

Synthesis of DL- α -(Dihexanoyl-, Dioctanoyl-, and Didecanoyl)-Phosphatidyl DL-Serines

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Condensation of monophenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester monosilver salt with the appropriate α -iodo diglyceride by the procedure of Bevan, Malkin, and Tiplady for the synthesis of racemic α -(distearoyl)-phosphatidyl serine, afforded dihexanoyl-, dioctanoyl-, and didecanoyl-DL- α -glycerylphenylphosphoryl N-carbobenzyloxy DL-serine benzyl esters. These esters, upon removal of the protective phenyl, benzyl, and carbobenzyloxy groups by catalytic hydrogenolysis, gave the short-chain fatty acid, DL- α -phosphatidyl DL-serines. In contrast to α -(distearoyl) phosphatidyl serine, these three phosphatidyl serines easily form an emulsion with water and should prove to be suitable substrates for biological and chemical studies.

SYNTHETIC PROCEDURES are available for the preparation of phosphatidyl serine containing a long-chain fatty acid (1, 2). However, investigation of the biological properties and effects of this compound is hampered by its insolubility in water and by the difficulty with which it forms aqueous emulsions (3). The work presented here describes the synthesis of phosphatidyl serines containing short-chain fatty acids. Such serines can be easily emulsified; therefore, they may be of value in biological investigations.

The synthesis reported in this paper utilizes the monophenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester monosilver salt described by Bevan, Malkin, and Tiplady (2). The use of the monosilver salt eliminates the possibility of the formation of bis-phosphatidic acid which is obtained as a by-product in the method of Baer and Maurukas (1). The procedure shown in the reaction scheme is as follows: N-carbobenzyloxy DL-serine benzyl ester (I) was phosphorylated with phenylphosphoryl dichloride in the presence of anhydrous quinoline and anhydrous ethanol-free chloroform in a -10° bath. The product, consisting of phenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester chloride (II) and bis-(N-carbobenzyloxy DL-serine benzyl ester)-phenyl phosphate (III), was caused to react further with potassium carbonate in water and pyridine to yield phenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester monopotassium salt (IV). The potassium salt was converted into the monosilver salt V and purified by crystallization from boiling 95% ethanol. Condensation of DL- α -iodo diacylglycerol (VI) with phenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester monosilver salt (V) in boiling xylene

yielded diacyl-DL- α -glycerylphenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester (VII). Hydrogenolysis in the presence of mixed platinum and palladium catalyst yielded phosphatidyl serines VIII in overall yields of 57–62%. This procedure should also prove suitable for the synthesis of both enantiomeric forms of the phosphatidyl serines.

EXPERIMENTAL

DL- α -(Dihexanoyl)-phosphatidyl DL-Serine

Condensation.—Five Gm. (8.46 mmoles) of phenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester monosilver salt (2), 85 ml. of boiling anhydrous xylene, and 2.70 Gm. (6.78 mmoles) of DL- α -iodo dihexanoylglycerol¹ were placed in a 200-ml. two-necked round-bottom flask equipped with a reflux condenser and mechanical stirrer. The mixture was stirred and refluxed in the dark for 15 minutes with the exclusion of moisture. After a short cooling period under running tap water, the mixture was centrifuged to remove silver iodide, and the clear yellowish supernatant was evaporated under reduced pressure at a water bath temperature of 35–40°. The resulting oil (6.5 Gm.) was dissolved in 150 ml. of ether and washed successively with saturated sodium bicarbonate solution and water until the water was neutral to litmus. After drying over anhydrous sodium sulfate, the ethereal solution was evaporated to dryness under reduced pressure at a bath temperature not exceeding 40°. The residue was suspended in 40 ml. of warm petroleum ether, refrigerated for 1 hour at 0°, and separated from the supernatant petroleum ether by centrifugation. The residue was dried *in vacuo* (0.025 mm.) to constant weight. The dihexanoyl-DL- α -glycerylphenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester, a viscous oil, weighed 4.0 Gm. (78.0% of theory). At room temperature this compound was highly soluble in ether, acetone, methanol, ethanol, ethyl acetate, and acetic acid, but insoluble in petroleum ether and water. For analysis, 0.5 Gm. of this product was further purified by dissolving in 5 ml. of chloroform

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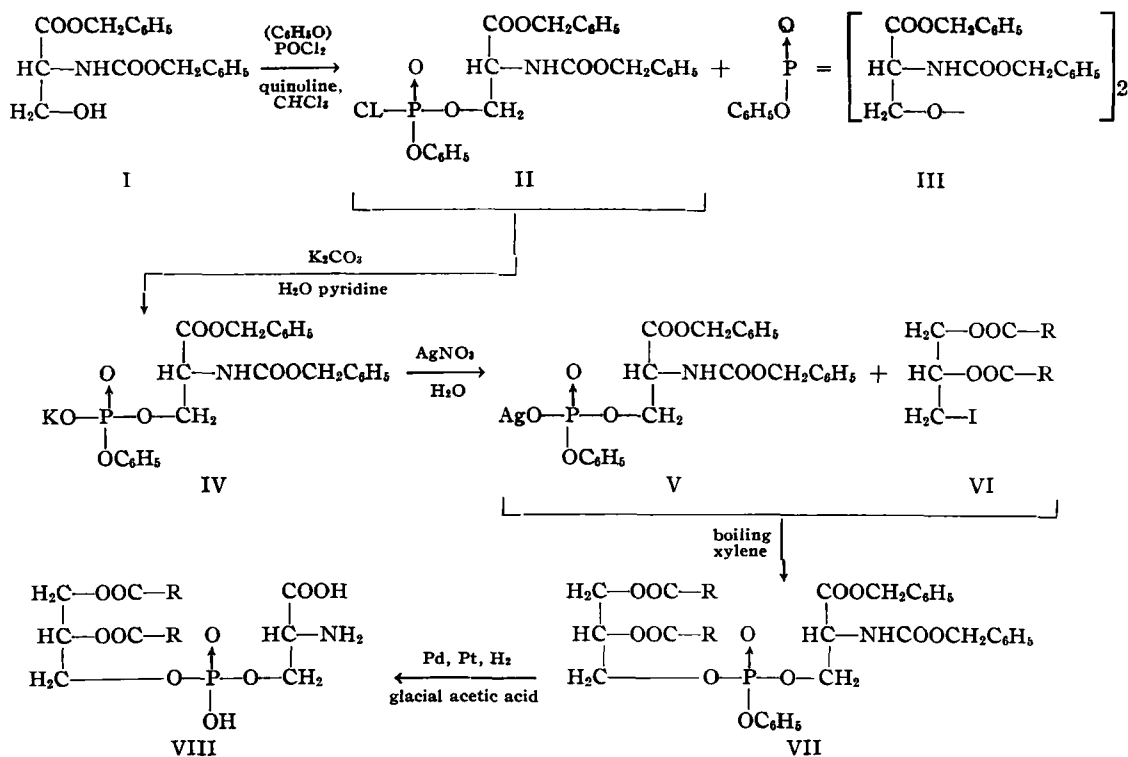
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¹ The three required α -iodo diglycerides containing hexanoic, octanoic, and decanoic acids have not been described in the literature. They were prepared from racemic α -iodo glycerol (4) *via* acetone glycerol (5) and esterified with the appropriate fatty acid chloride as described for the preparation of α -benzyl ether didecanoyl glycerol (6).

Reaction Scheme



DL- α -(diacyl)-phosphatidyl DL-serine
 R = C_6H_{11} , R = C_7H_{15} , R = C_8H_{17}

and passing it through a chromatographic column (20 \times 380 mm.) containing a mixture of 20 Gm. of silicic acid (Merck) and 10 Gm. of Celite (Johns-Manville).² The substance was eluted with 200 ml. of chloroform; the solvent evaporated to dryness at reduced pressure and at a water bath temperature not exceeding 40°. The recovery of dihexanoyl-DL- α -glycerylphenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester was 0.46 Gm., N₂ 1.5158.

Anal.—Calcd. for $C_{39}H_{60}NO_{12}P$ (755.78): C, 61.97; H, 6.67; N, 1.85; P, 4.10. Found: C, 61.20; H, 6.50; N, 1.76; P, 4.08.

Catalytic Hydrogenolysis.—A solution of 4.0 Gm. of dihexanoyl-DL- α -glycerylphenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester in 75 ml. of glacial acetic acid with 3 Gm. of platinum oxide-palladium black catalyst (1 : 1) (7, 8) was placed in an all-glass hydrogenation vessel of 200-ml. capacity. The mixture was shaken vigorously in an atmosphere of pure hydrogen at an initial pressure of 40 cm. of water until the absorption of hydrogen ceased. One liter was consumed in approximately 2 hours. Hydrogen was replaced by nitrogen, an additional gram of mixed catalyst was added, and hydrogenation was continued until absorption ceased. Approximately 0.8 L. of hydrogen was consumed in 1 hour. Hydrogen was again replaced by nitrogen. The catalyst was removed

by centrifugation and washed with a small amount of glacial acetic acid. The supernatant solutions were combined, and the acetic acid was removed by distillation under reduced pressure at a bath temperature of 35–40°. To complete the removal of traces of acetic acid, the substance was dissolved in 25 ml. of ether, the solution was diluted with 100 ml. of petroleum ether, and placed in a refrigerator at 0°. After 1 hour the crystalline material was centrifuged, washed with a small volume of ice-cold petroleum ether, and dried to constant weight *in vacuo* (0.025 mm.) over sodium hydroxide. The DL- α -(dihexanoyl)-phosphatidyl DL-serine weighed 1.93 Gm. (80% of theory); overall yield based on α -iodo diglyceride, 62.5%; m.p. 100°.³ This waxy substance at room temperature is highly soluble in ether, chloroform, acetone, methanol, ethanol, ethyl acetate, and acetic acid, but insoluble in petroleum ether. It readily forms an emulsion in water.

Anal.—Calcd. for $C_{18}H_{34}NO_{10}P$ (455.44): C, 47.46; H, 7.53; N, 3.07; P, 6.80. $C_{18}H_{34}NO_{10}P$. Found: C, 47.29; H, 7.47; N, 3.02; P, 6.77.

DL- α -(Diocanoyl)-phosphatidyl DL-Serine

DL- α -(dioctanoyl)-phosphatidyl DL-serine was prepared as described for the C_6 homolog. The condensation of 10.2 Gm. (17.3 mmoles) of phenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester

² The mixture of silicic acid and Celite was washed successively with methanol and chloroform before placing it on the column.

³ Melting points reported in this work were determined on the Fisher-Johns melting point apparatus.

monosilver salt with 6.2 Gm. (13.2 mmoles) of DL- α -iodo dioctanoylglycerol in 170 ml. of boiling dry xylene yielded 9.1 Gm. (82% of theory) of dioctanoyl-DL- α -glycerylphenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester. This viscous oil at room temperature is highly soluble in acetone, methanol, ethanol, ethyl acetate, chloroform, ether, petroleum ether, and acetic acid, and is insoluble in water.

For analysis, 0.5 Gm. of substance was placed on a chromatographic column described for the C₄ homolog and was eluted with 210 ml. of chloroform. The recovery of analytically pure dioctanoyl-DL- α -glycerylphenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester was 0.47 Gm.; N_D²⁰ 1.5058.

Anal.—Calcd. for C₄₃H₅₈NO₁₂P (811.88): C, 63.61; H, 7.20; N, 1.73; P, 3.82. Found: C, 63.51; H, 7.41; N, 1.71; P, 3.76.

The hydrogenolysis of 9.1 Gm. of dioctanoyl DL- α -glycerylphenylphosphoryl N-carbobenzyloxy benzyl DL-serine ester yielded 6.07 Gm. of crude phosphatidyl serine. This oil (containing traces of acetic acid) was dissolved in 116 ml. of warm ether and was precipitated with 350 ml. of petroleum ether. After 16 hours of refrigeration at -12°, the precipitate was centrifuged, washed with ice-cold petroleum ether, and dried *in vacuo* (0.025 mm.) over sodium hydroxide. The dioctanoyl-DL-glycerylphosphoryl DL-serine (weighing 4.0 Gm.) was obtained in a yield of 69.5% of theory (overall yield 57.0%), m.p. 160–161°. At room temperature this phosphatidyl serine was very soluble in chloroform, acetone, methanol, ethanol, ethyl acetate, acetic acid, and benzene, but insoluble in ether and petroleum ether. It readily formed an emulsion in water.

Anal.—Calcd. for C₂₂H₃₂NO₁₀P (511.55): C, 51.85; H, 8.28; N, 2.74; P, 6.06. Found: C, 51.68; H, 8.36; N, 2.73; P, 6.02.

DL- α -(Didecanoyl)-phosphatidyl DL-Serine

DL- α -(didecanoyl)-phosphatidyl DL-serine was prepared as described for the C₈ homolog. The condensation of 10.0 Gm. (16.9 mmoles) of phenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester monosilver salt with 7.0 Gm. (13.6 mmoles) of α -iodo didecanoylglycerol in 170 ml. of boiling dry

xylene gave 9.5 Gm. (80% of theory) of didecanoyl-DL- α -glycerylphenylphosphoryl N-carbobenzyloxy DL-serine benzyl ester. At room temperature it was readily soluble in acetone, methanol, ethanol, ethyl acetate, chloroform, ether, petroleum ether, and acetic acid, but insoluble in water. For analysis, 0.5 Gm. was passed through a silicic acid chromatographic column as described for the C₄ homolog. The recovered substance weighed 0.44 Gm.; N_D²⁰ 1.5030.

Anal.—Calcd. for C₄₇H₆₆O₁₂NP (867.99): C, 65.03; H, 7.67; P, 3.57; N, 1.61. Found: C, 64.51; H, 7.57; P, 3.52; N, 1.56.

The catalytic hydrogenolysis of 9.5 Gm. of didecanoyl-DL- α -glycerylphenylphosphoryl N-carbobenzyloxy-DL-serine benzyl ester yielded 5.0 Gm. of a glass-like phosphatidyl serine. This product (containing trace amounts of acetic acid) was dissolved in 115 ml. of petroleum ether and was evaporated to dryness. The amorphous residue was recrystallized from 47 ml. of absolute ethanol by immersion in an acetone dry ice bath at -40°, centrifuged, washed several times with cold ethanol, and dried *in vacuo* over sodium hydroxide. The final product, DL- α -(didecanoyl)-phosphatidyl DL-serine, weighed 4.72 Gm. (85.5% of theory). The overall yield based on α -iodo diglyceride was 59%; m.p. 164–165°. At room temperature this waxy substance was highly soluble in chloroform, absolute ethanol, ethyl acetate, acetic acid, and benzene, moderately soluble in methanol, and insoluble in acetone, ether, and petroleum ether. It readily formed an emulsion in water.

Anal.—Calcd. for C₂₆H₃₆NO₁₀P (576.65): C, 55.01; H, 8.88; N, 2.47; P, 5.46. Found: C, 55.19; H, 8.75; N, 2.46; P, 5.43.

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