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Note

Silicic acid column chromatography of phosphonolipids

VII*. Separation of 1-O-acetyl-3-O-glycerylphosphonate, 1-O-oleyl-2,3-glycerylbiphosphonate and 1,2-dioleyl-3-O-glycerylphosphonate from their phosphoryl analogues and other lipids and phospholipids

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The silicic acid column chromatography of a variety of phosphonolipid classes has been reported previously, and in some instances effective separations have been achieved[1–6].

In this paper the silicic acid column chromatographic separation of 1-O-acetyl-3-O-glycerylphosphonate, 1-O-oleyl-2,3-glycerylbiphosphonate and 1,2-dioleyl-3-O-glycerylphosphonate from their phosphoryl analogues, other lipids and phospholipids is reported. For comparison purposes cardiolipin, cephalin, lecithin and sphingomyelin were included in this elution experiment. The collected fractions were analysed by thin-layer chromatography (TLC) and IR spectroscopy to confirm species identification.

EXPERIMENTAL

Intrumentation

IR spectra were recorded on a Perkin-Elmer 197 double-beam grating IR spectrophotometer. A glass column (40 cm × 2.4 mm I.D.) was employed for the separations.

Reagent

The solvents for column chromatography and TLC were of analytical-reagent grade (Merck, Darmstadt, F.R.G.) and were distilled before use.

Standards

1,2-Dioleylglyceryl ether, 1,2-distearylglyceryl ether and 1-monooleylglyceryl ether were purchased from Serdary Research Laboratories (London, Ontario, Canada). Phosphatidylethanolamine, cardiolipin, sphingomyelin and 1-O-acetylglycerol were purchased from Koch-Light (Colnbrook, U.K.). Phosphatidylcholine was ob-

* For Part VI, see ref. 6.

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TABLE I

ELUTION OF THE CHROMATOGRAPHIC COLUMN WITH (40 cm × 2.4 mm I.D.) LOADED WITH 10.0 g OF SILICIC ACID TO A HEIGHT OF 5.6 cm AND A TOTAL COLUMN VOLUME OF 25 ml

Flow-rate: 1.8–2.1 ml/min. Fractions of approximately 5.0 ml were collected

Methanol in chloroform (%)	*Column volumes*	*Total volume of solvent (ml)*	*Fractions collected*
0	2	50	1–10
5	3	75	11–27
20	5	125	28–55
40	7	170	56–90
80	5	125	91–118

tained from Merck and silicic acid for column chromatography from Sigma Co (St. Louis, MO, U.S.A.).

All the phosphono- and phospholipids were synthesized in this laboratory.

Procedure

The procedure was similar to that described earlier[1–6]. Column elution was effected with methanol–chloroform mixtures as indicated in Table I.

IR spectra of the various pilot fractions were run as 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) and also on plates coated in this laboratory to a thickness of 0.30 mm. Development of the chromatograms was carried out in two

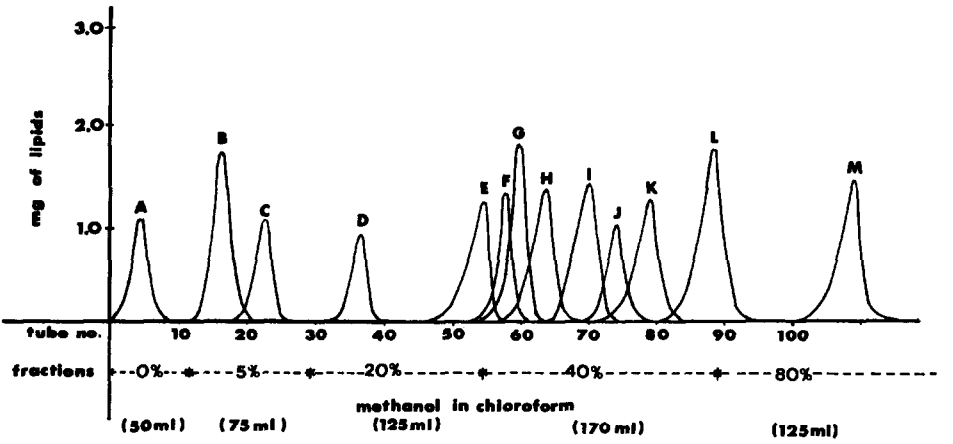

Fig. 1. Chromatography of phosphonolipids and phospholipids on silicic acid. Solvents: methanol in chloroform: (a) 5%, (b) 20%, (c) 40% and (d) 80%. The lipids were applied to the column in 5.0 ml of chloroform. Composition of lipids: (A) 1,2-oleylglyceryl ether, 2.5 mg; (B) 1-O-acetylglycerol, 4.8 mg; (C) 1-O-oleylglyceryl ether, 2.7 mg; (D) cardiolipin, 1.7 mg; (E) 1-O-acetylglycerylphosphonate, 2.9 mg; (F) 1-O-oleylglycerylphosphonate, 2.3 mg; (G) phosphatidylethanolamine, 5.3 mg; (H) 1-O-oleylglycerylbiphosphonate, 2.8 mg; (I) 1-O-acetylglycerylphosphorylcholine, 4.0 mg; (J) 1,2-oleylglycerylphosphorylcholine, 2.4 mg; (K) 1-O-oleylglycerylbiphosphorylcholine, 2.4 mg; (L) phosphatidylcholine, 6.4 mg; (M) sphingomyelin, 4.7 mg.

chambers of dimensions 8 × 20.5 cm; the run normally took about 45 min. The plates were developed in chloroform–methanol–water (65:25:4) (system A) and methanol–water (2:1) (system B)[7]. The spots were rendered visible with molybdenum blue, iodine vapour, UV irradiation and the Stillway–Harmon procedure[8]. Standards were also spotted on the plates to aid in the detection of the developed spots.

RESULTS

Column elution was effected with combinations of methanol in chloroform as indicated in Fig. 1. Fractions were identified by TLC and IR spectroscopy (Table II) and the nature of the fractionation pattern of the phosphonolipids is depicted in Fig. 1.

With the solvents used, 100.0% of the lipids applied could be recovered.

DISCUSSION

The results show that the dialkyl ethers are eluted from the column with chloroform, whilst 1-O-acetylglycerol and 1-monooleyl glyceryl ether are eluted in the cardiolipin fraction and prior to the latter lipid.

TABLE II

COMPOSITIONS OF FRACTIONS OBTAINED FROM CHROMATOGRAPHY OF LIPIDS ON SILICIC ACID

A 44.9-mg amount of phospho and phospholipids was applied to the column. The total recovery was 44.9 mg (100%)).

Methanol in methanol–chloroform solvent (%)	*Fractions collected*	*TLC R_F value*		*Component identified from IR spectra*
		System A	*System B*	
0	1–9	0.97	–	1,2-Dioleylglyceryl ether
5	11–22	0.97	0.49	1-O-Acetylglycerol
	16–25	0.98	–	1-O-Oleylglyceryl ether
20	31–40	0.68	–	Cardiolipin
	47–57	0.64	0.25	1-O-Acetylglycerylphosphonate
40	55–64	0.26	0.72	1,2-Dioleylglyceryl ether phosphonate
		0.69	–	Phosphatidylethanolamine
	58–69	0.59	–	1-O-Oleylglyceryl ether biphosphonate
	66–76	0.10		1-O-Acetylglycerylphos-
	71–80	0.43	–	phorylchloline
	71–80	0.43	–	1,2-Oleylglyceryl ether phosphorylcholine
	73–84	0.34	–	1-O-Oleylglyceryl ether biphosphorylcholine
	82–93	0.32	–	Phosphatidylcholine
80	103–114	0.17	–	Sphingomyelin

1-O-Acetylglyceryl ether phosphonate and the monoether glyceryl phosphonate are eluted in the phosphatidylethanolamine fraction, whilst the phosphoryl compounds are eluted in the lecithin fraction, with 20% and 40% methanol in chloroform respectively.

The elution experiments also confirmed that the silicic acid column chromatographic pattern of the 1,3-dialkylglyceryl ether phosphonates and of their phosphoryl analogues is similar to that exhibited by the 1,2-dialkylglyceryl ether phosphonates and phosphoryl analogues.

It is therefore possible, under the experimental conditions used, to separate effectively the above phosphonolipids from their phospholipid analogues and isolate them from natural sources.

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