DIOL LIPIDS

COMMUNICATION 9.* SYNTHESIS OF DIOL CEPHALINS

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Not long ago it was shown that chromatographically pure phosphatidylethanolamine isolated from the organs of mammals contains a small amount of ethylene glycol and 1, 3-propanediol [1]. Results were obtained showing that these dihydric alcohols are present in the tissues in combined form, possibly in the form of analogs of glycerophosphatides. Since these "diol phospholipids" had not yet been isolated individually, it was interesting to synthesize the diol analogs of phosphatidylethanolamine and investigate their chromatographic behavior and the other properties

$$\underset{(I)}{RCOOCH_2(CH_2)_nCH_2OP(O)(OH)OCH_2CH_2NH_2}$$

Ethylene glycol (I, n = 0) and 1, 3-propanediol (I, n = 1) analogs of phosphatidylethanolamine containing residues of fatty acids have been described [2-5]. We carried out a synthesis of three diol analogs of phosphatidylethanolamine containing the same fatty acid (stearic) and residues of three different diols: ethylene glycol, 1, 3-propanediol and 1, 4-butanediol (V, n = 0, 1, 2). The synthesis of these "diol cephalins" was carried out by the following process

$$\underset{(II)}{C_{17}H_{35}COO-CH_2-(CH_2)_n-CH_2I} + \underset{(III)}{AgOP(O)(OC_6H_5)OCH_2CH_2NHOCOCH_2C_6H_5} \rightarrow$$

$$\rightarrow \underset{(IV)}{C_{17}H_{35}COO-CH_2-(CH_2)_n-CH_2-OP(O)(OC_6H_5)OCH_2CH_2NHOCOCH_2C_6H_5} \xrightarrow{H_2/Pt,Pd}$$

$$\rightarrow \underset{(V)}{C_{17}H_{35}COO-CH_2-(CH_2)_n-CH_2-OP(O)(OH)OCH_2CH_2NH_2}$$

The starting substances for the synthesis of diol cephalins were stearic esters of mono(deoxyiodo) derivatives of the glycols (II, n = 0, 1, 2) and silver 2-[(benzyloxycarbonyl)amino]ethyl phenyl phosphate (III) [6]. By their condensation the corresponding phosphoric triesters (IV, n = 0, 1, 2) were obtained. The latter were converted into diol cephalins (V, n = 0, 1, 2) by hydrogenation over a mixed platinum-palladium catalyst. For comparison, by a similar method starting from DL-1-deoxy-1-iododistearoylglycerol and the silver salt (III), via distearoylglycerol 2-[(benzyloxycarbonyl)amino]ethyl phenyl phosphate (VI), we synthesized the DL-distearoylglycerylphosphorylethanolamine (VII) [6] in accordance with the scheme

* For Communication 8 see [1].

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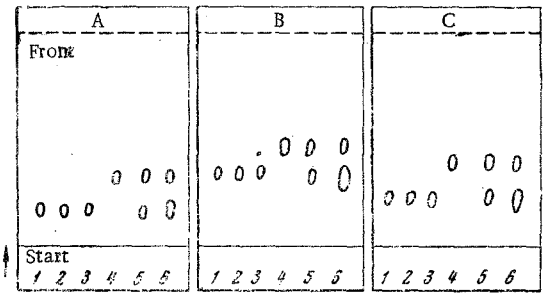
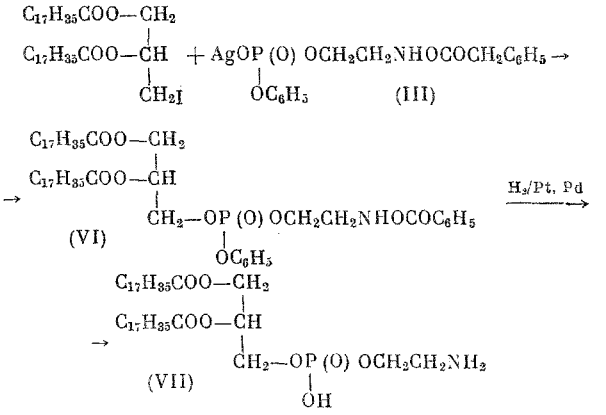


Fig. 1. Thin-layer chromatography of phosphatidylethanolamine and its diol analogs. A) In chloroform – methanol – water (65 : 25 : 4); B) in diisobutyl ketone – acetic acid – water (40 : 25 : 5); C) in chloroform – methanol – 4 N NH_4OH (17 : 7 : 1): 1) (V, n = 0); 2) (V, n = 1); 3) (V, n = 2); 4) (VII); 5) (VII) + (V, n = 1); 6) (VII) + mixture of all diol cephalins.



The diol cephalins which we obtained are finely crystalline colorless substances with high melting points. In their behavior in thin-layer chromatography (Fig. 1 and Table 1) they are much closer to lysophosphatidylethanolamine than to phosphatidylethanolamine: in the solvent systems which we tested the R_f values of the diol cephalins are ~ 0.6 of the R_f of phosphatidylethanolamine. The differences in solubility are also considerable (Table 2): diol cephalins are much less soluble in all tested solvents (except water) than phosphatidyl ethanolamine. The 1,4-butanediol analog (V, n = 2) is something of a peculiarity. It dissolves in heptane, chloroform, and methanol better than the remaining diol cephalins, but in acetone and in water better than phosphatidylethanolamine. The results obtained in a study of the chromatographic behavior of the diol phospholipids synthesized show that the lipids containing ethylene glycol and 1, 3-propanediol, which were observed earlier [1] in the cephalin fraction of organs of mammals, are not diol analogs of phosphatidylethanolamine.

The results of the biochemical and biophysical studies of diol cephalins will be published later.

EXPERIMENTAL

The melting points were determined on a Kofler block. For the diol analogs of phosphatidylethanolamine the temperature of formation of a meniscus in the capillary (cf. [6]) was taken as the melting point.

The infrared spectra were determined on a UR-10 spectrograph. Thin-layer chromatography was carried out on a bound layer of KSK silica gel (fraction smaller than 150 mesh) washed free from traces of metals with nitric acid.

3-Iodopropyl Stearate (II, n = 1). A solution of 14.5 g of stearoyl chloride in freshly distilled $CHCl_3$ was added dropwise over 40 min at 0° to 4.5 g of 3-chloro-1-propanol [7] in 3.9 ml of pyridine and 10 ml of $CHCl_3$, and the reaction mixture was allowed to warm up to room temperature and was then kept at 40° for 4 h. The mixture was then cooled, diluted with 400 ml of ether, washed with water, dried over Na_2SO_4, and filtered. The filtrate was evaporated in vacuo, and the residue was crystallized from a mixture of alcohol and ether. We obtained 16 g (97%) of crude 3-chloropropyl stearate.

From 16 g of the latter and 7.21 g of dry NaI in 75 ml of dry acetone, 15 g (74.4%) of 3-iodopropyl stearate (II, n = 1) was obtained, in the form of colorless needles with a double melting point of 29-30° and 35.5-36°, by the method described for 4-iodobutyl stearate (II, n = 2). Found %: C 56.36; H 8.90; I 27.46. $C_{21}H_{41}IO_2$. Calculated %: C 55.78; H 9.07; I 28.00.

4-Iodobutyl Stearate (II, n = 2). A mixture of 10 g of stearoyl chloride, 2 g of zinc chloride, and 12 g of tetrahydrofuran was kept for 1 h at 80°. The mixture was cooled and diluted with 30 ml of benzene, and the solution was washed in turn with water, 5% aqueous $NaHCO_3$, and again water, dried over Na_2SO_4 and filtered. The filtrate was evaporated in vacuo, and the residue was crystallized from a mixture of alcohol and ether. We obtained 7 g (59.3%) of 4-chlorobutyl stearate, mp 36-37°. Found %: C 70.87; H 11.21; Cl 8.96. $C_{22}H_{43}ClO_2$. Calculated %: C 70.52; H 11.48; Cl 9.46.

A solution of 6.75 g of 4-chlorobutyl stearate and 2.9 g of dry NaI in 30 ml of dry acetone was kept in a sealed ampule for 14 h at 90°. After cooling, the contents were poured into a mixture of 70 ml of water

TABLE 1. Thin-Layer Chromatography of Phosphatidylethanolamine and Its Diol Analogs

Compound	R_f in the system		
	$CHCl_3$ – methanol – water, 40 : 25 : 4	Diisobutyl ketone – acetic acid – water, 40 : 25 : 5	$CHCl_3$ – methanol – 4 N NH_4OH, 17 : 7 : 1
(V, n = 0)	0.11	0.25	0.12
(V, n = 1)	0.10	0.27	0.10
(V, n = 2)	0.09	0.24	0.11
(VII)	0.19	0.37	0.25

TABLE 2. Solubility of Phosphatidylethanolamine and Its Diol Analogs at 20° (mg/ml)

Solvent	Compound			
	(V, n = 0)	(V, n = 1)	(V, n = 2)	(VII)
Mixture of chloroform and methanol, 2:1	1.363	12.030	51.7	>60
Chloroform	0.350	1.175	>60	>60
Methanol	1.033	1.410	5.5	>60
Acetone	0.025	<0.01	0.530	0.430
Heptane	<0.01	<0.01	0.053	0.073
Water	<0.01	<0.01	0.400	<0.01

and 30 ml of ether. The ether layer was separated, washed with a 5% solution of sodium thiosulfate and with brine, dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was crystallized from a mixture of alcohol and ether; the yield of the iodide (II, n = 2) was 6.15 g (91.5%), mp 39-39.5°. Found %: C 56.34; H 8.68; I 26.80. $C_{22}H_{43}IO_2$. Calculated %: C 56.68; H 9.23; I 27.23.

2-[(Benzyloxycarbonyl)amino]ethyl Phenyl 3-(Stearoyloxy)propyl Phosphate (IV, n = 1). To a suspension of 45.2 mg of silver 2-[(benzyloxycarbonyl)amino]ethyl phenyl phosphate (III) [6] in 5 ml of dry benzene, 50 mg of 3-iodopropyl stearate (II, n = 1) was added in the dark with stirring. The mixture was boiled until the reaction was complete (~8 h; the progress of the reaction was followed by means of thin-layer chromatography in a 3 : 1 benzene–ethyl acetate system; the developer was conc. H_2SO_4). The mixture was cooled, and the precipitate was separated by centrifugation and washed with dry $CHCl_3$. The combined filtrates were evaporated in vacuo, and the residue was chromatographed in a column charged with 6 g of silica gel in benzene. Elution with benzene containing 30-60% of ethylacetate gave 61.7 mg (82.2% of the phosphoric triester (IV, n = 1) in the form of a waxy mass. Infrared spectrum (film): 3350 (s, b), 1740 (s), 1610 (m), 1540 (m), 1275 (s, b), 1025 (m, b). Found %: C 66.60; H 9.79; N 1.8; P 3.99. $C_{37}H_{58}NO_8P$. Calculated %: C 65.77; H 8.85; N 2.09; P 4.54.

2-[(Benzyloxycarbonyl)amino]ethyl Phenyl 2-(Stearoyloxy)ethyl Phosphate (IV, n = 0). By the method described for (IV, n = 1), 1.27 g (86%) of the phosphoric triester (IV, n = 0) was obtained in the form of a waxy mass from 1 g of 2-iodoethyl stearate [8] and 1.24 g of the silver salt 3310 (s), 1750 (s), 1730 (s), 1610 (m), 1270 (m), 1040 (s). Found %: C 65.56; H 8.82; N 2.52; P 4.38. $C_{36}H_{56}NO_8P$. Calculated %: C 65.33; H 8.561 N 2.20; P 4.67.

2-[(Benzyloxycarbonyl)amino]ethyl Phenyl 4-(Stearoyloxy)butyl Phosphate (IV, n = 2). 1.3 g (88.2%) of the phosphoric triester (IV, n = 2) was obtained by the method described above from 1.13 g of the iodide (II, n = 2) and 1.24 g of the silver salt (III). Infrared spectrum (film): 3350 (m, b), 1740 (vs), 1610 (m), 1540 (s), 1270 (s, b), 1020 (s, b). Found %: C 66.09; H 9.29; N 2.05; P 4.99. $C_{38}H_{60}NO_8P$. Calculated %: C 66.17; H 8.78; N 2.03; P 4.48.

2-Aminoethyl 2-(Stearoyloxy)ethyl Hydrogen Phosphate (V, n = 0). Under the conditions of the hydrogenolysis of (V, n = 1) 49.7 mg (83%) of the phosphoric diester (V, n = 0), mp 202° (becomes wet at 85°), was obtained from 60 mg of the phosphoric triester (IV, n = 0). Found %: C 59.07; H 10.19; N 3.20; P 7.13. $C_{22}H_{46}NO_6P$. Calculated %: C 58.51; H 10.27; N 3.10; P 6.86.

2-Aminoethyl 3-(Stearoyloxy)propyl Hydrogen Phosphate (V, n = 1). We added 50 mg of 5% palladium oxide on barium sulfate [9] and 18 mg of platinum oxide to a solution of 53.8 g of the phosphoric triester (IV, n = 1) in 3 ml of dioxane and 0.1 ml of acetic acid. Hydrogenolysis was carried out under a pressure slightly exceeding 760 mm and at a temperature of 23° until the absorption of hydrogen ceased (~6 h). After removal of excess hydrogen 10 ml of a mixture of $CHCl_3$ and methanol (2:1) was added the mixture was filtered, and the residue was washed with a mixture of $CHCl_3$ and methanol (2:1). The filtrate was evaporated to dryness, and the residue was crystallized from alcohol. We obtained 49.7 mg (92.3%) of the phosphoric diester (V, n = 1), mp 203.5 – 204° (becomes wet at 85°). Infrared 3310 (m, b), 1740 (s), 1230 (s), 1100 (s), 1043 (s). Found %: C 59.27; H 10.40; N 2.90; P 6.68. $C_{23}N_{48}NO_6P$. Calculated %: C 59.33; H 10.61; N 3.01; P 6.69.

2-Aminoethyl 4-(Stearoyloxy)butyl Hydrogen Phosphate (V, n = 2). From 102 mg of the phosphoric triester (IV, n = 2) 85.5 mg (84%) of the phosphoric diester (V, n = 2), mp 194-195° (becomes wet at 85°), was obtained by hydrogenolysis over 100 mg of palladium oxide on barium sulfate 3390 (m, b), 1740 (s), 1220 (s), 1095 (s), 1040 (s). Found %: C 59.71; H 10.95; N 2.88; P 6.15. $C_{24}H_{50}NO_6P$. Calculated %: C 60.10; H 10.51; N 2.92; P 6.45.

Determination of the Solubility of Phosphatidylethanolamine and Its Analogs. A suspension of a weighed portion (30-50 mg) of carefully ground substance, in 2-3 ml of solvent was stirred vigorously at 40° for 15 min cooled to 20°, kept at this temperature for 20 min, and filtered. The concentration of the substance in the filtrate was found from the phosphorus content, which was determined by the method described in [10] (see Table 2).

CONCLUSIONS

Diol analogs of phosphatidylethanolamine, namely, 2-aminoethyl 3-(stearoyloxy)propyl hydrogen phosphate and 2-aminoethyl 4-(stearoyloxy)butyl hydrogen phosphate, were synthesized; their solubilities and behaviors in thin-layer chromatography were studied.

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