Synthesis of 1,2-Dipalmitoyloxypropyl-3-(2-ammoniumethyl) Phosphinate

Michael C. Moschidis

SANCORIN Laboratories, 6 Aglawrou Street, Koukaki 117 41, Athens, Greece

Z. Naturforsch. **40c**, 595–598 (1985); received April 22, 1985

Phosphinolipids, Phosphatidyl Ethanolamine, 1,2-Dipalmitoyl-sn-glycerol Bromohydrin, Phosphino Analog of Phosphatidyl Ethanolamine

The total synthesis of 1,2-dipalmitoyloxypropyl-3-(2-ammoniumethyl) phosphinate, the phosphino-analog of phosphatidyl ethanolamine is described.

The phosphinate analog of phosphatidyl ethanolamine has been prepared by an Arbusov type of reaction between a diacyl-glycerol bromohydrin and 2-bromo-ethylphosphonic acid dimethyl ester for 48 h at 178 °C, followed by a reaction with aq. ammonia to yield the final product.

The product was characterized by elemental analysis, phosphono-phosphorus determinations and IR spectroscopy.

Introduction

Phosphinolipids have not been shown to exist in nature. The synthesis, however, of one direct diether phosphinate analog of lecithin [1] and two isosteric diether phosphinate analogs of lecithin [2] have been undertaken by Rosenthal *et al.* The synthesis of one direct diester phosphinate analog of lecithin has also been reported [3].

Rosenthal *et al.* synthesized the phosphinate analogs of lecithin starting from 1,2,5,6-diisopropylidene D-mannitol to obtain through a series of steps the intermediates L-3,4-dioctalecoxybutyl bromide and 2-hexadecoxy-3-octadecoxyiodopropane which were then reacted with diisopropyl allylphosphonite to produce finally the isosteric and completely isosteric phosphinate analogs of lecithin.

The direct diacyl phosphinate analog of lecithin was prepared by an Arbusov type reaction between dipalmitin bromohydrin and 2-bromoethyl phosphonic acid dimethyl ester [3]. Essentially the same procedure has been employed for the preparation of the phosphatidyl ethanolamine analog. This work has been undertaken to provide reference compounds of known structure and configuration which could facilitate the isolation of phosphinolipids from biological materials, aid in their identification and also for other research purposes.

Materials and Methods

Materials

1,2-dipalmitoyl-sn-glycerol was purchased from Fluka Buchs (Switzerland); trimethylchlorosilane (99%) from Serva and trimethyl phosphite from Koch-Light (Colnbrook, U.K.).

Ethylene dibromide was AR Merck (Darmstadt, F.R.G.) and all other reagents used were AR or pro-analysis E. Merck, Ferak (F.R.G.) and Vioryl (Kifisia, Athens, Greece). All organic solvents were purified and dried following established procedures; solid reagents were dried in vacuum dessicator over P_2O_5 for several days prior to use.

Analytical methods

Phosphono phosphorus determinations were carried out according to the procedure of Kapoulas [4]. Thin layer chromatography (TLC) was carried out on silica gel G plates of 0.25 mm thickness using solvent systems: A, $CHCl_3/CH_3OH/H_2O$ (65:25:4, by vol.) and B, CH_3OH/H_2O (2:1, v/v). The lipids were visualised with iodine spray, ammonium molybdate spray and the Stillway-Harmon procedure (5). IR spectra were recorded on a Perkin-Elmer 197 infrared spectrophotometer.

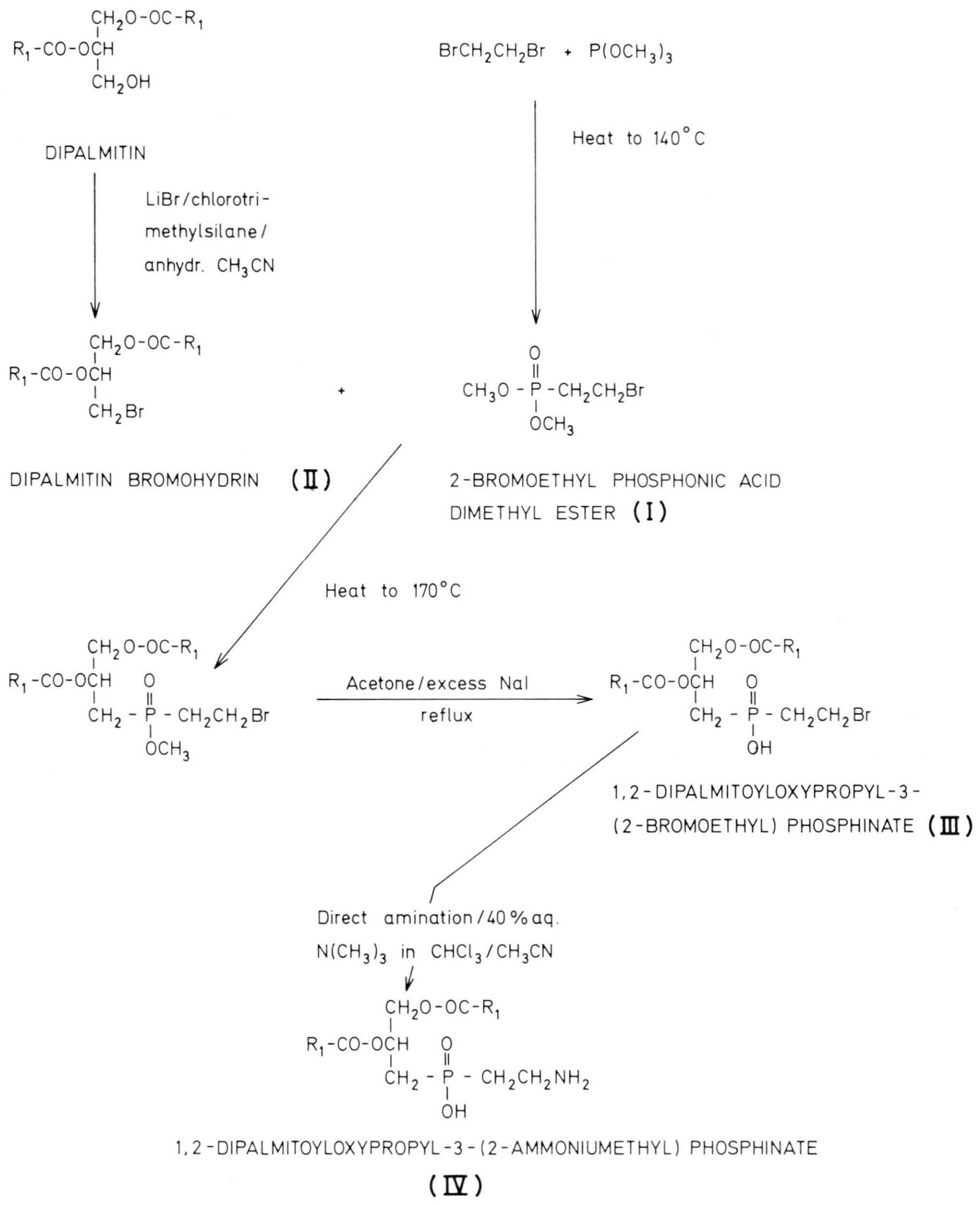
2-Bromoethyl phosphonic acid dimethyl ester (Scheme 1, *I*)

1,2-Dibromoethane (150 g, 0.8 M) was heated with trimethyl phosphite (24.8 g, 0.2 M) in an oil bath to 135–140 °C for 3 h. The product was obtained by

---

Reprint requests to Dr. M. C. Moschidis, 5 Thermopilon Street, Amarousion 151 24, Athens, Greece.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/85/0009–0595 $ 01.30/0



Scheme 1. Reaction scheme for the preparation of the phosphino analog of phosphatidyl ethanolamine. $R_1 = CH_3(CH_2)_{14}-$.

vacuum distillation [13 mm Hg) and the fraction boiling at 84.5 °C was collected.

2-Bromoethyl phosphonic acid dimethyl ester was a colourless oily substance which had a b.p. of 179−180 °C at 760 mm Hg. 26.80 g of pure substance were obtained (yield 66.70% of theory).

*1,2-Dipalmitoyl-sn-glycerol bromohydrin (Scheme 1, **II**)*

This was prepared as follows: chlorotrimethylsilane (2.7 g, 25 mmol) was added to a solution of lithium bromide (1.74 g, 20 mmol) in dry acetonitrile

(20 ml) with good stirring. Then 1,2-dipalmitoyl-sn-glycerol (5.68 g, 10 mmol) was added and the reaction mixture was heated under reflux for 23 h. At the end of this period the reaction mixture was taken up in ether, washed successively with water (25 ml × 2), sodium bicarbonate solution (10%, 50 ml) and finally with sodium chloride solution (5%, 50 ml) and dried over anhydrous sodium sulfate. The pure bromohydrin was obtained on evaporation of the ether. Repeated crystallisations from *n*-hexane, gave a white solid, m.p. = 40.5–41.0 °C (5.99 g, yield 95% of theory).

*1,2-Dipalmitoyloxypropyl-3-(2-bromoethyl) phosphinate (Scheme 1, **III**)*

1,2-Dipalmitoyl-sn-glycerol bromohydrin (**II**) (2.25 g, 3.56 mmol) was heated in an oil bath under reflux, with 2-bromoethyl phosphonic acid dimethyl ester (**I**) (5.70 g, 28.30 mmol) to 170 °C for 48 h. The reaction mixture, which solidified on cooling, was diluted with acetone and an excess of thoroughly dried NaI was added. The whole was refluxed for 24 h. The reaction mixture became clear on warming, and after the lapse of a few hours a precipitate was formed. At the end of the specified time, the precipitate was filtered off and dissolved in the minimum amount of water and passed through a column of Amberlite IR 120 (H+) ion-exchange resin. The aqueous solution was evaporated to dryness under vacuum and the resulting solid was dissolved in methanol. The final product was obtained chromatographically pure through preparative TLC on silica gel H using solvent system A. 1.70 g of 1,2-dipalmitoyloxypropyl-3-(2-bromoethyl) phosphinate, m.p. = 98.0 °C, were obtained (yield 66.30%). Elemental analysis gave the following results:

Found:	C 61.62	H 10.01	O 13.08 %
Expected:	C 61.41	H 9.96	O 13.28 %

The IR spectrum gave the following bands: ester carbonyl, at 1740 cm^{-1}; P – C stretch, at 1320 cm^{-1}; $(P = O)_v$ stretch, at 1240 cm^{-1} and $(P - C)_v$ stretch at 715 cm^{-1}.

*1,2-Dipalmitoyloxypropyl-3-(2-ammoniumethyl) phosphinate (Scheme I, **IV**)*

The direct ammination procedure of Eibl *et al.* was followed [6]: 1,2-Dipalmitoyloxypropyl-3-(2-bromoethyl) phosphinate (**III**) (1.0 g, 1.40 mmol) were dissolved in 20.0 ml of chloroform at 50 °C. Isopropanol (20 ml) and acetonitrile (20 ml) were added and these were followed by 11.0 ml of 25% ammonia solution in water. The reaction was completed in 9 h and the solvents were removed by evaporation under vacuum. The residue was dissolved in 30 ml of water, 30 ml of chloroform and 40 ml of methanol. After phase separation the chloroform waslayer was taken to dryness. The residue was dissolved in chloroform and precipitated with a 7-fold excess of acetone. It was kept in a refrigerator at 0 °C and the precipitate formed was collected accordingly.

1,2-Dipalmitoyloxypropyl-3-(2-ammoniumethyl) phosphinate, m.p. = 186–188 °C (547.80 mg) were obtained (yield 60.10%).

Elemental analysis gave the following results:

Found:	C 67.50	H 11.17	O 14.60	P 4.68%
Expected:	C 67.37	H 11.23	O 14.57	P 4.71%

Phosphono-phosphorus determinations indicated that the compound was a phosphonolipid. $[\alpha]_D^{24} = +1.65$ ($C = 5.6$, $CHCl_3 : CH_3OH$ (4:1)). The IR spectrum of the phosphinate analog of phosphatidyl ethanolamine is shown in Fig. 1.

Results and Discussion

The phosphinate analog of phosphatidyl ethanolamine has been synthesized by a three-step procedure, as outlined on Scheme 1. A double Michaelis-Arbusov type reaction [7, 8] has been employed with success and yields comparable to those reported in the literature.

TLC of the recrystallized product gave the following R_F values using solvent systems A: silica gel G plates, 0.74 and silica gel H plates, 0.66; and using solvent system B: silica gel G plates, 0.93. The phosphinate analog of phosphatidyl ethanolamine thus possesses chromatographic properties similar to those of the phosphoryl and phosphono analogs.

No inorganic phosphate was released even after rigorous acid hydrolysis (6 N HCl, 6 days, 11O °C), which pointed to the fact that the lipid was a phosphonolipid.

The phosphinate analog of phosphatidyl ethanolamine has been prepared in order to determine its column chromatographic properties (including other physical properties) and to facilitate the possible isolation of this compound from biological materials.

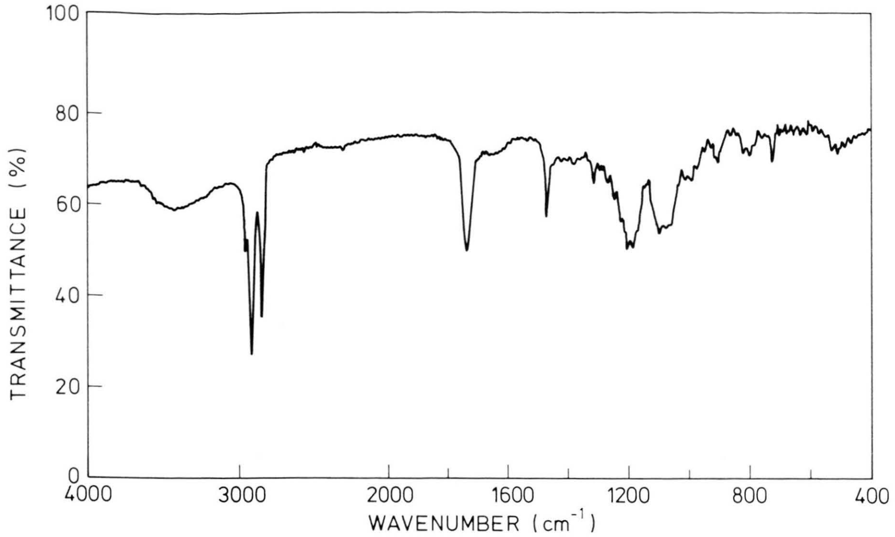


Fig. 1. IR spectrum of 1,2-dipalmitoyloxypropyl-3-(2-ammoniumethyl) phosphinate (KBr disc).

[1] A. F. Rosenthal, Methods in Enzymology **XXXV** (48), 520–525.
[2] A. F. Rosenthal, L. Vargas, and S. C. H. Han, Biochem. Biophys. Acta **260,** 369 (1972).
[3] M. C. Moschidis, Chem. Phys. Lipids, in press.
[4] V. M. Kapoulas, Post-Doctoral Thesis, University of Athens, 1969.
[5] L. W. Stillway and S. J. Harmon, J. Lipid Res. **21,** 1141 (1980).
[6] H. Eibl and A. Nicksch, Chem. Phys. Lipids **22,** 1 (1978).
[7] A. Michaelis and R. Koehne, Ber. **31,** 1048 (1898).
[8] A. E. Arbusov, J. Russ. Phys. Chem. Soc. **38,** 687 (1906).