METHOD FOR PREPARING PHOSPHATIDYLETHANOL

D. V. Arsenov, V. I. Dolgopalets, and M. A. Kisel′ UDC 547.915

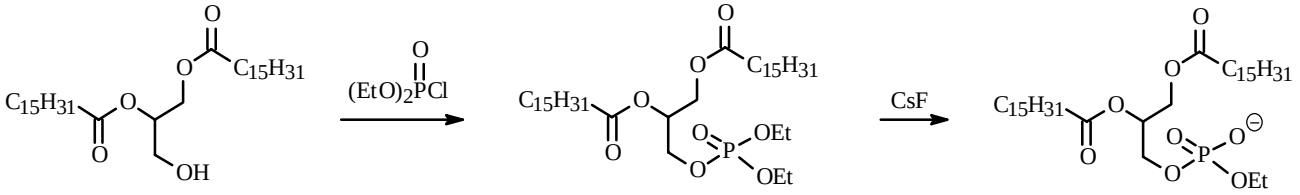
A method has been developed for preparing the unique phospholipid phosphatidylethanol, which is a marker of human and animal alcohol intoxication and acts as an effective component of liposomal forms of medicinal compounds. The method consists of preparation of the diethyl ester of phosphatidic acid with subsequent removal of one ethyl by CsF in t-butanol.

Key words: phosphatidylethanol, CsF.

The unique phospholipid phosphatidylethanol (PET) is synthesized in kidneys, brain, liver, and skeletal muscle of mice treated with ethanol [1, 2] and makes up 10% of the total fraction of anionic phospholipids. In the blood of human alcohol abusers, PET occurs at a concentration that persists at a level reliable for detection using HPLC for two weeks from the time of alcohol abstinence [3]. Analysis of the blood of volunteers found that PET is a more sensitive marker for alcohol intoxication that desialated transferrin [4]. It has been found that liposomes containing PET effectively encapsulate insulin [5, 6] and antibiotics [7]. Thus, PET can be used not only for biochemical research on alcoholism but also for resolving practical problems of pharmacology and medicine.

The principal method for producing PET is enzymatic and uses readily available phosphatidylcholine and phospholipase D [5, 8]. In spite of the high yield, the reaction mixture contains a side product, phosphatidic acid (PA), that is difficult to separate from PET. Synthetic methods for preparing PET by alkylation of PA using diazoethane [2] and condensation of PA with ethanol in the presence of trichloroacetonitrile [9] or triisopropylbenzenesulfonyl chloride [10] in pyridine are not widely used. The phospholipid obtained by these methods was isolated using TLC. The yield of the target product was not reported.

The preparation of 1,2-dipalmitoyl-*rac*-glycero-3-phosphoethanol (dipalmitoylphosphatidylethanol, **3**) from diglyceride **1** was used as an example of a simple and effective synthetic method for preparing PET in high yield without chromatographic purification of the target product. The method is based on selective removal of one of the ethyl groups from the phosphotriester 1,2-dipalmitoyl-*rac*-glycero-3-diethylphosphate (**2**) using anhydrous CsF in *t*-butanol.



1 **2** **3**

Fluoride ion is used for selective removal of trichloroethyl, phenyl, and *m*-chlorophenyl groups in phosphotriester derivatives of glycerophospholipids [11]. However, removal of only one ethyl from diethyl esters of PA using fluoride ion has not been reported. Diethyl esters of PA were used previously as intermediates in its synthesis [12, 13]. In our instance, according to TLC, PA did not form. Selective removal of one methyl or benzyl group was achieved earlier using *t*-butylamine [14] or NaI [13], respectively. However, these reagents are ineffective for removing one ethyl. According to TLC, fluoride under the reaction conditions does not affect the ester and phosphoester bond to the glycerine hydroxyl.

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 220141, Minsk, ul. Kuprevicha, 5/2, e-mail: kisel@iboch.bas-net.by. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 48-50, January-February, 2004. Original article submitted January 5, 2004.

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TABLE 1. Phase-Transition Parameters for Gel—Liquid Crystals of Liposomes from Dipalmitoyl Analogs of PET

PET preparation method	Buffer for preparing liposome	Tc, °C	$\Delta T_{1/2}$, °C	ΔH, kJ/mol	Ref.
This work	0.005 M Na phosphate (pH 7.4)	41.8	2.4	36.8	
Phospholipase D	0.05 M Tris-HCl (pH 7.4)	40.4	2.1	35.2	[8]
Phospholipase D	0.01 M Tris-HCl (pH 8.0)	41.6	n.d.	n.d.	[5]

n.d.: the data have not been indicated in [5].

Spots with R_f values corresponding to the mobilities of lysophosphatidylethanol and 1,2-dipalmitoylglycerine were not observed among the reaction products on the chromatography plate. It should be emphasized that the catalyst and reaction mixture should be anhydrous since fluoride is a powerful nucleophile and strong base only in the absence of a solvation sphere [15]. Primary and secondary alcohols should not be used as solvents because of possible transesterification of phosphotriester **2**.

The structure of phospholipid **3** prepared by this method was confirmed by PMR spectra and its direct synthesis from the dipalmitoyl analog of phosphatidylcholine using phospholipase D [5]. The synthesized **3**, like PET, was obtained by an enzymatic method and is easily solubilized in aqueous solutions to form liposomes, the thermotropic behavior of which is an important characteristic of the phospholipid purity. Table 1 lists the phase-transition parameters for gel—liquid crystals of **3** that were calculated using thermal-absorption curves of liposomes formed from it. It can be seen that the temperature (T_c), half-width ($\Delta T_{1/2}$), and enthalpy (ΔH) of the phase transition in liposomes of **3** obtained using both our and enzymatic methods agree with the literature data [5, 8].

EXPERIMENTAL

Diethylchlorophosphate and CsF were obtained commercially (Aldrich, USA). 1,2-Dipalmitoyl-*rac*-glycerine was prepared by the literature method [16]. TLC was performed on Sil G-25 plates (Macherey-Nagel, Germany) using solvent systems benzene:ethylacetate (4:1, system 1) and $CHCl_3$:CH_3OH:NH_4OH (7 N) (13:5:1, system 2). Chromatograms were developed using either molybdate reagent [17] or heating to 200°C after spraying with H_2SO_4 in CH_3OH (10%). The P content in the samples was determined by the Vaskovsky method [17]. PMR spectra were recorded on a Bruker AC-200 instrument. Chemical shifts are given relative to TMS as an internal standard. Thermal absorption curves were recorded on a DASM-1M instrument (Russia).

1,2-Dipalmitoyl-*rac*-glycero-3-diethylphosphate (2) was prepared by the method described for the synthesis of its distearoyl analog [13]. A mixture of diglyceride (**1**, 0.57 g), diethylchlorophosphate (0.69 g), and anhydrous pyridine (0.40 g) in anhydrous benzene (7 mL) was stirred at 40°C for 3 h. The resulting pyridinium chloride was separated by filtration. Phosphotriester **2** was purified using chromatography over silica gel with elution by benzene:ethylacetate (4:1) to afford **2** (0.49 g), yield 70%, R_f 0.3 (system 1).

PMR (200 MHz, $CDCl_3$, ppm): 0.86 (6H, t, $2\underline{CH_3}CH_2$), 1.20 [54H, m, $2CH_3(\underline{CH_2})_{12}$, $2\underline{CH_3}CH_2O$], 1.56 (4H, m, $2\underline{CH_2}CH_2CO$), 2.34 (4H, t, $\underline{CH_2}CO$), 3.85-4.15 (8H, m, $2CH_2OCO$, $2CH_2OP$), 5.21 (1H, m, CHOCO).

1,2-Dipalmitoyl-*rac*-glycero-3-phosphoethanol (3). A suspension of **2** (0.07 g) in anhydrous *t*-butanol (5 mL) was treated with anhydrous CsF (0.15 g), stirred at 50°C for 1 h, diluted with $CHCl_3$, and evaporated. The solid was dissolved in $CHCl_3$:CH_3OH (2:1) and filtered through silica gel to remove CsF. The eluate was evaporated. The solid was crystallized from boiling acetone to afford **3** (0.077 g), yield 95% (calculated as the Cs^+ form). TLC: R_f 0.77 (system 2).

PMR (200 MHz, $CDCl_3$, ppm): 0.86 (6H, t, $2\underline{CH_3}CH_2$), 1.20 [51H, m, $2CH_3(\underline{CH_2})_{12}$, $\underline{CH_3}CH_2O$], 1.60 (4H, m, $2\underline{CH_2}CH_2CO$), 2.32 (4H, t, $\underline{CH_2}CO$), 3.90-4.20 (6H, m, $2CH_2OCO$, CH_2OP), 5.20 (1H, m, CHOCO). P content of the Na^+ form of **3**: Found (%) P 4.41; calc. (%) P 4.43.

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