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Note

Silicic acid column chromatography of phosphinolipids

II*. Study of the column chromatographic properties of bis(diacyloxypropyl) phosphinate

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The column chromatographic properties of the direct phosphinate analogues of lecithin and cephalin have been reported[1], where it was noted that the two analogues were eluted rapidly with 20% methanol in chloroform. In this paper is reported the column chromatographic behaviour of bis(diacyloxypropyl) phosphinate in the presence of the phosphonate analogue of bis(diacylglycero) phosphate, of the direct phosphinate analogues of lecithin and cephalin, of phosphatidylcholine and of the phosphono analogue of cephalin. The collected fractions were analysed by thin-layer chromatography (TLC) and IR spectroscopy to confirm identification of the components.

EXPERIMENTAL

Instrumentation

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

Reagents

Solvents for column chromatography and TLC were of analytical-reagent grade and were purchased from Merck (Darmstadt, F.R.G.) and Vioryl (Kifissia, Athens, Greece). All solvents were distilled before use. Silicic acid for column chromatography was purchased from Sigma (St. Louis, MO, U.S.A.).

Standards

The phosphino analogues of lecithin, cephalin and bis(diacylglycero) phosphate and the phosphonate analogues of cephalin and bis(diacylglycero) phosphate were synthetic products. Phosphatidylcholine was purchased from Koch-Light (Colnbrook, U.K.).

* For Part I, see ref. 1.

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TABLE I

CHROMATOGRAPHIC CONDITIONS

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 volume of 26 ml. Flow-rate, 1.4–1.8 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	1–18
20	5	130	19–48
40	7	180	49–78

Procedure

The procedure used was similar to that reported earlier[1,2]. Column elution was effected with methanol–chloroform mixtures as indicated in Table I.

IR spectra of the various pilot fractions were recorded as thin films from dry chloroform or KBr discs. Thin-layer chromatograms were obtained on 20 × 20 cm silica gel G plates coated in this laboratory to a thickness of 0.30 mm. Development of the chromatograms was carried out in a chamber of dimensions 20 × 8 cm and the run normally took 55 min. The solvents used were chloroform–methanol–water (65:25:4) (system A) and methanol–water (2:1) (system B). The spots were rendered visible with molybdenum blue and iodine sprays and the Stillway–Harmon procedure[3]. Standards were also spotted to aid in the detection of the developed spots.

RESULTS AND DISCUSSION

Column elution was effected with various methanol–chloroform mixtures. The

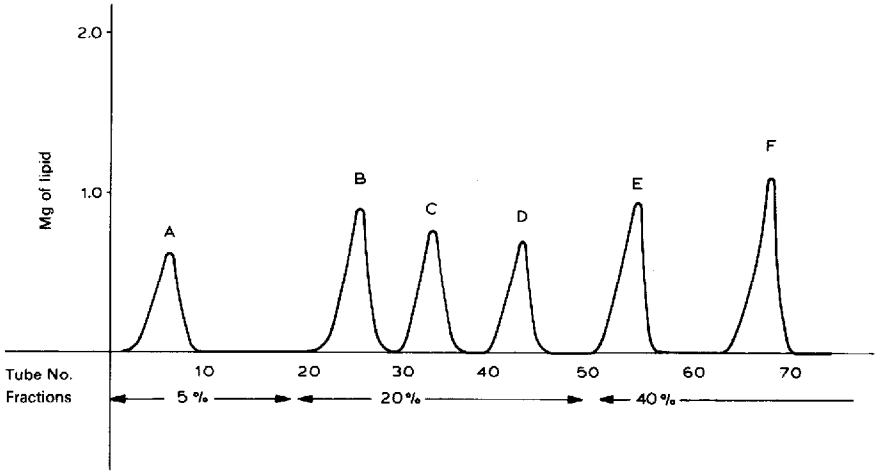

Fig. 1. Chromatography of phosphinolipids and phosphonolipids on silicic acid. Solvents as indicated. (A) Phosphono analogue of bis(diacylglycero) phosphate, 2.1 mg; (B) bis(diacyloxypropyl) phosphinate, 2.4 mg; (C) phosphinate analogue of cephalin, 2.4 mg; (D) phosphinate analogue of lecithin, 2.0 mg; (E) phosphonocephalin, 3.2 mg; (F) phosphatidylcholine, 4.5 mg. The lipids were applied in 3.5 ml of chloroform.

TABLE II

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

16.6 mg of lipids were applied to the column. The total recovery was 100%.

Solvent	*Fractions collected*	*TLC R_F values*		*Components identified by IR spectroscopy*
		System A	*System B*	
5% methanol in chloroform	3–8	0.71*	0.93	1,2-Diacyloxypropyl-3-(1',2'-diacyl-*sn*-glycero) phosphonate
20% methanol in chloroform	22–27	0.73*	0.95	Bis(diacyloxypropyl) phosphinate
	32–36	0.60	0.85	Phosphinate analogue of cephalin
	39–45	0.46	0.90	Phosphinate analogue of lecithin
40% methanol in chloroform	52–57	0.65	0.86	Phosphonocephalin
	64–72	0.42	0.00	Phosphatidylcholine

* Silica gel H.

fractionation pattern of the phosphino- and phosphonolipids is depicted in Fig. 1. Fractions were identified by TLC and IR spectroscopy (Table II).

Under the above experimental conditions and with the solvents used, 100% of the lipids applied to the column could be recovered.

Bis(diacyloxypropyl) phosphinate was eluted with 20% methanol in chloroform and very early in the process. This feature distinguishes this compound from its structurally related phosphono analogue of bis(diacylglycero) phosphate, as both compounds exhibit similar thin-layer chromatographic properties[4,5].

This project, concerned with the silicic acid column chromatography of phosphinolipids, is continuing.

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