phosphonocephalin from their phosphoryl analogues and other related phospholipids

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During the course of a more general project concerned with the synthesis of various phosphonolipids and phospholipids and the determination of their biological activity, a number of phospholipids and their phosphonolipid analogues have been, and are being, synthesized and tested[1–4].

In the present study the column chromatographic properties of phosphonolecithin and phosphonocephalin were investigated with the aim of the separation of these phosphonolipids from their phospholipid analogues. For comparison purposes, cardiolipin and lysolecithin were included. Collected fractions were analysed by thin-layer chromatography (TLC) and IR spectroscopy to confirm species identification.

EXPERIMENTAL

Instrumentation

IR spectra were recorded on a double-beam Perkin-Elmer 197 grating IR spectrophotometer. A glass column (35 × 1.6 cm I.D.) was employed for the separations.

Reagents

Solvents for column chromatography and TLC were analytical reagent grade (Merck) and were distilled before use. TLC was conducted on 20 × 20 cm chromato-plates of 0.25 mm thick silica gel G or 60 F_{254} (Merck).

Standards

Both natural and synthetic lecithins were employed and were either isolated and/or synthesized in this laboratory. Phosphonolecithin and phosphonocephalin were synthetic compounds. Cardiolipin and phosphatidylethanolamine were purchased from Koch-Light (Colnbrook, U.K.) and lysolecithin from E. Merck (Darmstadt, F.R.G.).

Silicic acid for column chromatography was purchased from Sigma (St. Louis, MO, U.S.A.).

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Procedure

The chromatographic column, which was fitted at the bottom with a glass-wool plug, was loaded with a slurry of 11 g of silicic acid in 50 ml of chloroform, to height of 10 cm and a total column volume of 26 ml. The column was washed with two column volumes of chloroform and the flow-rate maintained in the elution was 1.0–1.7 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 20 or 30 ml of the eluates was obtained by pooling of fractions (Table I). Evaporation of the solvents was accomplished under vacuum at a bath temperature of 35°C or under nitrogen. IR spectra of the various pilot fractions were run as chloroform solutions or KBr discs.

TABLE I

ELUTION OF THE CHROMATOGRAPHIC COLUMN

Dimensions 35 × 1.6 cm I.D., loaded with 11.0 g of silicic acid to a height of 10.0 cm and a total column volume of 26 ml. Flow-rate: 1.0–1.7 ml/min. Fractions of *ca.* 5.0 ml were collected.

% methanol in chloroform	*No. of column volume*	*Total volume of solvent (ml)*	*Fractions collected*
5	3	75	1– 19
20	5	130	20– 44
40	7	180	45– 75
80	5	140	76–104

Thin-layer chromatograms were run on silica gel G F_{254}, Merck plates and also on glass plates coated in this laboratory to a thickness of 0.30 mm. Development of the chromatograms was effected in two chambers of dimensions 8 × 20.5 cm (Desaga) and each run normally took about 45 min. The plates were developed in chloroform–methanol–water (65:25:4, v/v/v) (system A) or chloroform–methanol–acetic acid–water (25:15:4:2, v/v/v/v) (system B). Visualization was effected with molybdenum blue, iodine vapour or UV irradiation. Standards were also spotted on the plates to aid in the detection of the developed spots.

RESULTS

The chromatographic column was loaded with phospholipids and eluted with combinations of methanol in chloroform as indicated on Fig. 1. Fractions were identified by TLC and IR spectroscopy (Table II). With the solvents used, 100.0% of the lipids applied to the column could be recovered.

DISCUSSION

Phosphonocephalin is eluted with its phosphoryl analogue and prior to the appearance of the latter. The same order is obtained in the case of phosphonolecithin, and thus the normal pattern is followed in both cases.

Phosphonocephalin and phosphonolecithin exhibit R_F values which differ from

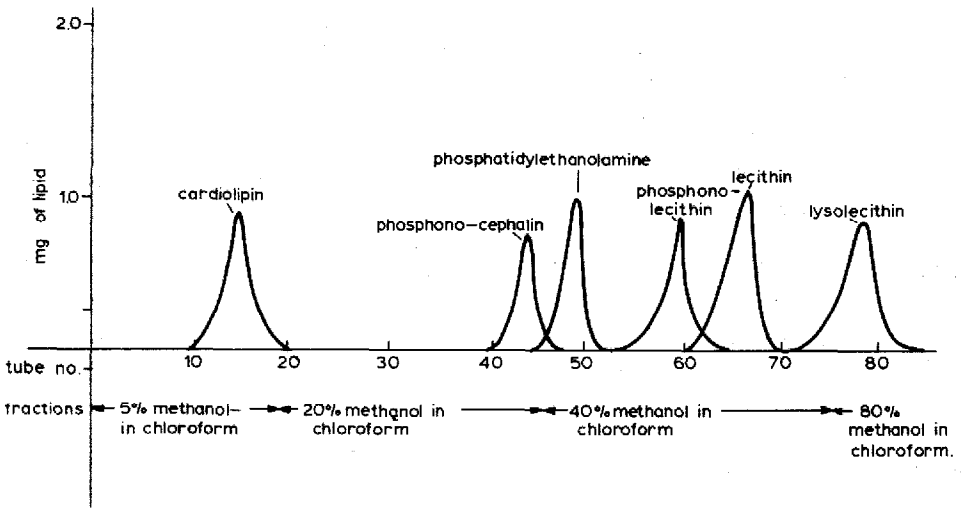

Fig. 1. Chromatography of various indicated phosphono- and phospholipids on a column of silicic acid. Solvents used were various percentages of methanol in chloroform. The composition of the phosphono- and phospholipids was: 3.0 mg cardiolipin; 4.0 mg phosphatidylethanolamine; 4.5 mg phosphatidylcholine; 4.0 mg lysolecithin; 2.5 mg phosphonophosphatidylethanolamine; 4.0 mg phosphonolecithin. The lipids were applied to the column in 5.0 ml of chloroform.

TABLE II

COMPOSITION OF FRACTIONS OBTAINED FROM CHROMATOGRAPHY OF PHOSPHONO- AND PHOSPHOLIPIDS ON SILICIC ACID

22.0 mg of lipids were applied to the column. Total recovery was 100.0%.

Solvent	*Fractions collected*	*TLC R_F value*			*Compound identified by IR spectra*
		System A	*System B*	*System C*	
5% Methanol in chloroform					
20% Methanol in chloroform	20–27	0.68	0.00		Cardiolipin
	42–46		0.86		Phosphono-phosphatidyl-ethanolamine
40% Methanol in chloroform	44–51	0.74	0.00		Phosphatidyl-ethanolamine
	52–64		0.80		Phosphonolecithin
	61–70	0.31	0.00		Phosphatidylcholine
80% Methanol in chloroform	72–83			0.10	Lysolecithin

those of the phosphoryl analogues [for system A: R_F for phosphatidylcholine, 0.31; for phosphatidylethanolamine, 0.74; for methanol–water (2:1, v/v) (system C): R_F for phosphonolecithin, 0.80; for phosphonocephalin, 0.86] and thus complete TLC separation can also be achieved[5].

It can therefore be concluded that phosphonocephalin and phosphonolecithin can be separated chromatographically from their phosphoryl analogues and other related phospholipids.

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