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Note

Silicic acid column chromatography of phosphonolipids

V. Separation of phosphono-amide AGEPC, phosphono-amide AGEPE, monoether biphosphono-, diether phosphono-glycerides and diacetyl phosphono-glycerides from their phosphoryl analogues and other related lipids and phospholipids

MICHAEL C. MOSCHIDIS
A. Dedoussi E. E. Pharmaceutical Co., Schimatari, Viotias (Greece)
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In a series of recent notes[1–4] the column chromatographic behaviour of phosphono-sphingomyelin, 1-O-alkyl-2-acetyl-*sn*-glyceryl-3-phosphonocholine (phosphono-AGEPC), phosphonolecithin, phosphonocephalin and of dihydroceramide phosphonates has been detailed.

During the course of an investigation into the synthesis of biologically active compounds, a number of phosphonolipids together with their phospholipid analogues are being synthesized. In the present note is described the silicic acid column chromatographic separation of 1-O-hexadecyl-2-N-acetylaminodeoxy-*sn*-glyceryl-3-phosphonocholine (phosphono-amide AGEPC), 1-O-hexadecyl-2-N-acetylaminodeoxy-*sn*-glyceryl-3-phosphonoethanolamine (phosphono-amide AGEPE), 2-monopalmitylglyceryl-1,3-biphosphonate, 1,2-dipalmityl-*sn*-glyceryl-3-phosphonate and diacetylglyceryl-3-phosphonate from their phosphoryl analogues, cardiolipin, phosphatidylethanolamine (PE), phosphatidylcholine (lecithin PC), sphingomyelin, 2-monopalmityl glyceryl ether, 1,2-dipalmityl glyceryl ether and diacetylglycerol. Collected fractions were analysed by thin-layer chromatography (TLC) and IR spectroscopy to confirm species identification.

By this method it is thus possible to separate, isolate and identify the aforementioned phosphonolipids from their phospholipid analogues.

EXPERIMENTAL

Instrumentation

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

Reagents

Solvents for column chromatography were analytical reagent grade (Merck) and were distilled before use. TLC was conducted on 20 × 20 cm chromatoplates of 0.25 mm thick silica gel G or 60 F_{254} (Merck) and visualization was effected with molybdenum blue, iodine vapour, UV irradiation, ninhydrin spray and the Stillway–Harmon procedure[5].

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TABLE I

CHROMATOGRAPHIC CONDITIONS

The column (40 × 2.4 cm I.D.) was packed with 10 g of silicic acid (Sigma) to a height of 6.0 cm and total column volume of 26 ml. Flow-rate: 1.7–1.9 ml/min. Fractions of *ca.* 5.0 ml were collected.

% Methanol in chloroform	*Column volumes*	*Total ml of solvent*	*Fractions collected*
5	3	75	1–14
20	5	125	15–36
40	7	175	37–72
80	5	110	73–99

Standards

Phosphono-AGEPC[6], phosphono-acetyl glyceryl ether phosphatidylethanolamine (AGEPE), the amide analogues, phosphono-diether glyceride, biphosphono-monoether glyceride and their phosphoryl analogues were all synthetic products. Cardiolipin, phosphatidylethanolamine and sphingomyelin were purchased from Koch-Light (Colnbrook U.K.). Phosphatidylcholine was purchased from E. Merck (Darmstadt, F.R.G.). 1,2-dipalmityl glyceryl ether and 2-monopalmityl glyceryl ether were purchased from Serdary Research Labs. (Ontario, Canada).

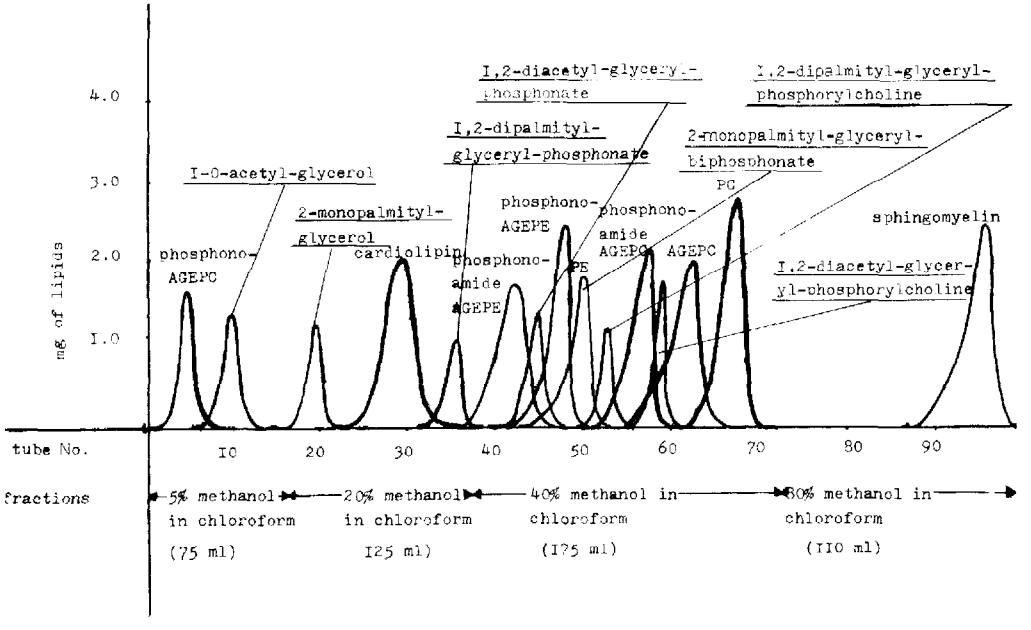

Fig. 1. Chromatography of various phosphono- and phospholipids on silicic acid. Solvents used are indicated. Lipids: phosphono-AGEPC, 4 mg; 1-O-acetylglycerol, 3.5 mg; 2-monopalmitylglycerol, 3.0 mg; cardiolipin, 6.0 mg; phosphono-amide AGEPE, 5.0 mg; 1,2-dipalmitylglyceryl-phosphonate, 2.7 mg; 1,2-diacetylglyceryl-phosphonate, 3.1 mg; phosphono-AGEPE, 6.0 mg; phosphatidylethanolamine (PE), 4.0 mg; 2-monopalmityl-glyceryl-biphosphonate, 3.0 mg; 1,2-dipalmitylglycerylphosphorylcholine, 2.2 mg; phosphono-amide AGEPC, 4.0 mg; AGEPC, 5.0 mg; 1,2-diacetylglycerylphosphorylcholine, 3.1 mg; lecithin, 8.0 mg and sphingomyelin, 6.0 mg. The lipids were applied to the column in 5.0 ml of chloroform.

Silicic acid for column chromatography was purchased from Sigma (St. Louis, MO, U.S.A.).

Procedure

The method adopted was similar to that described previously[1-4]. 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.

TLC chromatograms were run on silica gel G or 60 F_{254} plates (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 chambers of dimensions 8 × 20.5 cm (Desaga) and each analysis 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]. Visualization was effected as described under *Reagents*. Standards were also spotted on the plates to aid in the detection of the developed spots.

RESULTS

Column elution was effected with methanol–chloroform mixtures as indicated

TABLE II

COMPOSITIONS OF FRACTIONS OBTAINED FROM CHROMATOGRAPHY OF LIPIDS ON SILICIC ACID

68.60 mg of phospho- and phosphonolipids were applied to the column. Total recovery was 68.53 mg (99.9%).

Solvent	*Fractions collected*	*TLC R_F values*		*Component identified by IR*
		System A	*System B*	
5% Methanol in chloroform	2–6	0.78	–	Phosphono-AGEPC
	6–14	0.97	0.49	1-O-Acetylglycerol
20% Methanol in chloroform	16–24	0.99	–	2-Monopalmitylglycerol
	24–33	0.68	–	Cardiolipin
	32–37	0.39	0.73	1,2-Dipalmitylglyceryl-phosphonate
40% Methanol in chloroform	37–47	0.65	0.89	Phosphono-amide AGEPE
	43–47	0.36	0.68	1,2-Diacetylglyceryl-phosphonate
	43–51	–	0.96	Phosphono-AGEPE
	45–54	0.74	0.00	Phosphatidylethanolamine
	45–54	0.59	–	2-Monopalmitylglyceryl-biphosphonate
	50–56	0.43	0.00	1,2-Dipalmitylglyceryl-phosphorylcholine
	52–58	0.29	0.78	Phosphono-amide AGEPC
	56–64	0.30	0.44	AGEPC
	56–62	0.30	0.41	1,2-Diacetylglyceryl-phosphorylcholine
	62–70	0.32	0.00	Phosphatidylcholine
80% Methanol in chloroform	89–97	0.17	0.00	Sphingomyelin

in Fig. 1. Fractions were identified by TLC and IR spectroscopy (Table II) and the nature of the fractionation pattern of the phosphonolipids under examination is depicted on Fig. 1. With the solvents used, 99.9% of the lipids applied could be recovered.

DISCUSSION

From the results it is evident that no significant abnormalities in silicic acid column chromatographic behaviour are exhibited by the respective phosphonolipids. Cardiolipin, phosphatidylcholine, 1-O-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (AGEPC), AGEPE and sphingomyelin are eluted in their corresponding fractions with 20, 40 and 80% methanol in chloroform. Similarly, the phosphono-amide analogues of AGEPC and AGEPE are eluted in their fractions with 20 and 40% methanol in chloroform respectively and prior to the appearance of the corresponding phospholipids. 2-Monopalmitylglyceryl-biphosphonate is eluted with 40% methanol in chloroform together with phosphatidylethanolamine, and 1,2-palmitylglyceryl-phosphonate and diacetylglyceryl-phosphonate are eluted before and after the phosphono-amide AGEPE respectively. The behaviour of phosphono-AGEPC has been noted previously[2].

It is possible, therefore, to separate completely and effectively the phosphonolipids from their phospholipid analogues and thus render possible their isolation from natural sources.

REFERENCES

1 M. C. Moschidis, *J. Chromatogr.*, 259 (1983) 356.
2 M. C. Moschidis and C. A. Demopoulos, *J. Chromatogr.*, 259 (1983) 504.
3 M. C. Moshidis, *J. Chromatogr.*, 261 (1983) 181.
4 M. C. Moschidis, *Chim. Chron. New Ser.*, submitted for publication.
5 L. W. Stillway and S. J. Harmon, *J. Lipid Res.*, 21 (1980) 1141.
6 M. C. Moschidis, C. A. Demopoulos and L. G. Kritikou, *Chem. Phys. Lipids*, in press.
7 C. A. Demopoulos, M. C. Moschidis and L. G. Kritikou, *J. Chromatogr.*, 256 (1983) 378.