Diether Analogues of Lecithins

SYNTHESIS OF DI-O-OCTADECYL-L-α-GLYCERYLPHOSPHORYLCHOLINE

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Considerable interest has developed recently in naturally occurring phosphatides containing ether groups. Two classes of ether-containing phosphatides can be envisaged, i.e. monoether, monoester phosphatides, and diether phosphatides. To date, the only monoether, monoester phosphatides known to occur naturally are analogues of phosphatidylethanolamine (1-4) and phosphatidylycholine (5, 6). A chemical synthesis of phosphatidylethanolamine (1-4) and phosphatidylycholine (5, 6) have not yet been synthesized.

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Experimental Procedure

The procedure for the synthesis of the diether analogue of lecithin is based on that developed by Baer et al. (12-14) for the synthesis of the diether forms of lecithin, except that as starting material an α,β-dialkyl glycerol ether is used (see Scheme 1). Earlier investigations by Baer and Fischer (15, 16) have shown that the α-monoalkyl ethers of glycerol, such as butyl-, chloro-, and selachyl alcohol, which occur in bound form in liver oils of various marine animals, possess the α,β-dialkyl glycerol configuration. It is most likely that the α,β-dialkyl glycerol moieties of naturally occurring diether phosphatides will possess the same configuration. Hence, α,β-dioctadecyl glycerol (I) was selected for the starting material. It was phosphorylated with monophenylphosphoryl dichloride in the presence of pyridine. This reaction gave rise to the formation of dioctadecyl-L-α-glycerylphenyl phosphorocholine chloride (II) and tetra-O-octadecyl bis-L-α-glycerylphosphoric acid phenyl ester (III). Without isolating Intermediate II, it was esterified with choline iodide. The mixture of reaction products then was freed from bis-Compound III and from water-soluble material, and the crude dioctadecyl L-α-glyceryl(phenyl)phosphoryl choline iodide (IV) was purified by crystallization from acetone. Removal of the phenyl group of Compound IV by catalytic hydrogenolysis was slow and incomplete. The reaction mixture contained, in addition to Compound IV and starting material, considerable amounts of the ether analogues of phosphatic acid-like compounds. The latter most likely were formed during the reduction by acid hydrolysis (HI) of the ether lecithin and its phenyl ester. This difficulty was overcome by removing the iodide ion of Compound IV with silver carbonate prior to the reduction. The catalytic hydrogenolysis of the iodine-free Compound IV proceeded smoothly and gave in a good yield the desired diether lecithin (V).

Results

Materials—Phenylphosphoryl dichloride was prepared by the procedure of Zenftman and McGillivray (17), and the material was purified by vacuum distillation, collecting the fraction-distilling from 103-106°C at 9 mm (bath, 125-130°C). The choline iodide was obtained as described by Baer and Kindler (18). Before use, it was powdered finely and dried thoroughly under vacuum. Ethanol-free, anhydrous chloroform was prepared immediately before use, by distilling chloroform over phosphorus pentoxide. Anhydrous pyridine was obtained by refluxing pyridine of good commercial grade over finely powdered barium oxide, and distilling the pyridine under normal pressure with the exclusion of moisture. The silicic acid was Mallincrodt, 100 mesh powder, analytical reagent. It was freed from fine particles going through a sieve of 150 mesh per linear inch.
The platinic acid (Adams catalyst) was prepared as described by Adams, Voorhees, and Shriner (19), except that the sodium nitrate was replaced by an equimolecular amount of potassium nitrate (20). \( \alpha_\beta \)-Di-\( \alpha \)-octadecyl glycerol (I) was prepared as described by Kates et al. (11); m.p. 55-57°, \([\alpha]_D -6.95°\) in chloroform (c, 4.3); reported (11) m.p. 53.5-54.5°, \([\alpha]_D -6.85°\) in chloroform (c, 7.5). The infrared spectra of both preparations were identical.

**Di-\( \alpha \)-Octadecyl \( \alpha \)-Glyceryl(phenyl)phosphorylcholine Iodide (IV)**

**Phosphorylation**—Into a thoroughly dried 250-ml three-necked flask, equipped with an addition funnel, oil-sealed stirrer, and calcium chloride tube, were placed 1.50 ml (10 mmoles) of monophosphoryl dichloride, 0.81 ml (10 mmoles) of anhydrous pyridine, and 10 ml of anhydrous and ethanol-free chloroform, and the flask was immersed in a water bath at 10°. To the vigorously stirred solution was added in the course of 15 minutes a solution of 5.97 g (10 mmoles) of \( \alpha_\beta \)-Di-\( \alpha \)-octadecyl glycerol (I) in 50 ml of dry chloroform. The temperature of the bath then was raised within 15 minutes to 35°, and 30 minutes later, 12.5 ml (0.15 mole) of anhydrous pyridine were added. This was followed after 10 minutes by the addition of 2.31 g (10 mmoles) of choline iodide. The reaction mixture was stirred at room temperature (about 25°) for 48 hours. At the end of this time, practically all the choline iodide had gone into solution. The reaction mixture was freed from a small amount of insoluble material, and the pale yellow solution was evaporated to dryness under reduced pressure at a bath temperature of 30-35°. The residue was dried under reduced pressure and extracted with four 50-ml portions of anhydrous ether. (The ether extract contains several by-products. Their isolation and identification is described under "Isolation of By-products of Phosphorylation Procedure.") The ether-insoluble material was washed in succession with three 25-ml portions each of water, 99% ethanol, and acetone, separating the mixtures by centrifugation. The remaining white solid on drying under reduced pressure weighed 2.81 g (29% of theory); m.p. 128-129° with sintering at 95°. Found: P 3.50, N 1.51. For purification, the material was dissolved in 50 ml of boiling acetone, the solution was filtered while hot, and the filtrate was kept at 4°. The crystalline material, weighing 2.73 g (m.p. 129-130°), was crystallized once more from 50 ml of acetone to which 3 ml of chloroform had been added. The di-\( \alpha \)-octadecyl \( \alpha \)-glyceryl(phenyl)phosphorylcholine iodide (IV), after drying at room temperature over phosphorus pentoxide in a vacuum of 0.2 mm, weighed 2.67 g (27.6% of theory); m.p. 129.5-130.5° with sintering at 95°.

The combined ethanol extracts (90 ml) on standing at room temperature for several days deposited an additional 247 mg of Compound IV (m.p. 128-129° with sintering at 95°), raising the total yield to 30.2% of theory. A second preparation, on the same scale, gave Compound IV in a yield of 31.7%.

**Isolation of Compound IV**—The reaction mixture was freed from a small amount of insoluble material, and the pale yellow solution was evaporated to dryness under reduced pressure at a bath temperature of 30-35°. The residue was dried under reduced pressure and extracted with four 50-ml portions of anhydrous ether. (The ether extract contains several by-products. Their isolation and identification is described under "Isolation of By-products of Phosphorylation Procedure.") The ether-insoluble material was washed in succession with three 25-ml portions each of water, 99% ethanol, and acetone, separating the mixtures by centrifugation. The remaining white solid on drying under reduced pressure weighed 2.81 g (29% of theory); m.p. 128-129° with sintering at 95°. Found: P 3.50, N 1.51. For purification, the material was dissolved in 50 ml of boiling acetone, the solution was filtered while hot, and the filtrate was kept at 4°. The crystalline material, weighing 2.73 g (m.p. 129-130°), was crystallized once more from 50 ml of acetone to which 3 ml of chloroform had been added. The di-\( \alpha \)-octadecyl \( \alpha \)-glyceryl(phenyl)phosphorylcholine iodide (IV), after drying at room temperature over phosphorus pentoxide in a vacuum of 0.2 mm, weighed 2.67 g (27.6% of theory); m.p. 129.5-130.5° with sintering at 95°.

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**C\(_{46}\)H\(_{70}\)O\(_5\)N\(_4\)P** (966.2)

Calculated: C 62.15, H 10.12, N 1.45, P 3.21, I 13.14

Found: C 62.98, H 10.13, N 1.78, P 3.37, I 13.73

The infrared spectrum of Compound IV in chloroform showed bands at 3000 (medium), 1590 (medium), 1490 (strong) cm\(^{-1}\) (phenyl), 2830 (strong), 2860 (strong), 1465 (medium), and 1375 (weak) cm\(^{-1}\) (CH\(_2\)- and CH\(_3\)- groups), 1280 cm\(^{-1}\) (free P=O), 1220 cm\(^{-1}\) (P=O-C, aryl), 1040 cm\(^{-1}\) (P-O=C, aliphatic), 1095 cm\(^{-1}\) (C=O-C, ether), and other bands at 925, 910, and 870 cm\(^{-1}\).
Dioctadecyl L-α-glycerylphosphorylcholine (V)

Removal of Iodide Ion—A solution of 2.4 g (2.46 mmoles) of Compound IV in 240 ml of a mixture of 95% ethanol-chloroform (3:1, v/v) and 30 ml of distilled water, to which 3.0 g of silver carbonate had been added, was shaken for 1 hour. The hydrogen was replaced by nitrogen, the solvents were displaced into an all-glass vessel with a capacity of 1 liter, and 120 ml of 95% ethanol, and 6 ml of glacial acetic acid were added. The mixture was filtered, the catalyst was washed with the final wash alcohol, the solution of the iodine-free Compound IV, prepared as described above (2), was added to the catalyst, and the mixture was shaken vigorously in an atmosphere of pure hydrogen at an initial pressure of 30 to 40 cm of water until the theoretical amount of hydrogen (4 moles) had been consumed. The hydrogen was replaced by nitrogen, the reaction mixture was filtered, the catalyst was washed with 60 ml of a mixture of chloroform and ethanol (1:1), and the combined filtrates were evaporated to dryness under reduced pressure from a bath at 3&35°. The dry residue was suspended with stirring in 30 ml of 10% aqueous solution of acetic acid. After 10 minutes, the mixture was filtered with suction, and the material on the filter was washed with several small portions of acetone. The dry material was dissolved in 60 ml of chloroform and precipitated by the gradual addition of 300 ml of diethyl ether. The mixture was filtered with suction, and the dioctadecyl L-α-glycerylphosphorylcholine (V), a fine white powder, was dried for 6 hours in a vacuum of 0.1 mm at 58° for 8 hours. "

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Preparation of Platinum Catalyst—Platinic oxide (600 mg), 120 ml of 95% ethanol, and 6 ml of glacial acetic acid were placed into an all-glass vessel with a capacity of 1 liter, and the platinic oxide was reduced with hydrogen to platinum black. The hydrogen was replaced by nitrogen, the solvents were decanted, and the catalyst was washed in the reduction vessel with two 50-ml portions of 95% ethanol, and one 50-ml portion of 99% ethanol.

Removal of Phenyl Group by Reductive Cleavage—After
canting the final wash alcohol, the solution of the iodine-free Compound IV, prepared as described above (2), was added to the catalyst, and the mixture was shaken vigorously in an atmosphere of pure hydrogen at an initial pressure of 30 to 40 cm of water until the theoretical amount of hydrogen (4 moles) had been consumed. The hydrogen was replaced by nitrogen, the reaction mixture was filtered, the catalyst was washed with 60 ml of a mixture of chloroform and ethanol (1:1), and the combined filtrates were evaporated to dryness under reduced pressure from a bath at 3&35°. The dry residue was suspended with stirring in 30 ml of a 10% aqueous solution of acetic acid. After 10 minutes, the mixture was filtered with suction, and the material on the filter was washed with several small portions of acetone. The dry material was dissolved in 60 ml of chloroform and precipitated by the gradual addition of 300 ml of diethyl ether. The mixture was filtered with suction, and the diether lecithin weighed 1.34 g (69% of theory); m.p. 201-202° (varies slightly with rate of heating). It was found to be readily soluble in chloroform at room temperature and in warm ethyl acetate, slightly soluble in boiling petroleum ether (b.p. 35-60°), and insoluble in ether, acetone, and benzene.

\[
\text{C}_{44}\text{H}_{84}\text{O}_{7}\text{NP} \quad (780.2)
\]

Calculated: C 67.73, H 12.14, N 1.79, P 3.97

Found: C 67.24, H 12.00, N 1.73, P 3.87, 4.08

The diether lecithin was a homogenous substance. When chromatographed on silicic acid-impregnated paper with diisobutyl ketone-acetic acid-water (40:25:5), it gave on staining with Rhodamine 6G, a single spot that showed yellow fluorescence in ultraviolet light. The infrared spectrum of the diether lecithin had bands at 3250 (strong), 2930 (strong), 2850 (strong), 1650 (medium), and 1375 (weak) cm\(^{-1}\) (CH\(_3\)- and CH\(_2\)-), 1095 cm\(^{-1}\) (C-O-C, ether), 1245 cm\(^{-1}\) (P=O, hydrogen-bonded), 1040 cm\(^{-1}\) (P-O-C, aliphatic), and 975 cm\(^{-1}\), 865 cm\(^{-1}\). No ester (C=O) or phenyl bands were present.

Attempts to remove the phenyl group of Compound IV by...
catalytic hydrogenolysis without previous removal of the iodine ion were not successful as the reduction did not go to completion. Analysis of the reduction product by paper chromatography showed that it consisted of about equal amounts of starting material (IV), the diether lecithin (V), and a fast moving compound which was identified as di-O-octadecyl glycerylphosphoric acid. By treating the reduction product with silver carbonate, to remove the iodide ion, and repeating the hydrogenolysis, a product was obtained which, after washing with 10% acetic acid and acetone and precipitation from chloroform with ether, gave in moderate yields (32%) di-O-octadecyl L-α-glycerylphosphorylcholine (V); m.p. 201–202° (varies slightly with rate of heating).

Isolation of By-products of Phosphorylation Procedure

The combined ether extracts of the phosphorylation product were evaporated to dryness under reduced pressure at 30–35°, and the remaining material was fractionated by column chromatography on silicic acid. A solution of the residue (2.0 g) in 50 ml of chloroform was applied to a column of 40 g of silicic acid (2.2 x 20 cm), and the column was washed first with 200 ml of chloroform (U.S.P.), followed by a mixture of chloroform and methanol (1:1, v/v), yielding Eluates 1 and 2, respectively. Both eluates were evaporated to dryness under reduced pressure at 30–35°.

The residue of Eluate 1, a yellow oil, on addition of 50 ml of acetone gave a crystalline material which on drying at room temperature over phosphorus pentoxide in a vacuum of 0.1 mm weighed 1.33 g, and gave analytical values for carbon, hydrogen, and phosphorus which suggested that the material was a mixture of 80% of tetra-O-octadecyl bis-(L-a-glyceryl)phosphoric acid phenyl ester and 20% of d-α,β-di-O-octadecyl glycerol.

Di-O-Octadecyl L-α-Glycerylphosphoric Acid Monophenyl Ester

The residue of Eluate 2, a white solid, on recrystallization from 25 ml of acetone, gave a crystalline material which, when dried at room temperature over phosphorus pentoxide in a vacuum of 0.1 mm for 20 hours, weighed 337 mg. It analyzed for the di-O-octadecyl L-α-glycerylphosphoric acid monophenyl ester; m.p. 68°.

CaH₄O₃P (733.1)
Calculated: C 71.77, H 11.38, P 4.11
Found: C 71.21, H 11.34, P 3.68
C 71.30, H 11.35

On silicic acid-impregnated paper and development with disobutyl ketone-acetic acid and water, the substance gave a single spot with Rhodamine 6G showing blue fluorescence in ultraviolet light.

DISCUSSION

Table I summarizes some of the physical properties of the L-α-(dioctadecyl)lecithin, its ester analogue, and the chemical precursors of both. The ether compounds have rotations that are from 1 to 5° more negative than those of the corresponding ester compounds. The melting points of the ether compounds in general are lower than those of the corresponding ester compounds. The Rₚ values of the ether- and ester lecithins in disobutyl ketone-acetic acid-water (40:25:5) are, however, virtually the same. This result is not unexpected, since the solvent system is too polar to allow a separation on the basis of the relatively small differences in polarity between the ether and ester groups of these lecithins. The diether lecithin appears to be somewhat more soluble than the corresponding ester lecithin in the same organic solvent. Fig. 1 shows the infrared spectra of L-α-(dioctadecyl)lecithin (A) and L-α-(dioctadecanoyl)lecithin (B) in chloroform solution. Both spectra are similar, except that the ester lecithin (B) has a strong ester C=O absorption band at 1725 cm⁻¹, which is not shown by the ether lecithin. This provides a simple means to distinguish ester lecithins from ether lecithins.

SUMMARY

The first synthesis of a diether analogue of a lecithin is described. The L-α-(dioctadecyl)lecithin (di-O-octadecyl-L-α-glycerylphosphorylcholine) was obtained by phosphorylation of d-α,β-di-O-octadecyl glycerol with monophosphoryl dichloride and pyridine, esterification of the resulting di-O-octadecyl L-α-glyceryl(phenyl)phosphoryl chloride with choline iodide, conversion of di-O-octadecyl L-α-glyceryl(phenyl)phosphoryl choline iodide into the corresponding carbonate, and removal of the phenyl group by catalytic hydrogenolysis with platinum catalyst.

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