INVESTIGATIONS IN THE FIELD OF COMPLEX LIPIDS

Synthesis of L-(+)-α'-oleoyl-β-linoleoyl-α-glycerylphosphorylethanolamine

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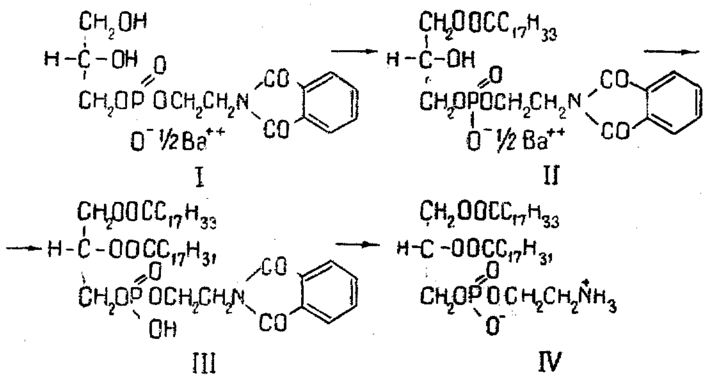
In a continuation of work [1] on the preparation of monoacid α-cephalins by the direct acylation of the barium salt of L-α-glycerylphosphoryl-N-phthaloylethanolamine, we have synthesized an unsymmetrical L-(+)-α-phosphatidylethanolamine (cephalin) with residues of oleic and linoleic acids, which are widely distributed in natural sources, using the different reactivities of the α- and β-hydroxyl groups of glycerol:



The esterification of the α'-hydroxyl group of the barium salt of L-α-glycerylphosphoryl-N-phthaloylethanolamine (I) [2] with oleoyl chloride in dimethylformamide in the presence of catalytic amounts of pyridine at 18°–20° C led to the α-monoacyl derivative of the barium salt (II). Acylation of the β-hydroxyl group in the same way but at 55°–60° C with linoleoyl chloride gave the corresponding N-phthaloylcephalin (III). Removal of the phthaloyl protection with hydrazine hydrate by boiling in methanol gave L-(+)-α'-oleoyl-β-linoleoyl-α-glycerylphosphorylethanolamine (IV).

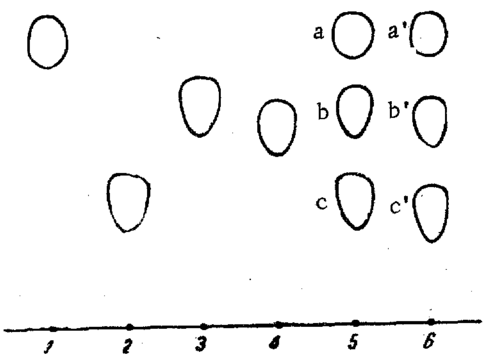


Fig. 1. Course of the synthesis of L-(+)-α'-oleoyl-β-linoleoyl-α'-glycerylphosphorylethanolamine as shown by chromatography in a thin layer of silica in the diisobutyl ketone–acetic acid–water (8 : 5 : 1) system. 1) Oleic or linoleic acid; 2) L-α'-glycerylphosphoryl-N-phthaloylethanolamine; 3) L-α'-oleoyl-β-lineoleoyl-α-glycerylphosphoryl-N-phthaloylethanolamine; 4) L-(+)-α'-oleoyl-β-linoleoyl-α-glycerylphosphorylethanolamine; 5) reaction mixtures in the formation of the β-mono- and α,β-diacyl derivatives of the barium salt (I): a) fatty acids; b) monoacyl derivatives; c) diacyl derivative; 6) hydrazinolysis reaction mixture: a') fatty acid; b') phosphatidylethanolamine, c') lysophosphatidylethanolamine.

The final cephalin (IV) and the intermediates (II) and (III) were isolated in the acid form by means of adsorption chromatography on silica. The course of the chromatography, the individuality of the substances, and the selectivity of the reactions were studied in a thin layer of silica with various mobile systems [ether-heptane (1 : 1); diisobutyl ketone-acetic acid-water (8 : 5 : 1)].

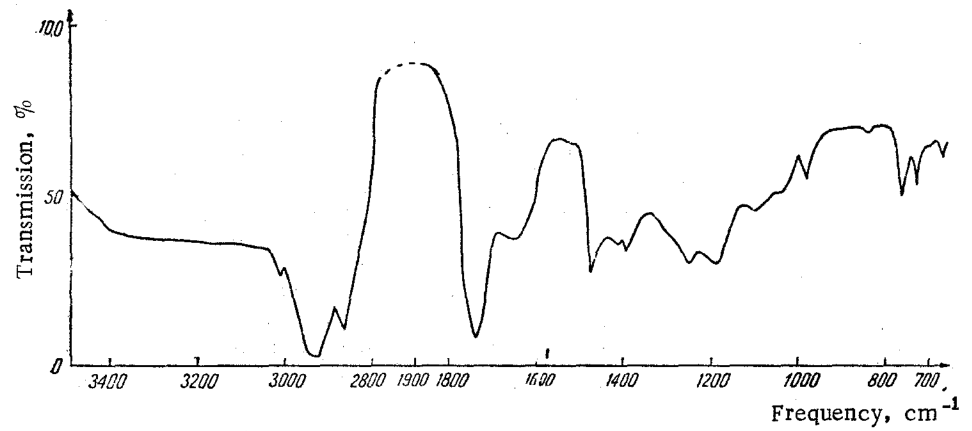


Fig. 2. IR spectrum of L-(+)-(α'-oleoyl-β-linoleoyl)-α-glycerylphosphorylethanolamine.

The study of the selective and direct acylation of the barium salts (I) and (II) and also that of the hydrazinolysis in the formation of the cephalins (III) → (IV) by means of thin layer chromatography show that these reactions take place in a single direction (Fig. 1).

In the selective esterification of the barium salt (I), the reaction was accompanied by the formation of considerable amounts of the fully acylated substance, which decreased the yield of the monacyl derivative of the barium salt (II) to 20.8% . Substitution of the β-hydroxyl group did not take place to completion. The yield of (III) was 73.5%. The reaction mixture was found to contain considerable amounts of the initial linoleic acid and of compound (II).

The hydrazinolysis (III → IV) took place with a low yield (44.1%) which is due to the splitting off of a fatty acid residue as a side reaction with the formation of a lysocephalin. On the basis of the data of [2], we assume that the ester bond which ruptures is that in the β position (Fig. 2).

The IR spectrum of α'-oleoyl-β-linoleoyl-α-glycerylethanolamine (IV) shows absorption bands characteristic for glycerol phosphatides [3] with well-defined frequencies at: 3000 cm^{-1} ($\overset{+}{N}H_3$), 3012 (CH in CH=CH), 2963, 2933, 2869, 1479, 1389, 725 (CH, CH_2, CH_3), 1741, 1178 (C=O and CO in COOR), 1660 (C=C in cis HC=CH), 1245 (P=O), 1096, 1040 (POC), 692 cm^{-1} (CH in cis HC=CH). The IR spectrum was taken on a UR-10 instrument with the substance in the form of a film 0.011 mm thick. NaCl prisms were used for the 680–2000 cm^{-1} region and LiF for the 2000–4000 cm^{-1} region.

Experimental

L-α'-Oleoyl-α-glycerylphosphoryl-N-phthaloylethanolamine (II). Oleoyl chloride (4.15 g; bp 156°–158° C at 0.5 mm) was added to 4.0 g of the barium salt of L-α-glycerylphosphoryl-N-phthaloylethanolamine (I), 4.83 ml of pyridine, and 60 ml of dimethylformamide, and the mixture was left for 60 hr at 18°–20° C. At the end of this time, 75 ml of 1 N hydrochloric acid was added to the reaction mixture with stirring. The substance was extracted with ether (three 60-ml portions), the solution was washed with 1 N hydrochloric acid (three 35-ml portions) and with saturated sodium hydrogen carbonate solution (two 25-ml portions), and was dried with sodium sulfate. The ether was distilled off, and the residue (4.0 g) was dissolved in 20 ml of chloroform and chromatographed on 45 g of hydrated silica calcined for 2 hr at 140°C. The substance was eluted with 70 ml of a mixture of chloroform and methyl alcohol (4 : 1). This gave an oily substance soluble in chloroform, ether, and methanol. Yield 1.31 g (20.8%).

The individuality of the compound was confirmed by thin-layer chromatography on silica supported on gypsum. In the heptane–ether (1 : 1) system, the substance remained at the start, and in the diisobutyl ketone–acetic acid–water (8 : 5 : 1) system it had R_f 0.57. The spots of the substances on the chromatograms were revealed by heating the plates at 120° -200° C after spraying them with 10% sulfuric acid.

Found, %: N 2.07; P 4.89. Calculated for $C_{31}H_{48}O_9NP$, % : N 2.29; P 5.09.

L-α'-Oleoyl-β-linoleoyl-α-glycerylphosphoryl-N-phthaloylethanolamine (III). A solution of 1.30 g of substance (II) in 25 ml of methanol was treated with a saturated methanolic solution of barium hydroxide until the pH was 8–9. The excess of barium ions was removed with carbon dioxide. The solution was filtered, the methanol was distilled off, and the residue was dried at 0.15 mm for 45 min. A mixture of the resulting 1.46 g of the barium salt of L-α'-oleoyl-α-glycerylphosphoryl-N-phthaloylethanolamine (II) and 5.5 ml of pyridine in 70 ml of dimethylformamide was treated with 3.7 g of linoleoyl chloride (bp 145°–147° C at 0.21 mm). The subsequent treatment was the same as in the case of (II). The resulting oily substance was readily soluble in chloroform, benzene, and ether, soluble to a limited extent in ethyl and methyl alcohols and gave stable emulsions with water. Yield 1.21 g (73.5%). In the heptane–ether (1:1) system, the substance remained at the start and in diisobutyl ketone–acetic acid–water (8:5:1) it had R_f 0.85.

Found, %: N 1.32; P 3.49. Calculated for $C_{49}H_{78}O_{10}NP$, %: N 1.61; P 3.56.

L-(+)-α'-Oleoyl-β-linoleoyl-α-glycerylphosphorylethanolamine (IV). Hydrazine hydrate (0.61 ml) was added to 1.20 g of (III) in 82 ml of methanol. The temperature of the reaction mixture was raised to 60° C over 1.5 hr and was kept there for 1 hr. The mass was filtered at 50° C, acidified with 7.5 ml of acetic acid, and evaporated at 20° C. A solution of the residue in 10 ml of chloroform was cooled to –20° C and filtered, and was chromatogoraphed on 40 g of hydrated silica. The cephalin (IV) was eluted with 75 ml of a mixture of chloroform and methanol (9:1). The solvents were removed, and the residue was dried at 0.15 mm for 1 hr. The resulting wax-like substance was readily soluble in chloroform, benzene, and ether and soluble to a limited extent in acetone and ethyl and methyl alcohols, while with water it gave stable emulsions. Yield 0.45 g (44.1%). In the heptane–ether (1:1) system, the substance remained at the start and in the diisobutyl ketone–acetic acid–water (8:5:1) system it had R_f 0.78; $[\alpha]_D^{20}$ + 7.32° (c 10, chloroform).

Found, %: N 2.11; P 3.87. Calculated for $C_{11}H_{76}O_8NP$, %: N.89; P 3.75.

Summary

1. The synthesis of L-(+)-α'-oleoyl-β-linoleoyl-α-glycerylphosphorylethanolamine has been effected.

2. During the investigations the following substances were isolated and characterized: L-α'-oleoyl-α-glycerylphosphoryl-N-phthaloylethanolamine and L-α'-β-linoleoyl-α-glycerylphosphoryl-N-phthanoylethanolamine.

REFERENCES

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