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Note

Silicic acid column chromatography of phosphonolipids

X. Some phosphono analogues of 1-O-alkylethylene glycol

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The silicic acid column chromatographic properties of the 1-O-alkylethylene glycol phosphono analogues of lecithin and cephalin have been examined[1]. The synthesis of 1-O-alkylethylene glycol phosphonic acid analogues of lecithin and cephalin was subsequently reported[2]. In this note the silicic acid column chromatographic properties of the latter phosphonolipids are examined and comparisons drawn with those of other phosphonolipids and phospholipids.

In particular, the column chromatographic behaviour has been studied, in two separate experiments, of (a) 1-O-dodecylethylene glycol-2-(2-trimethylammonioethyl)-phosphonate and 1-O-dodecylethylene glycol-2-(2-aminoethyl)phosphonate in the presence of their phosphoryl analogues and (b) the above two phosphonolipids in the presence of cardiolipin, cephalin and the phosphono analogues of cephalin and lecithin. Collected fractions were analysed by thin-layer chromatography (TLC) and IR spectroscopy to confirm species identification.

EXPERIMENTAL

Instrumentation

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

Reagents

Solvents for column chromatography and TLC were of analytical reagent grade (Merck, Darmstadt, F.R.G.) and were distilled before use. TLC was conducted on 20 cm × 20 cm chromatographic plates with a 0.25-mm layer of silica gel G or 60 F_{254} (Merck).

Standards

All phosphonolipids employed in these experiments were synthetic compounds. Cardiolipin and cephalin were obtained from Koch-Light (Colnbrook, U.K.), ethylene glycol monododecyl ether from Lancaster Synthesis (U.K.) and silicic acid for column chromatography from Sigma Chemical (St. Louis, MO, U.S.A.).

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TABLE I

ELUTION OF THE CHROMATOGRAPHIC COLUMN

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

Methanol–chloroform	*No. of column volumes*	*Total volume of solvent (ml)*	*Fractions collected*
0:100	2	50	1–9
1:99	2	50	10–19
5:95	3	75	20–36
10:90	3	75	37–53
20:80	5	130	54–81
40:60	9	225	82–127

Procedure

The procedure was similar to that described earlier[3]. Column elution was effected with methanol–chloroform mixtures as indicated in Tables I and II. IR spectra of the various pilot fractions were recorded as chloroform solutions or KBr discs.

TLC was performed on silica gel G or 60 F_{254} plates (Merck). The chromatograms were developed in two chambers of dimensions 20.5 × 8 cm (Desaga) and each analysis usually took 45 min. The plates were developed in chloroform–methanol–water (65:25:4) (system A) and methanol–water (2:1) (system B). Visualization was effected with molybdenum blue, iodine vapour, UV irradiation and the Stillway-Harmon procedure[4]. Standards were also spotted on the plates to aid in the detection of the developed spots.

RESULTS AND DISCUSSION

As mentioned above, column elution was effected with methanol–chloroform mixtures and the fractionation pattern of the lipids is depicted in Figs. 1 and 2.

Fractions were identified by TLC abd IR spectroscopy as indicated in Tables III and IV. With the solvents used, (a) 100% and (b) 99.9% of the lipids applied to the column could be recovered.

From the experimental data, 1-O-alkylethylene glycol-2-(2-aminoethyl)phosphonate is eluted with methanol–chloroform (20:80) and towards the end of the

TABLE II

CHROMATOGRAPHIC CONDITIONS

The column (35 cm × 1.6 cm I.D.) was packed with 12.0 g of silicic acid to a height of 10.5 cm and a total volume of 27 ml. Flow-rate: 1.0–1.5 ml/min. Fractions of *ca.* 5.0 ml were collected.

Methanol–chloroform	*No. of column volumes*	*Total volume of solvent (ml)*	*Fractions collected*
5:95	3	75	1–18
20:80	5	140	19–48
40:60	9	225	49–95

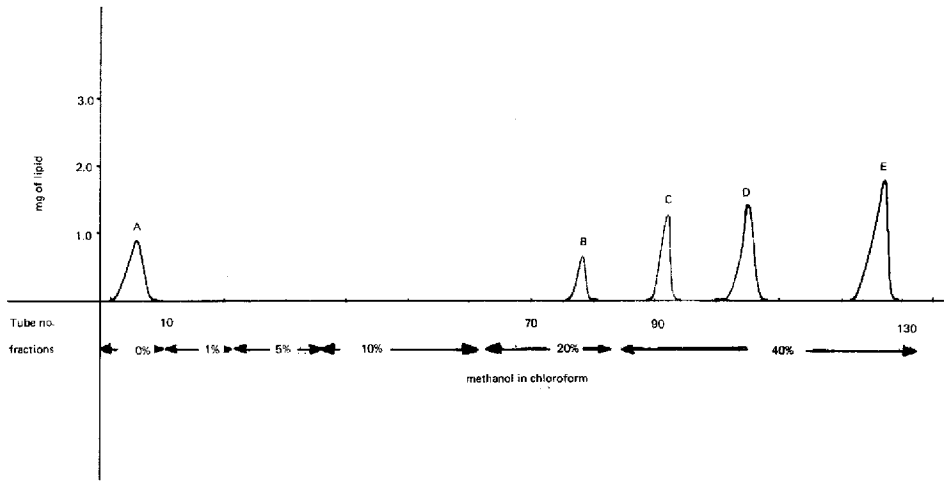

Fig. 1. Chromatography of ethylene glycol phosphono- and phospholipids on a column of silicic acid with methanol–chloroform as the eluent. Composition of the lipids: A = 1-O-dodecylethylene glycol, 2.8 mg; B = 1-O-alkylethylene glycol-2-(2-aminoethyl)phosphonate, 1.7 mg; C = 1-O-alkylethylene glycol-2-phosphorylethanolamine, 3.2 mg; D = 1-O-alkylethylene glycol-2-(2-trimethylammonioethyl)phosphonate, 3.8 mg; E = 1-O-alkylethylene glycol-2-phosphorylcholine, 5.3 mg. The lipids were applied to the column in 3.0 ml of chloroform.

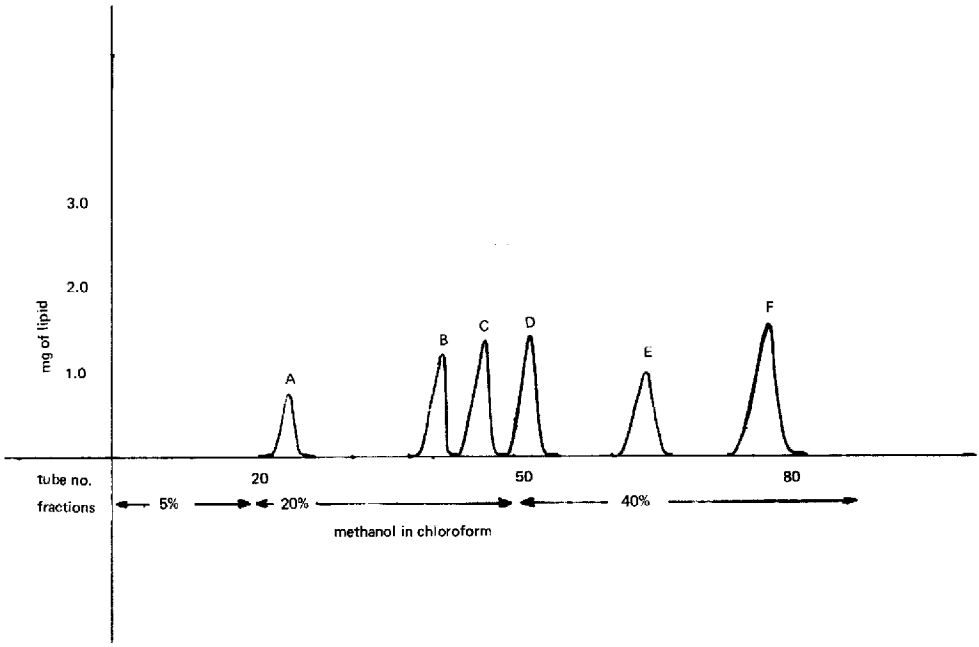

Fig. 2. Chromatography of phosphono- and phospholipids on a column of silicic acid with methanol–chloroform as the eluent. Composition of the lipids: A = cardiolipin, 2.3 mg; B = 1-O-alkylethylene glycol-2-(2-aminoethyl)phosphonate, 2.8 mg; C = phosphono analogue of cephalin, 3.4 mg; D = cephalin, 4.6 mg; E = 1-O-alkylethylene glycol-2-(2-trimethylammonioethyl)phosphonate, 3.2 mg; F = phosphono analogue of lecithin, 5.1 mg. The lipids were applied to the column in 4.0 ml of chloroform.

TABLE III

COMPOSITION OF FRACTIONS OBTAINED BY CHROMATOGRAPHY OF LIPIDS ON SILICIC ACID

A 16.8-mg amount of lipids was applied to the column. The total recovery was 100%.

Solvent	*Fractions collected*	*TLC R_F value*		*Component identified by IR spectra*
		System A	*System B*	
Chloroform	4–9	0.94	0.85	1-O-Alkylethylene glycol
Methanol–chloroform (20:80)	77–80	0.75	0.92	1-O-Alkylethylene glycol-2-(2-aminoethyl)phosphonate
	90–93	0.73	0.00	1-O-Alkylethylene glycol-2-phosphorylethanolamine
Methanol–chloroform (40:60)	102–107	0.44	0.86	1-O-Alkylethyleneglycol-2-(2-trimethylammonioethyl)phosphonate
	123–129	0.42	0.00	1-O-Alkylethylene glycol-2-phosphorylcholine

elution process, whilst the corresponding lecithin phosphono analogue is eluted in the initial stages of the elution with methanol–chloroform (40:60). From the results of the second experiment, there is good separation of the 1-O-alkylethylene glycol phosphonolipids from the corresponding glycero phosphonolipids which are eluted in their respective fractions following the ethylene glycol phosphonolipids.

When compared, however, with the 1-O-acylethylene glycol phosphonolipids, considerable overlapping is noted in some instances, as , *e.g.*, for the 1-O-alkylethyleneglycol phosphono analogue of cephalin with the 1-O-acylethyl glycol analogue of cephalin: for the 1-O-acylethylene glycol phosphono analogue of lecithin with the 1-O-alkylethylene glycol phosphono analogue of cephalin and finally for the 1-O-alkylethylene glycol phosphono analogue of lecithin with the phosphono analogue of lecithin proper.

TABLE IV

COMPOSITION OF FRACTIONS OBTAINED BY CHROMATOGRAPHY OF LIPIDS ON SILICIC ACID

A 21.4-mg amount of lipids was applied to the column. The total recovery was 99.9%.

Solvent: methanol–chloroform	*Fractions collected*	*TLC R_F value*		*Component identified by IR spectra*
		System A	*System B*	
5:95	–	–	–	–
20:80	21–23	0.68	0.00	Cardiolipin
	38–42	0.75	0.92	1-O-Alkylethylene glycol-2-(2-aminoethyl)phosphonate
	45–47	0.72		
	45–47	0.72	0.89	Phisphono analogue of cephalin
40:60	49–52	0.73	0.00	Cephalin
	66–69	0.44	0.86	1-O-Alkylethylene glycol-2-(2-trimethylammonioethyl)phosphonate
	78–84	0.43	0.82	Phosphono analogue of lecithin

Problems will be experienced when attempting to differentiate these phosphonolipids from a multi-component system, which can be resolved only by conducting additional research along these lines.

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