INVESTIGATIONS IN THE FIELD OF COMPLEX LIPIDS

XXIV. Synthesis of Optically Active Homoacid and Heteroacid Lecithins

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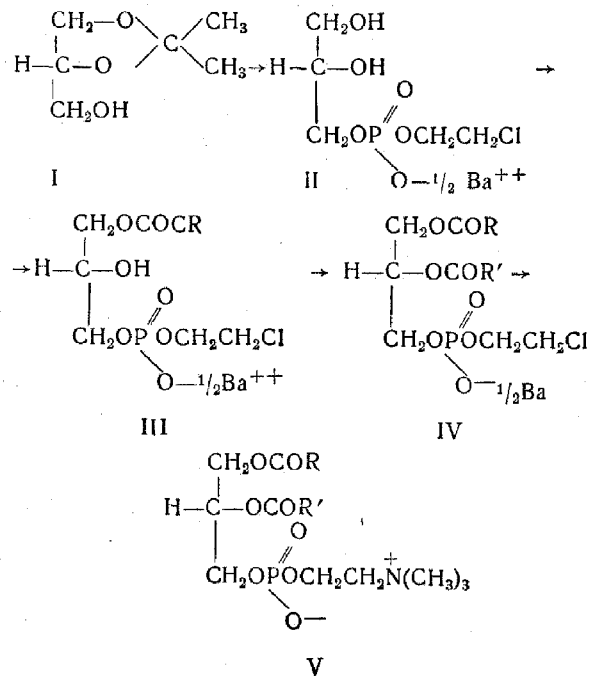
Khimiya Prirodnykh Soedinenii, Vol. 2, No. 4, pp. 225-229, 1966

The present communication describes the synthesis of symmetrical and unsymmetrical α-lecithins of the natural L-configuration with oleic and linoleic acid residues, which are widely distributed in lecithins isolated from animal cells [1].

This synthesis is based on the direct acylation of barium salts of glycerophosphoric derivatives, and therefore differs from the preparation of L-α-phosphatidylcholines via the intermediate formation of D-α, β-diglycerides that we have used previously [2].



D-α,β-Isopropylidineglycerol (I) was phosphorylated with phosphorus oxychloride in the presence of quinoline, and the resulting dichloride, without isolation, was condensed with ethylene chlorohydrin under the action of pyridine; the isopropylidine protection was removed by acid hydrolysis (pH 2-3) and the diphosphate was precipitated in the form of the barium salt (II). The esterification of substance (II) with linoleoyl chloride in dimethylformamide in the presence of pyridine at 55°-60° C led to the complete substitution of the hydroxy groups of the barium salt (II) with linoleic acid residues [(IV), R = R' = $C_{17}H_{31}$]. In view of the greater reactivity of the α-hydroxy group of glycerol, the same reaction with oleoyl chloride at 18°-20° C gave the barium salt of L-α-(α'-oleoyl)-glycerylphosphoryloxyethyl chloride [(III), R = $C_{17}H_{33}$]. The acylation with lineolyl chloride of substance [(III), R = $C_{17}H_{33}$] in dimethylformamide under the catalytic action of pyridine (55°-60° C) gave the heteroacid salt [(IV), R = $C_{17}H_{33}$, R' = $C_{17}H_{31}$].

As the results of thin-layer chromatography on silica show, the partial and selective acylation of the barium salts (II) and (III) take place nonuniformly and generally give mixtures of mono- and diacyl derivatives of the barium salts [(III), R = $C_{17}H_{33}$ and $C_{17}H_{31}$; (IV), R = R' = $C_{17}H_{31}$ and R = $C_{17}H_{33}$, R' = $C_{17}H_{31}$], which are separated by means of absorption chromatography on silica (Fig. 1). The reaction of the homoacid and heteroacid derivatives of L-α-glyceryl-phosphoryloxyethyl chloride [(IV), R = R' = $C_{17}H_{31}$ and R = $C_{17}H_{33}$, R' = $C_{17}H_{31}$] with trimethylamine in benzene at 60° C gave the L-(+)-α-phosphatidylcholines (lecithins) [(V), R = R' = $C_{17}H_{31}$ and R = $C_{17}H_{33}$, R' = $C_{17}H_{31}$]. In the synthesis of the lecithins (V), the trimethylaminolysis is accompanied by the splitting off of a fatty acid residue, apparently that located in the β-position, with the formation of a lysolecithin in a similar manner to that demonstrated by Baer, et al., [3], and to the hydrazinolysis reactions of the cephalins [4] (cf. Fig. 1).

The isolation of the final lecithins (V) and the intermediates (III) and (IV) in the acid form was carried out by means of absorption chromatography on silica. The course of the chromatographic process, the purity of the substances, and the occurrence of the reactions were studied by means of thin-layer chromatography on silica with the mobile systems ether–heptane (1:1) and diisobutyl ketone–acetic acid–water (40:25:5).

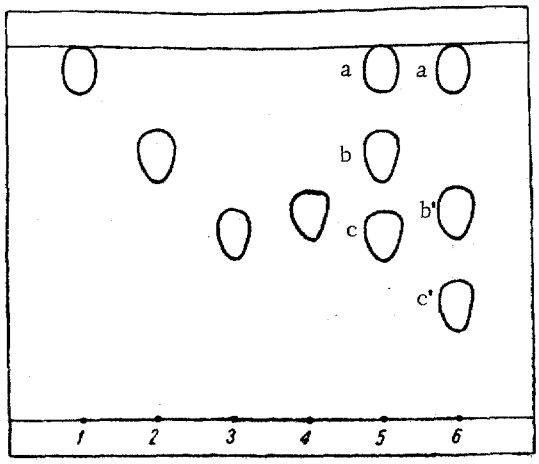


Fig. 1. Course of the synthesis of L-(+)-lecithins as shown by thin-layer chromatography on silica in the diisobutyl ketone–acetic acid–water (40: 25: 5) system. 1) Oleic or linoleic acid; 2) L-α-(α'-oleoyl-β-linoleoyl) glycerylphosphoryloxy-ethyl chloride and L-α-(α'-β-dilinoleoyl) glycerylphosphoryloxylethyl chloride; 3) L-α-(α'-oleoyl) glycerylphosphoryloxyethyl chloride; 4) L-α-(α'-oleoyl-β-linoleoyl) glycerylphosphorylcholine and L-α-(α', β-dilinoleoyl) glyceryl-phosphorylcholine; 5) reaction mixtures from the formation of α-mono- and α, β-diacyl derivatives of the barium salt (II): a) fatty acids; b) monoacyl derivative; c) diacyl derivative; 6) trimethylaminolysis reaction mixture: a') fatty acid; b') phosphatidylcholines; c') lysophosphatidylcholines.

The IR spectra of the α-phosphatidylcholines [(V), R = R' = $C_{17}H_{31}$ and R = $C_{17}H_{33}$, R' = $C_{17}H_{31}$] (Fig. 2) are practically identical and differ little from the spectra of the cephalins that we prepared previously [4], with the exception of the fact that the P=O band (1245 cm^{-1}) has undergone a certain displacement in the lecithins (1275 cm^{-1}).

The spectra of the substances in the form of films 0.011 mm thick were taken on a UR-10 instrument with NaCl prisms for the 680–2000 cm^{-1} region and LiF prisms for the 2000–4000 cm^{-1} region.

Experimental

Barium salt of L-α-glyceryphosphoryloxyethyl chloride (II). With stirring, 19.4 g of quinoline and 15.5 g of D-(+)-α, β-isopropylidineglycerol [(I), bp 91°–92°C at 25 mm, $[\alpha]_D^{20}$ +13.6° (in substance), n_D^{20} 1.4348] were added to 18.4 g of phosphorus oxychloride cooled to −15° C [5]. After 20 min, a mixture of 66.7 ml of pyridine and 13.4 ml of ethylene chlorohydrin (bp 128°–129° C) was added and the resulting mixture was stirred for 30 min at −10° C and for 1 hr at 18°–20° C. Then it was treated with 150 ml of water and left for 24 hr. It was extracted with chloroform (5 × 70 ml) and the pH of the aqueous solution was brought to 8–9 by the addition of a saturated aqueous solution of barium hydroxide. The excess of barium ions was eliminated with carbon dioxide and the precipitate that had deposited was filtered off. The solution was evaporated to dryness. The residue was treated with 100 ml of methanol. The insoluble residue was separated off and the solvent was distilled off. The substance was treated twice more in the same way and was dried at 20°–25°C/0.05 mm for 3 hr. The compound was soluble in water, methyl and ethyl alcohols, and dimethylformamide and insoluble in ether and chloroform. Yield 6.5 g (20.1%).

Found, %: C 19.60; H 3.64; Ba 22.20; Cl 11.70. Calculated for $C_5H_{11}O_6Ba$ 1/2 ClP, %: C 19.87; H 3.67; Ba 22.72; Cl 11.79.

L-α (α', β-Dilinoleoyl) glycerylphosphoryloxyethyl chloride [(IV), R = R' = $C_{17}H_{31}$]. A mixture of 1.5 g of the barium salt (II), 1.47 ml of pyridine, and 50 ml of dimethylformamide was treated with 3.2 g of linoleoyl chloride (bp 152°–154° C at 0.5 mm) and heated at 50° C for 60 hr. With stirring, the reaction mixture was poured into 100 ml of 1 N hydrochloric acid at 0° C and the substance was extracted with ether (3 × 50 ml). The organic layer was washed with 0.15 N hydrochloric acid (4 × 30 ml) and with saturated sodium hydrogen carbonate solution (2 × 25 ml), and was dried with sodium sulfate. The ether was distilled off. The residue (4.35 g) in 25 ml of chloroform was chromatograph-

ed on 50 g of silica gel activated at 140° C for 2 hr. The L-α-(α', β-dilinoleoyl) glycerylphosphoryloxyethyl chloride [(IV), R = R' = $C_{17}H_{31}$] was eluted with a mixture of 80 ml of methanol and 320 ml of chloroform. The solvent was eliminated and the residue was dried at 0.15 mm for 2 hr. This yielded an oily substance soluble in chloroform, ether, and benzene, and insoluble in water. Yield 2.8 g (66.7%).

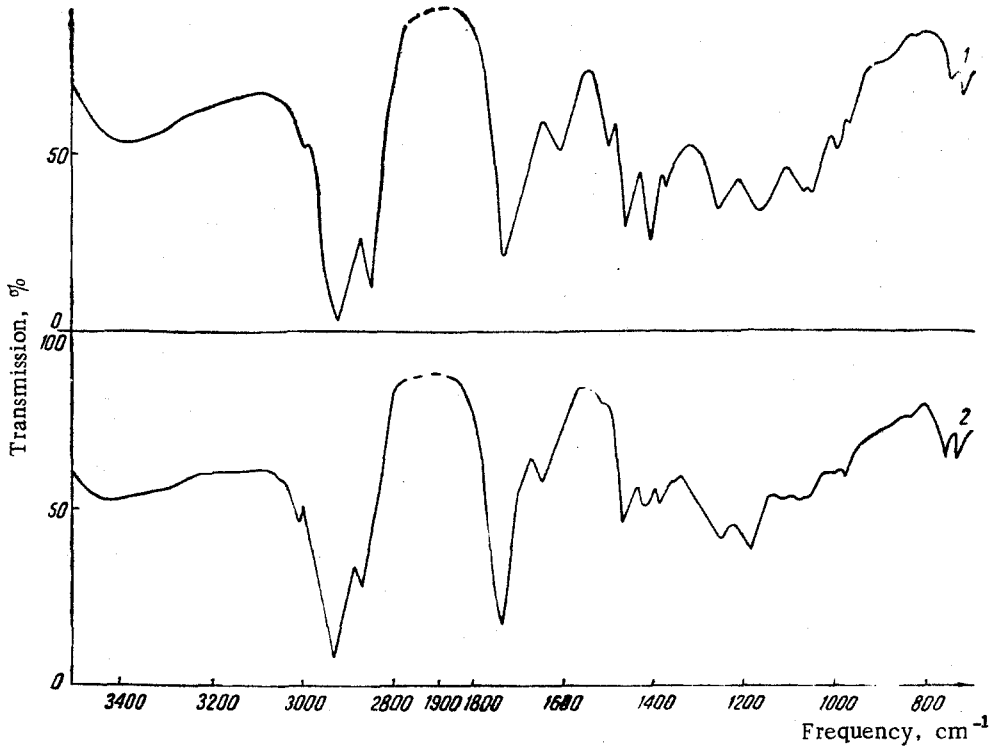


Fig. 2. IR spectra of L-(+)-α-phosphatidylcholines: 1) α-(α'-oleoyl-β-linoleoyl)glyceryl-phosphorylcholine; 2) α-(α', β-dilinoleoyl)glycerylphosphorylcholine.

The purity of the substance was checked by thin-layer chromatography on silica fixed with calcium sulfate in the ether–heptane (1:1) system (substance at the start) and the diisobutyl ketone–acetic acid–water (40:25:5) system (R_f 0.70). In all cases, the spots on the chromatogram were revealed by spraying the plates with 10% sulfuric acid with subsequent heating at 200°–250° C.

Found, %: Cl 4.17; P 3.75. Calculated for $C_{41}H_{72}O_8ClP$, %: Cl 4.65; P 4.09.

L α-(α'-Oleoyl) glycerylphosphoryloxyethyl chloride [(III), R = $C_{17}H_{33}$]. A solution of 1.0 g of the barium salt (II) in 50 ml of dimethylformamide and 2 ml of pyridine was treated with 2.6 g of oleoyl chloride (bp 149°–151° C at 0.42 mm) and left for 72 hr at 20° C. The subsequent treatment was carried out as described for substance [(IV), R = =R' = $C_{17}H_{31}$].

The product was an oily substance which was readily soluble in chloroform, benzene, ether, and ethyl and methyl alcohols and formed a stable emulsion with water. Yield 0.7 g (53.5%). It was chromatographed in the systems ether–heptane (1:1) (substance at the start) and diisobutyl ketone–acetic acid–water (40:25:5) (R_f 0.50).

Found, %: Cl 6.95; P 6.07. Calculated for $C_{23}H_{44}O_7ClP$, %: Cl 7.12; P 6.20.

L-α-(α'-Oleoyl-β-linoleoyl) glycerylphosphoryloxyethyl chloride [(IV), R = $C_{17}H_{33}$, R' = $C_{17}H_{31}$]. A saturated solution of barium hydroxide in methyl alcohol was added to 0.7 g of substance [(III), R = $C_{17}H_{33}$] in 30 ml of methanol to give a pH of 8–9. The excess of barium ions was eliminated with carbon dioxide. The solution was filtered and the methyl alcohol was evaporated off. Then the reaction was carried out under the conditions described for compound [(IV), R = R' = $C_{17}H_{31}$], starting from 2.5 g of the barium salt [(III), R = $C_{17}H_{33}$], 2.8 ml of pyridine in 50 ml of dimethylformamide, and 2.0 g of linoleoyl chloride.

The product was an oily substance which was readily soluble in benzene, chloroform, ether, methanol, and acetone and formed stable emulsions with water. Yield 0.7 g (35.1%). On chromatography in the ether–heptane (1:1) system the substance remained at the start, and in the diisobutyl–acetic acid–water (40:25:5) system, it had

R_f 0.70.

Found, %: Cl 4.41; P 3.89. Calculated for $C_{41}H_{74}O_8ClP$, %: Cl 4.67; P 4.08.

L-(+)-α-(α'-β-Dilinoleoyl) glycerylphosphorylcholine [(V), R = R' = $C_{17}H_{31}$]. A solution of 2.9 g of substance [(IV), R = R' = $C_{17}H_{31}$] in 12 ml of benzene and 15 ml of trimethylamine was heated in a sealed tube (70 × 25 mm) for 48 hr at 60° C. Then the tube was opened and the unchanged trimethylamine was eliminated. The solvent and the remaining traces of trimethylamine were distilled off. The substance was dried at 0.18 mm for 2 hr. The residue (4.05 g) in 25 ml of benzene was chromatographed on 45 g of silica gel activated at 140° C for 2 hr. The lecithin [(V), R = R' = = $C_{17}H_{31}$] was eluted with a 20% solution of methanol in chloroform (85 ml). The solvent was eliminated and the substance was dried at 0.12 mm for 2 hr. The product was an oily substance which was readily soluble in chloroform, ethyl and methyl alcohols, ether, and acetone and formed stable emulsions with water. Yield 0.9 g (30.2%).

The purity of the substance was checked by thin-layer chromatography on silica fixed with calcium sulfate in the systems ether–heptane (1:1) (substance at the start) and diisobutyl ketone–acetic acid–water (40:25:5) (R_f 0.55), $[\alpha]_D^{20}$ + 4.89° (c 10; chloroform).

Found, %: N 1.67; P 3.94. Calculated for $C_{44}H_{80}PN$, %: N 1.85; P. 3.91.

L-(+)-α(α'-Oleoyl-β-linoleoyl) glycerylphosphorylcholine [(V), R = $C_{17}H_{33}$, R' = $C_{17}H_{31}$]. This substance was obtained by the method described for the lecithin [(V), R = R' = $C_{17}H_{31}$], starting from 0.13 g of substance [(IV), R = $C_{17}H_{33}$, R' = $C_{17}H_{31}$] in 10 ml of benzene and 5 ml of trimethylamine. It formed an oily substance which was readily soluble in chloroform, ethyl and methyl alcohols, benzene, and ether and gave stable emulsions with water. Yield 0.07 g (51.9%).

When chromatographed in the ether–heptane (1:1) system the substance remained at the start, and in the diisobutyl ketone–acetic acid–water (40:25:5) system it had R_f 0.55; $[\alpha]_D^{20}$ + 4.55° (c 10; chloroform).

Found, %: N 1.71; P 3.18. Calculated for $C_{22}H_{82}O_8NP$, %: N 1.85; P 3.90.

Summary

1. The syntheses of L-(+)-α-(α', β-dilinoleoyl) and -(α'-oleoyl-β linoleoyl) glycerylphosphorylcholines have been effected.

2. During the work involved, the barium salts of L-α-glycerylphosphoryloxyethyl chloride, L-α-(α', β-dilinoleoyl) glycerylphosphoryloxyethyl chloride, L-α-(α'-oleoyl) glycerylphosphoryloxyethyl chloride, and L-α-(α'-oleoyl-β-linoleoyl) glycerylphosphoryloxyethyl chloride have been isolated and characterized.

REFERENCES

1. D. Green and J. Hefefi, Sci., 133, 7, 1961.
2. V. I. Shvets, et al., ZhOKh, 34, 3303, 1964.
3. E. Baer, D. Buchnea, and A. Newcombe, J. Am. Chem. Soc. 78, 232, 1956.
4. V. I. Shvets, V. A. Klopova, and N. A. Preobrazhenskii, KhPS [Chemistry of Natural Compounds], 80, 1965.
5. E. Baer and H. Fischer, 128, 463, 1939.

8 October 1965

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