BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 48 (3), 1077—1078 (1975)

A Novel Method for the Synthesis of Cytidine Diphosphate-Choline

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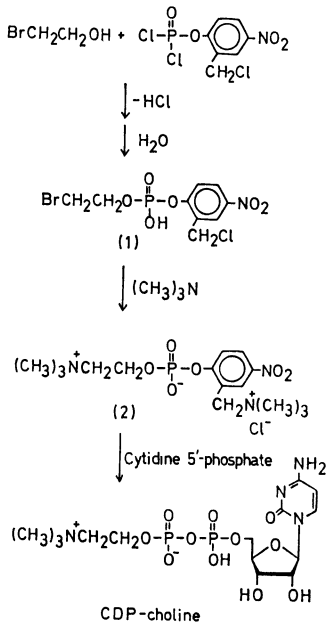
(Received November 18, 1974)

Synopsis. Cytidine diphosphate choline was synthesized by the reaction of cytidine 5′-phosphate with 2-bromoethyl 2′-(chloromethyl)-4′-nitrophenyl phosphate in the presence of trimethylamine.

Derivatives of cytidine 5′-phosphate are known to participate in the biosynthesis of phospholipids. Cytidine diphosphate choline (CDP-choline) is one of the important intermediates for the enzymatic incorporation of choline phosphate into lecithin. Although several methods for the synthesis of CDP-choline are described in the literature,[1] most synthetic methods give many by-products that are difficult to remove. An important consideration in the synthesis of CDP-choline is the method of introduction of the choline residue. Sanno and Tanaka synthesized CDP-choline with a 35% yield by the ring-opening reaction of ethyleneimine with cytidine 5′-diphosphate followed by methylation.[1b]

In this paper, we wish to report a novel method for the synthesis of CDP-choline using 2-bromoethyl 2′-(chloromethyl)-4′-nitrophenyl phosphate (**1**) as a precursor for choline phosphate as shown in Scheme 1.



Scheme 1.

In this method, the 2-(chloromethyl)-4-nitrophenoxy group is activated and the choline residue is formed by attack of trimethylamine together with the corresponding ammonium derivative (**2**), which in turn reacts with cytidine 5′-phosphate to afford CDP-choline.[2]

In addition, it should be noted that the intermediate (**2**) reacts selectively with the phosphate, leaving the amino group on the cytosine residue and the hydroxyl groups on the sugar moiety of the cytidine 5′-phosphate. Further, it was found that P^1-(2-bromoethyl) P^2-cytidine 5′-pyrophosphate and unreacted cytidine 5′-phosphate were only detected as the by-products which were easily separated from CDP-choline.

Experimental

Methods. For paper chromatography, the descending technique using Toyo Roshi No. 51 and No. 51A papers was employed. The solvent system was ethanol–1 M ammonium acetate (7: 3 v/v). Paper electrophoresis was carried out with an apparatus similar to that described by Markham and Smith.[3] The buffer solution was 0.05 M phosphate (pH 8.0). The yields of the reported compounds were most frequently estimated spectrophotometrically after elution of spots from paper chromatograms run. The preparation of 2-(chloromethyl)-4-nitrophenyl phosphorodichloridate has been described previously.[4] Commercial 2-bromoethanol was purified by distillation. Cytidine 5′-phosphate was purchased from Yamasa Shoyu co. A dry solution of trimethylamine in dimethylformamide (DMF) was prepared as follows: Gaseous trimethylamine generated from the commercially available aqueous solution by adding sodium hydroxide was passed through a soda-lime tube and bubbled into dry DMF.

Preparation of 2-Bromoethyl 2′-(Chloromethyl)-4′-nitrophenyl Phosphate (1). A mixture of 2-bromoethanol (62.4 mg, 0.5 mmol) and pyridine (39.6 mg, 0.5 mmol) was added to a solution of 2-(chloromethyl)-4-nitrophenyl phosphorodichloridate (1.52 g, 0.5 mmol) in 10 ml of tetrahydrofuran (THF) cooled to 0 °C for 2 hr. Then, it was allowed to stand at room temperature for 7 hr. The mixture was poured into an aqueous solution containing pyridine (39.6 mg, 0.5 mmol) and stirred for 30 min. After removal of THF under reduced pressure, the aqueous solution was washed with three 40 ml portions of chloroform and then with two 20 ml portions of chloroform. The chloroform extracts were combined and washed with water (5 ml) and the aqueous solution was washed again with chloroform. All of the chloroform extracts were combined and finally dried over sodium sulfate. After evaporation of chloroform, 2-bromoethyl 2′-(chloromethyl)-4′-nitrophenyl phosphate (1.66 g, 89%) was obtained as a pale yellowish oil which was homogeneous on a paper chromatogram.

Synthesis of CDP-choline. Compound **1** (0.5 mmol) was dissolved in 7 ml of DMF saturated with dry trimethylamine and containing a catalytic amount of sodium iodide. The solution was stirred at room temperature in a pressure bottle for 3 hr and then heated at 55 °C for 1 hr. After evaporation of the DMF under reduced pressure, further anhydrous trimethylamine in DMF was added and evaporation was repeated. A hot (75 °C) solution of the 4-morpholine *N,N′*-dicyclohexylcarboxamidinium salt of cytidine

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5′-phosphate (0.1 mmol) in dry DMF (5 ml) was added to the residue. The mixture was heated at 95 °C for 10 hr. After addition of water, the precipitate was removed. The aqueous solution was concentrated and chromatographed on a DEAE cellulose column. First, CDP-choline was eluted with a linear gradient of triethylammonium bicarbonate solution from 0 to 0.1 M. The eluate was concentrated. CDP-choline was obtained in 54% yield based on cytidine 5′-phosphate. It was homogeneous on a paper chromatogram. The CDP-choline was characterized by its electrophoretic mobility (0.25 at pH 8.0) relative to cytidine 5′-phosphate and by phosphorus analysis (phosphorus: cytidine=2.00: 1.00).

The authors wish to heartily thank Professor Teruaki Mukaiyama for his encouragement and comments throughout the investigation and to Dr. Yoshitaka Mushika for his kind advice given during the course fo this experiment.

References

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2) A few years ago, we reported a similar type of reaction for the synthesis of mixed diesters of phosphoric acid: T. Hata, Y. Mushika, and T. Mukaiyama, *Tetrahedron Lett.*, **1970**, 3505. Also Mushika and Yoneda synthesized α-glycerophosphoryl choline by the reaction of 2-chloroethyl 2′-(chloromethyl)-4′-nitrophenyl isopropyridene-α-glyceryl phosphate with aqueous trimethylamine: Y. Mushika and N. Yoneda, *Chem. Pharm. Bull.* (Tokyo), **19**, 696 (1971).

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