# Synthesis of α-Cephalins by a New Procedure. II. Dioleoyl and Dilinoleoyl L-α-Cephalins*

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The synthesis of L-α-(dioleoyl)cephalin and L-α-(dilinoleoyl)cephalin by a new procedure is described. They were obtained by treating the barium salt of L-α-glycerylphosphoryl-2′-hydroxyethylphthalimide with the chloride of oleic acid or linoleic acid and pyridine, and freeing the *N*-phthaloyl cephalins from their protective phthaloyl group by hydrazinolysis in an atmosphere of nitrogen.

Recently Baer *et al.* (1963) reported a new, simpler procedure for the synthesis of the enantiomeric as well as racemic forms of α-cephalins, and applied it to the preparation of distearoyl- and dipalmitoyl L-α-cephalin. It was hoped that the procedure would be applicable also to the synthesis of unsaturated α-cephalins. We now wish to report the modifications necessary to prepare two typical unsaturated representatives, viz., dioleoyl- and dilinoleoyl L-α-glycerylphosphoryl ethanolamine. They were obtained as chromatographically homogeneous substances in over-all yields of 22.7% and 18.9% of theory, respectively.

## Experimental Procedure

*Materials.*—References to procedures for the preparation of D-acetone glycerol, 2′-hydroxyethylphthalimide, and anhydrous pyridine, quinoline, dimethylformamide, chloroform, and hydrazine will be found in the publication of Baer *et al.* (1963) reporting the new procedure. Oleic acid with a purity of better than 99% was prepared by the method of Rubin and Paisley (1962). The oleic acid and linoleic acid were converted into the chlorides by the method of Fierz-David and Kuster (1939). The silicic acid 100-mesh powder, analytical reagent (Mallinckrodt) was suspended with stirring in 10 parts of water, the mixture was allowed to settle, and the wash water was decanted. The process was repeated five times. The silicic acid then was air-dried, first at room temperature then at 120° for 16 hours.

*Dioleoyl L-α-Glycerylphosphoryl-2′-hydroxyethylphthalimide.*[1]—Into a dry flask were placed 1.67 g (2.0 mmoles) of thoroughly dried barium salt of L-α-glycerylphosphoryl-2′-hydroxyethylphthalimide (Baer *et al.*, 1963), 1.61 ml (20 mmoles) of dry pyridine, 20 ml of dry dimethylformamide, and 3.0 g (10 mmoles) of freshly distilled oleoyl chloride, and the mixture was kept under anhydrous conditions at 60° for 42 hours. It then was poured into a separatory funnel containing 50 g of crushed ice and 50 ml of water, and the suspension was extracted with four 75-ml portions of ether by gentle shaking; if emulsions formed they were separated by centrifugation. The combined ethereal extracts were washed as rapidly as possible with 100 ml of ice-cold 0.2 N sulfuric acid and two 100-ml portions of water. The ether solution was dried with anhydrous sodium sulfate and filtered, and the ether was distilled off under reduced pressure at 30°. The remaining brown oil, weighing 3.33 g, was dissolved in 25 ml of a mixture of petroleum ether (bp 60–80°) and benzene (1:1, v/v), and the solution was applied to a column of silicic acid (3 × 50 cm). The column was eluted successively with approximately 1 liter of the same solvent mixture (eluate 1), followed by 500 ml of a 1:3 mixture of petroleum ether and benzene (eluate 2), and 1 liter of benzene (eluate 3); removal of the solvents of eluates 1, 2, and 3 by distillation under reduced pressure from a bath at 35–40° gave 1.6 g of essentially pure oleic acid (eluate 1), 0.2 g of a material low both in nitrogen and phosphorus (eluate 2), and 1.36 g (38.5% of theory) of an oil (eluate 3) which gave an infrared spectrum consistent with the structure of dioleoyl L-α-glycerylphosphoryl-2′-hydroxyethylphthalimide, and whose physical constants agreed with those reported for this compound by Baer and Buchnea (1959). $[\alpha]_D^{23}$ + 3.0° in dry chloroform (*c*, 10). $n_D^{31}$ 1.4891.

*Anal.* Calcd for $C_{49}H_{80}O_{10}NP$ (874.1): C, 67.33; H, 9.22; N, 1.60; P, 3.55. Found: C, 68.08; H, 9.35; N, 1.51; P, 3.66.

*Dilinoleoyl L-α-Glycerylphosphoryl-2′-hydroxyethylphthalimide.*—The acylation of the barium salt of L-α-glycerylphosphoryl-2′-hydroxyethylphthalimide (1.67 g) with linoleoyl chloride (3.0 g), the isolation of the reaction products, and their separation by column chromatography on silicic acid were carried out as described for the oleoyl compound, using the same amounts of reagents. The dilinoleoyl L-α-glycerylphosphoryl-2′-hydroxyethylphthalimide, a slightly colored, viscous oil, after drying at room temperature in a vacuum of 0.2 mm for 18 hours, weighed 1.29 g (36.7% of theory). $n_D^{23}$ 1.4947; $[\alpha]_D^{23}$ + 3.1° in dry and ethanol-free chloroform (*c*, 5).

*Anal.* Calcd for $C_{49}H_{76}O_{10}NP$ (870.1): C, 67.63; H, 8.81; N, 1.61; P, 3.56. Found: C, 67.78; H, 9.07; N, 1.66; P, 3.61.

*L-α-(Dioleoyl)cephalin.*—To a solution of 1.05 g (1.2 mmoles) of dioleoyl L-α-glycerylphosphoryl-2′-hydroxyethylphthalimide in 30 ml of 95% ethanol at 0° was added 0.51 ml of 12.5% solution of hydrazine hydrate in 95% ethanol. After the solution attained room temperature (approximately 30 minutes), a further 0.76 ml of the hydrazine solution was added, and the mixture was stored under nitrogen for 2 days at 40°. The solvent then was distilled off under reduced pressure from a bath at 40°, and the residual oil was taken up in 30 ml of ether. To the solution were added 2.5 ml each of methanol and water, and 2.0 g of Amberlite IRC-50 ($H^+$), and the mixture was shaken for 1 hour. The resin was filtered off and washed with ether, the combined filtrates were evaporated under

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[1] All operations where feasible were carried out under nitrogen.

reduced pressure at 40°, and the residue was dried at room temperature in a vacuum of 0.1 mm. A solution of the crude cephalin in a 1:1 (v/v) mixture of benzene and chloroform was passed through a column of silicic acid (3 × 35 cm). Exhaustive elution of the column with a 1:2 mixture of benzene and chloroform (eluate 1), followed by a 1:3 mixture of benzene and chloroform (eluate 2) and evaporation of the eluates under reduced pressure from a bath at 30–35°, gave a small amount of material low in both nitrogen and phosphorus (eluate 1) and 0.76 g (eluate 2) of fairly pure cephalin (found: N, 1.81; P, 4.09). Rechromatography of the latter on silicic acid, elution of the product with a 1:3 mixture of benzene and chloroform, evaporation of the eluate under reduced pressure at 30–35°, and drying of the waxy, slightly yellowish solid for 3 days at room temperature in a vacuum of 0.02 mm gave 0.53 g (59% of theory) of chromatographically pure L-α-(dioleoyl)cephalin. $[\alpha]_D^{23}$ +5.8° in ethanol-free anhydrous chloroform (*c*, 5). Reported (Baer and Buchnea, 1959) $[\alpha]_D$ +6.0° in chloroform (*c*, 7).

*Anal.* Calcd for $C_{41}H_{78}O_8NP$ (744.05): N, 1.88; P, 4.18; iodine number, 68.3. Found: N, 1.82, 1.84; P, 4.15, 4.12, iodine number, 66.0, 66.3.

L-α-(*Dilinoleoyl*)*cephalin.*—The hydrazinolysis of dilinoleoyl L - α - glycerylphosphoryl - 2′ - hydroxyethylphthalimide (1.0 g), and the separation of the reaction products by column chromatography on silicic acid was carried out as described for the oleoyl compound. The L-α-(dilinoleoyl)cephalin, a slightly yellowish-colored waxy solid, weighed 0.44 g (51.7% of theory). On exposure to air it becomes a viscous gum. $[\alpha]_D^{23}$ +5.8° in anhydrous ethanol-free chloroform (*c*, 5). Reported by Dorofeeva *et al.* (1963): $[\alpha]_D$ +6°. The L-α-(dilinoleoyl)cephalin is soluble in all of the commonly used organic solvents.

*Anal.* Calcd for $C_{41}H_{74}O_8NP$ (740.0): C, 66.56; H, 10.08; N, 1.89; P, 4.18; iodine number, 137.2. Found: C, 66.12; H, 10.35; N, 1.79; P, 4.07; iodine number, 133.1, 132.9.

L-α-(*Distearoyl*)*cephalin.*—Hydrogenation of L-α-(dilinoleoyl)cephalin (91 mg) in ethanol with platinum dioxide as catalyst, and purification of the reaction product as described by Baer (1957) gave 63 mg (69.2% of theory) of pure L-α-(distearoyl)cephalin; mp 180–182°. Reported (Baer, 1957) mp 180–182°.

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# On the Structure of Cardiolipin*


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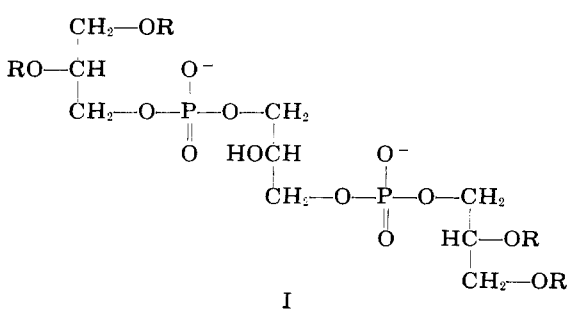
Cardiolipin (diphosphatidylglycerol) from beef heart and from *Mycobacterium phlei* has been deacylated and degraded to glycerol-1,3-diphosphate by the action of sodium metaperiodate and 1,1-dimethylhydrazine. The results establish that the lipid has the 1,3-diphosphatidylglycerol structure, a point that was still open to question. For references, crystalline cyclohexylamine salts of glycerol-1,3-diphosphate and L-glycerol-1,2-diphosphate have been prepared. The reported optical activity of the triglyceroldiphosphate obtained by deacylation of cardiolipin has been confirmed, and is found to be identical with that of synthetic 1,3-di-*O*-(L-glycerol-3′-phosphoryl)-glycerol synthesized by P. Plackett (*Australian J. Chem. 17*, 101, 1964), thus establishing the over-all absolute configuration of the cardiolipin molecule.


Cardiolipin was first isolated from beef heart by Pangborn (1942), who demonstrated that the substance was essential for the reactivity of beef heart antigens in the serologic test for syphilis. On hydrolysis, cardiolipin yielded fatty acids and a "phosphorylated polysaccharide" which was shown to be a polyglycerolphosphate (Pangborn, 1947). From the analytical data, she proposed a formula in which four glycerol residues were connected by three phosphate groups in diester linkage, the fatty acids being esterified to the remaining hydroxyl groups of the glycerol molecules.

McKibbin and Taylor (1952) later isolated from dog liver a polyglycerolphosphatide in which the ratio of glycerol to phosphorus was 3:2, a result in disagreement with Pangborn's formula. In 1956, Faure and Morelec-Coulon repeated the preparation of cardiolipin from cardiac muscle, and found molar ratios for glycerol–phosphorus–fatty acids of 3:2:4. This result was confirmed by MacFarlane and Gray (1957) and Gray and MacFarlane (1958), who examined preparations of cardiolipin obtained by various methods. These authors (1958), as well as Faure and Morelec-Coulon (1958a) showed that glyceroldiphosphate could be obtained as one of the degradation products of cardiolipin. Based on these results, MacFarlane and Gray



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