INVESTIGATIONS IN THE FIELD OF COMPLEX LIPIDS

XXV. Synthesis of 1,4-Diphosphoinositides

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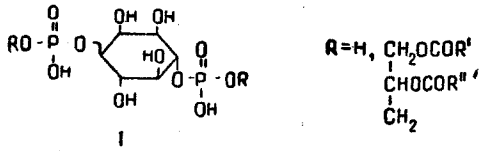
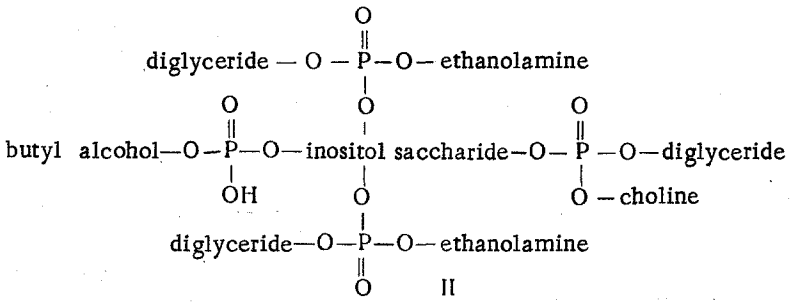
Khimiya Prirodnykh Soedinenii, Vol. 2, No. 4, pp. 230-233, 1966

In mammals, besides the phospholipids of the brain, blood, etc., important physiological functions are performed by diphosphoinositides. Regarding the structure of these compounds it is known that their composition includes myoinositol diphosphates in which the phosphoric acid residues are predominantly in positions 1 and 4 of the inositol ring [1] and, connected with them, α, β-diglycerides containing considerable amounts [2] of stearic acid, together with other fatty acids [1].



I

Investigations of recent years have shown that diphosphoinositide diesters of this type (I) are fragments of more complex compounds present in the animal brain, glycophospholipids (II), which, in their turn, are combined with proteins by various types of bonds [3]:

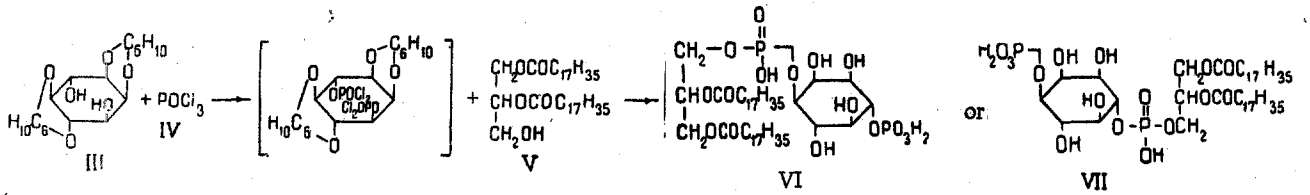


II

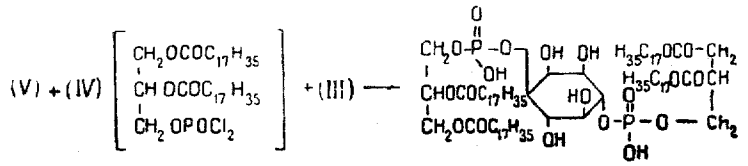
We have synthesized 1,4-diphosphoinositide structures [4] containing both one (VI) or (VII) and two (VIII) molecules of an α, β-diglyceride, the acyl radicals in which are stearic acid residues.

The method of synthesis was based on the condensation of the α, β-diglycerides (V) and the 1,2: 4,5-dicyclohexylidene derivative of myoinositol (III) with phosphorus oxychloride (IV).

By the interaction of a molar amount of the 1,2: 4,5-di-O-cyclohexylidene derivative of myoinositol (III) with phosphorus oxychloride [(IV), 2 moles] and subsequent condensation with a half-molar amount of α, β-distearoylglycerol (V) and elimination of the cyclohexylidene protection by hydrolysis, we obtained a predominantly 1,4-diphosphoinositide structure in which the myoinositol 1,4-diphosphate was connected through a phosphoric ester bond with one molecule of the α, β-diglyceride [(VI) or (VIII)]:

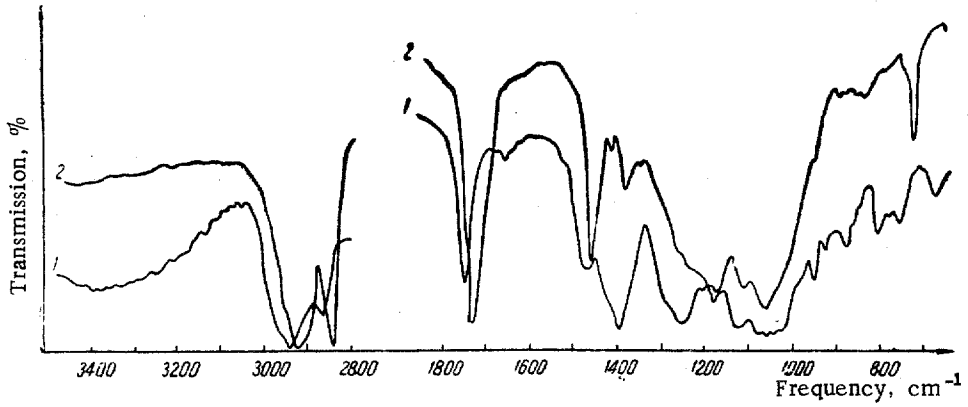


The action on a molar amount of substance (III) of phosphorus oxychloride [(IV), 2 moles] and the same amount of α, β-diglyceride (V), with subsequent removal of the protecting groups at pH 2-3, gave mainly 1,4-bis-[α-α',β-distearoyl)-glycerylphosphoryl]myoinositol (VIII).



VIII

In both cases, the complex mixtures of reaction products were separated by means of adsorption chromatography on silica. Thin-layer chromatography on silica was used to confirm the purity of compounds (VI) or (VII) and (VIII).



IR spectra of the α-(α', β-distearoyl)glyceryl ester of myoinositol 1,4-diphosphate (I) and of 1,4-bis-[α', -β-distearoyl) glycerylphosphoryl]myoinositol (2).

In the main, the IR spectra of the diphosphoinositides (VI) or (VII) and (VIII) have similar absorption frequencies, which confirms their structure: 3400-3200 cm^{-1} (OH), 2960, 2931, 2865, 1475, 1387, 720 (CH, CH_2, CH_3), 1750, 1185 (COOR), 1250 (P=O), 1129, 1050 (POC), 960 cm^{-1} (P-OH), but they differ by the fact that, in contrast to the spectrum of substance (VI) or (VII), the spectrum of (VIII), which contains more active methylene groups (-CH_2CO-), includes their deformation vibrations (1440 cm^{-1}) between the usual bands of the -CH deformation vibrations (1387 and 1475 cm^{-1}). The IR spectra were taken on a UR-10 instrument in tablets of KBr [1.61 mg of substance (VI) or (VII) in 18.4 mg of KBr, and 1.56 mg of substance (VIII) in 75.3 mg of KBr]. NaCl prisms were used for the 680-2000 cm^{-1} region and LiF prisms for the 2000-4000 cm^{-1} region.

Experimental

The α-(α', β-distearoyl) glyceryl ester of myoinositol 1,4-diphosphate (VI) or (VII). Over one hour, a mixture of 1.0 g of 1,2: 4,5-dicyclohexylidene myoinositol [(III), mp 173-174° C] [5] in 20 ml of chloroform containing 5 ml of pyridine was added to a solution of 0.9 g of phosphorus oxychloride (IV) in 15 ml of chloroform cooled to 2° C. The mixture was stirred for one hour at 25° C and was then cooled to 10° C after which a solution of 0.92 g of α, β-distearoylglycerol [(V), mp 70-71° C] [6] in 20 ml of cyclohexane and 3 ml of quinoline was added over 1.5 hr. The mixture was left for 3 hr at 18-20° C, after which 0.5 ml of water was added and the process was continued for another 1.5 hr. Then it was diluted with 150 ml of ether and was shaken with 150 ml of 2 N sulfuric acid at 18-20° C for 2 hr. The ethereal layer was washed with a saturated solution of sodium hydrogen carbonate (2 × 25 ml) and dried with sodium sulfate. The resulting compound (1.1 g) was chromatographed on a column containing 20 g of silica gel that had been heated for 4 hr at 120°-140° C. Substance (VI) was eluted with 300 ml of a mixture of methyl alcohol and chloroform (1 : 1). After elimination of the solvent and drying at 0.4 mm at 20°-22° C for 2.5 hr, a colorless crystalline substance was obained. Yield 0.21 g (16.6%), mp 161°-163° C. IR spectrum*: 3400-3200 s., 2960 v.s., 2931 s., 2865 s., 1750 v.s., 1475 m., 1385 v.s., 1250 s., 1185 w., 1120 m., 1050 m., 960 m., and 720 m. cm^{-1}, R_f 0.15

*s-strong, vs-very strong, m-medium, w-weak

[thin-layer chromatography on silica in the chloroform–methyl alcohol–water (80 : 25 : 5) system; in all cases, the spots on the chromatograms were revealed by spraying the plate with 10% sulfuric acid with subsequent heating at 200°–250° C].

Found, %: C 58.32; H 9.49; P 6.67. Calculated for $C_{45}H_{88}O_{16}P_2$, %: C 58.00; H 9.52; P 6.61.

1, 4-Bis-[α-(α', β-distearoyl)glycerylphosphoryl] myoinositol (VIII). With stirring, a mixture of 4.81 g of α, β-distearoylglycerol (V) in 40 ml of cyclohexane and 8 ml of quinoline was added slowly to a solution of 1.17 g of phosphorus oxychloride (IV) in 20 ml of cyclohexane cooled to 0° C. The mixture was stirred at 20° C for 3 hr and, after being cooled to 5° C, it was treated over one hour with a solution of 1.3 g of 1, 2: 4, 5-dicyclohexylidenemyoinositol (III) in 25 ml of chloroform and 6 ml of pyridine. Stirring was continued at 20° C for 1 hr, and then a few drops of water was added and the mixture was left for 2.5 hr at 20°–22° C. The reaction mixture was diluted with 250 ml of ether and was shaken with 200 ml of 2 N sulfuric acid for 1.5 hr at 18°–20° C. The ethereal layer was separated off, washed with a saturated solution of sodium hydrogen carbonate (2 × 50 ml) and dried with sodium sulfate. The solvent was eliminated and the residue was dried for 2 hr at 0.6 mm and 20°–25° C. The substance obtained (4.36 g) was chromatographed on a column containing 80 g of hydrated silica that had been heated for 4 hr at 120°–140° C. Substance (VIII) was eluted with 500 ml of ether. After elimination of the solvent and drying for 1.5 hr at 20°–23° C/0.4 mm, a colorless crystalline substance was obtained. Yield 0.88 g (14.8%), mp 131°–131.5° C. IR spectrum: 3400–3200 w., 2959 v.s., 2933 s., 2861 v.s., 1745 v.s., 1480 v.s., 1440 w., 1382 m., 1240 w., 1180 s., 1120 m., 1065 s., 960 w., 718 m. cm^{-1}, R_f 0.69 [thin-layer chromatography on silica in the chloroform–methyl alcohol–water (80 : 25 : 5) system]. Substance (VIII) is readily soluble in chloroform and ether, sparingly soluble in alcohol, and insoluble in water.

Found, %: C 64.98; H 10.36; P 4.02. Calculated for $C_{84}H_{162}O_{20}P_2$, %: C 64.91; H 10.49; P 3.38.

Summary

The synthesis of the following 1, 4-diphosphoinositides has been effected: the α-(α', β-distearoyl) glyceryl ester of myoinositol 1, 4-diphosphate (VI) or (VII) and 1, 4-bis-[α-(α', β-distearoyl) glycerylphosphoryl] myoinositol (VIII).

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8 October 1965

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