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An Improved Procedure for the Synthesis of Enantiomeric α-Lecithins

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The procedure by Baer and Kates for the synthesis of the racemic and enantiomeric forms of fully saturated α-lecithins has been shortened considerably and the modified parts of the procedure are described herein. The molecular weight of synthetic L-α-(dipalmitoyl)-lecithin in methanolic solution was determined by the vapor pressure method and the values observed were close to that calculated for the monomolecular form. It is therefore concluded that our method of synthesis produces monomeric α-lecithins.

A recent publication from this Laboratory[1] has described a generally applicable procedure for the synthesis of the enantiomeric forms of α-lecithins. The synthesis was as follows: The α,β-diglyceride (I) was phosphorylated with monophenylphosphoryl dichloride (II) and bis-α,α-(diacylglyceryl)-phenylphosphate in the presence of one mole of pyridine, giving rise to the formation of diacyl-α-glycerylphenylphosphoryl chloride (IIa). Without isolating the intermediate compound II, the reaction mixture was immediately treated with choline chloride in the presence of a large excess of pyridine. The water insoluble part of the reaction product consisted almost entirely of a mixture of IIa and of diacyl-α-glycerylphenylphosphorylcholine chloride (III), which was separated *via* the reinecke salt. The α-lecithins were obtained by replacing the reineckate ion of compound III with the sulfate ion, and by removing the phenyl group by catalytic hydrogenolysis. This procedure had served its purpose in giving the first pure synthetic α-lecithins. It was realized, however, that the isolation and purification of compound III by means of the reineckate and its subsequent conversion into the sulfate was too cumbersome for routine preparations of the α-lecithins. It has now been found that the separation of IIa and III can be accomplished more conveniently by the use of the appropriate organic solvents. The experimental details of the procedure vary slightly with the length of the carbon chain of the fatty acid substituents, The new procedure has the further advantage that the diacyl-α-glycerylphenylphosphorylcholine is obtained directly with an anion (Cl') which does not interfere with the subsequent removal of the phenyl group by catalytic hydrogenolysis. For the purpose of comparison, the sequence of reactions for both the original and modified procedure is shown in the reaction scheme. The α-lecithins prepared by the modified procedure started to sinter at higher temperatures than those prepared by the original method, but in all other respects their properties were as reported previously.

For reasons of brevity only the synthesis of L-α-lecithins and, furthermore, only those parts in which the synthesis of these substances differs from the original procedure, will be described in the experimental part of this paper. With regard to the other steps of the synthesis our earlier publication has to be consulted.[1] The modified procedure is equally well suited for the preparation of D- and D,L-α-lecithins. D-α-(Dimyristoyl)-lecithin, the first representative member of the D-series to become known, was prepared and its use as a component of the antigen in the serodiagnosis of syphilis was studied. The experimental details of its synthesis and serological investigation will be reported elsewhere.

The authors have had the opportunity of observing the stability of the synthetic α-lecithins under normal conditions of storage over a period of several years. It was noticed that the lecithin preparations which had been recrystallized from hot (60–80°) diisobutyl ketone were inclined to decompose more readily, with the liberation of trimethylamine, than either crude lecithins or lecithins which had been reprecipitated at room temperature from chloroform by the addition of acetone. In general the modified procedure yields the lecithins in such a state of purity that there is no need for further purification by recrystallization.

Recently Fleury and Guitard,[2,3] studying the hydrolysis of egg lecithin in cold methanolic potassium hydroxide, observed the formation of a polymeric glycerophosphoric acid (glycérophosphatogèn, GPG) which in contrast to the normally found products of hydrolysis, α- and β-glycerophosphoric acid, does not form an insoluble lead salt. During a study of the hydrolysis of synthetic α-lecithins in methanolic sodium hydroxide at 37° we had also observed the formation of an organic phosphate which could not be precipitated as a lead salt.[4]

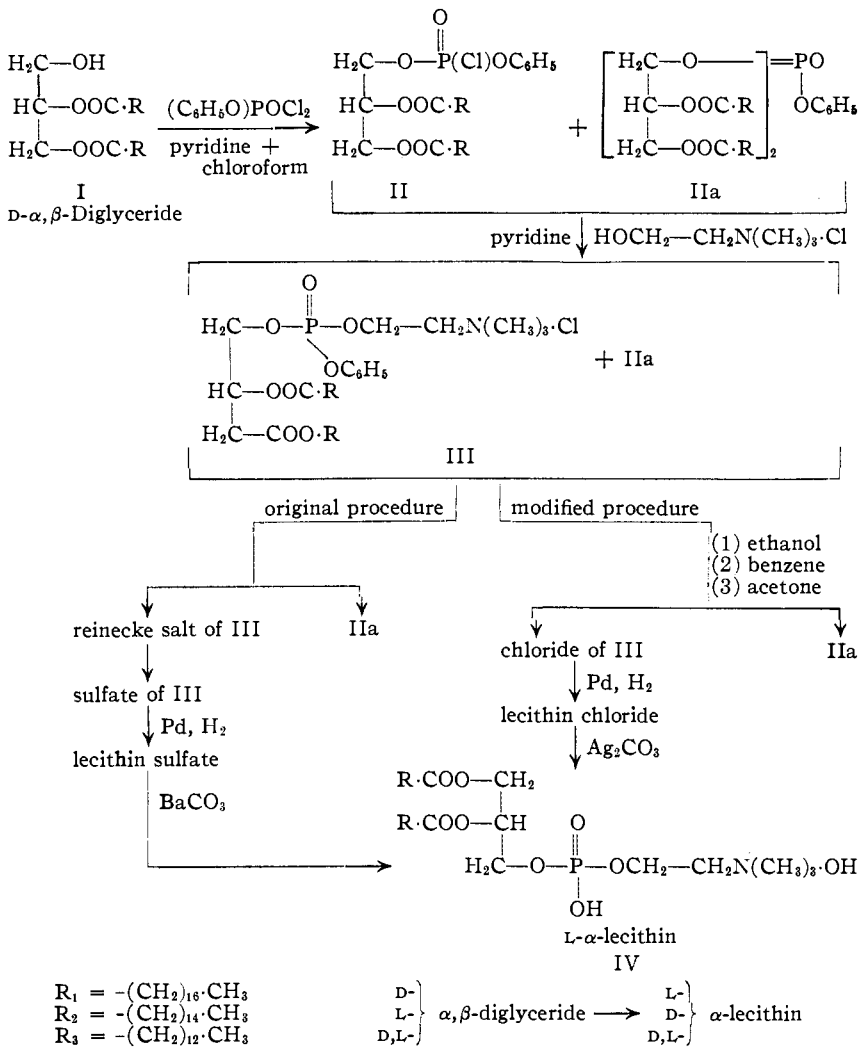
Although no investigation of its structure was made at the time there seems to be little doubt that this compound is either identical with or very similar to the GPG of Fleury and Guitard. The formation of GPG from a natural lecithin induced Fleury to propose a polymeric structure for these substances. Although the lecithins obtained by our method of synthesis were identical with their natural prototypes they were assumed to be monomolecular and thus it became necessary to determine their molecular size. The dipalmitoyllecithin (DPL) was selected for this purpose because it occurs in nature and occupies the central position of the three homologous α-lecithins synthesized in this Laboratory. Dr. I. E. Puddington of the National Research Council, Ottawa, kindly consented to carry out a molecular weight determination of the DPL by the vapor pressure method and gave us permission to report his results. Dr. Puddington found that the molecular weight of DPL in methanol at low concentration (0.9%) was close to the theoretical value of the lecithin monomer, and that even at a comparatively high concentration (4.7%) it was not associated excessively. This confirmed our assumption that our method of synthesis yields

(1) E. Baer and M. Kates, THIS JOURNAL, 72, 942 (1950).

(2) P. Fleury, *Bull. soc. chim. biol.*, 30, 519, 521 (1948).

(3) P. Fleury and H. Guitard, *ibid.*, 30, 525, 528 (1948).

(4) E. Baer and M. Kates, *J. Biol. Chem.*, 185, 615 (1950).



the α-lecithins in the monomolecular form. Furthermore the values obtained by Dr. Puddington for the synthetic lecithins are in agreement with those reported for a catalytically hydrogenated liver lecithin in boiling methanol (700, 810) by Levene and Simms,[5] and for egg lecithin in boiling ethanol (766, 797) by Price and Lewis,[6] or in ethanol at room temperature (approx. 800) by Faure and Legault-Demare.[7]

Experimental Part

The two-step phosphorylation of the D-α,β-diglycerides (0.02 mole of distearin, 0.021 mole of dipalmitin and 0.02 mole of dimyristin) and the isolation of the mixture of phosphorylation products IIa and III were carried out as described in our former publication[1] on page 945, second column, line 20 to 53; page 946, second column, line 68 to page 947, first column, line 14, and page 947, second column, line 66 to page 948, first column, line 10, respectively. The separation of the phosphorylation products and the isolation of the diacyl-L-α-glycerylphenylphosphorylcholine chlorides (III, $R_1 \rightarrow R_3$) were carried out as follows.

Distearoyl-L-α-glycerylphenylphosphorylcholine Chloride (III, R_1).—The mixture of phosphorylation products (IIa R_1 and III R_1; 13.4 g.) suspended in 55 ml. of 99% ethanol was placed in a water-bath at 40° and stirred for a period of 10 minutes. The solid[8] and extract were separated by centrifugation, and the extraction with ethanol (55 ml.) was repeated twice more. The combined extracts (approx. 165 ml.) were brought to dryness under reduced pressure at a bath temperature of 35–40°.[9] The residue (8.05 g.) was dissolved in 32 ml. of warm (50–60°) benzene, and the solution, while still warm, was centrifuged to remove small amounts of insoluble material. The precipitate was washed twice with 10-ml. portions of warm benzene. The combined benzene solutions were permitted to attain room temperature (25°) gradually and were placed in an ice-box (+6°) to complete the crystallization. The solid which was collected with suction on a buchner funnel, washed with 25 ml. of cold (+6°) benzene, and dried *in vacuo* (0.1 mm.) over solid sodium hydroxide, weighed 6.64 g. (36.8% of theory) and was already fairly pure distearoyl L-α-glycerylphenylphosphorylcholine chloride. (Found: N, 1.50; P, 3.29; m.p. 130–131°; $[\alpha]^{25}D$ +2.3° in anhydrous chloroform, *c* 7.5). For analytical purposes a small amount of the substance (400 mg.) was recrystallized from a boiling mixture (25 ml.) of acetone (20 vol.) and chloroform (1 vol.). The recovery of compound III, R_1 was 87.5%. The substance started to sinter at 80°, and melted with the formation of a meniscus at 131–132°; $[\alpha]^{25}D$ +2.5° in anhydrous and ethanol-free chloroform (*c* 7.5). *Anal.* Calcd. for $C_{50}H_{93}O_8NPCl$ (902.7): C, 66.52; H, 10.38; N, 1.55; P, 3.43. Found: C, 66.01; H, 10.63; N, 1.55; P, 3.42.

Dipalmitoyl-L-α-glycerylphenylphosphorylcholine Chloride (III, R_2).—The mixture of III, R_2 and IIa, R_2 (11.8 g.) suspended in 60 ml. of 99% ethanol at 24° was stirred for a period of 10 min. The solid and extract were separated by centrifugation. The ethanol extraction was repeated twice more using the same volumes of ethanol. The combined extracts were brought to dryness *in vacuo* (bath temperature 35–40°) and the residue (7.96 g.) was dissolved in 50 ml. of warm benzene. The solution, while still warm, was cleared by centrifugation and set aside for crystallization (ice-box at +6°). The solid was collected with suction on a buchner funnel and washed with 25 ml. of cold (+6°) benzene. The dipalmitoyl-L-α-glycerylphenylphosphorylcholine chloride, after drying *in vacuo* (0.1 mm.) over solid sodium hydroxide, weighed 7.31 g. (41% of theory). It started to sinter at 80° and melted at 130–131°; $[\alpha]^{25}D$ +2.0° in anhydrous and ethanol-free chloroform (*c* 7.6). *Anal.* Calcd. for $C_{46}H_{85}O_8NPCl$ (846.6): C, 65.25; H, 10.12; N, 1.65; P, 3.66; Cl, 4.19. Found: C, 65.15; H, 9.93; N, 1.70; P, 3.64; Cl, 4.23.

Dimyristoyl-L-α-glycerylphenylphosphorylcholine Chloride (III, R_3).—The mixture of III, R_3 and IIa, R_3 (11.4 g.[10]) suspended in 30 ml. of 99% ethanol at 24° was stirred for 10

(5) P. A. Levene and H. S. Simms, *J. Biol. Chem.*, **48**, 185 (1921).

(6) H. I. Price and W. C. McCullagh Lewis, *Biochem. J.*, **23**, (2) 1030 (1929).

(7) M. Faure and J. Legault-Demare, *Bull. soc. chim. biol.*, **32**, 509 (1950).

(8) The alcohol-insoluble solid was mainly bis-α,α-(distearoylglyceryl)-phenylphosphate, IIa R_1. The substance can be obtained in a pure state by recrystallization from boiling acetone.

(9) To facilitate the removal of the solids from the distillation vessel all evaporations under reduced pressure were carried out in short-necked round flasks.

(10) On repeating the phosphorylation of 0.02 mole of dimyristin, 11.4 g. of the phosphorylation product (III, R_3 + IIa, R_3) were obtained. This yield is considerably higher than that previously reported by us (6.6 g.).

minutes, and the solid and extract were separated by centrifugation. The precipitate was extracted as described above with two further 30-ml. portions of 99% ethanol. The combined extracts were brought to dryness under reduced pressure at 35–40° bath temperature, and the residue (8.15 g.) was dissolved in a boiling mixture of 180 ml. of anhydrous ether and 6 ml. of 99% ethanol. After keeping the solution overnight in the ice-box (+6°), the mixture was filtered with suction and the substance was washed with a small volume of cold ether. The solid, which after drying *in vacuo* weighed 6.90 g., was dissolved in 27 ml. of warm (40°) benzene, and the solution, after clearing by centrifugation, was cooled gradually to room temperature and placed in the ice-box (+6°). The substance was collected with suction on a buchner funnel, washed with 10 ml. of cold benzene and freed of solvent *in vacuo*. For further purification the substance (6.36 g., P, 3.80; N, 2.16) was recrystallized from 67 ml. of warm acetone by cooling to +6°. The dimyristoyl-L-α-glycerylphenylphosphorylcholine, after washing with cold acetone and drying *in vacuo* (0.2 mm.), weighed 5.68 g. (35.9% of theory). The substance started to sinter at 65° and melted with the formation of a meniscus at 126–127°; $[\alpha]^{25}D$ +1.7° in anhydrous and ethanol-free chloroform (*c* 10). *Anal.* Calcd. for $C_{42}H_{77}O_8NPCl$ (790.5): C, 63.81; H, 9.82; P, 3.92; N, 1.70. Found: C, 63.88; H, 10.07; P, 3.90; N, 1.74.

Distearoyl-L-α-glycerylphosphorylcholine (IV, R_1).—The distearoyl-L-α-glycerylphenylphosphorylcholine chloride (6.64 g.) was dissolved in a mixture of 125 ml. of 99% ethanol and 8 ml. of chloroform by warming to 45° and the solution, together with 0.8 g. of platinum dioxide (Adams catalyst), was placed in an all-glass hydrogenation vessel of 300-ml. capacity. The mixture was shaken vigorously at room temperature in an atmosphere of hydrogen at an initial pressure of 50 cm. of water until the absorption of hydrogen had practically ceased. In approx. two hours 1055 ml. of hydrogen were consumed. After displacing the hydrogen by nitrogen and adding 30 ml. of chloroform the catalyst was removed by centrifugation and was washed with 15 ml. of a chloroform–ethanol mixture (1:1). To the combined supernatant solutions were added 11 ml. of water and 2.62 g. of finely powdered silver carbonate, and the mixture was stirred vigorously for one-half hour. At the end of this period the silver salts were removed by centrifugation and washed on the centrifuge with a mixture of chloroform–ethanol (1:1). The combined supernatants were brought to dryness under reduced pressure at 35–38° bath temperature,[11] and the residue was kept for several hours at this temperature in a vacuum of 0.1 to 0.4 mm. To obtain the lecithin in a finely powdered form it was dissolved in 15 ml. of chloroform and the solution, after adding 30 ml. of petroleum ether (b.p. 35–60°), was brought to dryness under reduced pressure at a bath temperature not exceeding 20°. The L-α-(distearoyl)-lecithin after drying *in vacuo* (0.1 mm.) over calcium chloride weighed 5.60 g. which corresponds to a yield of 94% based on the phenyl ester or an over-all yield of 34.7% based on distearin. The lecithin started to sinter at 120°,[12] forming individual droplets. On further heating (20° per min. up to 210° and from there on 10° per min.) the droplets gradually darkened and coalesced suddenly with the formation of a meniscus at 230–232°[13]; $[\alpha]^{25}D$ +6.1° in chloroform–methanol (1:1), *c* 9.7. *Anal.* Calcd. for $C_{44}H_{90}O_8NP$ (808.2): C, 65.38; H, 11.22; N, 1.73; P, 3.83. Found: C, 65.36; H, 11.14; N, 1.78; P, 3.90.

Dipalmitoyl-L-α-glycerylphosphorylcholine (IV, R_2).—The dipalmitoyl-L-α-glycerylphenylphosphorylcholine chloride (7.31 g.) was freed of its phenyl group as described for the stearoyl compound. The yield of L-α-(dipalmitoyl)-lecithin was 5.84 g. (90% based on the phenyl compound and 37% based on the dipalmitin); $[\alpha]^{25}D$ +7.0° in dry and ethanol-free chloroform, (*c* 5.6). The dipalmitoyl lecithin started to sinter at 120° forming individual droplets. On further heating as described above the droplets gradually darkened and coalesced suddenly with the formation of a meniscus at 235 to 236°. *Anal.* Calcd. for $C_{40}H_{82}O_9NP$ (752): C, 63.89; H, 11.00; N, 1.86; P, 4.12. Found: C, 63.81; H, 11.15; N, 1.87; P, 4.20.

Molecular Weight Determination of L-α-(Dipalmitoyl)-lecithin by the Vapor Pressure Method.—(1) Sample, 2.03 mg. of lecithin; solvent, 40.2 mg. of anhydrous methanol; % concn. by wt. 4.7; 20°; mol. wt., found, 1020, 1050, 1070, 1020, 1030; average mol. wt., 1040. (2) Sample, 0.32 mg. of lecithin; solvent, 34.5 mg. of anhydrous methanol; % concn. by wt. 0.9; 20°; mol. wt., found, 790, 820, 780; average mol. wt. 795. Calcd. mol. wt. for dipalmitoyl lecithin, 752.

Dimyristoyl-L-α-glycerylphosphorylcholine (IV, R_3).—The solution of 5.68 g. of dimyristoyl-L-α-glycerylphenylphosphorylcholine chloride in 95 ml. of 99% ethanol, together with 0.8 g. of platinum dioxide, was vigorously shaken at room temperature in an atmosphere of hydrogen at the initial pressure of 50 cm. of water until the absorption of hydrogen ceased. After replacing the hydrogen by nitrogen and removing the catalyst by centrifugation, the decanted supernatant solution was diluted with 15 ml. of ethanol and 14 ml. of distilled water and, in the presence of 2.4 g. of finely powdered silver carbonate, was stirred vigorously for 30 minutes. The silver salts were removed by centrifugation, the alcoholic solution was brought to dryness under reduced pressure at 35–40°, and the solid residue was freed of organic solvents and dried *in vacuo* (0.1 mm.) over calcium chloride. The yield of L-α-(dimyristoyl)-lecithin was 4.55 g. (91% based on the phenyl compound and 32.7% on the dimyristin). The dimyristoyl lecithin started to sinter at about 90°, forming individual droplets. On further heating as described above the droplets gradually darkened and coalesced suddenly with the formation of a meniscus at 236–237°; $[\alpha]^{24}D$ +7.0° in dry ethanol–chloroform (1:1), (*c* 8). *Anal.* Calcd. for $C_{36}H_{74}O_9NP$ (695.9): C, 62.13; H, 10.72; N, 2.01; P, 4.46. Found: C, 62.16; H, 10.81; N, 2.04; P, 4.51.

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(11) If at this stage the lecithin was discolored by silver, it was dissolved in a mixture of ethanol–chloroform 1:1 and the solution was centrifuged until clear. The lecithin was recovered by removing the solvents *in vacuo* at 35°.

(12) It was observed that the α-lecithins prepared by the modified procedure started to sinter 20 to 30 degrees higher than those prepared by our original procedure (ref. 1). However, the temperatures at which the meniscus is formed remained the same.

(13) All melting point determinations reported in this and earlier papers by us were carried out in capillary tubes using an electrically heated bath of *n*-butyl phthalate and short-stem thermometers with a range of 50 degrees.