alkyl(or aralkyl)-β-chloroethylamines has been described. These compounds possess adrenergic blocking activity; N-(9-fluorenyl)-N-ethyl-β-chloroethylamine hydrochloride being outstanding in this regard.

PHILADELPHIA, PENNSYLVANIA RECEIVED JUNE 3, 1949

[CONTRIBUTION FROM THE BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO]

Synthesis of Enantiomeric α-Lecithins[1]

BY ERICH BAER AND MORRIS KATES[2]

A critical survey of the pertinent literature reveals the surprising fact that it was not until 1941 that the first individual lecithin, (dipalmitoyl) lecithin[3] (DPL), was isolated from a natural source (*Cysticercus fasciolaris*).[4] The same compound was later isolated also from brain, lung and spleen.[5]

The great difficulties encountered in obtaining individual lecithins from natural sources have prompted numerous attempts to obtain these compounds by synthesis. The properties of these racemic "synthetic lecithins" were in some respects similar to those of natural lecithins, but they differed widely in others. On examining critically the earlier work in the field of "synthetic lecithins"[6-8] it becomes evident that the reported compounds were not, as claimed, pure individual lecithins but most likely mixtures of α- and β-lecithins and/or the choline salts of α- and β-phosphatidic acids.

During the past twelve years methods have been developed in this Laboratory which permit the synthesis of the pure enantiomeric forms of asymmetrically substituted glycerol derivatives of predetermined constitution and configuration.[9]

It was thought that these methods might be applicable to the preparation of individual lecithins.

In a previous communication it was shown that α-glycerylphosphorylcholine[9m] (α-GPC) obtained from natural lecithins belongs to the L-series. The introduction of two acyl groups into L-α-GPC therefore should yield α-lecithins with the configuration of the natural products. Various attempts to esterify the synthetic L-α-GPC by means of stearoyl chloride and pyridine did not, however, produce the desired α-lecithin but instead yielded products which consisted mainly of mono stearoyl-GPC (lyso-lecithin), as indicated by the analytical values and a strong hemolytic activity. When further efforts to improve the acylating procedure remained unproductive the method was abandoned.

In the second approach to the synthesis the order of introducing the various substituents into the glycerol molecule was reversed, *i. e.*, the fatty acids were introduced first. After overcoming several technical difficulties this procedure proved to be successful. The sequence of reactions and the steric relationships of the various intermediary compounds are illustrated in the accompanying reaction scheme.

The synthesis is as follows. The D-α,β-diglyceride (I)[10] is phosphorylated with monophenylphosphoryl dichloride in the presence of *one mole* of pyridine[11] giving rise to the formation of diacyl L-α-glycerylphenylphosphoryl chloride (II) and bis-(diacylglyceryl)-phenylphosphate (IIa). Without isolating the intermediate compound (II) the reaction mixture is immediately treated with choline chloride in the presence of a large excess of pyridine. The ether- and water-insoluble part of the reaction product consists almost entirely of a mixture of diacyl α-glycerylphenylphosphorylcholine chloride (III) and bis-(diacylglyceryl)-phosphoric acid phenyl ester (IIa).[12] The choline ester (III) is isolated and

(1) A preliminary report of the subject matter of this paper has appeared in *Science*, **109**, 31 (1949). Patents applied for.

(2) This paper forms part of a thesis submitted by M. Kates to the Department of Chemistry, University of Toronto, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, November, 1948. Present address: National Research Council, Ottawa, Canada.

(3) Since the name *lecithin* has been used for generations to describe the whole molecule, prefixes such as distearoyl—cannot be applied correctly in the chemical sense since this suggests substitution; it is therefore proposed to place these purely descriptive prefixes within parentheses.

(4) Lesuk and Anderson, *J. Biol. Chem.*, **139**, 457 (1941).

(5) Thannhauser, Benotti and Boncoddo, *ibid.*, **166**, 669 (1946); Thannhauser and Boncoddo, *ibid.*, **172**, 135 (1948).

(6) Grün and Limpächer, *Ber.*, **59**, 1345, 1350 (1926); *ibid.*, **60**, 147 (1927).

(7) Kabashima and Suzuki, *Proc. Imp. Acad. (Tokyo)*, **8**, 492 (1932); *C. A.*, **27**, 1634 (1933); Kabashima, *Ber.*, **71B**, 76 (1938).

(8) Obata, *Bull. Inst. Phys. Chem. Research (Tokyo)*, **22**, 115 (1943); *C. A.*, **42**, 522 (1948).

(9) Baer and Fischer, (a) *J. Biol. Chem.*, **128**, 463 (1939); (b) **128**, 475 (1939); (c) **128**, 491 (1939); (d) **135**, 321 (1940); (e) **140**, 397 (1941); (f) **145**, 61 (1942); (g) Baer, Cushing and Fischer, *Can. J. Res.*, **21B**, 119 (1943); (h) Baer, Rubin and Fischer, *J. Biol. Chem.*, **155**, 447 (1944); (i) **170**, 337 (1947); (j) Baer and Fischer, THIS JOURNAL, **61**, 761 (1939); (k) **67**, 944 (1945); (l) **67**, 2031 (1945); (m) Baer and Kates, *ibid.*, **70**, 1394 (1948).

(10) Prepared according to the method of Sowden and Fischer, *ibid.*, **63**, 3244 (1941).

(11) In contrast to the phosphorylation of D-acetone-glycerol with monophenylphosphoryl dichloride[9m] it was found that the phosphorylation of the diglyceride had to be carried out in the presence of the more strongly basic pyridine rather than quinoline and at temperatures ranging from +10 to +35°.

(12) These compounds are being converted to the corresponding bis-(diacylglyceryl)-phosphoric acids which may have some relationship to the complex phosphatidic acid, cardiolipin, of M. C. Pangborn (*J. Biol. Chem.*, **168**, 351, 1947). On separating the phosphorylation mixtures derived from distearin, dipalmitin and dimyristin, it was found that they contain compounds (IIa) and (III) in the approximate molar ratios of (1:8), (1:4) and (1:3), respectively. Apparently the formation of bis-(diacylglyceryl) phosphoric acid phenyl esters increases with decreasing length of the fatty acid.

obtained in a pure state by means of its ethyl acetate-soluble reineckate. This in turn is converted to the sulfate and the protective phenyl group is removed by catalytic hydrogenolysis. After elimination of the sulfate ion the resulting L-α-lecithin (IV) is purified by crystallization from a suitable solvent such as diisobutyl ketone.

In this manner L-α-(distearoyl) lecithin (DSL), L-α-(dipalmitoyl) lecithin and L-α-(dimyristoyl) lecithin (DML) were prepared. By means of the same series of reactions but starting with L- or D,L-α,β-diglycerides the D- or D,L-α-lecithins are obtainable. In the course of the present investigation D,L-α-(distearoyl) lecithin and D,L-α-(dipalmitoyl) lecithin were also prepared and their synthesis is described briefly in the experimental part.

The three synthetic L-α-lecithins were obtained as white powders in over-all yields of 37% (DSL), 35% (DPL) and 23% (DML). Their analytical values for carbon, hydrogen, nitrogen and phosphorus agreed well with those calculated for the open formula

$$[R{-}O{-}\underset{\underset{OH}{|}}{P(O)}{-}OCH_2{-}CH_2\underset{OH}{N(CH_3)_3}]$$

but not for the endo-salt form

$$[R{-}O{-}\underset{O^-}{P(O)}{-}OCH_2{-}CH_2N^+(CH_3)_3]$$

of Grün and Limpächer.[6] The molecular ratios of choline:phosphoric acid:fatty acids were very close to the theoretical values of 1:1:2. The three lecithins gave cadmium chloride addition compounds all of which were obtained in excellent yields. On the basis of the analytical data the general formula $[\text{L-}\alpha\text{-lecithin}]_2[CdCl_2]_3$ had to be assigned to these compounds.

The three synthetic lecithins after recrystallization from diisobutyl ketone, did not exhibit a definite crystalline form under the microscope; they gave, however, distinct X-ray diffraction patterns (Plate 1) and exhibited birefringence colors under polarized light. The crystalline structure of the synthetic lecithins obtained from diisobutyl ketone is thus established. Later it was found that by slow crystallization from dioxane all three lecithins can be obtained in form of crystals large enough to be seen even at low magnification.

As may be gathered by a comparison of the X-ray diffraction patterns on Plate 1, a difference of two carbon atoms in the length of the fatty acid is sufficient to cause a distinct and measurable shift in the position of three of the six lines. Thus the pattern of the synthetic L-α-DPL, while identical with that of the natural DPL, differs distinctly from those of DSL and DML. These X-ray diffraction patterns, therefore, should provide excellent means for the identification of the corresponding natural α-lecithins.

The solubilities of the synthetic α-lecithins in several solvents were investigated. In a given solvent the solubilities of the homologous lecithins increase markedly with decreasing length of the fatty acid chain. The observed solubilities of the synthetic DSL and DPL were in accord with those reported for DSL[13,14] obtained from hydrogenated egg lecithin and DPL[4,5] also obtained from natural sources.

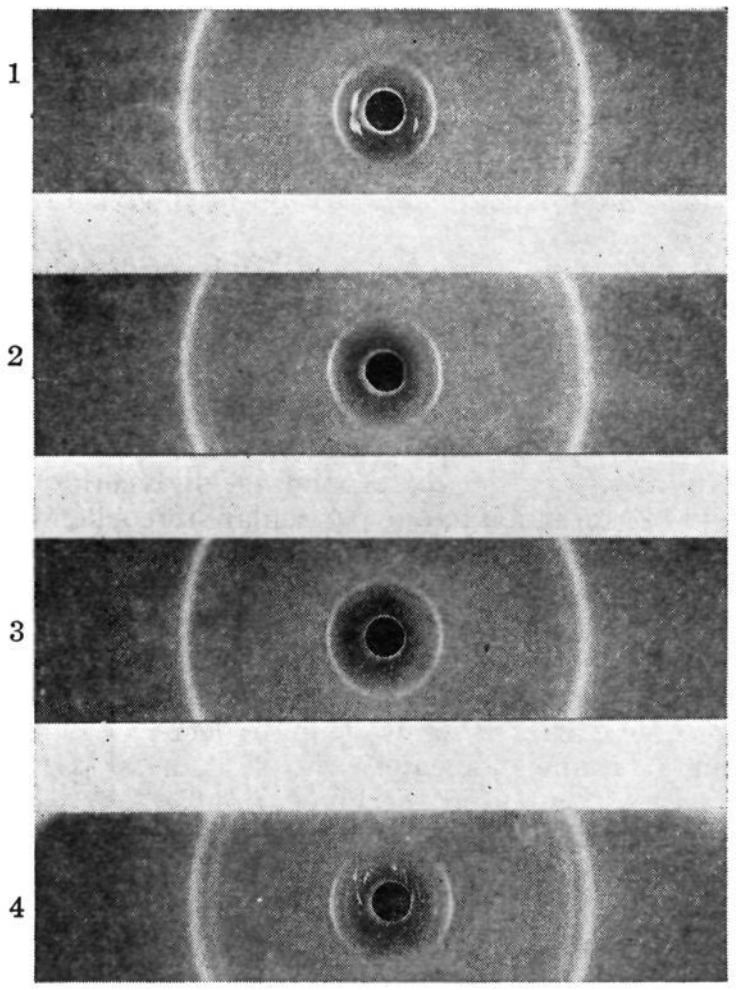


Plate 1.—X-Ray powder diffraction patterns of DSL, DPL and DML: Debye–Scherer powder camera (114.5 mm.); radiation, CrK_α, (λ, 2.287 $K\chi$); V_2O_5 filter; actual diameters in cm. as measured on the original photographs: (1) synth. L-α-(distearoyl) lecithin, 1.58, 2.35, 3.19, 4.19, 5.82, 6.38; (2) synthetic L-α-(dipalmitoyl) lecithin, 1.78, 2.65, 3.54, 4.20, 5.79, 6.37; (3) natural L-α-(dipalmitoyl) lecithin, 1.78, 2.67, 3.58, 4.20, 5.80, 6.36; (4) synthetic L-α-(dimyristoyl) lecithin, 1.89, 2.79, 3.77, 4.14, 5.72, 6.34.

The optical and constitutional purity of the synthetic α-lecithins although assured by the method of synthesis, in the case of the synthetic L-α-DPL could be checked and confirmed by comparison with the natural product. The specific and molecular rotations of the synthetic product ($[\alpha]_D + 6.6°$, $[M]_D + 49.5°$) were found to be similar to those reported by Lesuk and Anderson[4] ($[\alpha]_D + 7.1°$, $[M]_D + 53°$[15]) and by Thannhauser and associates[5] ($[\alpha]_D + 6.25°$, $[M]_D + 47.0°$[15]) for the natural product.

Since the molecular rotations of L-α-DSL ($[M]_D + 49.3°$) and L-α-DML ($[M]_D + 48.7°$)

(13) Paal and Oehme, *Ber.*, **46**, 1296 (1913).

(14) Ritter, *ibid.*, **47**, 530 (1914).

(15) The molecular rotations are calculated from the specific rotations reported by the authors.

agree well with that of L-α-DPL the optical purity of these two compounds is also confirmed.

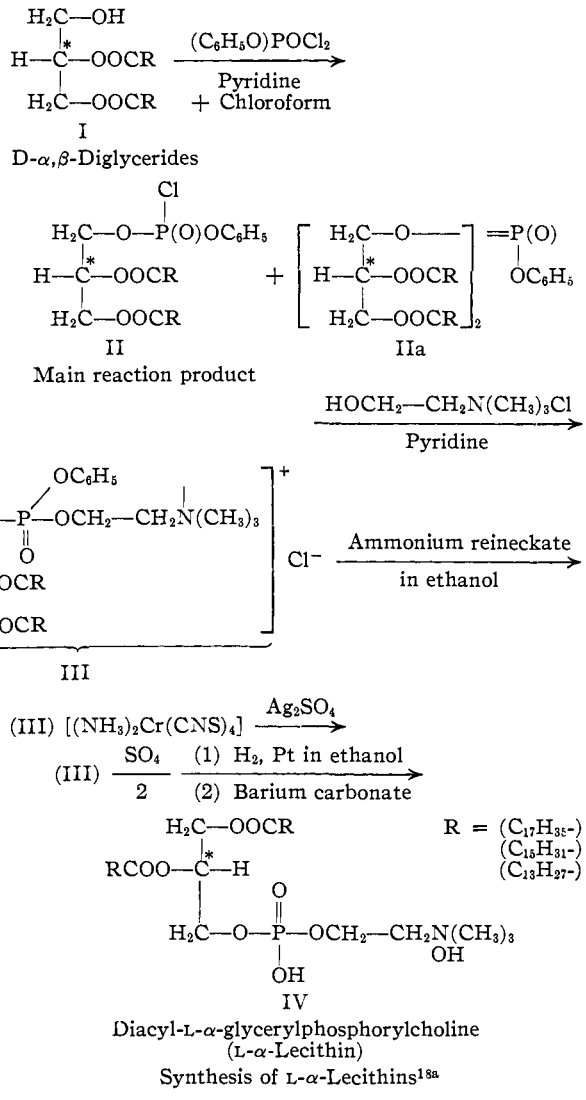
The steric classification of asymmetrically substituted mono- and diglycerides offers no particular difficulties. It is established by relating these compounds to D- or L-glyceraldehyde.[16]

In the case of mixed acid triglycerides, however, the steric classification is arbitrary, but can be applied without ambiguity if one of the α-substituents is given preference, the choice depending on chemical or biochemical considerations. Thus, if one considers a lecithin as a derivative of the corresponding diglyceride, it would be assigned to one particular stereochemical series, whereas if one considers the same compound as a derivative of the glycerylphosphorylcholine moiety, it would be assigned to the opposite stereochemical series. Since the diglyceride portion of the molecule varies from lecithin to lecithin, it seemed logical to choose as a reference compound the glycerylphosphorylcholine moiety because it is common to all lecithins. Thus, by definition, lecithins containing the L-α-glycerylphosphorylcholine belong to the L-series and those containing the D-α-glycerylphosphorylcholine to the D-series. The synthetic lecithins prepared according to the reaction scheme from D-acetone-glycerol contain the L-α-glycerylphosphorylcholine and according to the definition are members of the L-series. It has long been suspected that the α-GPA of glycolysis is a precursor in the *in vivo* formation of lecithins. In the event of conformation of this role of GPA, the choice of α-GPC as compound of reference would bring into better focus the stereochemical relationship between the carbohydrates and the lecithins.

The synthetic L-α-(dipalmitoyl) lecithin and the natural, *dextro*-rotatory (dipalmitoyl) lecithin[4,5] were found to have the same elementary composition, melting points, solubilities, X-ray diffraction pattern and optical rotation. This establishes the identity of these two compounds beyond doubt and therefore the α-constitution and the L-configuration must be assigned to the natural DPL. The same constitution and configuration must also be assigned to the lecithin isolated by from egg[17] and from brain[18] since on catalytic reduction both yielded dextrorotatory distearoyl lecithin ($[\alpha]_D \sim +6.0°$), which appears to be identical with the synthetic L-α-DSL.

The procedures described in this paper make available for the first time individual lecithins of desired constitution and configuration. These compounds should prove useful in elucidating the chemical, biochemical and physiological properties of the natural lecithins.

(16) Fischer and Baer, *Chem. Rev.*, **29**, 287 (1941).
(17) Levene and West, *J. Biol. Chem.*, **34**, 111 (1918).
(18) Levene and Rolf, *ibid.*, **46**, 353 (1921).



Synthesis of L-α-Lecithins[18a]

The synthesis of several enantiomeric α-phosphatidic acids and of α-cephalins along similar lines has been carried out successfully in this Laboratory and will be described in the near future.

Experimental Part

D-α,β-Diglycerides

D-α,β-Distearin and D-α,β-Dipalmitin.—Both compounds were prepared essentially as described by Sowden and Fischer[10] except for the following changes: (1) The D-acetone glycerol was prepared by catalytic reduction (Raney nickel) of D-acetone-glyceraldehyde[9a] at atmospheric pressure.[9m] (2) The hydrolysis of the benzyl ether of D-acetone-glycerol was carried out in a mixture of ethanol and water (1:1) which was 0.2 *N* with regard to sulfuric acid. (3) The acylation of L-α-benzyl glycerol ether was made in absolute carbon tetrachloride as solvent. (4) The benzyl ethers[19] of both the distearin and dipalmi-

(18a) The D-α-lecithins and D,L-α-lecithins are obtained by starting the synthesis with L- or D,L-α,β-diglycerides, respectively.

(19) To obtain the benzyl ethers in a pure state for the catalytical hydrogenolysis their recrystallization from ether should include a treatment with decolorizing charcoal.

tin were catalytically cleaved in dry pure cyclohexane (6.5 ml./1 g. of diglyceride-benzyl ether) using palladium black (0.27–0.25 g. of Pd/1 g. of benzyl ether). Our products had the physical constants: D-α,β-distearin, recrystallized from a mixture of chloroform–low boiling (40–60°) petroleum ether (1:1.5) (17 ml./1 g. of diglyceride): m. p. 76–77°; $[\alpha]_D$ −2.8° in absolute chloroform (*c*, 6.3), $[M]_D$ −17.5°. D-α,β-Dipalmitin, recrystallized from a mixture of chloroform–low boiling petroleum ether (1:10) (17 ml./1 g. of diglyceride), m. p. 68–69°; $[\alpha]_D$ −2.9° in absolute chloroform (*c*, 8.0); $[M]_D$ −16.5°.

D-α,β-Dimyristin.—In preparing this diglyceride, not described by Sowden and Fischer, we followed their general method, incorporating, however, the above-mentioned changes.

Acylation.—L-α-Benzyl-glycerol ether (11.0 g.) on acylation with 30.0 g. of myristoyl chloride in a mixture of 29.5 ml. of dry quinoline and 50 ml. of absolute carbon tetrachloride yielded 27.5 g.[20] of crude dimyristin benzyl ether, m. p. 32–34°. On recrystallization from 70–80 ml. of dry ether (−10°), 24.5 g. (67%) of pure dimyristin benzyl ether was obtained, m. p. 33.5–34°. For analytical purposes a small amount of the compound was crystallized a second time from dry ether, m. p. 33.5–34°; $[\alpha]_D$ + 5.4° in abs. chloroform (*c*, 7.1). The benzyl ether is soluble in ether, ethanol, chloroform, glacial acetic acid, acetone, benzene or dioxane at room temperature. *Anal.* Calcd. for $C_{38}H_{66}O_5$ (602.5): C, 75.6; H, 11.0; 3.32 ml. of 0.1 *N* NaOH per 100 mg. Found: C, 75.5; H, 11.1; 3.25 ml. of 0.1 *N* NaOH per 100 mg.

Catalytic Hydrogenolysis.—A solution of 17 g. of pure dimyristin benzyl ether in 110 ml. of dry cyclohexane was shaken together with 4.6 g. of palladium black[21] in an atmosphere of hydrogen at an initial pressure of approximately 50 cm. of water until the uptake of hydrogen ceased (approx. two hours). The hydrogenation mixture was diluted with 100 ml. of ether, the catalyst removed by centrifugation and the ether solution concentrated *in vacuo* to dryness. A solution of the residue in a lukewarm mixture of 12 ml. of chloroform and 180 ml. of petroleum ether (b. p. 40–60°) yielded after standing overnight at 18°, 8.2 g. of fairly pure D-dimyristin; m. p. 57.5–59.0°. The crystallization was repeated and yielded 7.7 g. of pure D-myristin (53%); plates, m. p. 58–59°; $[\alpha]_D$ −3.3° in abs. chloroform (*c*, 7.3); $[M]_D$ −17°. *Anal.* Calcd. for $C_{31}H_{60}O_5$ (512.5): C, 72.6; H, 11.8; 3.90 ml. of 0.1 *N* NaOH per 100 mg. Found: C, 72.8; H, 11.8; 3.93 ml. of 0.1 *N* NaOH per 100 mg.

Proof of the Optical Purity of the D-α,β-Dimyristin.—To verify the optical purity of the new diglyceride we followed the procedure used by Sowden and Fischer[10] to demonstrate the optical purity of D-α,β-distearin and D-α,β-dipalmitin. The dimyristin was converted to the *p*-nitrobenzoate (1) and the rotation of this compound compared with the rotation of the product obtained from the reaction of L-α-*p*-nitrobenzoyl-glycerol and myristoyl chloride (2). (1) D-α,β-dimyristin (0.5 g.) yielded 0.38 g. (59%) of D-α,β-dimyristin-*p*-nitrobenzoate, m. p. 51–52°; $[\alpha]_D$ −2.3° in abs. chloroform (*c*, 8.8). *Anal.* Calcd. for $C_{38}H_{63}O_8N$ (662): C, 69.0; H, 9.6; N, 2.12; 4.53 ml. 0.1 *N* NaOH per 100 mg. Found: C, 69.2; H, 9.6; N, 2.15; 4.44 ml. 0.1 *N* NaOH per 100 mg.

(2) L-α-*p*-nitrobenzoyl-glycerol[9k] ($[\alpha]_D$ −18.4° in ethanol) yielded 17.8% of D-α,β-dimyristin *p*-nitrobenzoate, m. p. 51.5–52.0°. $[\alpha]_D$ − 2.3° in abs. chloroform (*c*, 8.7). *Anal.* Calcd. for $C_{38}H_{63}O_8N$ (662): C, 69.0; H, 9.6; N, 2.12. Found: C, 69.2; H, 9.4; N, 2.18.

D,L-α,β-Diglycerides

The synthesis of D,L-α,β-distearin and dipalmitin is identical with that described for the corresponding D-forms, except that the D,L-acetone-glycerol[22] is used as starting material. Only yields, physical and analytical data of the various compounds will be reported.

(20) For washing purposes, ethyl ether cooled to −20° was used.

(21) Tausz and v. Putnoky, *Ber.*, **52**, 1573 (1919).

(22) Newman and Renoll, THIS JOURNAL, **67**, 1621 (1945).

D,L-Acetone-glycerol α-Benzyl Ether.—Yield 78%, b. p. (0.1 mm.) 93–96°.

D,L-α-Benzyl Glycerol Ether.—Yield 81%, b.p. (0.8 mm.) 141–143°; $n^{23}D$ 1.5310. *Anal.* Calcd. for $C_{10}H_{14}O_3$ (182): C, 65.9; H, 7.7. Found: C, 65.7; H, 7.7.

D,L-α,β-Distearin Benzyl Ether.—Yield 71%, m. p. 53.5–54°. *Anal.* Calcd. for $C_{46}H_{82}O_5$ (715): C, 77.2; H, 11.5. Found: C, 77.3; H, 11.4.

D,L-α,β-Distearin.—Yield 82%, m. p. 71.5–72.5°. *Anal.* Calcd. for $C_{39}H_{76}O_5$ (625): C, 75.0; H, 12.3. Found: C, 75.2; H, 12.1.

D,L-α,β-Dipalmitin Benzyl Ether.—Yield 48%, m. p. 45–46.5°. *Anal.* Calcd. for $C_{42}H_{74}O_5$ (659): C, 76.5; H, 11.3. Found: C, 76.45; H, 11.3.

D,L-α,β-Dipalmitin.—Yield 81%, m. p. 65–66°. *Anal.* Calcd. for $C_{35}H_{68}O_5$ (569): C, 73.9; H, 12.1. Found: C, 74.4; H, 11.7.

L-α-Lecithins

L-α-(Distearoyl) Lecithin

Distearoyl-L-α-glycerylphenylphosphorylcholine: (1) **Phosphorylation.**—In a 500-ml. round-bottomed, two-necked and thick-walled flask equipped with a mercury-sealed, motor-driven stirrer and dropping funnel were placed 3.0 ml. (0.02 mole) of monophenylphosphoryl dichloride, 1.7 ml. (0.02 mole) of anhydrous pyridine and 8 ml. of dry and alcohol-free chloroform. The flask was immersed in a water-bath at 9–10° and a solution of 12.5 g. (0.02 mole) of D-distearin in 100 ml. of absolute chloroform was added dropwise in the course of fifteen minutes to the vigorously stirred phosphorylating mixture. The stirring was continued and the temperature of the water-bath was raised slowly during the next fifteen minutes to 35° where it was kept for a period of ten minutes. Then 25 ml. (0.3 mole) of dry pyridine, followed after ten minutes by 2.82 g. (0.02 mole) of dry choline chloride[23] were added. The reaction mixture was allowed to cool to room temperature and the stirring was continued for at least forty hours.

(2) **Isolation of the Phosphorylation Product as the Reineckate.**—The resulting solution was freed by centrifugation from a small amount of suspended material and brought to dryness *in vacuo*. The solid was triturated at the centrifuge with three 90 ml. portions of anhydrous ether. The dried ether-insoluble material (19.8 g.) was freed from pyridine hydrochloride and unreacted choline chloride by stirring with 250 ml. of a cold (+ 10°) 0.5% solution of sodium bicarbonate in acetone–water (1:2) for one-half hour. The suspension was centrifuged sharply and the residue was washed with 80 ml. of cold acetone (+ 5°) and dried *in vacuo* over potassium hydroxide and calcium chloride. The crude distearoyl-L-α-glyceryl-phenylphosphorylcholine chloride (13.4 g.) was converted directly to the reineckate.

To a solution of the crude chloride in 175 ml. of 99% ethanol–chloroform (3:4) were added gradually and with stirring 125 ml. of an ethanolic solution of ammonium reineckate.[24] After standing overnight in the ice-box, the precipitate was filtered off, washed with ethanol and ether, and dried *in vacuo*.

The crude reineckate (15.0 g.) was extracted at the centrifuge with seven 100-ml. portions of dry ethyl acetate[25]

(23) The choline chloride was thoroughly dried *in vacuo* over phosphorus pentoxide at 56°.

(24) This solution was prepared immediately before use by extracting the ammonium reineckate with ethanol (20 to 25 ml./1 g. of reineckate).

(25) The ethyl acetate-insoluble residue contaioed considerable amounts of bis-α,α-(D-distearoylglyceryl)-phenyl phosphate. The slightly colored solid (3.6 g.) was extracted twice with 45 ml. of benzene, the combined extracts were centrifuged and the supernatant liquid filtered with suction through a super-cel coated filter. The filter was washed with benzene and the combined filtrates concentrated to dryness *in vacuo*. The solid (1.6 g.) was taken up in 60 ml. of chloroform–ethanol (1:1) and the solution decolorized by the dropwise addition of a 1% aqueous silver sulfate solution. The colored precipitate was centrifuged off, and the supernatant, warmed

and the combined extracts were concentrated *in vacuo* to a small volume (approx. 40–50 ml.). The reineckate was brought into solution at 35–40° with a minimum of ethyl acetate (40–45 ml.) and reprecipitated by the gradual addition of 250 ml. of 95% ethanol. After standing overnight in the ice-box, the reineckate was filtered off, washed with ethanol and ether and dried *in vacuo;* yield of distearoyl-L-α-glycerylphenylphosphorylcholine reineckate, 11.1 g. (46.5% calcd. for distearin), m. p. 137–138°.

For analytical purposes, 270 mg. of the reineckate was recrystallized from 7.5 ml. of an ethanol–chloroform mixture (2:3); yield, 200 mg.; m. p. 139–139.5°. The reineckate is a pink, crystalline solid which is soluble in acetone, chloroform or ethyl acetate and insoluble in ethanol, ether or water. *Anal.* Calcd. for $C_{84}H_{99}O_8N_7PCrS_4$ (1185): C, 54.7; H, 8.4; N, 8.27; P, 2.62. Found: C, 55.7; H, 8.2; N, 8.31; P, 2.58.

(3) **Conversion of the Reineckate to the Sulfate.**—To a vigorously stirred solution of 11.1 g. (0.0094 mole) of the reineckate in 180 ml. of chloroform, diluted with 120 ml. of ethanol, was added simultaneously 145 ml. of a 1% aqueous silver sulfate solution (0.0047 mole) and 250 ml. of ethanol. The silver reineckate was removed by centrifugation and washed with a total of 50 ml. of a chloroform–ethanol mixture (1:1). The combined supernatants were cleared by filtration, if necessary, and concentrated *in vacuo* as rapidly as possible to a volume of about 180 ml. The concentrate was cooled in ice for one half-hour and centrifuged. The precipitate was washed with a small amount of cold acetone and dried *in vacuo* over calcium chloride; yield of distearoyl-L-α-glycerylphenylphosphorylcholine sulfate, 8.1 g. (94% based on the reineckate). At this stage the sulfate is pure enough for further processing. For analytical purposes, 250 mg. of the sulfate was purified by reprecipitation from its solution in 3 ml. of a chloroform–99% ethanol mixture (1:1) by the addition of 3 ml. of anhydrous acetone; yield, 240 mg. The sulfate is a non-hygroscopic white powder which is soluble in chloroform, slightly soluble in ethanol and insoluble in acetone, ether or water.

Anal. Calcd. for $C_{100}H_{186}O_{20}N_2P_2S$ (1830): C, 65.5; H, 10.24; N, 1.53; P, 3.39. Found: C, 65.5; H, 10.6; N, 1.63; P, 3.26.

Distearoyl-L-α-glycerylphosphorylcholine: Catalytic Removal of the Phenyl Group.—A warm solution (35–40°) of 8.1 g. of the sulfate in 80 ml. of a mixture of 99% ethanol–chloroform (3:1) was cleared by centrifugation and the precipitate was washed with a total of 40 ml. of the chloroform–ethanol mixture. The combined supernatants together with 1.6 g. of platinum dioxide (Adams catalyst) were vigorously shaken in an atmosphere of hydrogen at an initial pressure of approx. 50 cm. of water. In fifty minutes the absorption of hydrogen had practically ceased and 1200 ml. (N. T. P.) of hydrogen were consumed (calculated for reduction of the platinum oxide and the sulfate, 1090 ml.). After the hydrogen was displaced by nitrogen, the precipitate of lecithin was brought into solution by the addition of 25 ml. of chloroform, and the catalyst was removed by centrifugation and washed with 30 ml. of a chloroform–ethanol mixture (1:1). The combined supernatants were diluted with 20 ml. of water and stirred for one-half hour in the presence of 2.5 g. of finely powdered barium carbonate. The barium salts were removed by centrifugation, washed with chloroform–ethanol (1:1) and the combined supernatants were concentrated *in vacuo* (35–40°) to a volume of approximately 130 ml. The precipitate was redissolved at 35–40° by adding a minimum amount (15 ml.) of chloroform and was reprecipitated by pouring the chloroform solution into 200 ml. of a cold (10°) acetone–water (1:1) mixture. The mixture was centrifuged and the precipitate was washed with 50 ml. of cold acetone. The lecithin, after drying *in vacuo* over calcium chloride, weighed 6.6 g. It was obtained in a pure state by crystallization from diisobutyl ketone. The material (6.6 g.) was dissolved in diisobutyl ketone (20 ml./1 g.) at 65° and the solution, while still warm, was cleared quickly by centrifugation. The decanted supernatant liquid was allowed to cool slowly to room temperature (three hours). After two hours in the ice-box the lecithin (thick clusters of spherules) was filtered with suction and dried *in vacuo* (0.01 mm.) over paraffin and phosphorus pentoxide for three days; yield of pure L-α-(distearoyl) lecithin, 5.9 g. (84% based on the sulfate); over-all yield from distearin 37.0%. It was also obtained in crystalline state by allowing a warm solution (65 to 70°) of L-α-DSL in dioxane (56 ml./1 g.) to cool slowly to room temperature.

The lecithin sintered at 84–90° forming individual transparent droplets which did not coalesce. On further heating (20°/min. up to 210° and from there on at 10°/min.) the droplets gradually darkened and suddenly formed a meniscus at 230.5–231.5°.[26] When immersed in a bath of 200° and heated at a rate of 3°/min. the substance formed the meniscus at 222–223°; $[\alpha]^{26}D$ + 6.1° in chloroform–methanol (1:1), *c*, 4.2; [M]D + 49.3°.

Anal. Calcd. for $C_{44}H_{90}O_9NP$ (808): C, 65.4; H, 11.2; N, 1.73; P, 3.84. Found: C, 64.6; H, 10.84; N, 1.85; P, 3.81.

Recovery of Choline and Stearic Acid.—A solution of 309.2 mg. of (distearoyl) lecithin in a mixture of 20 ml. of ethanol and 20 ml. of 2 *N* aqueous potassium hydroxide was refluxed for four hours (90–95°). The hot solution was acidified with 4 ml. of 12 *N* sulfuric acid and cautiously concentrated at 95° in a stream of air to a volume of approx. 20 ml. To the cooled solution were added 5 ml. of water and 25 ml. of low-boiling petroleum ether and the mixture transferred quantitatively to a separatory funnel, using 10 ml. of water and 10 ml. of petroleum ether for washing. The aqueous solution was withdrawn and the petroleum ether solution washed once with 10 ml. of water.

(1) **Choline.**—The combined aqueous solutions were neutralized with concentrated ammonium hydroxide to the congo red end-point and the choline determined gravimetrically in the form of its reineckate. Calcd. choline reineckate: 161.0 mg. Found: 152.0 mg. (94.5% recovery).

(2) **Stearic Acid.**—The petroleum ether solution was concentrated to dryness at 50–60° in a stream of air. The residue (214.7 mg.) was titrated according to the method of Stetten[27] using, however, thymol blue as indicator. Calcd. stearic acid: 218.0 mg. Found: 207 mg. (95% recovery).

Cadmium Chloride Compound of L-α-(Distearoyl) Lecithin.—A solution of 0.35 g. of cadmium chloride (2.5 H_2O) in 0.25 ml. of water, diluted with 9 ml. of 99% ethanol, was added gradually and with stirring to a solution of 0.5 g. of L-α-(distearoyl) lecithin in a mixture of 15 ml. of chloroform–ethanol (1:2). After standing in the ice-box for a short time the mixture was centrifuged and the precipitate transferred to a small Büchner funnel using 5 ml. of ethanol. The precipitate was washed with ether and dried in a high vacuum; yield of the amorphous cadmium chloride addition compound 0.61 g. (91%). *Anal.* Calcd. for $[C_{44}H_{90}O_9NP]_2 \cdot [CdCl_2]_3$ (2166): C, 48.7; H, 8.3; N, 1.30; P, 2.86. Found: C, 46.9; H, 8.2; N, 1.27 (Kjeldahl); P, 2.92.

L-α-(Dipalmitoyl) Lecithin

Dipalmitoyl L-α-Glycerylphenylphosphorylcholine.—(1) **Phosphorylation.**—The two-stage phosphorylation of 12 g. (0.021 mole) of D-dipalmitin was carried out as described for distearin, using the same molecular ratios but keeping

to 40°, was diluted with 25 ml. of ethanol and allowed to cool to room temperature. The precipitate after filtering, washing with ethanol and drying, weighed 1.4 g. For analytical purposes, recrystallized from a warm mixture of benzene–ethanol (3:2) (25 ml./g.); (needles) m. p. 80.5–81°; [α]n +3.9° in benzene (*c*, 4.7); [M]D +54°. *Anal.* Calcd. for $C_{84}H_{154}O_{13}P$ (1387): C, 72.7; H, 11.2; P, 2.24. Found: C, 72.64; H, 10.96; P, 2.27.

(26) The choline salt of the distearoyl-L-α-glycerophosphoric acid on heating shows a similar behavior; however, it forms a meniscus at temperatures approximately 20 to 25° lower than that of the corresponding choline ester (lecithins).

(27) Stetten and Grail, *Ind. Eng. Chem. Anal. Ed.*, **15**, 300 (1943)

the cold bath in the first step of the phosphorylation at 7–8°.

(2) **Isolation of the Phosphorylation Product as the Reineckate.**—The reaction mixture was cleared by centrifugation and brought to dryness *in vacuo*. The residue was triturated at the centrifuge with three 100-ml. portions of anhydrous ether. After a few minutes the ether extracts began to deposit a white precipitate which after several hours was centrifuged off, washed with 70 ml. of ether and added to the original ether-insoluble residue. The combined, dried solids (18.7 g.) were freed from water-soluble substances as described for the stearoyl compound, and the dried crude dipalmitoyl-L-α-glycerylphenylphosphorylcholine chloride (11.8 g.) was converted directly to the reineckate. The material was triturated with 120 ml. of warm (40°) 99% ethanol and the mixture was centrifuged. Extraction of the solid was repeated twice with 50 ml. of warm ethanol.[28] To the combined alcoholic solutions were added gradually and with stirring, 120 ml. of an ethanolic ammonium reineckate solution.[24] After standing overnight in the ice-box, the reineckate was filtered off, washed with ethanol and ether, and dried *in vacuo*.

The crude reineckate (11.1 g.) was extracted at the centrifuge with several portions of ethyl acetate totalling 300 ml. and the combined extracts were concentrated *in vacuo* to a small volume. The precipitate was brought into solution at 40° with 9–10 ml. of ethyl acetate and reprecipitated by adding 140 ml. of 99% ethanol. After several hours in the ice-box, the reineckate was recovered and weighed 10.2 g. (43% yield based on dipalmitin); m. p. 136–137°. For analytical purposes 145 mg. of the reineckate was crystallized from 6 ml. of chloroform–ethanol (3:4); yield, 135 mg.; m. p. 136.5–137.5° (cubes).

The solubilities of the dipalmitoyl-L-α-glycerylphenylphosphorylcholine reineckate are similar to those of the corresponding stearoyl compound.

Anal. Calcd. for $C_{50}H_{91}O_8N_7PCrS_4$ (1129): C, 53.2; H, 8.1; N, 8.68; P, 2.75. Found: C, 53.7, 54.0; H, 8.3, 8.1; N, 8.63, 8.68; P, 2.74, 2.75.

(3) **Conversion of the Reineckate to the Sulfate.**—The reineckate (10.2 g.) was dissolved in 160 ml. of a mixture of chloroform–ethanol (1:1) and was converted to the sulfate as described for the corresponding stearoyl compound; yield of the sulfate, 7.0 g. (90%). For analytical purposes, a small amount of the sulfate was reprecipitated from its solution in 99% ethanol by the gradual addition of dry acetone. The sulfate is soluble in chloroform or warm ethanol and insoluble in acetone, ether or water.

Anal. Calcd. for $C_{92}H_{170}O_{20}N_2P_2S$ (1718): C, 64.3; H, 10.0; N, 1.65; P, 3.66. Found: C, 63.8; H, 9.9; N, 1.59 (Kjeldahl); P, 3.62.

(4) **Dipalmitoyl-L-α-glycerylphosphorylcholine: Catalytic Removal of the Phenyl Group.**—A solution of 7.0 g. of the sulfate in 90 ml. of warm (40°) 99% ethanol was cleared by centrifugation and shaken together with 1.8 g. of platinum dioxide in an atmosphere of hydrogen. After one hour the absorption of hydrogen had practically ceased with the consumption of 1040 ml. (N.T.P.); calcd. for the cleavage and the reduction of the catalyst 1060 ml. (N.T.P.).

The catalyst was removed by centrifugation and washed twice with 40 ml. of ethanol–chloroform (1:2). The combined solutions were diluted with 17 ml. of water and stirred in the presence of 2.7 g. of finely powdered barium carbonate for one half-hour. The barium salts were removed by centrifugation and washed with 25 ml. of ethanol–chloroform (1:1). The combined solutions were concentrated *in vacuo* (bath temperature 35°) to about 90 ml. and the precipitate was dissolved at 40° with a minimum of chloroform. The solution was poured into 240 ml. of an ice-cold acetone–water mixture (1:1).

After fifteen minutes the precipitate was removed by centrifugation, washed with cold acetone (45 ml.) and dried over calcium chloride and sodium hydroxide; yield of crude (dipalmitoyl) lecithin, 5.75 g. The material was obtained in a crystalline state from hot (60°) diisobutyl ketone (23 ml./g.) or dioxane (21 ml./g.) as described for the diasteroyl lecithin; yield of pure L-α-(dipalmitoyl)-lecithin, 5.6 g. (91% based on the sulfate); over-all yield, based on dipalmitins 35%.

The substance changed at 75–79° to transparent droplets which on further heating (20°/min. up to 210°, then at 10°/min.), formed a meniscus at 234–235°. When immersed at 200° and heated at a rate of 3°/min. the meniscus formed at 225–226°. The softening points and melting points of the natural and synthetic (dipalmitoyl) lecithin are identical when both substances are heated simultaneously in the same apparatus; $[\alpha]^{23}D$ + 6.6° in chloroform–methanol (1:1), *c*, 4.2; [M]D + 49.5°; reported [α]D + 6.25° in menthanol–chloroform[5] and [α]D + 7.1° in chloroform.[4]

Anal. Calcd. for $C_{40}H_{82}O_9NP$ (752): C, 63.9; H, 11.0; N, 1.86; P, 4.12. Found: C, 63.9; H, 11.1; N, 1.87 (Dumas), 1.81 (Kjeldahl); P, 4.13.

Recovery of Choline and Palmitic Acid.—The lecithin (296.7 mg.) was hydrolyzed and the amounts of liberated choline and palmitic acid in the hydrolysate determined as described for the distearoyl lecithin. Choline reineckate calcd.: 166.7 mg. Found: 160.5 mg. (96.3%); palmitic acid calcd.: 202.3 mg. Found: 196.0 mg. (97.0%).

Cadmium Chloride Compound of L-α-(Dipalmitoyl) Lecithin.—The cadmium chloride compound was prepared as described for the distearoyl lecithin, except that instead of the chloroform–ethanol mixture an equal volume of 99% ethanol was used; yield 98%.

The substance was hydrolyzed (100°, two hours) in 2.5 *N* hydrochloric acid (60 ml./1 g.) for the determination of cadmium and choline, and in 2.5 *N* nitric acid (75 ml./1 g.) for the determination of chlorine. Each hydrolysate was quantitatively separated into a petroleum ether-soluble fraction and a water-soluble fraction. The petroleum ether fractions were brought to dryness and the palmitic acid in each residue determined titrimetrically according to Stetten.[27] The cadmium in the hydrochloric acid solution was precipitated as cadmium carbonate and determined gravimetrically as sulfate. The choline in the filtrate of the cadmium carbonate precipitate was determined in the form of its reineckate. The chloride was determined gravimetrically as silver chloride using the nitric acid solution.

Anal. Calcd. for $[C_{40}H_{82}O_9NP]_2[CdCl_2]_3$ (2053.4): C, 46.7; H, 8.0; N, 1.36; P, 3.02; Cd, 16.4; Cl, 10.4; palmitic acid, 49.8; choline, 11.8. Found: C, 46.2; H, 8.0; N, 1.27 (Kjeldahl); P, 3.00; Cd, 16.4; Cl, 10.8; palmitic acid, 47.6; choline, 11.2. Ratios of P:Cd: Cl calcd.: 1.00:1.50:3.00. Found: 1.00:1.51:3.15.

L-α-(Dimyristoyl) Lecithin

Dimyristoyl-L-α-glycerylphenylphosphorylcholine: (1) Phosphorylation.—The two-stage phosphorylation was carried out as described for the corresponding palmitoyl compound starting with 10.3 g. (0.02 mole) of D-α,β-dimyristin.

(2) **Isolation of the Phosphorylation Product as the Reineckate.**—The chloroform solution containing the phosphorylation products was cleared by centrifugation and evaporated to dryness *in vacuo*. The solid was triturated at the centrifuge with three 80-ml. portions of anhydrous ether. The combined extracts, which contained

(28) The ethanol-insoluble residue (2.9 g.) consisted almost entirely of bis-α,α-(D-dipalmitoylglyceryl)-phenyl phosphate. The substance was obtained in a pure state by dissolving the residue in 20 ml. of chloroform, diluting the solution with 40 ml. of ether, and clearing the solution by filtration. The filtrate was warmed to 35°, diluted with 30 ml. of 99% ethanol and cooled gradually to 18°. After filtering with suction, washing with ethanol and drying *in vacuo*, the substance weighed 2.5 g.; needles, m. p. 75–76°; [α]D +4.0°, in dry benzene (*c*, 4.9); [M]D +51°. *Anal.* Calcd. for $C_{76}H_{139}O_{12}P$ (1275): C, 71.5; H, 10.98; P, 2.43. Found: C, 71.5; H, 10.91; P, 2.46.

most of the dimyristoyl compound, were diluted with 150 ml. of dry ether, cooled to 10° and centrifuged. The precipitate was washed with cold ether and dried *in vacuo;* weight 5.6 g. The residue of the ether extraction still contained some of the desired product. By suspending the residue in 110 ml. of a 0.5% solution of sodium bicarbonate in acetone–water (1:4), keeping the suspension in ice overnight, centrifuging sharply, washing the precipitate with ice-cold acetone and ether, and drying *in vacuo* an additional 1.0 g. of product was obtained. The combined solids (6.6 g.) were suspended in 80 ml. of 99% ethanol and the solution was centrifuged.[29] The alcohol-soluble dimyristoyl L-α-glycerylphenylphosphorylcholine chloride was converted to the reineckate and the reineckate was purified as described for the corresponding dipalmitoyl compound. Yield of dimyristoyl-L-α-glycerylphenylphosphorylcholine reineckate was 6.9 g. (32% based on dimyristin); m. p. 138–139°. For analytical purposes the reineckate was recrystallized from a mixture of chloroform–ethanol (3:4); m. p. 138–138.5°. *Anal.* Calcd. $C_{46}H_{83}O_8N_7PCrS_4$ (1073): C, 51.5; H, 7.8; N, 9.13; P, 2.89. Found: C, 51.5; H, 8.0; N, 9.07; P, 2.88.

(3) **Conversion of the Reineckate to the Sulfate.**—The reineckate (6.9 g.) was dissolved in 110 ml. of a chloroform–ethanol mixture (1:1) and was converted to the sulfate as described for the distearoyl compound, except that to the concentrate of the combined supernatant liquids (130 ml.) acetone (28 ml.) was added; yield 4.5 g. (87%) of sulfate. For analytical purposes the sulfate was precipitated from its solution in ethanol by gradual addition of dry acetone. At room temperature the sulfate is readily soluble in ethanol or chloroform and insoluble in acetone, ether or water. *Anal.* Calcd. for $C_{84}H_{154}O_{20}N_2P_2S$ (1605): C, 62.8; H, 9.7; N, 1.74; P, 3.86. Found: C, 62.1; H, 9.9; (Kjeldahl) N, 1.69; P, 3.76.

Dimyristoyl-L-α-glycerylphosphorylcholine.—The conversion of the sulfate (4.5 g.) to the lecithin was carried out as described for the dipalmitoyl compound, except for the following changes: the spent catalyst was washed with ethanol alone; the ethanolic solution of the lecithin was concentrated *in vacuo* until foaming prevented further concentration; and more of the cold (−5°) acetone–water mixture (220 ml.) was used for the precipitation of the lecithin; yield of crude (dimyristoyl) lecithin 3.4 g. Recrystallization from hot (50°) diisobutyl ketone (80 ml.) as described for the dipalmitoyl compound yielded 3.2 g. of pure L-α-(dimyristoyl) lecithin (82%); over-all yield 23%. The substance changes at 60–70° to a clear, viscous mass which at 237–237.5° suddenly forms a meniscus and darkens; $[\alpha]_D$ + 7.0° in abs. chloroform–methanol (1:1), *c*, 3.9; $[M]_D$ + 48.7°. *Anal.* Calcd. for $C_{36}H_{74}O_9NP$ (695.6): C, 62.1; H, 10.7; N, 2.01; P, 4.46. Found: C, 62.0; H, 11.1; N, 1.97; P, 4.43.

Recovery of Choline and Myristic Acid.—The substance (247.2 mg.) was hydrolyzed and the liberated choline and myristic acid determined as described above: choline reineckate, calcd., 149.5 mg. Found: 145.5 mg. (97.5%). Myristic acid, calcd., 162.0 mg. Found: 157.0 mg. (97.2%).

Cadmium Chloride Compound of L-α-(Dimyristoyl) Lecithin.—Prepared as described for the dipalmitoyl compound; yield 98%. *Anal.* Calcd. for $[C_{36}H_{74}O_9NP]_2[CdCl_2]_3$ (1942): C, 44.5; H, 7.7; N, 1.44; P, 3.19. Found: C, 45.3; H, 7.74; N, 1.38 (Kjeldahl); P, 3.26.

Solubilities of the Synthetic Lecithins

In order to estimate the solubilities in ethanol, ether and acetone, stoppered test-tubes containing the lecithin and the solvent were shaken in a water-bath at 22–23° for eighteen to twenty hours, care being taken that the amount of solvent was insufficient to dissolve all of the lecithin present. The suspensions were centrifuged, known volumes of the supernatant solutions brought to dryness and the residues weighed. The solubilities in glacial acetic acid, in pyridine and in methanol were estimated by adding to known amounts of lecithin just sufficient solvent to effect solution. The values thus obtained (see Table I) while not precise, indicate the approximate magnitude of the solubilities of the three lecithins.

TABLE I

SOLUBILITY OF THE SYNTHETIC LECITHINS AT 22–23° IN G./100 ML. OF SOLUTION

Solvent (dry)	DSL	DPL	DML
Ethanol	0.8	1.5	>15[a]
Ether	0.02	0.02	0.03
Acetone	0.01	0.02	0.06
Pyridine	0.5[b]	1.1[b]	1.3[b]
Acetic acid	1.3[b]	4.0[b]	>10[a]
Methanol	0.8[b]	1.4[b]	24[a]

[a] The solubility lies above the stated amount. For lack of material the saturation point was not attained. [b] Not more than the amount stated.

All three synthetic lecithins are readily soluble in chloroform, hot diisobutyl ketone or hot dioxane. They are readily emulsified in water; it is noteworthy that the stability of the emulsions increases with decreasing chain length of the fatty acids.

D,L-α-Lecithins

The synthesis of the racemic α-lecithins is identical with that described for the corresponding L-forms, except that D,L-α,β-diglycerides are used as starting materials. Only the physical and analytical data of the various compounds will be reported here.

D,L-α-(Distearoyl) Lecithin

Distearoyl-D,L-α-glycerylphenylphosphorylcholine: (a) **Reineckate.**—M. p. 138–139° (recrystallized from ethanol–chloroform (1:1)). *Anal.* Calcd. for $C_{54}H_{99}O_8N_7PCrS_4$ (1185): C, 54.7; H, 8.42; N, 8.26; P, 2.62. Found: C, 55.0; H, 8.13; N, 8.52; P, 2.56.

(b) **Sulfate.**—Obtained from the reineckate in a yield of 88%. *Anal.* Calcd. for $C_{100}H_{186}O_{20}N_2P_2S$ (1829.6): C, 65.5; H, 10.2; N, 1.53; P, 3.38. Found: C, 66.1; H, 10.4; N, 1.43; P, 3.26.

Distearoyl-D,L-α-glycerylphosphorylcholine.—The substance sinters from 75–81° to a clear viscous mass which forms a meniscus and darkens at 224–225°. *Anal.* Calcd. for $C_{44}H_{90}O_9NP$ (808): C, 65.4; H, 11.2; N, 1.73; P, 3.84. Found: C, 66.2; H, 10.5; N, 1.47; P, 3.76.

D,L-α-(Dipalmitoyl) Lecithin

Dipalmitoyl-D,L-α-glycerylphenylphosphorylcholine: (a) **Reineckate.**—M. p. 137–138°, recrystallized from ethanol–chloroform (1:1). *Anal.* Calcd. for $C_{50}H_{91}O_8N_7PCrS_4$ (1129): C, 53.2; H, 8.1; N, 8.68; P, 2.75. Found: C, 53.3; H, 8.17; N, 8.63; P, 2.71.

(b) **Sulfate.**—Obtained from the reineckate in a yield of 85%. *Anal.* Calcd. for $C_{92}H_{170}O_{20}N_2P_2S$ (1718): C, 64.3; H, 10.0; N, 1.63; P, 3.61. Found: C, 65.0; H, 9.8; N, 1.73; P, 3.61.

Dipalmitoyl-D,L-α-glycerylphosphorylcholine.—The substance sinters at 73–75° to a clear viscous mass, which forms a meniscus and darkens at 227–229°. *Anal.* Calcd. for $C_{40}H_{82}O_9NP$ (752): C, 63.8; H, 11.0; N, 1.86; P, 4.12. Found: C, 64.5; H, 10.9; N, 1.77; P, 4.08.

Acknowledgments.—This work has been supported by grants, at various times, from the Ontario Research Commission, the Banting Re-

(29) The ethanol-insoluble material (1.2 g.) was mainly bis-α,α-(D-dimyristoylglyceryl)-phenyl phosphate. It was purified by dissolving it in 50 ml. of warm (40°) acetone, clearing the solution by centrifugation and allowing the supernatant liquid to cool gradually to room temperature (20–22°). After filtering, washing with acetone, and drying, the substance weighed 1.0 g.; needles; m. p. 67.5–68°; $[\alpha]_D$ +4.5°, in dry benzene, (*c*, 4.5); $[M]_D$ +52°. *Anal.* Calcd. for $C_{68}H_{133}O_{12}P$ (1163): C, 70.4; H, 10.65; P, 2.66. Found: C, 70.4; H, 10.65; P, 2.68.

search Foundation and the Nutrition Foundation, Inc., to whom we express our sincerest thanks. We wish also to express our appreciation to Professor M. A. Peacock, Professor G. F Wright and Mr. A. Brook for the X-ray diffraction photographs, to Professor S. J. Thannhauser for a gift of the natural dipalmitoyl lecithin, to Mr. F. M. Martin for preparing some of the starting materials and to Mrs. E. Mason for the combustion analyses.

Summary

1. A method for the synthesis of both enantiomeric forms of fully saturated α-lecithins of assured constitutional and configurational purity has been developed.

2. The synthesis of three homologous α-lecithins of the L-series, namely, (distearoyl)-, (dipalmitoyl)- and (dimyristoyl)- lecithin is described.

3. The X-ray diffraction patterns, the solubilities and other physical data of these pure, individual, crystalline lecithins are reported.

4. The synthetic L-α-(dipalmitoyl) lecithin and natural (dipalmitoyl) lecithin were found to be identical, thus establishing the α-constitution and L-configuration of this natural lecithin.

TORONTO 5, CANADA RECEIVED JUNE 23, 1949

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY PASADENA 4, CALIFORNIA, No. 1261]

The Crystal Structure of DL-Alanine. II. Revision of Parameters by Three-Dimensional Fourier Analysis[1]

BY JERRY DONOHUE

Introduction

The crystal structure of DL-alanine was determined over seven years ago by Levy and Corey.[2] Crystals of DL-alanine are isomorphous with the space group C_{2v}^9–Pna, having no center of symmetry. Because of the low symmetry of the crystal and the pecularities of its atomic arrangement, only limited use could be made of the customary Fourier projections or structure factor plots, and the refinement of the parameters of all atoms but one was carried out primarily by trial and error methods. Most use was made of reflections of the type (*hk*0) in the determination of the structure, since for this zone alone the structure factors are real. With the exception of the methyl carbon atom, which was resolved in a Fourier projection on (001), the *x*- and *y*-parameters were arrived at by the use of plots of the trigonometric portion of the structure factors as guides in obtaining the best agreement between observed and calculated values of F_{hk0}. The *z*-parameters of all atoms were refined by trial and error adjustment of all six atoms in accord with intensity data from (0*kl*) and (*h*0*l*) reflections. Because of the molecular arrangement, no resolution of atomic peaks could be expected in asymmetric Fourier projections on either (100) or (010), and the calculation of a three-dimensional Fourier function was prohibited by the excessive time and labor which would have been involved.

The calculation of three-dimensional plots of interatomic vectors and electron densities has been made feasible recently through the use of punched card methods[3a] and especially by the design[3b] of a file of cards to correspond to a set of Beevers–Lipson strips. These methods have made possible the rapid calculation of one- and two-dimensional Patterson and electron density functions and have reduced to the order of several days the time required for the calculation of corresponding three-dimensional functions. In connection with a determination of the crystal structure of L-threonine[4] based on three-dimensional Fourier functions a new punched card method was devised[5] which greatly reduces the time required for the calculation of complex structure factors, a very significant item in the analysis of crystals having no center of symmetry.

The dimensions of the L-threonine molecule as determined by this very exhaustive X-ray investigation[4] are, with one exception, in good agreement with the corresponding dimensions found by Levy and Corey in the molecule of DL-alanine. In their analysis of alanine the C–N distance was found to be 1.43 Å., about 0.04 Å. shorter than that calculated from the generally accepted covalent radii.[6] In threonine the C–N distance was established as 1.49 Å., close to the normal value. Since methods are now available for the calculation of three-dimensional plots of electron density, this discrepancy in the C–N distances suggested the desirability of a redetermination of the parameters of DL-alanine so as to

(1) Aided by a grant from the National Foundation for Infantile Paralysis.

(2) H. A. Levy and R. B. Corey, THIS JOURNAL, **63**, 2045 (1941).

(3) (a) P. A. Shaffer, Jr., V. Schomaker, and L. Pauling, *J. Chem. Phys.*, **14**, 648 (1946); (b) V. Schomaker, unpublished work.

(4) D. Shoemaker, J. Donohue, V. Schomaker, and R. B. Corey, submitted to THIS JOURNAL.

(5) J. Donohue and V. Schomaker, *Acta Cryst.*, **2**, 344 (1949).

(6) L. Pauling, "The Nature of the Chemical Bond," second edition, Cornell University Press, Ithaca, N. Y., 1940. For a compilation of C–N bond distances observed in various compounds see E. W. Hughes and W. N. Lipscomb, THIS JOURNAL, **68**, 1970 (1946).