Evidence for the Presence of Glycerophosphonolipids in the Land Snail *Eobania vermiculata*

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Glycerophosphonolipids, Land Snail

Total phosphonolipids from the land snail have been isolated by thin-layer chromatography (TLC) using the solvent system methanol–water (2:1) and have been subjected to silicic acid column chromatography. The fraction eluted with 40% methanol in chloroform has been obtained, the single species isolated by these means has been identified as the phosphono analog of phosphatidyl choline and has been fully characterized by TLC, IR spectroscopy, elemental analysis and phosphono-phosphorus determinations.

Introduction

The presence of phosphonolipids in water and land snails is well established [1–11]. Only sphingophosphonolipids, however, have been isolated from these species and in particular ceramide aminoethyl phosphonate and cerebroside phosphonate; the presence of glycero-phosphonolipids has not been proved or disproved to date.

In this paper evidence is provided for the presence of the phosphono analog of phosphatidyl choline in the land snail; the total phosphonolipids were isolated by preparative TLC in methanol–water (2:1) [12], were subjected to silicic acid column chromatography and the thus fractionated phosphono-lecithin was fully identified and characterized by TLC, IR spectroscopy, elemental analysis and phosphono-phosphorus determinations.

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, FRG) and silicic acid for column chromatography from Sigma (St. Louis, MO, USA). Snails, *Eobania vermiculata* species originated from Crete, Greece.

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Methods

The shell-free snails were homogenized in chloroform–methanol (2:1, v/v) with a Sorvall homogenizer; the snails weighed 184 g as fresh tissue. Preparative TLC was performed on glass plates coated with silica gel G to a thickness of 0.80 mm. The chromatograms were developed in methanol–water (2:1, v/v) as the solvent, and the run took approximately 80 min for full development. Chloroform–methanol–water (65:25:4, v/v/v) was also used for identification purposes.

The spots and bands were rendered visible with iodine, ammonium molybdate and ninhydrin spray reagents and by the Stillway and Harmon procedure [13].

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

Total phosphorus and phosphono-phosphorus were determined by the procedure of Kapoulas [14].

A glass column of length 40 cm and I.D. 2.4 cm was used for the chromatographic separation of the inherent phosphono-lecithin.

Procedure

The lipids from the homogenized whole snail sample were extracted according to the procedure of Bligh and Dyer [15] and the solvents were evaporated under vacuum and a bath temperature of 35 °C. The residue was dissolved in 150 ml of chloroform and rapidly extracted twice with a 5% aqueous NaCl solution. The chloroform layer was filtered dry through anhydrous sodium sulphate and again evaporated to dryness. The total lipids were subsequently extracted with acetone to constant phospholipid weight and were dried in a vacuum desiccator over phosphorus pentoxide for 24 h.

The total phospholipids were dissolved in 20 ml of chloroform–methanol (2:1) and subjected to preparative TLC in methanol–water (2:1). The band whose R_F ranged from 0.80 to 0.98 was scraped off and the phosphonolipids were obtained from the silica gel with chloroform. The phosphonolipids were checked for purity by rechromatographing a small sample in the same solvent system; no phosphorus could be detected at the origin.

The phosphonolipids were then subjected to TLC in chloroform–methanol–water (65:25:4) on glass plates coated with silica gel G to a thickness of

0.25 mm. The absence of carbohydrates and amino-acids was confirmed by the application of the respective sprays on the chromatograms (α-naphthol-sulphuric acid and ninhydrin sprays, respectively).

After initial identification the phosphonolipids were fractionated on a silicic acid column as described previously [16]. The amount of silicic acid was 10 g, the column was loaded to a height of 6 cm and a total column volume of 24 ml. The flow rate was maintained at about 1.8 ml/min. Tubes No. 56–67 of the corresponding fraction comprising 40% methanol in chloroform were collected and analysed accordingly.

Results and Discussion

The IR spectrum of the total phosphonolipids is given in Fig. 1, and the IR spectrum of the phosphono analog of phosphatidyl choline, fractionated

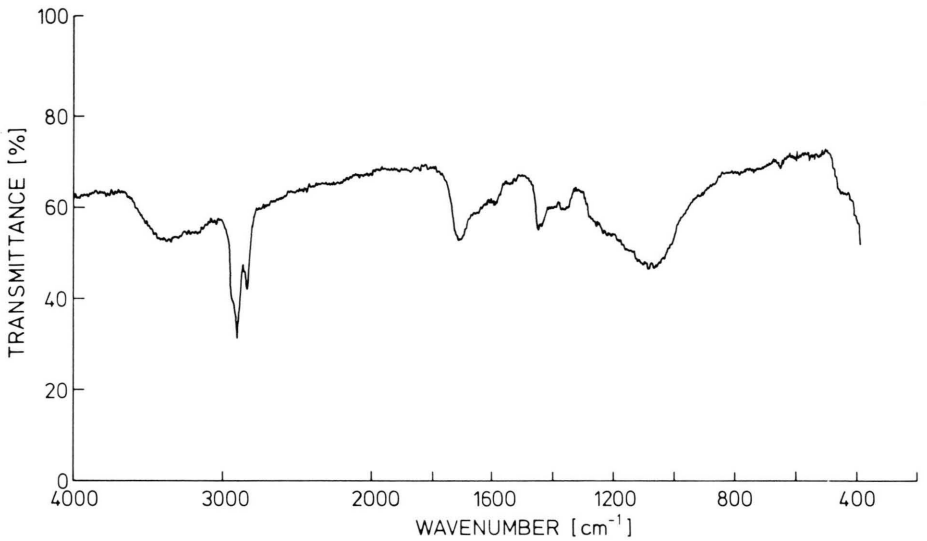

Fig. 1. The IR spectrum of total phosphonolipids isolated from the land snail, as thin film from dry chloroform.

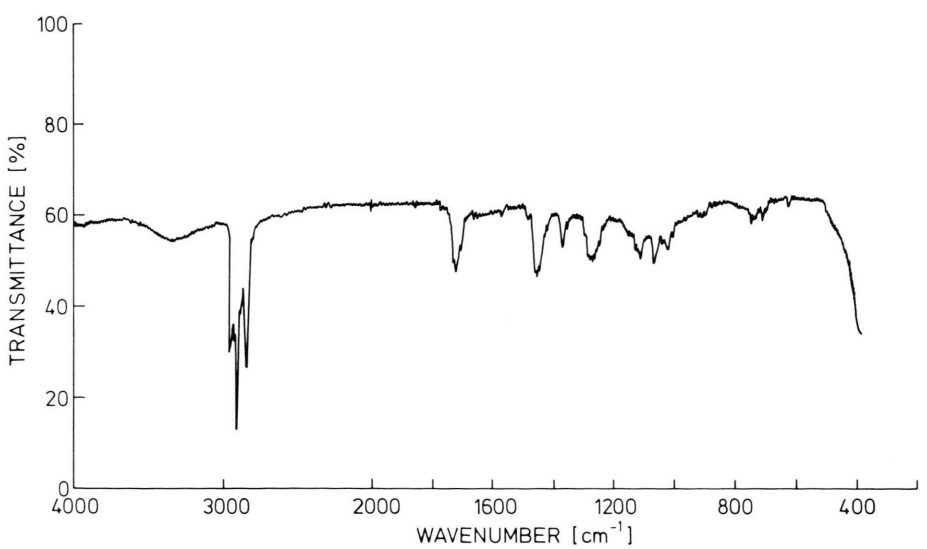

Fig. 2. IR spectrum of the phosphono analog of phosphatidyl choline fractionated on a silicic acid column with 40% methanol in chloroform, from the total phosphonolipids of the land snail. Taken on sodium chloride optics as a thin film from dry chloroform.

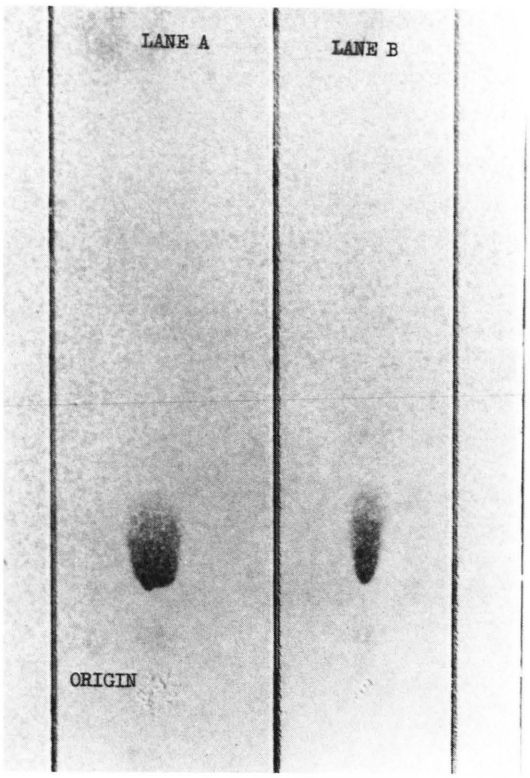

Fig. 3. Thin layer chromatographic profile of the phosphono analog of phosphatidyl choline, in chloroform–methanol–water (65:24:4) as solvent system. R_F value: 0.43. Lane A: phosphatidyl choline; lane B: phosphono analog of phosphatidyl choline. Visualization was effected with the ammonium molybdate spray reagent.

on the silicic acid column with 40% methanol in chloroform, is shown in Fig. 2.

Other analytical co-ordinates for total phospholipids and phosphonolipid content are well documented and resemble those found in the present case [1–11]. The phosphono-lecithin isolated in accordance with the procedure set out above amounted to 3.1% of the total phosphonolipids. Lipid nitrogen amounted to 2.04% and total phosphorus to 4.43%.

The phosphonolipid possessed an R_F value of 0.43 in chloroform–methanol–water (65:25:4) (Fig. 3).

Phosphorus determinations were carried out on the phospholipid and phosphonolipid fractions separated by preparative TLC and were shown to be free from interfering phosphonolipids and phospholipids, respectively. The chromatograms were also examined for interfering amino-acids and sugars by spraying with ninhydrin and α-naphthol-sulphuric acid reagents; none were detected.

The phosphono analog of phosphatidyl choline was obtained by collecting tubes No. 56–67, which contained a single species and which, after pooling of the solutions together and evaporating the solvents, was submitted to analyses.

By the joint application of preparative TLC, silicic acid column chromatography, TLC, IR spectroscopy, elemental analyses and phosphono-phosphorus determinations has been provided evidence for the presence of the phosphono analog of phosphatidyl choline in the land snail.

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