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Note

Silicic acid column chromatography of phosphinolipids

I. Phosphinate analogues of lecithin and cephalin, phosphonic acid and the phosphonate analogue of bis(diacylglycero)phosphate: their separation from other phospholipids and related phosphonolipids

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The column chromatographic behaviour and other properties of various phosphonolipids have been reported in a series of notes[1–7] and their separation from other related phospholipids has been accomplished. The column chromatographic behaviour and other properties of phosphinolipids are largely unknown and their chromatographic separation from structurally related phospholipids and phosphonolipids has never been reported.

In this note the column chromatographic behaviours of 1,2-dipalmitoyloxypropyl-3-(2-trimethylammoniumethyl)phosphinate, of 1,2-dipalmitoyloxypropyl-3-(2-ammoniumethyl)phosphinate, of phosphonic acid and of 1,2-diacyloxypropyl-3-(1′,2′-diacyl-*sn*-glycero)phosphonate in the presence of cardiolipin, phosphatidylethanolamine, phosphatidylcholine and sphingomyelin are reported. In a separate chromatographic experiment, satisfactory separation was achieved of the above phosphinolipids and phosphonolipids from the phosphono analogues of phosphatidylethanolamine, phosphatidylcholine and sphingomyelin. Collected fractions were analyzed 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 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. Silicic acid for column chromatography was purchased from Sigma (St. Louis, MO, U.S.A.).

Standards

The phosphino analogues of cephalin, of lecithin[8], the phosphono analogue of bis(diacylglycero)phosphate[9] and phosphonic acid were synthetic products. Cardiolipin, cephalin and lecithin were purchased from Koch-Light (Colnbrook, U.K.).

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Procedure

The procedure was similar to that described earlier[1-7]. Column elution was effected with methanol–chloroform mixtures as indicated in Tables I and III.

IR spectra of the various pilot fractions were recorded for chloroform solutions or KBr discs. Thin-layer chromatograms were run on 20 × 20 cm silica gel G or F_{254} plates (0.25 mm layer) (Merck), 20 × 5 cm silica gel H plates (0.30-mm layer) (Merck) and also on plates of both types coated in this laboratory to a thickness of 0.30 mm. Development of the chromatograms was carried out in two chambers of dimensions 20 × 8 cm; the procedure normally took about 50 min. The solvents were chloroform–methanol–water (65:25:4) (system A) and methanol–water (2:1) (system B)[10]. The spots were rendered visible with molybdenum blue, iodine vapour, UV irradiation or the Stillway–Harmon[11] procedure. Standards were also spotted to aid in the detection of the developed spots.

TABLE I

CHROMATOGRAPHIC CONDITIONS

The column (35 × 1.6 cm I.D.) was packed with 10.0 g of silicic acid to a height of 9.5 cm and a total column volume of 25 ml. Flow-rate: 1.0–1.7 ml/min. Fractions of *ca.* 5.0 ml were collected.

% Methanol in chloroform	*No. of column volumes*	*Total volume of solvent (ml)*	*Fractions collected*
5	3	75	1–20
20	5	135	21–45
40	7	180	46–76
80	5	140	77–102

TABLE II

COMPOSITIONS OF FRACTIONS OBTAINED BY CHROMATOGRAPHY OF LIPIDS ON SILICIC ACID

52.0 mg of phosphino, phosphono and phospholipids were applied to the column. Total recovery was 52.0 mg (100%).

Solvent	*Fractions collected*	*TLC R_F values*		*Component identified by IR spectroscopy*
		System A	*System B*	
5% Methanol in chloroform	1–4	0.72*	0.90	1,2-Diacyloxypropyl-3-(1′,2′-diacyl-*sn*-glycero)phosphonate
	3–6	0.75	0.87	Diacylglycerophosphonic acid
	11–18	0.67	0.00	Cardiolipin
20% Methanol in chloroform	22–27	0.63 (0.55)*	0.83	Phosphinate analogue of cephalin
	29–37	0.48 (0.35)*	0.88	Phosphinate analogue of lecithin
	42–53	0.69	0.00	Phosphatidylethanolamine
40% Methanol in chloroform	58–69	0.38	0.00	Phosphatidylcholine
80% Methanol in chloroform	82–98	0.17	0.00	Sphingomyelin

* On silica gel H plates coated to a thickness of 0.30 mm.

RESULTS

Column elution was effected with various methanol–chloroform mixtures. The fractionation pattern of the phosphinolipids is depicted in Fig. 1; that for the separation of the phosphinolipids from their phosphonolipid analogue is depicted in Fig. 2. Fractions were identified by TLC and IR spectroscopy (Tables II and IV).

Under the above experimental conditions and with the solvents used, *ca.* 100% of the lipids applied to the two separate columns could be recovered.

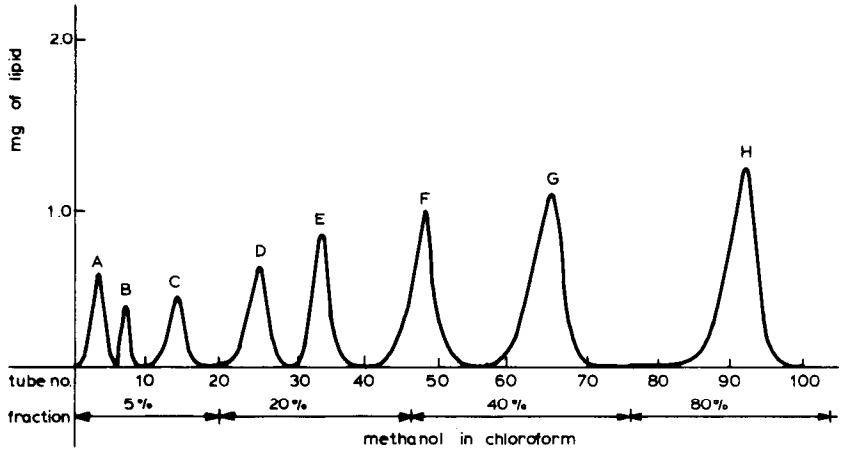

Fig. 1. Chromatography of various phosphino, phosphono and phospholipids on a column of silicic acid. The solvents used were various percentages of methanol in chloroform. Lipids: A, phosphono analogue of bis(diacylglycero)phosphate, 2.7 mg; B, diacylglycerophosphonic acid, 1.8 mg; C, cardiolipin, 3.6 mg; D, phosphinate analogue of cephalin, 4.8 mg; E, phosphinate analogue of lecithin, 6.7 mg; F, phosphatidylethanolamine, 7.9 mg; G, phosphatidylcholine, 10.6 mg; H, sphingomyelin, 14.9 mg. The lipids were applied in 5.0 ml of chloroform.

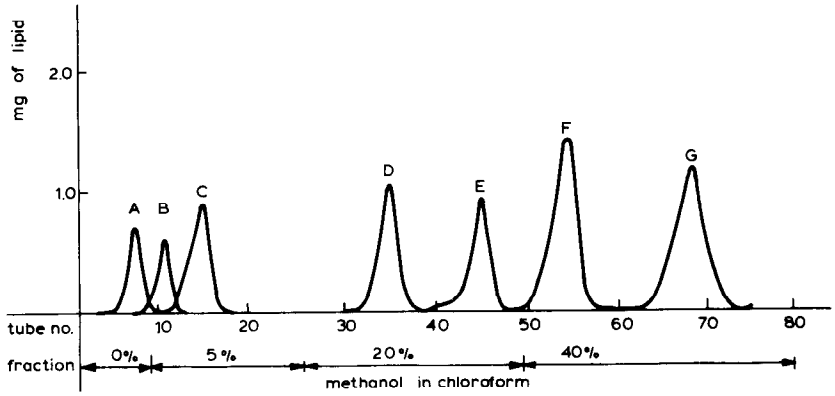

Fig. 2. Chromatography of various phosphinolipids and phosphonolipids on silicic acid. Solvents as indicated. Lipids: A, phosphono analogue of bis(diacylglycero)phosphate, 2.0 mg; B, diacylglycerophosphonic acid, 1.5 mg; C, phosphono-sphingomyelin, 3.0 mg; D, phosphinate analogue of cephalin, 4.0 mg; E, phosphinate analogue of lecithin, 3.5 mg; F, phosphono cephalin, 6.0 mg; G, phosphono lecithin, 4.0 mg. The lipids were applied in 3.5 ml of chloroform.

TABLE III

ELUTION OF THE CHROMATOGRAPHIC COLUMN

The column (35 × 1.6 cm I.D.) was packed with 11.0 g of silicic acid to a height of 10.0 cm and a total column volume of 26 ml. Flow-rate: 1.6–1.9 ml/min. Fractions of *ca.* 5.0 ml were collected.

% Methanol in chloroform	*No. of column volumes*	*Total volume of solvent (ml)*	*Fractions collected*
0	1.5	30	1–7
5	2.8	55	8–25
20	5	130	26–50
40	7	180	51–79
80	5	140	80–104

DISCUSSION

In the first column chromatographic experiment, the two phosphono analogues of bis(diacylglycero)phosphate and diacylglycerophosphoric acid appeared in the initial fractions eluted with 5% methanol in chloroform. The two phosphinate analogues of cephalin and lecithin are eluted successively with 20% methanol in chloroform. There followed phosphatidylethanolamine and phosphatidylcholine and lastly sphingomyelin.

The elution of the two phosphinate analogues of cephalin and lecithin with 20% methanol in chloroform proved to be an interesting observation. In the second chromatographic experiment, these two phosphinate analogues appeared in the initial fractions upon elution with 20% methanol in chloroform and prior to the appearance of the two structurally related phosphono analogues of cephalin and lecithin, which

TABLE IV

COMPOSITION OF FRACTIONS OBTAINED BY CHROMATOGRAPHY OF PHOSPHINOLIPIDS AND PHOSPHONOLIPIDS ON SILICIC ACID

21.0 mg of lipids were applied to the column. Total recovery was 100%.

Solvent	*Fractions collected*	*TLC R_F values*		*Component identified by IR spectroscopy*
		System A	*System B*	
0% Methanol in chloroform	5–9	0.71*	0.94	1,2-Diacyloxypropyl-3-(1′,2′-diacyl-*sn*-glycero)phosphonate
	7–13	0.75	0.88	Diacylglycerophosphonic acid
5% Methanol in chloroform	11–16	0.94	0.90	Phosphono-sphingomyelin
20% Methanol in chloroform	32–37	0.61 (0.58*)	0.85	Phosphinate analogue of cephalin
	38–48	0.47 (0.33*)	0.90	Phosphinate analogue of lecithin
40% Methanol in chloroform	50–57		0.86	Phosphono cephalin
	64–73		0.81	Phosphono lecithin

* On silica gel H plates of 0.30 mm thickness.

were eluted with 40% methanol in chloroform. The TLC properties (R_F values) of the phosphinate analogues closely resemble those of their phosphoryl analogues; it appears then that the absence of an additional oxygen atom from the phosphinolipids under examination has a marked effect on their column chromatographic properties.

Another interesting observation is that the phosphono analogues of bis(diacylglycero)phosphate and of diacylglycerophosphoric acid were eluted with 5% methanol in chloroform and prior to phospohono-sphingomylelin, with slight overlapping.

The silicic acid column chromatographic pattern of the above phosphinolipids and phosphonolipids has been established, and although phosphinolipids have not as yet been found in nature, the TLC and silicic acid column chromatographic properties of the phosphinate analogues of cephalin and lecithin are known and this could aid significantly in attempts at their isolation and identification.

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