Synthesis of an unsaturated diether analogue of phosphatidyl ethanolamine

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SUMMARY A synthesis of racemic diether analogues of glyceryl phosphatides is reported which is applicable to unsaturated aliphatic substituents. The "oleyl" diether analogue of phosphatidyl ethanolamine has been synthesized.

KEY WORDS unsaturated · glyceryl diether · phosphatidyl ethanolamine · diether analogue · di-*O*-octadec-9-enyl glycerophosphoryl ethanolamine

THE ISOLATION of aliphatic diether analogues of glycerides and phospholipids from natural sources has been reported by several authors.[1] Kates, Chan, and Stanacev (1) have described the synthesis of D-α,β-dialkyl glyceryl ethers containing fully saturated aliphatic side chains, and the synthesis of phosphatidyl choline and phosphonolipids from these has been described (2, 3). In our studies of the enzymatic synthesis of cyclopropane compounds (4), we were interested in examining as substrates diether analogues of phosphatides containing *un-*

Abbreviations: TLC, thin-layer chromatography (on Adsorbosil-1); NMR, nuclear magnetic resonance.

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[1] The literature in this field is summarized in ref. 1.

saturated aliphatic side chains. Synthesis of the phosphatides requires as starting materials the corresponding unsaturated glyceryl diethers. Since these cannot be made directly by the method of Kates et al., which employs catalytic hydrogenolysis to remove the blocking group, we wish to report our modification of this method, and the characterization of our intermediate, 2,3-(9-octadecenyloxy)-1-propanol, as well as our final product, the diether phosphatidyl ethanolamine.

The synthesis was accomplished by employing monotrityl glycerol, rather than monobenzyl glycerol, as the starting material. The catalytic hydrogenolysis is thus eliminated, since the trityl group can be removed easily with acid, without affecting the aliphatic ether groups. The diether was then esterified with 2-phthalimidoethyldichlorophosphate,[2] and the phthaloyl group was removed with hydrazine and hydrochloric acid to give the phosphatidyl ethanolamine.

3-Trityloxy-1,2-propanediol (monotrityl glycerol) was prepared from trityl chloride and excess glycerol by the method of Jackson and Lundberg (6). 1-Bromo-*cis*-9-octadecene ("oleyl bromide") was prepared by adding phosphorous tribromide to a solution of *cis*-9-octadecen-1-ol ("oleyl alcohol")[3] in carbon tetrachloride, as described by Loev and Dawson (7). As noted by those authors, some *trans* isomer is formed by this procedure, even at −10°C. This *cis* isomer can be recovered in at least 95% purity by fractional distillation (bp 160–162°C/0.5 mm).

1-Trityloxy-2,3-di-(9-octadecenyloxy)-propane (Trityl diether). In accordance with the method of Kates et al., oleyl bromide (11 g, 33 mmoles) was added to a three neck flask containing powdered potassium hydroxide (2.3 g, 50 mmoles) and monotrityl glycerol (2.7 g, 8 mmoles) in 50 ml of benzene. A condenser with a Dean-Stark water trap was attached, and the mixture was refluxed for 16 hr, with continuous mechanical stirring. At the end of this time, the contents of the flask were washed out with 200 ml of ethyl ether. The ether solution was washed with 2 × 50 ml each of water, ice-cold 1% hydrochloric acid, and water; and then was dried over anhydrous sodium sulfate. After filtration, the ether was removed in vacuo, and the residue was examined by TLC on silicic acid, developed in petroleum ether–benzene 1:1. The major spots consisted of excess oleyl bromide, at the solvent front, and the product, R_f 0.6. The latter spot was easily visible after exposure to iodine vapor, which indicated that lipophilic alkyl groups were present, and it gave a bright yellow color when sprayed with dilute sulfuric acid, which indicated the presence of the trityl group. Other minor spots were visible with one or the other of these reagents, but not with both. The crude product was used without purification for the next step.

2,3-Di-(9-octadecenyloxy)-1-propanol (Diether). The residue from the above procedure (about 12 g), containing the trityl diether, was dissolved in petroleum ether and treated with anhydrous hydrogen chloride, as described by Jackson and Lundberg (8) for trityl diglycerides. After this treatment, the solution was washed with water and 5% sodium bicarbonate, and dried over anhydrous sodium sulfate. Trityl alcohol was precipitated from the solution by cooling to −20°C, and was removed by filtration. The solvent was then removed in vacuo, and the residue (9 g) was applied to a 500 g column of activated silicic acid in hexane. The excess oleyl bromide was eluted with 5% ether in hexane, and the product was eluted with 20–30% ether in hexane.[4] This fraction was further purified by precipitation from acetone at −60°C, followed by rechromatography on silicic acid. The final product, which was obtained in 64% yield, based on the trityl glycerol, was a colorless oil at room temperature. It was homogeneous as judged by TLC.

Analysis:[5] $C_{39}H_{76}O_3$, 593; calculated: C, 78.99; H, 12.92
found: C, 78.99; H, 12.98

The infrared spectrum of the diether, taken on the oil, showed the expected absorption bands at 3550 (m, OH), 2950 (s, doublet, CH_2), 1480 (m, CH_2), 1110 (m, C—O—C), 1050 (m, OH), 965 (w, *trans* C═C), and 720 cm^{-1} (w, CH_2). Measurement of the absorption band at 2145 mμ on a Cary 14 spectrophotometer (Applied Physics Corp., Monrovia, Calif.) as described by Goddu (9) indicated that the diether had about 85–90% of the *cis* unsaturation found for the methyl oleate starting material. Some isomerization may have occurred in the base-catalyzed condensation. No further elaidinization would be expected in the additional steps leading to the diether glycerophosphatide. The nuclear magnetic resonance (NMR) bands, in ppm, taking tetramethylsilane as zero, (and their approximate relative intensity) are assigned as follows: 5.24(4), vinyl protons; 3.4(9), protons adjacent to oxygen; 1.9(8), protons allylic to double bond; 1.3(48), methylene protons; 0.9(6), terminal methyl protons. The splitting pattern of the band at 5.24 was qualitatively identical with that observed for methyl oleate, and distinct from that observed for

[2] This material was prepared as described by Hirt and Berchtold (5). All operations involving its preparation and use were carried out in a nitrogen-filled glove-bag to protect it from moisture.

[3] "Oleyl" alcohol was obtained from Sigma Chemical Co. (St. Louis, Mo.), or prepared by reduction of pure methyl oleate (Applied Science Labs., Inc., State College, Pa.) with lithium aluminum hydride.

[4] Column fractions were monitored by silicic acid TLC, using a solvent system of 15% ether in petroleum ether. The glyceryl diether had an *Rf* of 0.15–0.30 in this system.

[5] Analyses were done by Galbraith Labs., Inc. (Knoxville, Tenn.).

methyl elaidate. Since we would not determine, from these data, whether the diether contained a primary, and not a secondary, hydroxyl group, a portion of the product was acetylated with acetic anhydride and pyridine, at room temperature, for 48 hr. The acetyl derivative was extracted as usual and purified by precipitation from acetone at −60°C.

Analysis:[5] $C_{41}H_{78}O_4$, 635; calculated: C, 77.55; H, 12.38
found: C, 77.32; H, 12.34

The NMR spectrum of the acetyl diether differed from that of the diether in having an additional band at 4.05 (2) and a sharp peak at 1.98 (3). The latter is due to the protons of the acetyl methyl group; the band at 4.05 was assigned to the protons on the primary carbon at which the acetyl group was attached. From the previous work of Carter, Smith, and Jones (10), it is clear that the acetyl group cannot be on the secondary carbon.

1,2-Di-(9-octadecenyloxy)-3-(2'-aminoethyl)phosphoryl propane (*Diether GPE*). The glyceryl diether (0.5 g) was treated in 100 ml of dry chloroform with 2-phthalimidoethyl dichlorophosphate[2] (3 g) and pyridine (1.6 g) by the procedure of Hirt and Berchtold (5). The solution was allowed to stand 48 hr at room temperature; the chloroform was then removed in vacuo and the residue was dissolved in wet ether and allowed to stand 24 hr. The ether solution was then washed with 20 ml each of water, 2.5% hydrochloric acid, twice again with water, and dried over anhydrous sodium sulfate. The solution was filtered and the ether was evaporated in vacuo. The white solid residue was dissolved in 100 ml of 99% ethanol; 15 ml of 10% hydrazine in ethanol were added in small portions, with cooling, and the solution was then refluxed for 2 hr. After the solution was again cool, 10 ml of 20% hydrochloric acid was added, and the solution was allowed to stand 2 hr at room temperature.

The solvent was removed in vacuo, and the residue was taken up in 150 ml of chloroform–methanol 2:1. The insoluble material was removed by filtration, and the solution was washed with 30 ml of 1% sodium chloride. The upper layer was discarded, and the lower layer was again washed with 20 ml of 1% sodium chloride. The product, after evaporation of the chloroform, was purified by chromatography on DEAE-cellulose, as described by Rouser, Kritchevsky, Heller, and Lieber (11) for phosphatidyl ethanolamine. The sample (500 mg) was applied to a tightly packed, 3.5 × 20 cm column in chloroform–methanol 7:1; fraction 1 was eluted with 250 ml of chloroform–methanol 7:1 and fractions 2 and 3 with 250 ml of chloroform–methanol 7:3. Fraction 3 contained 440 mg of material. This gave a single ninhydrin-positive spot on silicic acid TLC (Adsorbosil-1, Applied Science Laboratories, Inc., State College, Pa.) running with or slightly ahead of diacyl phosphatidyl ethanolamine when developed in chloroform–methanol 2:1. The R_f of the diether cephalin is virtually the same as that of the diacyl compound. This fraction was dissolved in benzene and lyophilized to give the final product, a fluffy, snow-white powder, in 73% yield from the glyceryl diether. The diether glycerophosphatide is soluble in chloroform, ethanol, ether, and benzene, and slightly soluble in hexane. It does not exhibit a sharp melting point, but softens at around 200°C.

Analysis:[5] $C_{41}H_{81}NO_6P$, 715;

calculated: C, 68.86; H, 11.42; N, 1.96; P, 4.33
found: C, 68.75; H, 11.59; N, 1.89; P, 4.27

Note added in proof. The use of monotrityl glycerol for the synthesis of saturated glyceryl diethers was described in an article by M. Kates, B. Palmeta, and L. S. Yengoyan, *Biochemistry* **4:** 1595, 1965, which appeared after this manuscript was submitted.

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