The Total Synthesis of Phosphatidyl(Dioleoyl)Hydroxy-L-Proline and Its Activity in Blood-Clotting Systems

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ABSTRACT

The phthalimidomethyl ester of N-anisyl oxycarbonyl-hydroxy-L-proline was combined with phosphorus oxychloride and rac-1,2-diolein. The diolein was made by large-scale preparative application of the method of Krabisch and Borgström (1). The protected phosphatide, obtained by the phosphorylation reaction, was stripped of its protective groups under mild conditions. The phosphatidyl(dioleoyl)-hydroxy-L-proline was purified by TEAE cellulose (acetate) chromatography, as developed by Rouser (6), also by silicic acid chromatography. Aqueous dispersions of the material were tested for anticoagulant activity in the antithromboplastin test and the Hicks-Pitney test. The new phosphatide had about one-tenth of the activity of beef brain phosphatidylserine.

INTRODUCTION

The total synthesis of phosphatidyl(di-stearoyl) hydroxy-L-proline from an optically active 1,2-distearin was achieved by Baer and Zschocke (2). However, because saturated phosphatides are not dispersible in water, it is not possible to test the phosphatide of Baer and Zschocke in biological tests. From experience with phosphatidylserines and phosphatidylethanolamine it can be expected that unsaturated phosphatides would be sufficiently dispersible in water to be tested. In order to synthesize unsaturated phosphatides of this type, it is necessary to use protecting groups that can be removed without altering the double bonds. In a synthesis of unsaturated phosphatidylserines (3,4) we employed the phthalimidomethyl group to protect the carboxyl of serine, and the anisyloxycarbonyl group to protect the amino group. This technique has now been used with hydroxy-L-proline (Fig. 1).

Although phosphatidylhydroxy-L-proline has not been found in nature, it has sufficient similarity to phosphatidylserine to make its possible biological activity a matter of great interest. The relation between structure and activity might be clarified by the synthesis of a variety of phosphatidyl amino acids. This is particularly emphasized by the recent report that a phosphatide containing an unidentified hydroxy-amino acid (not serine) is a renin activator (5).

PROCEDURES AND RESULTS

Materials and Methods

Hydroxy-L-proline (M. A. grade) was obtained from Mann Laboratories, New York, and oleic acid (Purum) for the 1,2-diolein from Fluka A.G., Buchs, Switzerland. The TEAE cellulose (selectacel, No. 83, standard grade) was obtained from Carl Schleicher and Schuell Inc., Keene, N. H. A portion of 100 g was put into a 4-liter beaker, filled with distilled water. After most of the TEAE cellulose had settled, the water was decanted. This process was repeated 10 times to remove fines. The TEAE cellulose was then washed with 0.1 N sodium hydroxide, washed with water until neutral, cycled through the chloride form, and finally restored to the washed OH form. It was next washed with methanol, dried in a high vacuum, and converted to the acetate form with glacial acetic acid according to the earlier methods of Rouser (6,7).

Magnesium oxide (“light” grade) was obtained from British Drug Houses, Poole, England. Anisyl azideformate was made as described before (4). Peroxide-containing solvents, like tetrahydrofuran, were freed of peroxides by distilling over triphenyl phosphine. Solvents were dried, where necessary, over molecular sieves (4). Hydrazine (95% grade) was from Eastman Kodak. Silicic acid was Bio-sil of Bio-Rad Laboratories, Richmond, Calif.

All operations were conducted under nitrogen when unsaturated fatty acids or unsaturated phosphatides were treated. Transfers were made in the glove box of I2R Inc., Cheltenham, Pa. Infrared spectra were determined on Nujol mulls by using the Infracord (Perkin-Elmer Inc., Norwalk, Conn.). Melting points were determined on the Kofler hot bench.

rac-1,2-Diolein. The rac-1-(2'-tetrahydropranyl)-glycerol was made according to Barry and Craig (8), and it was acylated with oleoyl chloride to form rac-1,2-dioleoyl-3-(2'-tetrahydropranyl)-glycerol. The tetrahydropranyl group was removed from this in 10-g batches, according to Krabisch and Borgström (1). The
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Fig. 1. Scheme for the synthesis of phosphatidyl(dioleoyl)hydroxy-L-proline.

removal required 13.9 ml of concentrated hydrochloric acid, added to a solution of the tetrahydropyrylglyceride in 280 ml of U.S.P. ether and 280 ml of methanol. After 10 min at room temperature the mixture was diluted with water and extracted with heptane. The diglyceride was purified according to the methods of Crossley et al. (9,10) and was examined by TLC with the use of Silica Gel H treated with boric acid (11) and also silica gel treated with silver nitrate (with the solvent isopropyl alcohol-chloroform 1.5:98.5) (12) by comparison with authentic 1,2-diolein and 1,3-diolein. From this TLC investigation it appeared that the material was 1,2-diolein with traces of impurities.

p-Methoxybenzoyloxy carbonyl-hydroxy-L-proline. The protected amino acid was made by the general procedure of Weygand and Hunger (13) by the action of anisyl azidoformate on the amino acid in the presence of magnesium oxide. The product was an oil obtained in 73% yield. Its infrared spectrum was consistent with the structure expected, 5.65 µ (carboxy) 5.90 µ (urethane carbonyl), 2.9 µ and 9.2 µ (secondary OH).

The substance was characterized as a dicyclohexylammonium salt, obtained by adding dicyclohexylamine to a solution of the acid in dimethyl formamide and then precipitating the product with acetone and ether. This gave a gelatinous solid which was recrystallized from ethanol-ether, mp 165°C (dec.). The carbonyl bands in this material were now shifted to 5.87 µ and 6.08 µ.

Anal. Calcd. for C_{20}H_{24}N_2O_6: C, 65.52; H, 8.46. Found: C, 65.53; H, 8.41.

Phthalimidomethyl Ester of p-Methoxybenzoyloxy carbonyl-hydroxy-L-proline. The preceding dicyclohexylammonium salt (26 g) was put into 200 ml of dry dimethylformamide, and the mixture was heated to 70°C. An equimolar amount of phthalimidomethyl chloride was dissolved in the clear solution. The mixture was stored in a stoppered flask at 4°C over-night. The addition of water precipitated an oil, which was rubbed to induce crystallization. The crystals were filtered and washed thoroughly with water. They were dried and recrystallized from ethanol. The yield was 85%, mp 147°C [α]D = -20.1 (c= 1%, CHCl_3).

The infrared spectrum showed the typical spectrum of this type of compound (3) with four bands in the carbonyl region (5.39; 5.70; 5.80; 5.89 µ).

Anal. Calcd. for C_{40}H_{30}N_2O_4: C, 60.79; H, 4.88; N, 6.17. Found: C, 60.76; H, 4.80; N, 6.10.

Dioleoylglycerophosphoryl-N-anisoyloxy carbonyl-hydroxy-L-proline Phthalimidomethyl Ester. A solution of 7.85 g (17.3 mM) of the phthalimidomethyl ester of anisoyloxy carbonylhydroxy-L-proline and 2.6 ml (19 mM) of triethylamine in 100 ml of dry tetrahydrofuran