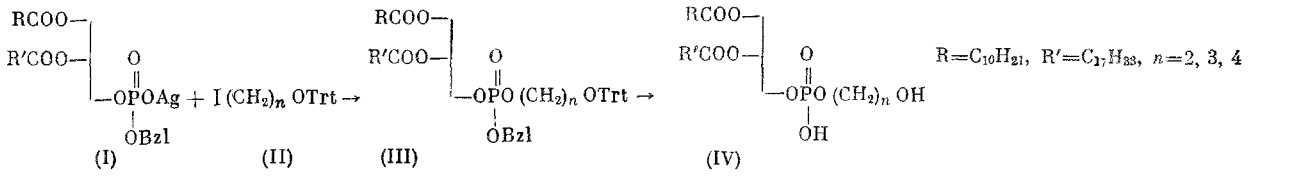
SYNTHESIS OF PHOSPHATIDYLDIOLS

Yul. G. Molotkovskii, V. I. Kozhukhov, and L. D. Bergel'son

UDC 542.91+547.42+661.718.1

As a continuation of our work on the synthesis and study of the properties of diol phospholipids [1, 2] we undertook the synthesis of some diol analogs of phosphatidylglycerol of general formula (IV), the presence of which in natural sources is deemed probable [3]. Previously the phosphatidyldiols (IV) had been obtained from egg lecithin by treatment with phospholipase D in the presence of an excess of the appropriate glycol [4]. The phosphatidyldiols obtained in this manner were not pure as regards their aliphatic acid composition, and were characterized only by chromatography.

The synthesis of the phosphatidyldiols (IV) described in this communication is based on the condensation of silver benzyl-(1-lauroyl-2-oleolyl-sn-glyceryl)phosphate with the trityl ethers of glycol iodohydrins (II). The cleavage of the benzyl group from the triphosphates (III) by treatment with sodium iodide in acetone, and subsequent removal of the trityl protection by acid hydrolysis, led to the formation of 1-lauroyl-2-oleoyl-sn-glycero-3-phosphorylethylene glycol (IV, n = 2), 1-lauroyl-2-oleoyl-sn-glycero-3-phosphoryl-1,3-propanediol (IV, n = 3), and 1-lauroyl-2-oleoyl-sn-glycero-3-phosphoryl-1,4-butanediol (IV, n = 4). The obtained phosphatidyldiols (IV) are soluble in most of the common organic solvents, and, as the calcium salts, are quite stable when stored (the free phospholipids decompose quite rapidly due to autocatalytic hydrolysis).



EXPERIMENTAL

The melting points were determined on a Koffler block and corrected. The angles of rotation were taken on an SPU-M spectropolarimeter in $CHCl_3$ at 18-22°. The TLC was run on a bound layer of KSK silica gel (fraction smaller than 150 mesh), while the column chromatography was run on the same silica gel (100-150 mesh). Silver benzyl-(1-lauroyl-2-oleoyl-sn-glyceryl)phosphate (I), mp 51-53°, $[\alpha]_D$ +5.0° (C 0.5), was obtained as described in [5].

1-Iodo-2-triphenylmethoxyethane (II, n = 2). A mixture of 2.35 g of ethylene iodohydrin, 15 ml of absolute pyridine and 6.4 h of $(C_6H_5)_3CCl$ was kept for a day at room temperature, diluted with benzene, washed with water, then with 1 N HCl solution, again with water, then in succession with saturated $KHCO_3$ solution, water and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was recrystallized from a 1:10 mixture of ether and petroleum ether. The yield of the trityl derivative was 80%, mp 134-134.5°. Found: C 60.7; H 4.6; I 30.0%. $C_{21}H_{19}OI$. Calculated: C 60.87; H 4.62; I 30.63%.

1-Iodo-3-triphenylmethoxypropane (II, n = 3). Obtained by the tritylation (see above) of trimethylene iodohydrin [6], yield 60%, mp 128-129°. Found: C 61.9; H 4.7; I 28.8%. $C_{22}H_{21}OI$. Calculated: C 61.68; H 4.52; I 29.63%.

Institute of the Chemistry of Natural Compounds, Academy of Sciences of the USSR. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No.1, pp.194-196, January, 1971. Original article submitted April 29, 1970.

1-Iodo-4-triphenylmethoxybutane (II, n = 4). To a suspension of 1.2 g of anhydrous $ZnCl_2$ in 7.6 ml of absolute THF at 0-5°C, with stirring, was added in 1 h 6.3 g of $(C_6H_5)_3CCl$ in portions. The mixture was kept for 1 h at 5° and then overnight at room temperature, diluted with 30 ml of benzene, washed in succession with water, saturated $KHCO_3$ solution, water and brine, dried over Na_2SO_4, and evaporated. The residue was transferred to a column containing 50 g of Al_2O_3 (III activity) and eluted with petroleum ether containing 20% benzene to give 4.7 g (59%) of 1-chloro-4-triphenylmethoxybutane, which, based on the data of TLC (system: petroleum ether – benzene, 2:1, developer conc. H_2SO_4) was pure. A solution of 5.2 g of this material, 2.3 g of anhydrous NaI and 50 mg of $NaHCO_3$ in 25 ml of absolute acetone was kept for 27 h at 80° in a sealed ampul, after which the mixture was poured into ether. washed in succession with water, 5% $Na_2S_2O_3$ solution and brine, dried over Na_2SO_4, and evaporated. The residue was filtered through Al_2O_3 (see above). The yield of the iodide (II, n = 4) was 86%, mp 114.5-115° (from ether – petroleum ether). Found: C 62.8; H 5.3; I 28.3%. $C_{23}H_{23}OI$. Calculated: C 62.44; H 5.20; I 28.73%.

Benzyl-(2-triphenylmethoxyethyl)-(1'-lauroyl-2'-oleoyl-sn-glyceryl)phosphate (III, n = 2). A mixture of 361 mg of the silver salt (I), 183 mg of the iodide (II, n = 2) and 5 ml of absolute toluene was refluxed for 2 h in the dark, with stirring, after which the precipitate was separated on a centrifuge, washed, and filtrate was evaporated. The residue was chromatographed on 30 g of silica gel, and elution with benzene containing 15-20% ethyl acetate gave 320 mg (72%) of the wax-like triphosphate (III, n = 2), which, based on the data of TLC, in the system: benzene – ethyl acetate, 9:1 (developer conc. H_2SO_4), was pure; $[\alpha]_D$ +1.1° (C 4.7). Found: C 74.5; H 9.2; P 3.0%. $C_{61}H_{87}O_9P$. Calculated: C 73.60; H 8.81; P 3.11%.

1-Lauroyl-2-oleoyl-sn-glyceryl-(2'-hydroxyethyl)phosphate (IV, n = 2). A solution of 320 mg of the triester (III, n = 2) and 58 mg of dry NaI in 10 ml of absolute acetone was refluxed in the dark for 1 h, evaporated in vacuo, and the residue was dissolved in ether, filtered, and evaporated. The residue was transferred to a column containing 25 g of siliga gel, and elution with chloroform containing 10% methanol gave 290 mg of 1-lauroyl-2-oleoyl-sn-glyceryl-(2'-triphenylmethoxyethyl)phosphate. A solution of the later in 10 ml of a mixture of $CHCl_3$ – methanol – CH_3COOH – water, 80:13:8:0.3, was stirred for 1 h with 1 ml of Amberlite IR-120 (H^+), after which the resin was suction-filtered and washed, while the filtrate was evaporated. We obtained 160 mg (76%) of the wax-like phospatidyldiol (IV, n = 2); $[\alpha]_D$ +5.9° (C 0.5). For conversion to the calcium salt a 5% solution of the phospholipid in a 3:1 mixture of chloroform and methanol was stirred with excess $CaCO_3$ for 1 h, filtered, evaporated, and dried at 0.01 mm over P_2O_5. Found: C 61.2; H 10.0; P + Ca 7.0%. $C_{34}H_{65}Ca_{0.5}OP$. Calculated: C 61.05; H 9.79; P + Ca 7.63%.

1-Lauroyl-2-oleoyl-sn-glyceryl-(3'-hydroxypropyl)phosphate (IV, n = 3). Using the above-described method, from the silver salt (I) and the 1-iodo-3-trityloxypropane (II, n = 3) was obtained benzyl-(1-lauroyl-2-oleoyl-sn-glyceryl)-(3'-triphenylmethoxypropyl)phosphate (III, n = 3) in 94% yield, $[\alpha]_D$ +2.0° (C 3.9). Found: C 73.6; H 9.0; P 3.1%. $C_{62}H_{89}O_9P$. Calculated: C 73.77; H 8.88; P 3.07%. After debenzylation and acid hydrolysis of the latter we obtained the diol phospholipid (IV, n = 3) in 81% yield; $[\alpha]_D$ +3.8° (C 0.9). For the calcium salt. Found: C 61.0; H 9.6; P + Ca 8.0%. $C_{35}H_{67}Ca_{0.5}O_9P$. Calculated: C 62.55; H 9.88; P + Ca 7.47%.

1-Lauroyl-2-oleoyl-sn-glyceryl-(4'-hydroxybutyl)phosphate (IV, n = 4). From the silver salt (I) and the iodide (II, n = 4) was obtained benzyl-(1-lauroyl-2-oleoyl-sn-glyceryl)-(4'-triphenylmethoxybutyl)phosphate (III, n = 4) in 71% yield; $[\alpha]_D$ +1.4° (C 3). Found: C 74.0; H 9.0; P 3.1%. $C_{63}H_{91}O_9P$. Calculated: C 73.93; H 8.96; P 3.03%. It was converted to the phosphatidyldiol (IV, n = 4) in 72% yield; $[\alpha]_D$ +6.0° (C 1.0). For the calcium salt: Found: C 63.3; H 10.1; P + Ca 7.7%. $C_{36}H_{69}Ca_{0.5}O_9P$. Calculated: C 62.03; H 9.97; P + Ca 7.32%.

All three phosphatidyldiols (IV) have the same R_f values during TLC: 0.54, in the system $CHCl_3$ – methanol – water, 65:26:4; 0.27, in the system $CHCl_3$ – methanol – conc. NH_4OH solution, 70:20:1.5; 0.35, in the system $CHCl_3$ – methanol – CH_3COOH – water, 80:13:8:0.3.

CONCLUSIONS

The following diol analogs of phosphatidylglycerol were synthesized: 1-lauroyl-2-oleoyl-sn-glyceryl-(2'-hydroxyethyl)phosphate, 1-lauroyl-2-oleoyl-sn-glyceryl-(3'-hydroxypropyl)phosphate, and 1-lauroyl-2-oleoyl-sn-glyceryl-(4'-hydroxybutyl)phosphate.

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