Synthesis of *sn*-glycero-1-phosphocholine

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Summary The preparation of *sn*-glycero-1-phosphocholine is described. 2,3-O-Isopropylidene-*sn*-glycerol is condensed first with the 1,2-dimethylethenylene phosphorochloridate in the presence of triethylamine to yield the cyclic phosphotriester derivative. Choline *p*-toluenesulfonate, as the second alcohol, is condensed with this intermediate to yield the phosphotriester bearing the 1-methylacetonyl blocking group. This is removed under basic conditions to afford the 2,3-O-isopropylidene-*sn*-glycerol-1-phosphocholine in 57% yield following silicic acid column chromatography. The acetone blocking group is then removed with aqueous HCl (pH 2.3) to give pure *sn*-glycero-1-phosphocholine.—**Kanda, P., and M. A. Wells.** Synthesis of *sn*-glycero-1-phosphocholine. *J. Lipid Res.* 1981. **22:** 879–882.

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1-*sn*-Phosphatidylcholines are useful as substrates or substrate analogues in studies with phospholipases and potentially could be valuable in model membrane studies. Being of the "unnatural" configuration, their chemical synthesis is required. Two general approaches have been taken. For example, one starts with a *rac*-1,2-diacylglyerol (1), phosphorylates it with 2-bromoethylphosphoryldichloride (2), and replaces the bromine with trimethylamine (3). Other phosphorylating agents have been used for this purpose (4–6). The resulting *rac*-lecithin is then subjected to phospholipase A_2 digestion. Since only the 3-*sn*-phosphatidylcholine is attacked (7), the 1-*sn*-isomer is left intact and can be separated from the 3-*sn*-lysophosphatidylcholine digestion product by column chromatography. A second approach may use, as intermediates, an optically active 2,3-diacyl-*sn*-glycerol (8) or 2,3-diacyl-*sn*-glycero-1-iodohydrin (9), both of which are somewhat more tedious to prepare than their racemic counterparts. Phosphorylation and introduction or completion of the choline moiety result in the desired enantiomer. 1-*sn*-Phosphatidylcholine can also be prepared by condensation of a 2,3-diacyl-*sn*-glycero-1-phosphate with choline tosylate (10). These syntheses are not without drawbacks. The number of reactions involved lower final yields considerably, especially in the case of the *rac*-phosphatidylcholines where the desired isomer constitutes only half of the final product. In many cases, purification of resulting lecithins is complicated by the presence of a number of by-products. With regard to short acyl chain derivatives, their appreciable water solubility prevents washing of organic layers containing these compounds with aqueous acid or base to remove impurities. Likewise, the extreme light sensitivity of the short acyl chain glyceroiodohydrin derivatives

Abbreviations: GPC, glycerophosphocholine; TLC, thin-layer chromatography; pmr, proton magnetic resonance; tosylate, *p*-toluenesulfonate.

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discourages their use in phosphatidylcholine syntheses.

In recent years, the advent of more effective phosphorylating agents (5, 6) as well as modifications of existing procedures (11–13) have improved yields of synthetic phosphatidylcholines appreciably. However, to circumvent the aforementioned problems in preparing short chain 1-*sn*-phosphatidylcholines, we directed our efforts toward the synthesis of the glycerophosphocholine moiety of the 1-*sn*-configuration. Subsequent acylation with the appropriate fatty acid would yield a lecithin readily purified by column chromatography. Thus, we describe here a synthesis of optically pure *sn*-glycero-1-phosphocholine, an intermediate in the preparation of 1-*sn*-phosphatidylcholines.

MATERIALS AND METHODS

Materials

Silicic acid (CC-7), acetonitrile (redistilled from $CaCl_2$), and anhydrous ethyl ether were from Mallinckrodt, St. Louis, MO. Chloroform, methanol, and Darco G-60 charcoal were from Matheson, Coleman, and Bell, Norwood, OH. Silica gel G for thin-layer plates was from E. M. Laboratories, Elmsford, NY. Total phosphorus was determined by the method of Fiske and Subba Row (14). AG 3x-4A was from Bio-Rad, Richmond, CA and was further purified (15) before being converted to hydroxide form. Choline *p*-toluenesulfonate was prepared according to Rosenthal (16) and was dried for 48 hr over P_2O_5 in vacuo immediately before use. 2,3-O-Isopropylidene-*sn*-glycerol was prepared immediately before use as described elsewhere (17). 1,2-Dimethylethenylene phosphorochloridate (2-chloro-4,5-dimethyl-1-oxo-1,3,2-dioxaphosphole) was synthesized immediately before use according to Ramirez et al. (18).

Synthesis of *sn*-glycero-1-phosphocholine

The procedure of Sarma et al. (19) was used with slight modification. Under a dry nitrogen atmosphere, a solution of 2,3-O-isopropylidene-*sn*-glycerol (26.5 mmol, 3.26 ml) and triethylamine (2.67 g, 1 molar equivalent) in 30 ml of dry ethyl ether was added dropwise to a stirred ether solution (95 ml) of 1,2-dimethylethenylene phosphorochloridate (26.5 mmol, 3.4 ml) at 22°C. After 2 hr at 22°C, the triethylammonium chloride (3.35 g) was filtered (under N_2) and washed with ether. The combined filtrates were evaporated and the residue (92% yield) was placed under vacuum (0.1 mm) for 1 hr. Sixty-five ml of dry acetonitrile was added, following by choline *p*-toluenesulfonate (26 mmol, 7.15 g) and triethylamine (5.24 g, 52 mmol). The mixture was stirred at 22°C for 36 hr under nitrogen. Water (170 ml) was added and the solution was heated to 70°C for 7 hr, followed by evaporation of the solvents under reduced pressure. The residue was taken up in a minimal amount of chloroform and loaded onto a column of 400 g silicic acid (CC-7) packed in chloroform. The column was eluted successively with chloroform (1.5 l), then mixtures of chloroform–methanol (100:2, 1.5 l; 100:7, 1.5 l; 7:1, 1.5 l; 4:1, 1.5 l; 1:1, 3 l). Methanol was passed through the column until all desired product was eluted. The solvent was evaporated under reduced pressure and the residue was taken up in 50 ml of methanol and treated with Darco G-60 charcoal. After filtration and evaporation, the product, a colorless, viscous syrup, was taken up in methanol and stored at −20°C until converted to 1-*sn*-GPC. TLC on silica gel G plates in the solvent system methanol–water 75:25 showed a single phosphorus- and choline-positive spot with $R_F = 0.23$. This was essentially identical to the R_F for *sn*-glycero-1-phosphocholine. The pmr spectrum (D_2O) compared with that of 3-*sn*-GPC except for the presence of two peaks: δ1.95 and δ2.03 (2s, 6H, $C(CH_3)_2$) corresponding to the acetone group.

Yield − 14.9 mmol (57%) $[\alpha]^{22}_{546}$

$= +4.8$ (C, 3.0 in H_2O).

To remove the acetone group, the 2,3-O-isopropylidene-*sn*-glycero-1-phosphocholine was dissolved in 50 ml of water and the pH was lowered to 2.3 with HCl (20). After 15 hr, the solution was neutralized with AG 3X-4A resin (20–50 mesh, OH form) and centrifuged. The supernatant was lyophilized and the *sn*-glycero-1-phosphocholine (14 mmol) was stored in methanol at −20°C.

$$[\alpha]^{22}_{546} = +3.0 \text{ (C, 2.5 in } H_2O)$$

(for 3-*sn*-GPC $[\alpha]^{22}_{546} = -3.0$ (C, 2.5 in H_2O)).[2]

This was converted to its cadmium chloride adduct for analysis of phosphorus. Calculated for $(C_8\ H_{22}\ O_7\ NP)_2 \cdot (CdCl_2)_3$ (1100:P, 5.62% found:5.55%.

RESULTS AND DISCUSSION

The successful use of the 1,2-dimethylethenylene phosphorochloridate function in preparing 1-*sn*-GPC

[2] This was the optical rotation found for 3-*sn*-GPC prepared by de-acylation of egg yolk lecithin by the method of Brockerhoff and Yurkowski (21).

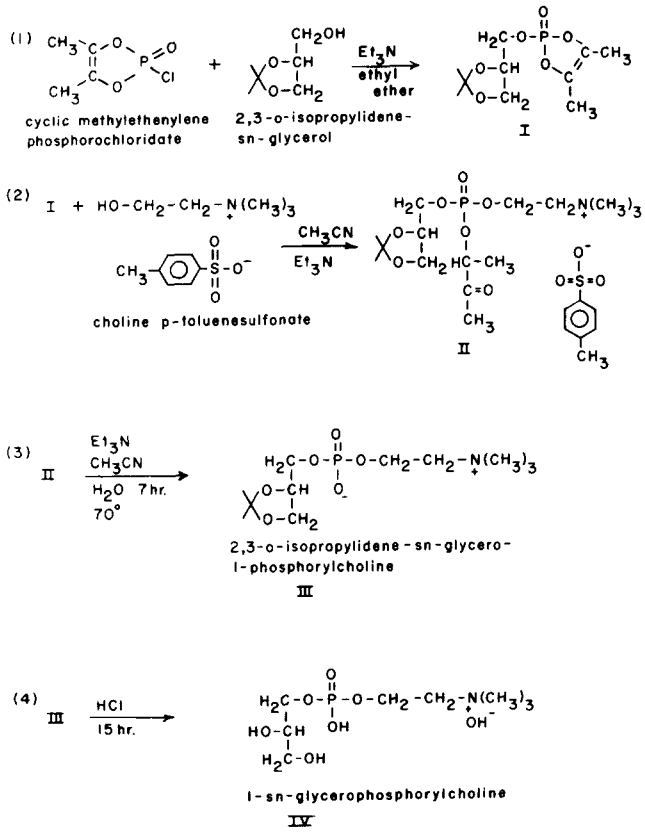

Fig. 1. Reaction scheme for the synthesis of 1-*sn*-glycerophosphocholine (IV). In reaction (1), the 1,2-dimethylethenylene phosphorochloridate is condensed with 2,3-O-isopropylidene-*sn*-glycerol in the presence of triethylamine to yield the cyclic phosphotriester (I). In reaction (2), the triethylamine catalyzed reaction of (I) with choline *p*-toluenesulfonate yields the acyclic phosphotriester bearing the 1-methyl acetonyl blocking group. This is removed by treatment of (II) with a mixture of water, triethylamine, and acetonitrile at 70°C as shown in reaction (3). The resulting 2,3-O-isopropylidene-*sn*-glycero-1-phosphocholine (III) is hydrolyzed with aqueous HCl at pH 2.3 to remove the acetone blocking group (reaction 4), yielding the 1-*sn*-glycero-phosphocholine (IV).

is in accordance with its application toward the synthesis of other similar unsymmetrical phosphodiesters (19). The synthetic scheme is outlined in **Fig. 1.** Due to the insolubility of choline tosylate in ether, the 2,3-O-isopropylidene-*sn*-glycerol was condensed first with the phosphorochloridate. The choline tosylate did, however, dissolve completely in acetonitrile in the second condensation reaction. Removal of the 1-methylacetonyl blocking group under slightly basic conditions afforded the 2,3-O-isopropylidene-1-*sn*-GPC in good yield following column chromatography. The presence of appreciable amounts of triethylammonium- and choline-containing salts in the reaction mixture sometimes required larger amounts of chloroform–methanol 1:1 than stated here to be eluted through the column to separate these from the desired 1-*sn*-GPC derivative.

Aside from facilitating access to short acyl chain 3-*sn*-phosphatidylcholines, the 1-*sn*-GPC in general makes available 1-*sn*-phosphatidylcholines of any desired fatty acid composition merely through acylation. The 2,3-dihexanoyl-*sn*-glycero-1-phosphocholine prepared[3] from 1-*sn*-GPC had an $[\alpha]^{22}_{546} = -11.4$ (C, 2.3 in $CHCl_3$–CH_3OH 1:1) and was completely inert toward phospholipase A_2 digestion. Finally, mixed acid 1-*sn*-phosphatidylcholines and 1-*sn*-lysophosphatidylcholines can be obtained as described by Smith and Kuksis (22).

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