SYNTHESIS OF ENANTIOMERIC α-PHOSPHATIDIC ACIDS

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Chibnall and Channon, investigating the cytoplasm of cabbage and spinach leaves (1), isolated a mixture of substances which resembled monophosphatides in their structure and composition, but were virtually free of nitrogenous bases. The main constituents were identified as the calcium, magnesium, and potassium salts of phosphoric acid esters of diglycerides. These compounds, constituting a new class of phosphatides, were named phosphatidic acids. Their fatty acid content differed from that of the lecithins and cephalins in that the greater part of the acids consisted of linolic and linolenic acids, whereas palmitic acid and stearic acid were only minor constituents. More recently phosphatidic acids have been found also in wheat germ (2) and in tubercle bacilli (3–6).

Doubts have been expressed as to the origin of the phosphatidic acids in the cytoplasm. Whereas Channon and Chibnall were inclined to believe that these substances are intermediate compounds in the metabolism of lecithins and cephalins, Levene and Komatsu expressed the view that they are formed after death of the tissue. The recent discovery by Hanahan and Chaikoff (7) of a phospholipide-splitting enzyme in fresh cabbage leaves, which is capable of attacking the nitrogenous base-phosphoric ester linkage, however, seems to add considerable weight to the contention of Channon and Chibnall.

As has been discussed more fully elsewhere (8–11) an asymmetrically substituted triglyceride can be assigned to either one of the two optical series. Hence in the specific case of α-phosphatidic acids any particular member can be considered as a derivative of either its diglyceride or of its glycerophosphoric acid (GPA) moiety and thus can be assigned to either the D or L series respectively. Since in every α-phosphatidic acid the glycerophosphoric acid is the same and its stereochemical relationship to glyceraldehyde has already been established (12), it is proposed as the stereochemical reference compound. Thus arbitrarily, but in conformity with the usage in the α-lecithin (8–10) and α-cephalin (13) series, an α-phosphatidic acid is assigned the L configuration if it contains L-α-GPA and vice versa.

The synthesis of the racemic forms of fully saturated α-phosphatidic acids has been reported by a number of investigators (14–21). In all
instances the starting material or the method of synthesis was not wholly satisfactory and, as a result, products of questionable identity were obtained.

This paper presents a method for the synthesis of both the racemic and enantiomeric forms of fully saturated \( \alpha \)-phosphatidic acids. The sequence of reactions is such that the asymmetry of the substituted glycerol molecule is maintained throughout, whereby the optical purity of the phosphatidic acids is assured. The synthesis is as follows (see the reaction scheme): The D or L isomer of a fully saturated \( \alpha,\beta \)-diglyceride (A), prepared according to the method of Sowden and Fischer (9, 22) is phosphorylated by means of diphenylphosphoryl chloride in the presence of pyridine, and the reaction product (B) is freed from its phenyl groups by catalytic hydrogenolysis. The desired phosphatidic acid (C) is obtained in analytically pure state in over-all yields ranging from 75 to 82 per cent. The configuration of the phosphatidic acid is determined by that of the starting material. Thus the phosphorylation of a D-, L-, or DL-\( \alpha \)-diglyceride yields a L-, D-, or DL-phosphatidic acid, respectively.

\[
\text{Reaction Scheme}
\]

\[
\begin{align*}
\text{H}_2\text{C} & \text{--OOC}\cdot \text{R} \\
\text{CH} & \text{--OOC}\cdot \text{R} \\
\text{H}_2\text{C} & \text{--OH} \\
\text{\( \alpha,\beta \)-Diglyceride} & \\
\text{R} & = -\text{C}_1\text{H}_6\text{H} \\
& -\text{C}_1\text{H}_2\text{H} \\
& -\text{C}_1\text{H}_4\text{H} \\
\text{(A)} \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_2\text{C} & \text{--OOC}\cdot \text{R} \\
\text{CH} & \text{--OOC}\cdot \text{R} \\
\text{H}_2\text{C} & \text{--OH} \\
\text{\( \alpha,\beta \)-Diglyceride} & \\
\text{R} & = -\text{C}_1\text{H}_6\text{H} \\
& -\text{C}_1\text{H}_2\text{H} \\
& -\text{C}_1\text{H}_4\text{H} \\
\text{(A)} \\
\end{align*}
\]

\[
\begin{array}{c}
\text{(1) D-} \\
\text{(2) L-} \\
\text{(3) DL-} \\
\end{array}
\]

\[
\begin{array}{c}
\text{\( \alpha,\beta \)-diglyceride} \\
\rightarrow \\
\text{\( \alpha \)-phosphatidic acid} \\
\text{(A)} \\
\end{array}
\]

\[
\begin{array}{c}
\text{(1) L-} \\
\text{(2) D-} \\
\text{(3) DL-} \\
\end{array}
\]

\[
\begin{array}{c}
\text{\( \alpha \)-phosphatidic acid} \\
\rightarrow \\
\text{\( \alpha \)-phosphatidic acid} \\
\text{(A)} \\
\end{array}
\]

\[
\begin{array}{c}
\text{(1) D-} \\
\text{(2) L-} \\
\text{(3) DL-} \\
\end{array}
\]

\[
\begin{array}{c}
\text{\( \alpha \)-phosphatidic acid} \\
\rightarrow \\
\text{\( \alpha \)-phosphatidic acid} \\
\text{(A)} \\
\end{array}
\]

From previous investigations in this laboratory it was known that the following biologically occurring substances, namely \( \alpha \)-GPA (12), \( \alpha \)-glycerylphosphorylcholine (8, 23), and distearoyl- and dipalmitoyl-\( \alpha \)-lecithin\(^1\) (9, 21).

\(^1\) So far these are the only two lecithins isolated in pure state from natural sources.
10) are members of the L series. It seemed reasonable therefore to assume that the biologically occurring α-phosphatidic acids would belong to the same series. For this reason the L-phosphatidic acids have been given first consideration from the standpoint of synthesis. The present report describes the synthesis of the distearoyl-, dipalmitoyl-, and dimyristoyl-L-α-glycerophosphoric acids and the preparation of their choline and ethanolamine salts. With the exception of the α-lysolecithins, α-lysocephalins, and L-α-glycerylphosphorylcholine, all the intermediate compounds which conceivably might occur in the biological synthesis and degradation of the fully saturated L-α-distearoyl-, dipalmitoyl-, or dimyristoyllecithin and the corresponding cephalins have now been synthesised (8, 10, 12, 22, 24) in pure state. The synthesis of L-α-glycerylphosphorylcholine is in progress in this laboratory.

The author has long been attracted by the hypothesis that L-α-glycerophosphoric acid is a possible precursor in the biosynthesis of the α-phospholipides and is supplied via the carbohydrate cycle, where it is presumably formed by the enzymatic asymmetric reduction of the dihydroxyacetone phosphate (12). This idea has received considerable support as work progressed, since the L-α-lecithins and all of their intermediates so far studied have constitutional and configurational relationships consistent with this hypothesis.

**EXPERIMENTAL**

Distearoyl-L-α-glycerophosphoric Acid Diphenyl Ester (I)—A solution of 12.5 gm. (0.02 mole) of L-α,β-distearin (m.p. 75–76°, [α]D = -2.7° (22)) in 125 ml. of dry pyridine was mixed with a solution of 5.92 gm. (0.022 mole) of diphenylphosphoryl chloride (25) in 30 ml. of dry pyridine, and the mixture, protected from moisture, was kept at 30° for 18 hours. 1 hour before the end of this period 2 ml. of water were added to destroy the excess of diphenylphosphoryl chloride. The reaction product was precipitated by the gradual addition of 100 gm. of chopped ice, followed by 650 ml. of ice-cold water. After standing in ice for 2 hours, the coarse material was filtered with suction, washed on the filter thoroughly with water, and dried in vacuo over phosphorus pentoxide to constant weight. The crude phosphatidic acid diphenyl ester, weighing 17.4 gm., was dissolved in 370 ml. of warm (30°) petroleum ether (b.p. 35–60°) and, after addition of a small amount of Hyflo Super-Cel, filtered while still warm. The filtrate was brought to dryness under diminished pressure and the residue freed in vacuo (0.005 mm.) from solvent and traces of pyridine. The yield of almost pure distearoyl L-α-glycerophosphoric acid diphenyl ester (I) was

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1. α indicates the position of the phosphoric acid.
2. The substance is pure enough at this stage for further processing.
16.1 gm. (94 per cent); m.p. 52–53°; \([\alpha]_s = +1.96^\circ\) in anhydrous and ethanol-free chloroform\(^4\) (c 10.2).

For further purification the substance (1.0 gm.) was triturated with ethyl acetate (15 ml.) at 22° and the solution, after being freed from a small amount of insoluble material, was centrifuged at 0° for \(\frac{1}{2}\) hour. The solid was dried \textit{in vacuo} (0.002 mm.) to constant weight. Recovery was 90 per cent of compound I. The substance started to sinter at 49.5°, melted at 50.5–51.5°, solidified at 52°, and melted again at 54.5–55°; \([\alpha]_s = +2.0^\circ\) in chloroform (c 0.9); \(M_s = +17.1^\circ\).

\textit{Analysis}—\(C_{41}H_{75}O_5P\) (856.7)

\begin{align*}
\text{Calculated.} & \quad C \ 71.43, \ H \ 10.00, \ P \ 3.62 \\
\text{Found.} & \quad \ \\
\end{align*}

The phosphorylation of \(D-\alpha,\beta\)-dipalmitin (22) and of \(D-\alpha,\beta\)-dimyristin (10) was carried out as described for distearin, with the same molecular ratios of diglyceride, diphenylphosphoryl chloride, and pyridine.

\textit{Dipalmitoyl-L-\alpha-glycerophosphoric Acid Diphenyl Ester (II)—}The yield of diphenyl ester was 90 per cent (found, P 3.80 per cent). For analysis the substance was recrystallized from ethyl acetate (5 ml. per 1 gm. of substance; cooling to \(-15^\circ\)) and dried in a high vacuum. Recovery 93 per cent; over-all yield 83.7 per cent; m.p. 47–48° (sintering at 46°); \([\alpha]_s = +2.4^\circ\) in chloroform (c 11.0); \(M_s = +19.2^\circ\).

\textit{Analysis}—\(C_{47}H_{77}O_5P\) (800.7)

\begin{align*}
\text{Calculated.} & \quad C \ 70.43, \ H \ 9.70, \ P \ 3.87 \\
\text{Found.} & \quad \ \\
\end{align*}

\textit{Dimyristoyl-L-\alpha-glycerophosphoric Acid Diphenyl Ester (III)—}The yield of diphenyl ester was 95 per cent (found, P 4.18 per cent). For analysis the substance was recrystallized from 99 per cent ethanol (15 ml. per 1 gm.; cooling to 0°) and dried in a high vacuum. Recovery 85 per cent; over-all yield 80.7 per cent; m.p. 38–39° (sintering at 37°); \([\alpha]_s = +2.6^\circ\) in chloroform (c 10); \(M_s = +19.4^\circ\).

\textit{Analysis}—\(C_{43}H_{80}O_5P\) (744.7)

\begin{align*}
\text{Calculated.} & \quad C \ 69.29, \ H \ 9.36, \ P \ 4.16 \\
\text{Found.} & \quad \ \\
\end{align*}

These phosphatidic acid diphenyl esters (I, II, III) are readily soluble at room temperature in ethyl ether, butyl ether, dioxane, ethyl acetate, acetone, chloroform, benzene, cyclohexane, and petroleum ether, moderately soluble in boiling methanol, and insoluble in water. In the same sol-

\(^4\) All optical rotations reported in this communication were determined in anhydrous ethanol-free chloroform (distilled from phosphorus pentoxide).
vent their solubility decreases with increasing length of the fatty acid. They decompose gradually, with the liberation of phenol.

_Distearoyl-L-α-glycerophosphoric Acid (IV)—_A solution of 17.1 gm. (0.0200 mole) of distearoyl-L-α-glycerophosphoric acid diphenyl ester (I) in 300 ml. of glacial acetic acid and 3.0 gm. of platinic oxide (Adams’ catalyst) was shaken vigorously in an atmosphere of pure hydrogen at an initial pressure of 40 to 50 cm. of water until the absorption of hydrogen ceased. Within 1 hour 8.30 moles of hydrogen had been consumed. After replacement of the hydrogen by nitrogen and addition of chloroform until all phosphatidic acid was dissolved (approximately 200 ml.), the platinum catalyst was removed by filtration and the clear filtrate brought to dryness _in vacuo_ (10 mm.) at a bath temperature of 30–40°. Yield of fairly pure distearoyl-L-α-glycerophosphoric acid (DS-L-α-GPA), 13.9 gm. or 98.6 per cent; total P found, 4.34 per cent. The substance was recrystallized from boiling acetone (40 ml. per gm.). Recovery 91 per cent; over-all yield, 89.7 per cent; \([\alpha]_\beta^{25} = +3.8°\) in chloroform (c 9.3); \(M_\phi = +26.8°\).

_Analysis_—C_{30}H_{72}O_{8}P (704.6)

| Calculated | C 66.42, H 11.01, P 4.42 |

The solubilities of DS-L-α-GPA in various anhydrous solvents, expressed as the weight\(^5\) of the solute in 100 ml. of solution at 21°, are <1 mg. in petroleum ether, 80 mg. in acetone, 150 mg. in benzene, 350 mg. in acetic acid, 270 mg. in methanol, 800 mg. in ethanol, and 1.6 gm. in ether.

_Dipalmitoyl-L-α-glycerophosphoric Acid (V)—_The catalytic hydrogenolysis of the dipalmitoyl-L-α-glycerophosphoric acid (DP-L-α-GPA) diphenyl ester (16.0 gm., 0.0200 mole) in glacial acetic acid (300 ml.) and isolation of the phosphatidic acid were carried out as described for the corresponding distearoyl compound. The crude product (12.7 gm. or 98 per cent, P 4.54 per cent) was dissolved in boiling acetone (15 ml. per gm.), and the solution cooled to 0° and filtered. Recovery of analytically pure dipalmitoyl-L-α-glycerophosphoric acid, 83 per cent (10.5 gm.); over-all yield 81 per cent; \([\alpha]_\beta^{25} = +4.0°\) in chloroform (c 9.6); \(M_\phi = +25.9°\).

_Analysis_—C_{55}H_{110}O_{8}P (648.6)

| Calculated | C 64.75, H 10.73, P 4.78 |
| Found      | “ 64.50, “ 10.50, “ 4.70 |

The solubilities of DP-L-α-GPA in various anhydrous solvents, expressed as the weight\(^5\) of the solute in 100 ml. of solution at 21°, are <1 mg. in

\(^5\) The values, while not very precise, give the approximate magnitude of solubility.
petroleum ether, 1.6 gm. in acetone, 4.2 gm. in benzene, 1.3 gm. in acetic acid, 2.0 gm. in methanol, 2.5 gm. in ethanol, and 1.5 gm. in ether.

Dimyristoyl-L-\(\alpha\)-glycerophosphoric Acid (VI)—The reductive cleavage of the dimyristoyl-L-\(\alpha\)-glycerophosphoric acid (DM-L-\(\alpha\)-GPA) diphenyl ester (0.0200 mole, 12.97 gm.) was carried out as described for the distearoyl compound. The crude phosphatidic acid (11.56 gm. or 97.6 per cent, P found 5.12 per cent) was purified by reprecipitation from anhydrous acetone (10 ml. per 1 gm.) by cooling to \(-35^\circ\). Recovery, 89.5 per cent (10.35 gm.); over-all yield, 87.3 per cent; \([\alpha]^2_{D} = +4.4^\circ\) in chloroform (c 11); \(M_\alpha = +26.1^\circ\).

**Analysis**—C\(_{31}\)H\(_{61}\)O\(_{10}\)P (592.5)

Calculated. C 62.78, H 10.38, P 5.23

Found. C 63.01, H 10.25, P 5.33, 5.26

The solubility of the DM-L-\(\alpha\)-GPA in acetone, benzene, glacial acetic acid, methanol, ethanol, and ether is considerably higher than that of the two other phosphatidic acids in the same solvents.

The melting points of the phosphatidic acids IV, V, and VI are not reproducible with the desired degree of accuracy and are therefore omitted.

The phosphatidic acids (IV, V, VI) are free from inorganic phosphoric acid and are relatively stable substances. On long standing, however, they show signs of change. The observation of Grün and associates (17, 18, 26) that the phosphatidic acids readily undergo disproportionation to inorganic phosphoric acid and bisdiglyceride phosphoric acid esters\(^*\) could not be confirmed. On exposure of one of the synthetic \(\alpha\)-phosphatidic acids in a stoppered test tube to the changing conditions of light and temperature of our southerly exposed laboratory for a period of 5 months, its content of inorganic phosphoric acid increased from the initial value of 0.35 per cent to 1.7 per cent of the organically bound phosphoric acid.

Assuming that this rate remains constant, it would take at least 14 years to complete the transesterification. It is obvious that the phosphatidic acids do not, when pure, possess the pronounced tendency to disproportionate attributed to them by Grün and his associates. Grün’s use of phosphorus pentoxide as the phosphorylating agent probably led to the formation of highly unstable organic polyphosphates which contaminated his preparations and were the source of the inorganic phosphate.

As was reported recently (27), the alkaline degradation of phosphatidic acids, in contrast to that of \(\alpha\)-glycerylphosphorylcholine (28) and of \(\alpha\)-lecithins (27), is not accompanied by migration of phosphoric acid and hence yields optically pure \(\alpha\)-GPA. On the other hand, acid hydrolysis of \(\alpha\)-phosphatidic acid yields (27) mixtures of \(\alpha\)- and \(\beta\)-glycerophosphoric acid.

\[^*\]2(C\(_{19}\)H\(_{36}\)COO)\(_{2}\cdot\)C\(_{16}\)H\(_{31}\)OPO\(_{3}\)H\(_{2}\) \(\rightarrow\) H\(_{2}\)PO\(_{4}\) \(+\) [(C\(_{19}\)H\(_{36}\)COO)\(_{2}\cdot\)C\(_{16}\)H\(_{31}\)O\(_{2}\)·PO(OH)].
acids, similar in composition to those obtained by acid hydrolysis of α-glycerylphosphorylcholine (28) and of α-lecithins (27).

Recent investigations by Rosenberg (29), Kline (30), and Allen have shown that in antigens for serological tests of syphilis the “purified natural lecithin” component can be replaced to advantage by a synthetic lecithin, such as the dipalmitoyl- and dimyristoyl-L-α-lecithins. Dr. R. H. Allen, of the Department of National Health and Welfare, Ottawa, has found that the choline salt of dipalmitoyl-L-α-glycerophosphoric acid, in contrast to the corresponding choline ester, is without serological activity in this respect.

Choline Salts—The choline salts were prepared by dissolving 1.0 gm. of DS-, DP-, or DM-L-α-GPA in 45, 25, or 20 ml., respectively, of warm (60°) 99 per cent ethanol and adding 0.38, 0.45, or 0.50 gm., respectively, of an aqueous solution of choline bicarbonate (8.8 per cent by base) dissolved in 5 ml. of 99 per cent ethanol. This corresponds to an approximate ratio of 1.1 mole of choline to 1.0 mole of phosphatidic acid. A larger excess of choline should be avoided. The solution of the choline salt of DS-L-α-GPA was filtered hot, while those of DP- and DM-L-α-GPA were cooled to room temperature before filtering. The clear filtrates were brought to dryness under diminished pressure. The solid residues were suspended in cold, dry acetone, filtered, and dried in vacuo. The crude choline salts of DS-, DP-, and DM-L-α-GPA were obtained in yields of 74, 67, and 86 per cent (0.85, 0.78, and 1.0 gm.), respectively.

Distearoyl-L-α-glycerophosphoric Acid Monocholine Salt (VII)—The crude choline salt (0.85 gm.) was dissolved in 30 ml. of a warm mixture of chloroform and acetone (1:1). The warm solution was filtered and cooled to room temperature (24°), and the crystals were collected on a Büchner funnel and washed with dry acetone. The vacuum-dried choline salt weighed 0.78 gm. (92 per cent recovery) and was analytically pure. The substance started to sinter slightly at 90° and, on being further heated, gradually turned into translucent droplets which suddenly formed a meniscus at 197–198°. [α]_D$^2$ = +9.1° in chloroform (c 2.4); M_p = +73.5°.

Analysis—C_{40}H_{63}O_{6}N_{3}P (807.7)

Calculated. C 65.37, H 11.23, N 1.73, P 3.84

Found. C 65.40, H 11.09, N 1.70, P 3.81

Dipalmitoyl-L-α-Glycerophosphoric Acid Monocholine Salt (VIII)—The crude choline salt (0.78 gm.) was recrystallized from 23 ml. of a warm

7 Private communication.
8 The author wishes to express his gratitude to Dr. J. K. Dale, Commercial Solvents Corporation, Terre Haute, Indiana, for his generous gift of a concentrated choline bicarbonate solution.
mixture of chloroform and acetone (1:1) as described for the other choline salt; recovery, 0.70 gm. (90 per cent). The salt sintered at 75°C, formed translucent droplets at about 85°C, and melted with the formation of a meniscus at 192.5-193°C. \([\alpha]_D^{25} = +10.8^\circ\) in chloroform (c 2); \(M_\infty = +81.2^\circ\).

**Analysis—**C_{45}H_{92}O_{18}N_P (751.7)
Calculated. C 63.85, H 11.00, N 1.86, P 4.12
Found. " 63.64, " 10.84, " 1.85, " 4.17

**Dimyristoyl-L-α-Glycerophosphoric Acid Monocholine Salt (IX)—**The crude salt (1.01 gm.) was recrystallized from 20 ml. of a warm mixture of chloroform and acetone (1:2). The recovery of analytically pure choline salt was 0.87 gm. (86 per cent). The substance sintered at 63-65°C, formed non-coalescing droplets at 70°C, and melted with the formation of a meniscus at 192-193°C. \([\alpha]_D^{25} = +10.8^\circ\) in chloroform (c 2); \(M_\infty = +75^\circ\).

**Analysis—**C_{46}H_{94}O_{18}N_P (695.6)
Calculated. C 62.10, H 10.72, N 2.01, P 4.46
Found. " 62.30, " 10.74, " 1.95, 2.00, P 4.45

On addition of ammonium reineckate to the ethanolic solutions of the choline salts VII, VIII, and IX, choline reineckate precipitated immediately.

At 21°C the following amounts of choline salt VII, VIII, or IX are contained in 100 ml. of solution: ether or petroleum ether, both <1 mg.; dry acetone, <1, 2, and 7 mg.; 95 per cent ethanol, 0.4, 2.4, and 5.3 gm.; dry ethanol, 0.5, 2.3, and 5.2 gm.; dry methanol, 1.1, 9.0, and —9 gm., respectively.

**Ethanolamine Salts—**The monoethanolamine salts of the phosphatidic acids were prepared by mixing the solution of the phosphatidic acid (1 mole) in dry chloroform (10 ml. of chloroform per 1 gm. of substance) with a solution of ethanolamine (1.05 mole, b.p. 169.5-171.5°C) in dry chloroform (20 ml. of chloroform per 1 gm. of ethanolamine) and gradually adding, with cooling, dry acetone to the mixture. The precipitates were filtered with suction, washed on the filter with dry acetone, and dried in vacuo (10 mm.). The crude ethanolamine salts were obtained in almost theoretical yields. For analysis they were rapidly recrystallized10 from warm 99 per cent ethanol (25 to 30 ml. per 1 gm. of substance). The analytically pure ethanolamine salts X, XI, and XII were obtained in yields of 93, 81, and 74 per cent, respectively.

**Distearoyl-L-α-glycerophosphoric Acid Monoethanolamine Salt (X)—**The salt started to sinter slightly at approximately 100°C. On being further

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9 Too soluble; not determined.
10 To avoid loss of fatty acids by transesterification.
heated, it changed gradually to a transparent mass and melted sharply at 172–173° with the formation of a meniscus. \([\alpha]_{D}^{25} = +6.8^\circ\) in chloroform \((c\ 2.0)\); \(M_n = +52.0^\circ\).

**Analysis**—\(C_{41}H_{46}O_{18}NP\) (765.7)  
Calculated. C 64.25, H 11.06, N 1.83, P 4.05  
Found. C 64.19, H 11.09, N 1.88, P 4.12

Dipalmitoyl-L-\(\alpha\)-glycerophosphoric Acid Monoethanolamine Salt (XI)—The salt started to sinter slightly at 90° and melted with the formation of a meniscus at 173–175°. \([\alpha]_{D}^{25} = +7.7^\circ\) in chloroform \((c\ 2.6)\); \(M_n = +54.6^\circ\).

**Analysis**—\(C_{37}H_{74}O_{18}NP\) (709.6)  
Calculated. C 62.57, H 10.79, N 1.97, P 4.37  
Found. C 62.85, H 10.68, N 1.87, P 4.42

Dimyristoyl-L-\(\alpha\)-glycerophosphoric Acid Monoethanolamine Salt (XII)—The salt sintered slightly at 100° and melted with formation of a meniscus at 178–180°. \([\alpha]_{D}^{27} = +8.8^\circ\) in chloroform \((c\ 2.4)\); \(M_n = +57.5^\circ\).

**Analysis**—\(C_{33}H_{68}O_{18}NP\) (653.5)  
Calculated. C 60.59, H 10.48, N 2.14, P 4.74  
Found. C 60.75, H 10.32, N 2.04, P 4.74, 4.79

When chloroform solutions of the phosphatidic acid ethanolamine salts were extracted with adequate amounts of ice-cold dilute \((0.05\ n)\) sulfuric acid, 90 to 95 per cent of the theoretical amounts of amino nitrogen was found in the acid extracts. The \(\alpha\)-cephalins, when treated under the same conditions, yielded only a few per cent of their nitrogenous material to the acid.

At 21° the following amounts of ethanolamine salt X, XI, or XII are contained in 100 ml. of solution: 5 dry acetone, ether, petroleum ether, or benzene, all <1 mg.; 95 per cent ethanol, 0.02, 0.07, and 0.23 gm.; dry ethanol 0.02, 0.04, and 0.15 gm.; dry methanol 0.09, 0.18, and 0.70 gm., respectively.

Distearoyl-L-\(\alpha\)-glycerophosphoric Acid Monopyridine Salt (XIII)—The phosphatidic acid (IV), suspended in 10 times its weight of dry pyridine, was brought into solution by warming to 45°. The solution was cleared by centrifugation, cooled, and diluted with 3 times its volume of dry acetone. After standing in ice for 30 minutes, the substance was collected with suction on a Büchner funnel, washed with dry acetone, and stored in a desiccator over pyridine to prevent decomposition of the pyridine salt. The pyridine salt was obtained in a yield of 85 per cent. It lost most of its pyridine either on being heated in vacuo or on repeated crystal-
lization from acetone. The salt started to sinter at 87°, gradually forming translucent droplets, which melted suddenly, with the formation of a meniscus, at 144–145°. The pyridine salt was not sufficiently soluble in chloroform at room temperature to permit an accurate determination of its optical activity.

Analysis—C₆H₁₃O₆NP (783.6)
Calculated. C 67.38, H 10.54, P 3.95
Found. " 67.48, " 10.47, " 3.96, 3.94

The pyridine salt is insoluble in ethyl acetate, acetone, benzene, ether, or carbon tetrachloride at room temperature, but moderately soluble in the same solvents at the boiling point.

Dipalmitoyl-α-L-α-glycerophosphoric Acid Monopyridine Salt (XIV)—The phosphatidic acid (V) was dissolved with warming to 45° in 4 times its weight of dry pyridine. To obtain the pyridine salt the solution was cooled in ice and the sludge of crystals was spread on a porous clay plate. The plate was placed in a desiccator over pyridine. The pyridine salt (XIV) was obtained in a yield of 55 per cent. It started to sinter at 90°, on being further heated formed translucent droplets and melted suddenly, with the formation of a meniscus, at 152–153°. [α]D₂⁰ = +3.8° in chloroform (c 4.4); M₉ = +27.6°. On standing in moist air, the salt gradually loses most of its pyridine.

Analysis—C₆₀H₁₃₀O₄NP (727.6)
Calculated. C 65.97, H 10.24, P 4.26
Found. " 65.64, " 10.50, " 4.28, 4.30

At room temperature the salt is practically insoluble in ethyl acetate, acetone, ether, or carbon tetrachloride, but is readily soluble in benzene or chloroform.

DISCUSSION

The data presented in this and various earlier publications from this laboratory now make it possible to compare the choline and ethanolamine salts and esters of structurally and configurationally pure DS-, DP-, and DM-L-α-glycerophosphoric acids. Table I lists the melting points, specific rotations, and solubilities of these compounds.

In 1926 Grün and Limpächer (31) reported the synthesis of DL-α-di-stearoyllecithin (m.p. 187°) and of distearoyl-DL-α-glycerophosphoric acid choline salt (m.p. 187°). It seemed odd that both the ester and the salt of choline should possess an identical melting point. A comparative study by King (32) of the hydrolysis of synthetic (Grün) and of natural lecithin by lecithinases revealed such considerable differences in the rates of hy-
# Table I

Data for Choline and Ethanolamine Salts and Esters of Distearoyl-, Dipalmitoyl-, and Dimyristoyl-L-α-glycerophosphoric Acids

<table>
<thead>
<tr>
<th></th>
<th>Starts to sinter at</th>
<th>M.p. with meniscus formation</th>
<th>$\alpha_D$ (+)</th>
<th>Solubility at 21–22°, gm. per 100 ml. solution*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C.</td>
<td>°C.</td>
<td>degrees</td>
<td>Ethanol</td>
</tr>
<tr>
<td>DS-L-α-GPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ch.E.</td>
<td>84–90</td>
<td>230–231†</td>
<td>6.1‡</td>
<td>0.8</td>
</tr>
<tr>
<td>Ch.S.</td>
<td>Slightly at 90</td>
<td>197–198</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>DP-L-α-GPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ch.E.</td>
<td>75–79</td>
<td>234–235†</td>
<td>6.6‡</td>
<td>1.5</td>
</tr>
<tr>
<td>Ch.S.</td>
<td>75</td>
<td>192–193</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>DM-L-α-GPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ch.E.</td>
<td>60–70</td>
<td>237–238</td>
<td>7.0‡          &gt; 15¶</td>
<td>0.03</td>
</tr>
<tr>
<td>Ch.S.</td>
<td>63–65</td>
<td>192–193</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>DS-L-α-GPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EA.E.</td>
<td>83</td>
<td>172–173.5</td>
<td>6.0**</td>
<td>8</td>
</tr>
<tr>
<td>EA.S.</td>
<td>Approximately 100</td>
<td>172–173</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>DP-L-α-GPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EA.E.</td>
<td>88</td>
<td>172.5–175</td>
<td>6.4¶</td>
<td>36</td>
</tr>
<tr>
<td>EA.S.</td>
<td>Approximately 90</td>
<td>173–175</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>DM-L-α-GPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EA.E.</td>
<td>88</td>
<td>175–177</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>EA.S.</td>
<td>Approximately 100</td>
<td>174–177</td>
<td>8.8</td>
<td></td>
</tr>
</tbody>
</table>

Ch.E. = choline ester; Ch.S. = choline salt; EA.E. = ethanolamine ester; EA.S. = ethanolamine salt.

* All solvents were anhydrous. In some of the solvents the solubility rises or falls sharply with slight variations in temperature.
† Rate of heating 20° per minute from 100–210°; from there on 10° per minute.
‡ In chloroform-methanol (1:1).
§ Immersed in a bath of 200° and heated at a rate of 3° per minute.
|| In chloroform at 26°.
¶ The solubility lies above the stated amount. For lack of material the saturation point was not attained.
** In chloroform-acetic acid mixture (7:1).
drolysis that doubts were expressed as to the nature of Grün's product. A recent and unambiguous synthesis of distearoyl-DL-α-lecithin (10) has shown that the pure phosphatide melts at 224–225°. A comparison of the data in Table I leaves no doubt that the choline ester and the choline salt of the same phosphatidic acid show considerable differences in melting point, ranging from 30–40°. It is apparent that Grün's synthetic lecithin was at best highly impure and probably consisted predominantly of the choline salt of the distearoylphosphoric acid.

It is of interest, however, that the ethanolamine ester and salt of each phosphatidic acid apparently possess the same melting point (Table I). It is conceivable that this phenomenon is caused by acyl migration during the period of heating, leading to the formation of identical products from both ester and salt. Acyl migrations from oxygen to nitrogen and the reverse are fairly common and have been studied extensively by von Auwers, Gabriel, and their associates (33). Choline esters and salts, possessing a completely methylated nitrogen atom, seem to be incapable of undergoing similar shifts and possess different melting points.

**SUMMARY**

1. A generally applicable method for the synthesis of the enantiomeric forms of fully saturated α-phosphatidic acids is reported.

2. The synthesis of distearoyl-, dipalmitoyl-, and dimyristoyl-L-α-phosphatidic acids and the preparation of their choline, ethanolamine, and pyridine salts are described.

3. A table containing the melting points, specific rotations, and solubilities of the choline and ethanolamine salts and esters of the three phosphatidic acids is presented. The melting points have been used to evaluate the synthetic products obtained by some of the earlier workers in this field.

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**BIBLIOGRAPHY**

16. Ulzer, F., and Batik, German patent 193,189 (1906); *Chem. Zentr.*, 1, 997 (1908).
SYNTHESIS OF ENANTIOMERIC $\alpha$-PHOSPHATIDIC ACIDS
Erich Baer


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