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Note

Silicic acid column chromatography of phosphonolipids

VIII. Separation of 1-O-acylethylene glycol-2-(2-trimethylammoniumethyl) and -(2-aminoethyl) phosphonates from their phosphoryl analogues and other phosphonolipids

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In this note the column chromatographic behaviours of 1-O-palmitoylethylene glycol-2-(2-trimethylammoniumethyl) and -(2-aminoethyl) phosphonates have been examined and their separation from their direct phosphoryl analogues has been achieved. The phosphono analogues of lecithin and cephalin were also included in the same elution experiment. The 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 spectrophotometer.

A glass column (35 × 1.6 cm I.D.) was employed for the separation.

Reagents

Solvents for column chromatography and TLC were analytical grade (Merck) and were distilled before use. Silicic acid for column chromatography was purchased from Sigma (St. Louis, MO, U.S.A.).

1-O-Acylethylene glycol-2-(2-trimethylammoniumethyl) and -(2-aminoethyl) phosphonates, their phosphoryl analogues and the phosphono analogues of lecithin and cephalin were synthetic products.

Procedure

The procedure adopted was similar to that reported earlier[1–7]. 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 chloroform or as KBr discs. Thin-layer chromatograms were run on 20 × 20 cm silica gel G or F_{254} plates (thickness 0.25 mm) (Merck), and on plates coated in this laboratory to a thickness of 0.35 mm. Development was carried out in chambers of dimensions 20 × 8 cm and each experiment normally took about 50 min. The solvents used were chloroform–

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TABLE I

ELUTION OF THE CHROMATOGRAPHIC COLUMN

The column (35 × 1.6 cm I.D.) was packed with 10.0 g of silicic acid to a height of 9.6 cm and a total column volume of 25 cm^3. Flow-rate: 1.2–1.7 ml/min. Fractions of *ca.* 4.5 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	21–48
40	7	175	49–76

methanol–water (65:25:4) (system A) and methanol–water (2:1) (system B). The spots were rendered visible with molybdenum blue, iodine vapour, UV irradiation and the Stillway–Harmon procedure[8]. Standards were also spotted to aid in the detection of the developed spots.

RESULTS

Column elution was effected with various methanol–chloroform mixtures (Table I). The fractionation pattern of the 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.

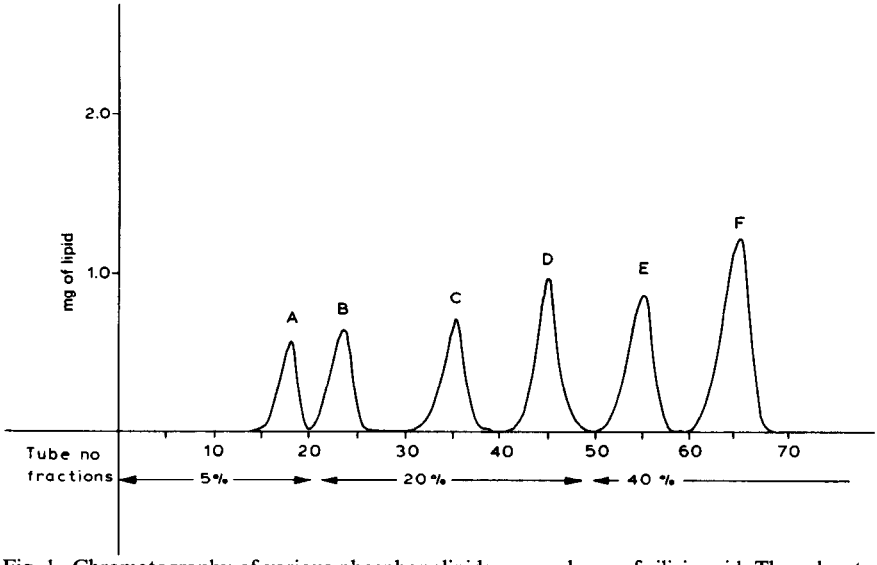

Fig. 1. Chromatography of various phosphonolipids on a column of silicic acid. The solvents used were various percentages of methanol in chloroform. Lipids: A, 1-O-acylethylene glycol-2-(2-aminoethyl) phosphonate (2.1 mg); B, 1-O-acylethylene glycol-2-(2-trimethylammoniumethyl) phosphonate (2.3 mg); C, 1-O-acylethylene glycol-2-phosphorylethanolamine (2.6 mg); D, 1-O-acylethylene glycol-2-phosphorylcholine (3.2 mg); E, phosphonocephalin (3.0 mg); F, phosphonolecithin (8.3 mg). The lipids were applied in 3.0 ml of chloroform.

TABLE II

COMPOSITIONS OF FRACTIONS OBTAINED BY CHROMATOGRAPHY OF PHOSPHOLIPIDS AND PHOSPHONOLIPIDS ON SILICIC ACID

A 21.5-mg amount of lipids was applied to the column. Total recovery was 100%.

Solvent	*Fractions collected*	*TLC R_F values*		*Components identified by IR spectroscopy*
		System A	*System B*	
5% Methanol in chloroform	14–19	0.62	0.87	1-O-Acylethylene glycol-2-(2-aminoethyl) phosphonate
20% Methanol in chloroform	21–26	0.43	0.90	1-O-Acylethylene glycol-2-(2-trimethylammoniumethyl) phosphonate
	32–38	0.61	0.00	1-O-Acylethylene glycol-2-phosphorylethanolamine
	42–48	0.41	0.00	1-O-Acylethylene glycol-2-phosphorylcholine
40% Methanol in chloroform	51–57	0.64	0.88	Phosphonocephalin
	60–67	0.43	0.90	Phosphonolecithin

DISCUSSION

The column chromatographic behaviours and properties of phosphonolipids have been examined previously[1–7]. Most of this work was concerned with phosphonolipids possessing a glycerol backbone. Silicic acid column chromatography of phosphonolipids possessing a backbone other than glycerol has not thus far been reported.

This communication is concerned principally with the examination of the column chromatographic properties of phosphonolipids with an ethylene glycol backbone. Unusual behaviour is exhibited by the two phosphono and the two phosphoryl derivatives of ethylene glycol. Thus, 1-O-acylethylene glycol-2-phosphorylethanolamine and 2-phosphorylcholine are eluted with 20% methanol in chloroform; the former is eluted in the middle of the elution, whilst the latter appears late in the elution and immediately precedes the phosphono analogue of cephalin, which can be rapidly eluted with 40% methanol in chloroform. Similar behaviour is exhibited by 1-O-acylethylene glycol-2-(2-trimethylammoniumethyl) and -(2-aminoethyl) phosphonates; the latter compound is eluted with 5% methanol in chloroform quite late in the process, followed immediately by the former compound which can be eluted rapidly with 20% methanol in chloroform. The differences in molecular weights and structure, compared to phosphatidylcholine and ethanolamine and their phosphono analogues, might contribute to this departure from the previously observed behaviour of phosphatidylcholine and phosphatidylethanolamine.

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