I. Synthesis of sn-Glycerol-Cyclic-Phosphodiester Isomers

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ABSTRACT

A procedure for the synthesis of stereoisomerically pure sn-glycerol-cyclic-phosphodiester has been developed. The process involves the following sequence of reactions: benzyl-sn-glycerol → benzyl-sn-glycerol-cyclic(phenyl)-phosphodiester → sn-glycerol-cyclic-phosphodiester. The following isomers have been synthesized: sn-glycerol-2,3-, 1,2-, 1,3-cyclic-phosphodiester and the racemic mixture. The 2,3- and 1,2-cyclic-phosphodiester of glycerol are optically active antipodes. They are five-membered ring asymmetrical compounds, with specific rotations of -1.6° ± 0.1° and +1.6° ± 0.1° respectively. These two enantiomers and their racemate are thick liquids and are unstable; therefore they were converted into Ba(glycerol-cyclic-phosphodiester)₂ salts, which can be better stored. The six-membered ring sn-glycerol-1,3-cyclic-phosphodiester is a crystalline, stable compound. The physical and chemical properties of these cyclic-phosphodiesters of glycerol are described and their chemical analyses are reported.

INTRODUCTION

Half a century ago, Octave Bailly (1) reacted sodium phosphate with epichlorohydrin and claimed during reaction the formation of a six-membered ring cyclic-phosphodiester of glycerol takes place. About 20 years later, Verkade and coworkers (2) studied the acid-catalyzed phosphate group migration of glycerol-phosphoric-acid-ester and postulated a mechanism by way of cyclic-phosphodiester. Later, Chargaff (3) demonstrated that this rearrangement indeed involves an intramolecular migration of the phosphate group. Baer and Kates (4) studied phosphate group migration of synthetic sn-glycerol-3-phosphoryl-choline, in acidic and alkaline media, and also postulated the formation of the cyclic-phosphodiester of glycerol as an intermediate during the migration.

A similar phosphate group migration occurs during the hydrolysis of nucleic acid. Markham and Smith (5) have isolated and identified cyclic-2',3'-nucleotides as intermediates in the hydrolysis of ribonucleic acid, and have shown that the formation of a cyclic-phosphodiester is responsible for the migration of the phosphate group from the 3'- to the 2'-hydroxyl of the ribose. Other cyclic-phosphodiesters have also been described, i.e., pantetheine-2',4'-cyclic-phosphodiester (6), glucose-cyclic-phosphodiester (7) and riboflavin-4',5'-cyclic-phosphodiester (8).

The possibility of isolation of a cyclic-phosphodiester of glycerol was investigated by Ukita et al. (9). They found that no evidence for accumulation of the cyclic-phosphodiester of glycerol during the hydrolysis of lecithin, but synthesized the cyclic-phosphodiester of glycerol by intramolecular cyclization of the sn-glycerol-2-phosphoric-acid-ester catalyzed with trifluoroacetic anhydride, according to the procedure of Brown and coworkers (10). Later, Maruo and Benson (11) prepared a radioactive glycerol-phosphate-ester by the method of McMurray et al. (12) and then cyclized this product by intramolecular phosphorylation (term introduced by Khorana et al. [13]) using dicyclohexylcarbodiimide (DCC) as catalyst, according to the procedure of Khorana and coworkers (13).

However no direct synthesis of isomerically pure cyclic-phosphodiesters of glycerol has been reported. This paper describes the synthesis of the stereo- and positional isomers of sn-glycerol-cyclic-phosphodiester.

EXPERIMENTAL PROCEDURE

Benzyl Glycerol Ethers

Reaction of 2,3-isopropylidene-sn-glycerol and 1,2-isopropylidene-sn-glycerol with 50% sodium hydroxide yielded the sodium alkoxides as described by Kaufmann and Förster (14). Without isolation these were reacted with benzyl chloride and hydrolyzed according to the method of Sowden and Fischer (15) to produce 1- and 3-benzyl-sn-glycerols. 2-Benzyl-sn-glycerol was prepared from 1,3-benzylidene-sn-glycerol. The latter was prepared by the method of Hibbert and Carter (16), but with the modification introduced by Verkade and van Roon (17). 2-Potassium-1,3-benzylidene-sn-glyceroxide was prepared according to the method described by Gupta and Kummerow (18), and this, without isolation, was reacted with benzyl chloride to
produce 2-benzyl-1,3-benzylidene-sn-glycerol, which on hydrolysis of the benzylidene group with 10% acetic acid yielded 2-benzyl-sn-glycerol.

Benzyl chloride and phenylphosphoryl dichloride were certified reagents, and both were redistilled before use. Pyridine was dried over calcium hydride and toluene with sodium wire. Both solvents were certified spectroanalyzed.

**Phosphocyclization**

The phenylphosphoryl dichloride was used as phosphocyclizing reagent, as shown in the reaction scheme (Fig. 1). The position of the benzyl-protective group in the glycerol moiety dictates the cyclization position of the phosphodiester residue.

The phosphocyclization was carried out in a 500 ml three-necked, round-bottom flask fitted with a magnetic stirrer, two dropping funnels and a calcium chloride tube. The reaction flask was kept in a cold bath of ice and salt (-10 to -15 C). An appropriate benzyl-sn-glycerol (18.2 g, 0.1 mol) was dissolved in dry toluene and brought to the same volume as the benzyl-sn-glycerol and pyridine mixture. The mixture of phenylphosphoryl dichloride and toluene was placed in the second dropping funnel.

Both reagents were added drop by drop at the same rate with stirring at -10 to -15 C in the reaction flask. The addition of the reagents was finished after ca. 30 min. The reaction mixture was stirred further for 2 hr at -10 C, and for 12 hr at room temperature (20-25 C). The reaction product was freed of the solvents, toluene and pyridine, by distillation in high vacuum at a bath temperature of 30-35 C. The residue, consisting of benzyl-sn-glycerol-cyclic(phenyl)-phosphodiester and pyridine hydrochloride, was dissolved in methanol or ethanol, and the alcoholic solution was then passed through an ion exchange column of Rexyn-101 (H⁺) in order to remove the pyridine hydrochloride from the reaction product. The column was 40 cm long, 4.5 cm wide and contained 400 g Rexyn-101 (H⁺).

The column was washed with either methanol or ethanol until the effluent was free of solute. The eluate was then concentrated to dryness under reduced pressure at a bath temperature of 30-35 C. The benzyl-sn-glycerol-cyclic(phenyl)-phosphodiester was recovered,