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Note

Thin-layer chromatographic and infrared spectral evidence for the presence of phosphonolipids in ground apricot kernel

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No information is available in the literature on the presence of phosphonolipids in plants, nor is there any reference of their isolation from plant materials. In addition, the presence of phosphonolipids in plants has been neither proved nor disproved. However, data are available on the lipid and phospholipid composition of plant materials[1–17] and especially seeds, without any reference to the presence of phosphonolipids.

In this study use has been made of preparative thin-layer chromatography (TLC)[18] for the isolation of the total phosphonolipids from ground apricot kernels, corn and barley seeds. The phosphonolipids, where appropriate, were preliminarily identified by TLC and IR spectral data.

EXPERIMENTAL

Materials

The solvents used were of pro analysi or analytical-reagent grade and were distilled before use.

Silica gel G was purchased from Merck (Darmstadt, F.R.G.) and fresh apricots, corn and barley seeds were purchased locally.

Most amino acids and sugars were chemically pure or analytical-reagent grade reagents from Mallinckrodt (St. Louis, MO, U.S.A.), Merck, BDH (Poole, U.K.) and Koch-Light (Colnbrook, U.K.). Balanced peptone and tryptone were purchased from London Analytical and Bacteriological Media (London, U.K.). Yeast exctract and Bacto-peptone were purchased from Difco Labs. (Detroit, MI, U.S.A.).

Methods

Fresh apricot kernels (100 g), dried corn (147.6 g) and dried barley (118 g) were ground and weighed out.

Preparative TLC was performed on glass plates coated with silica gel G to a thickness of 0.75 mm. The glass plates used for two-dimensional preparative TLC were coated to a thickness of 1.00 mm.

For the isolation of the seed phospholipids, acetone extraction was used for the ground apricot kernels and two-dimensional preparative TLC for the corn and barley. A concentrated solution of lipids was spotted on the glass plate, which was

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developed initially in methanol–water (2:1) (solvent A) and subsequently, after thorough air drying, in chloroform–methanol–water (65:25:4) (solvent B). Development in solvent A took approximately 80 min and that in solvent B approximately 45 min. Solvent B was also used for identification purposes.

The spots were rendered visible by spraying with iodine, ammonium molybdate, ninhydrin, α-naphtholsulphuric acid and Rhodamine 6G reagents. Phosphonolipids were also detected according to the Stillway and Harmon procedure[19].

IR spectra were recorded on a Perkin-Elmer 197 double-beam IR spectrophotometer as thin films from dry chloroform.

Total phosphorus and phosphono-phosphorus were determined according to the method of Kapoulas[20].

Procedure

The lipids from the seeds were extracted according to the procedure of Kates and Eberhardt[21] and the solvents were evaporated under reduced pressure and a bath temperature of 35°C. The phospholipids from apricot kernel were obtained by acetone extraction. With corn and barley two-dimensional preparative TLC was employed.

The phospholipids from apricot kernel were dissolved in 10 ml of chloroform–methanol (2:1) and subjected to preparative TLC in solvent A. The band whose R_F value ranged from 0.8 to 0.99 was scraped off and the phosphonolipids were extracted from the silica gel with chloroform. The phosphonolipids were checked for purity by re-chromatographing a small sample in solvent A and no phosphorus or other lipid could be detected at the origin. Preliminary TLC analysis of the isolated phosphonolipid fraction followed in solvent B.

A detailed TLC study was undertaken of the chromatographic behaviour of selected amino acids, peptides, lipids and carbohydrates in solvent A. Both TLC and preparative TLC were used for this purpose with detection as above.

RESULTS

Freshly ground apricot kernels (100 g) furnished, after extraction, 0.247 g of phospholipids, of which 6.47% were phosphonolipids. Chromatography of the phosphonolipid-free apricot kernel phospholipids (TLC in solvent B) provided evidence for the presence of the following phospholipids: lyso-lecithin, $R_F = 0.11$; sphingomyelin, $R_F = 0.31$; phosphatidylchloline, $R_F = 0.37$; phosphatidylglycerol, $R_F = 0.56$; phosphatidylethanolamine, $R_F = 0.65$; and an unidentified spot at $R_F = 0.84$ (probably cardiolipin).

Ground corn (147.6 g) gave, after extraction, 8.42 g of total lipids and similarly ground barley (118 g) gave 3.60 g of total lipids. Two-dimensional preparative TLC of corn and barley lipids furnished no evidence of the presence of phosphonolipids.

The IR spectrum of apricot kernel total phosphonolipids, together with the spectra of the phosphonolipids isolated from sheep spleen, beef brain, sheep brain and chicken liver, are shown in Fig. 1.

The isolated phosphonolipids released no phospholipid-phosphorus and was shown to contain phosphono-phosphorus when subjected to phosphorus determinations[20]. Also, the phosphonolipid extract was free from amino acids and sugars,

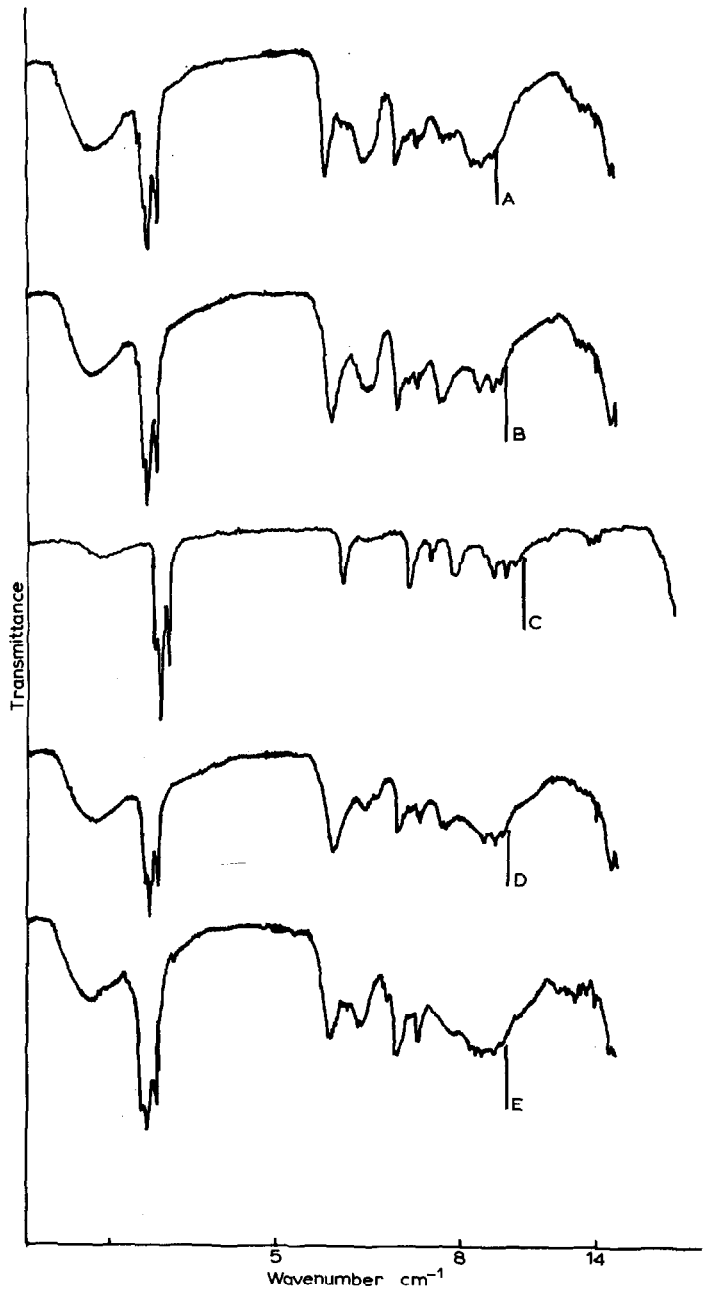

Fig. 1. IR spectrum of ground apricot kernel total phosphonolipids (A). The spectra of total phosphonolipids of sheep spleen (B), beef brain (C), sheep lung (D) and chicken liver (E) have been included for comparison purposes. The lines drawn on the individual spectra correspond to wavenumber 1000 cm^{-1}.

TABLE I

THIN-LAYER CHROMATOGRAPHY OF AMINO ACIDS, CARBOHYDRATES AND LIPIDS USING METHANOL–WATER (2:1). (SOLVENT A)

Chromatography was carried out on TLC and preparative TLC plates of thickness 0.25 and 0.75 mm, respectively. With most amino acids bands were obtained and the point of maximum advance is noted.

Compound	*R_F values solvent A*	*Iodine spray*	*Ninhydrin spray*	*α-Naphthol-sulphuric acid spray*	*Choline spray*	*Ammonium molybdate spray*
L-glutamine	0.70	+	+	–	–	–
dl-Lysine · HCl	0.65	+	+	–	–	–
L-Arginine	0.56	+	+	–	–	–
dl-Alanine	0.56	+	+	–	–	–
L-Histidine	0.56	+	+	–	–	–
dl-Serine	0.74	+	+	–	–	–
D-Alanine	0.75	+	+	–	–	–
Glycine	0.65	+	+	–	–	–
dl-Phenylalanine	0.75	+	+	–	–	–
L-4-Hydroxyproline	0.72	+	+	–	–	–
L-Tyrosine	0.67	+	+	–	–	–
Bacto-peptone	0.68	+	+	–	–	–
Balanced peptone	0.70	+	+			
Tryptone	0.68	+	+			
Yeast extract	0.72	+	+	+	–	–
Sucrose	0.72	+	–	+	–	–
Mannitol	0.65	+	–	+	–	–
D(+)-Mannose	0.00	+	–	+	–	–
D(–)-Fructose	0.74	+	–	+	–	–
Lactose	0.70	+	–	+	–	–
Dextrin	0.00	+	–	+	–	–
d-Glucose	0.73	+	–	+	–	–
D-Sorbitol	0.72	+	–	+	–	–
Sphingosine sulphate	0.00	+	–	–	–	–
α-Lecithin	0.00	+	–	–	+	+
Sphingomyelin	0.00	+	+	–	+	+
Dihydroceramide	0.00	+	+	–	–	–
3-O-Hexadecyl-1-O-acetylglycerol	0.00	+	–	–	–	–
Phosphonic acid, anilinium salt	0.82	+	+	–	–	+
Phosphonosphingosine	0.86	+	+	–	–	+
Phosphatidylserine	0.00	+	+	–	–	+
Glycerol tripalmitate	0.00	+	–	–	–	–
Glycerol dipalmitate	0.00	+	–	–	–	–
Cholesterol	0.00	+	–	–	–	–
1-O-Acetylglycerol	Band between 0.30 and 0.80	+	–	–	–	–
2-Bromoethyl phosphonic acid, dimethyl ester	0.86	+	–	–	–	+
Dipalmithinphosphonic acid, barium salt	0.00	+	–	–	–	+
Batyl alcohol	0.00	+	–	–	–	–
Gangliosides from beef brain	0.00	+	+	+	–	–
lyso-Lecithin	0.00	+	–	–	+	+
Phosphatidylethanolamine	0.00	+	+	–	–	+

as no indication was obtained fo their presence on the chromatograms when treated with the respective sprays. The following spots were obtained when the phosphonolipid extract was chromatographed in solvent B: (a) R_F = 0.19; (b) R_F = 0.44; (c) R_F = 0.70. Of these, spot (b) was tentatively assigned to the phosphono analogue of phosphatidylchloine.

Table I gives the R_F values and indicates the staining behaviour of the various amino acids, sugars and lipids tested in the TLC study. The substances were chromatographed in solvent A.

DISCUSSION

The results of the TLC study of the possible contaminating substances, *i.e.*, amino acids, peptides, carbohydrates and lipids, led to the conclusion that the phosphonolipid band with R_F values from 0.80 to 0.99 is virtually free from interfering substances. No amino acid, peptide, carbohydrate and/or lipid tested under these conditions possesses an R_F value greater than 0.75. Also, no amino acid and/or sugar was detected on the thin-layer chromatograms after development in solvent A, which indicates the effectiveness of the extraction procedure and the choice of solvent system. It is also noteworthy that all the phosphonolipids so far synthesized and/or isolated from natural sources have R_F values greater than 0.80.

The TLC method employed thus provides a unique means of quantitatively isolating and efficiently separating the inherent phosphonolipids from the total phospholipid fraction.

A simple examination of the spectral evidence reveals the striking similarity of the IR spectra of the total phosphonolipids of apricot kernels and those isolated from animal tissues.

TLC analysis in solvent B revealed the presence of three spots, one of which could be tentatively assigned to the phosphono analogue of phosphatidylcholine on account of its greater abundance in the total lipid extract. No evidence could be obtained for the presence of phosphonolipids in corn and barley seeds.

The identification of the other two phosphonolipids isolated from ground apricot kernel is at present under investigation, as also is the possibility of the presence of phosphonolipids in other plants and plant seeds.

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