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Short-Chain Fatty Acid (C_6,C_8,C_{10}) Phosphatidyl Ethanolamines*

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Received July 9, 1962

Condensation of the appropriate α-iodo diglyceride with monosilver salt of monophenylphosphoryl *N*-carbobenzoxyethanolamine gave dihexanoyl-, dioctanoyl-, and didecanoyl-DL-α-glycerylmonophenylphosphoryl *N*-carbobenzoxyethanolamines. Removal of the protective phenyl and carbobenzoxy groups by catalytic hydrogenolysis in the presence of palladium and platinum produced short-chain fatty acid phosphatidyl ethanolamines in yields ranging from 48.4% to 60.5% of theory. The required, but unknown, α-iodo diglycerides of hexanoic, octanoic, and decanoic acids are also described in detail. Dihexanoylphosphatidyl ethanolamine promises to be a suitable aqueous substrate for biological and chemical investigations.

Saturated long-chain fatty acid phosphatidyl ethanolamines have been synthesized by Rose (1947), Hunter *et al.* (1948), Bevan and Malkin (1951), Baylis *et al.* (1958), and Baer *et al.* (1952).

Recently Baer and Grof (1960) synthesized dihexanoyl L-α-phosphatidyl ethanolamine. Purification of the final product was achieved by the use of chromatography on a silicic acid column. The low absorptive capacity of silicic acid for phospholipids, the slow elution time, and the large volumes of eluting fluid required make this elegant method impractical for the preparation of the large quantities of phospholipids which are required for biological studies being carried out in this laboratory.

The procedure reported in this paper utilized monophenylphosphoryl dichloride as the phosphorylating agent (first applied to the synthesis of phospholipids and their biological intermediates by Baer and coworkers, 1948, 1950, 1952, 1953, 1955) and utilized the condensation of the monosilver salt of phosphoric acid diester with alkyl halide introduced by Malkin and co-workers for the synthesis of long-chain fatty acid phosphatidyl serine (Bevan *et al.*, 1957) and phosphatidyl ethanolamines (Baylis *et al.*, 1958).

The three required α-iodo diglycerides containing hexanoic, octanoic, and decanoic acids have not been described in the literature. They were prepared from α-iodo glycerol (Baer and Fischer, 1948) *via* acetone glycerol (Newman and Renall, 1945) and esterified with the appropriate fatty acid chloride as described by Baer and Mahadevan (1959) for the preparation of α-benzyl ether didecanoylglycerol. In the past, biological investigations of phospholipids have been limited to lecithins, since synthetic phosphatidyl ethanolamines were unsuitable because of their very low solubility in aqueous solutions. The DL-α-phosphatidyl ethanolamines described here containing hexanoic, octanoic, and decanoic acids were found to be highly soluble in methanol and ethanol, but only sparingly soluble in water. Of these three compounds, only DL-α-(dihexanoyl)-phosphatidyl ethanolamine promises to become useful as a substrate for biological studies in homogeneous aqueous systems. It was found to be soluble to the extent of 0.4 g per 100 ml of water. The racemic α-phosphatidyl ethanolamines have been obtained in over-all yields ranging from 48.4% to 60.5%. Studied by the technique of Marinetti and Stotz (1956), the three compounds described here appeared to be chromatographically pure.

Experimental Procedures and Results

Materials

Synthetic quinoline and barium oxide were shaken for eight hours and the quinoline distilled *in vacuo*.

* Supported by Grants from the USPHS (H-6408), United Health Foundation of Greater Elyria, and Cleveland Area Heart Society.

An ACS grade pyridine was refluxed over barium oxide and distilled with the exclusion of moisture. α-Iodo glycerol was prepared by the method of Baer and Fischer (1948). Acid chlorides of hexanoic, octanoic, and decanoic acids were prepared by the method of Fierz-David and Kuster (1939). Palladium black catalyst was prepared by the method of Tausz and von Putnoky (1919). Platinum dioxide was prepared as described in "Organic Synthesis" (1948) with the substitution of potassium nitrate for sodium nitrate as described by Cook and Linstead (1934). Melting points were determined on glass slides; Fisher's melting point apparatus and a long-stem thermometer with a range of 300° were used.

DL-α-Iodo Diglycerides

DL-α-Iodo dihexanoylglycerol. A solution of 40.4 g (0.200 mole) of α-iodo glycerol in 34.2 g (0.432 mole) of anhydrous pyridine and 100 ml of anhydrous benzene was added with stirring and ice-cooling to 58.2 g (0.432 mole) of hexanoyl chloride in 50 ml of anhydrous benzene during a 1-hour period. After being kept overnight at room temperature, the pyridine hydrochloride was removed by filtration and the filtrate evaporated to dryness under reduced pressure and at a bath temperature not exceeding 40°. The residue was dissolved in 400 ml of ether and successively washed twice with 250 ml of ice-cold water, 250 ml of cold 2 N hydrochloric acid, 250 ml of cold water, and 250 ml of saturated sodium bicarbonate. The ethereal solution was neutral to litmus paper. After the ethereal solution was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure and at a bath temperature of 35–40°, 76.7 g (96.2% of theory) of DL-α-iodo dihexanoylglycerol, an almost colorless oil, was obtained, N_D^{25} 1.4749.

Anal. Calcd. for $C_{15}H_{27}O_4I$ (398.29): C, 45.23; H, 6.83; I, 31.86. Found: C, 44.60; H, 6.32; I, 33.00.

DL-α-Iodo dioctanoylglycerol was prepared as was its dihexanoyl homolog. The yield was 98.7% of theory, based on α-iodo glycerol, N_D^{25} 1.4720.

Anal. Calcd. for $C_{19}H_{35}O_4I$ (454.39): C, 50.23; H, 7.76; I, 27.93. Found: C, 50.79; H, 7.41; I, 27.41.

DL-α-Iodo didecanoylglycerol was prepared as was its dihexanoyl homolog. The yield was 91.5% of theory based on α-iodo glycerol, N_D^{25} 1.4700.

Anal. Calcd. for $C_{23}H_{43}O_4I$ (510.49): C, 54.11; H, 8.49; I, 24.86. Found: C, 54.98; H, 8.42; I, 24.50.

Monophenylphosphoryl N - carbobenzoxyethanolamine monosilver salt was prepared as described by Baylis *et al.* (1958). Phosphorylation of 75.60 g (0.386 mole) of *N*-carbobenzoxyethanolamine with 82.05 g (0.386 mole) of monophenylphosphoryl dichloride, conversion to the potassium salt, and subsequent conversion to the silver salt yielded 70.0 g of monophenylphosphoryl *N*-carbobenzoxyethanolamine monosilver salt.

The over-all yield was 40% based on *N*-carbobenzoxyethanolamine. The silver salt was purified by dissolving in hot glacial acetic acid (3 ml of solvent per gram of substance) and precipitating with 99% ethanol (6 ml per gram of substance) m.p. 136–138°.

Anal. Calcd. for $C_{16}H_{12}O_6P$ N Ag (453.12): P, 6.83; N, 3.09. Found: P, 6.79; N, 3.04.

DL-α-Phosphatidyl Ethanolamines

DL-α-(Dihexanoyl)-phosphatidyl ethanolamine. A solution of 22.50 g (0.056 mole) of α-iodo dihexanoylglycerol in 1130 ml of anhydrous and thiophene-free benzene and 31.10 g (0.068 mole) of monophenylphosphoryl *N*-carbobenzoxyethanolamine monosilver salt was refluxed with vigorous stirring for 3 hours in the dark. At the end of this period the reaction mixture was quickly cooled to room temperature, the silver iodide removed by filtration, and the solvent was removed under reduced pressure in a 40° water bath. The slightly colored residue (39.10 g) was dissolved in 200 ml of ether and cleared of a small amount of insoluble material by centrifugation, and the ethereal solution was washed successively with two portions of 150 ml of saturated sodium bicarbonate and three portions of 200 ml of water. The ethereal solution was dried with anhydrous sodium sulfate and evaporated to dryness under reduced pressure at a bath temperature not exceeding 40°. The crude material, weighing 27.0 g, was purified by washing with two 150-ml portions of cold petroleum ether. After drying *in vacuo* to constant weight, 22.5 g (64.2% of theory based on α-iodo diglyceride) of DL-α-(dihexanoyl)-glyceryl monophenylphosphoryl *N*-carbobenzoxyethanolamine was obtained, N_D^{25} 1.4965.

Anal. Calcd. for $C_{31}H_{44}O_{10}NP$ (621.6): C, 59.89; H, 7.13; P, 4.98; N, 2.25. Found: C, 59.30; H, 7.28; P, 4.92; N, 2.11.

Removal of protective phenyl and N-carbobenzoxy groups. A solution of 22.5 g of DL-α-(dihexanoyl)-monophenylphosphoryl *N*-carbobenzoxyethanolamine in 340 ml of glacial acetic acid was placed in a polyethylene bottle together with 11.0 g of mixed platinum oxide and palladium black catalyst (1:1). The mixture was shaken vigorously in an atmosphere of pure hydrogen at room temperature and at an initial pressure of 42 cm of water until the absorption of hydrogen had ceased. A total of three successive hydrogenations, each time with 11 g of fresh catalyst, were required for the completion of catalytic hydrogenolysis. Total hydrogen uptake was 2300 ml. After the hydrogen was replaced with nitrogen the catalyst was removed by filtration and washed with glacial acetic acid, and the combined filtrates were evaporated to dryness under reduced pressure at a bath temperature not exceeding 40°. The residue was dissolved in 40 ml of ether and on addition of 860 ml of ether precipitated in an amorphous powdery form. After refrigeration at approximately −12° for 24 hours, the substance was isolated by centrifugation, washed with 75 ml of ice-cold ether, and dried to constant weight *in vacuo* over sodium hydroxide pellets. The DL-α-(dihexanoyl)-glycerylphosphorylethanolamine, weighing 11.25 g, was obtained in a yield of 75.5% based on *N*-carbobenzoxy-, phenyl - phosphatidyl ethanolamine. Over - all yield based on α-iodo dihexanoylglycerol, 48.4%, m.p. 134–136°. The DL - α - (dihexanoyl) - phosphatidyl ethanolamine was a white powdery material. At room temperature it was found to be soluble in methanol, ethanol, and chloroform, sparingly soluble in acetone and water (400 mg per 100 ml), and insoluble in ether and petroleum ether.

Anal. Calcd. for $C_{17}H_{34}O_8NP$ (411.43): C, 49.62; H, 8.33; N, 3.40; P, 7.53. Found: C, 49.61; H, 8.18; N, 3.48; P, 7.56.

DL-α-(Dioctanoyl)-phosphatidyl ethanolamine was prepared as described in the procedure for dihexanoyl phosphatidyl ethanolamine. The condensation of 5.0 g (0.010 mole) of diglyceride with 5.18 g (0.011 mole) of monosilver salt in 200 ml of anhydrous benzene produced 5.90 g of dioctanoyl-DL-α-glyceryl-monophenylphosphoryl *N* - carbobenzoxyethanolamine (79.0% of theory, based on diglyceride), N_D^{25} 1.4885.

Anal. Calcd. for $C_{35}H_{53}O_{10}NP$ (677.75): C, 62.02; H, 7.73; N, 2.07; P, 4.57. Found: C, 62.03; H, 7.17; N, 1.90; P, 4.43.

Hydrogenolysis of 5.9 g of dioctanoyl-DL-α-glyceryl-monophenylphosphoryl *N*-carbobenzoxyethanolamine with two 2.5-g portions of mixed catalyst and with a total hydrogen uptake of approximately 1600 ml produced 2.7 g (66.5% of theory) of dioctanoyl-phosphatidyl ethanolamine. The over-all yield based on α-iodo diglyceride was 52.6%, m.p. 216°. At room temperature it was highly soluble in chloroform, methanol, and glacial acetic acid, moderately soluble in ethanol, benzene, and dioxane, and insoluble in ether, petroleum ether, acetone, and ethyl acetate.

Anal. Calcd. for $C_{21}H_{42}O_8NP$ (467.53): C, 53.94; H, 9.06; N, 3.00; P, 6.63. Found: C, 53.27; H, 8.49, 8.25, 8.29; N, 2.98; P, 6.69.

DL-α-(*Didecanoyl*)-*phosphatidyl ethanolamine.* The condensation of DL-α-iodo didecanoylglycerol (12.54 g, 0.025 mole) with monophenylphosphoryl *N*-carbobenzoxyethanolamine monosilver salt (13.6 g, 0.030 mole) in anhydrous benzene (630 ml) and the isolation of didecanoyl-DL-α-glycerylmonophenylphosphoryl *N*-carbobenzoxyethanolamine (13.88 g, 77% of theory; N_D^{25} 1.4861) were carried out as described for the corresponding compound, dihexanoyl - phosphatidyl ethanolamine.

Anal. Calcd. for $C_{39}H_{60}O_{10}NP$ (733.90): C, 63.83; H, 8.24; N, 1.91; P, 4.23. Found: C, 63.47; H, 8.05; N, 1.89; P, 4.24.

Hydrogenolysis of 13.88 g of didecanoyl-DL-α-glyceryl-monophenylphosphoryl *N* - carbobenzoxyethanolamine with two 7-g portions of mixed catalyst (Pd-PtO_2, 1:1) and two crystallizations from ether yielded 7.16 g of didecanoyl - DL - α - phosphatidyl ethanolamine (60.5% of theory based on α-iodo diglyceride), m.p. 204–206°. Didecanoyl-phosphatidyl ethanolamine at room temperature is highly soluble in chloroform, methanol, and acetic acid, moderately soluble in ethanol, benzene, and dioxane, and insoluble in ethyl acetate, ether, petroleum ether, and acetone.

Anal. Calcd. for $C_{25}H_{50}O_8NP$ (523.68): C, 57.33; H, 9.62; N, 2.67; P, 5.92. Found: C, 57.25; H, 9.69; N, 2.66; P, 5.90.

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