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A New Synthetic Method for Cytidine Diphosphate-Ethanolamine

Cytidine diphosphate (CDP)-ethanolamine (Ⅲ), which was discovered in 1956 by Kennedy, *et al.*,[1] is well known to participate in the biosynthesis of phosphatidylethanolamine and to play an important rôle in the metabolism of phospholipid. In the same year, Kennedy, *et al.*[2,3] synthesized the compound from cytidine 5′-monophosphate (CMP) (Ⅴ) and ethanolamine phosphate (Ⅳ) by the dicyclohexylcarbodiimide (DCC) method, but the yield was as poor as 10%. The present writers, having observed that the product obtained by the above method was contaminated by numerous, hardly removable by-

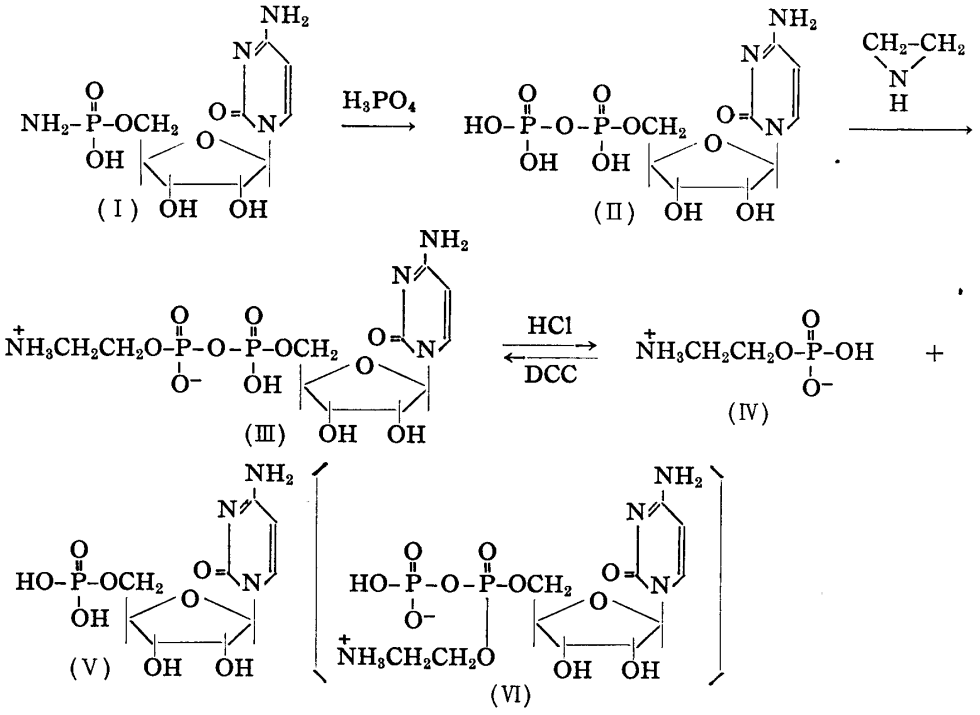

Chart 1.

products, tried another method according to that shown in Chart 1 and found that the expected compound (Ⅲ) was profitably obtained by this new method.

First, cyclohexylguanidinium cytidine 5′-phosphoramidate (CMP-NH_2) (I),[4,5] was condensed with orthophosphoric acid and the reaction mixture was adsorbed on Dowex-1 ×8 (200~400 mesh, chloride form). The unreacted CMP-NH_2 (I) and CMP (Ⅴ) were removed by treatment with 0.002*N* HCl and the resulting cytidine 5′-diphosphate (CDP) (Ⅱ) was eluted with 0.01*N* HCl. The eluate, after being treated with charcoal as usual, was passed through a column of Amberlite IR-120 (acid form) to convert the product into free acid, the effluent was concentrated, and resulting solid was recrystallized from water to give the product (Ⅱ) as colorless needles, m.p. 183°(decomp.), $[\alpha]_D^{22}$ +26°(c=0.5% in H_2O) (*Anal.* Calcd. for $C_9H_{15}O_{11}N_3P_2 \cdot H_2O$: C, 25.64; H, 4.07; N, 9.97; P, 14.74. Found: C, 25.59; H, 4.32; N, 10.41; P, 14.57).

1) E. P. Kennedy, S. B. Weiss: J. Biol. Chem., **222**, 193(1956).
2) E. P. Kennedy: *Ibid.*, **222**, 185(1956).
3) E. P. Kennedy, L. F. Borkenhagen, S. W. Smith: *Ibid.*, **234**, 1998(1959).
4) K. Tanaka, M. Honjo, Y. Sanno, H. Moriyama: This Bulletin, 8, 749(1960).
5) R. S. Chambers, P. Shapiro, V. Kurkov: J. Am. Chem. Soc., **82**, 970(1960).

An aqueous solution of 201 mg. of CDP (II) (0.5 m. mole) and 43 mg. of ethyleneimine (1.0 m. mole) was heated at 37° for 40 hr., the reaction mixture was adsorbed on a column of 40 cc. of Dowex-1 ×2 (200~400 mesh, formate form), and the resulting CDP-ethanolamine (III) was eluted with 0.04*N* formic acid. The eluate was concentrated and the scale-like crystals that separated (60 mg. or 28%) were confirmed to be the expected product (III) from the following results:

i) Paper partition chromatography (solvent: 60% EtOH containing 0.02*M* AcOH) gave one spot at the same Rf as (III) prepared by another method.[3]

ii) Paper electrophoresis (0.05*M* acetate buffer, pH 4.5, 11 V/cm., 500 V applied, 2 hr.) afforded one spot at R_{CMP} 0.96. Und er the same conditions R_{CMP}* of CDP-choline was 0.95, and under other conditions (0.1*M* phosphate buffer, pH 7.5, 11 V/cm., 500 V applied, 2 hr.) R_{CMP} values of (III) and CDP-choline were 0.53 and 0.51, respectively.

iii) 100.1% of nitrogen was determined by azotometry as against the amount of (III) calculated from its optical density at 280 mμ. Nitrogen values of CMP (V) and ethanolamine phosphate (IV) which were employed as control were 0% and 99.4%, respectively.

iv) 4 mg. of (III) was hydrolyzed by heating with *N* HCl at 100° for 40 min., the hydrolysate was subjected to paper partition chromatography (0.02*M* AcOH-60% EtOH) and paper electrophoresis (acetate buffer, pH 5; borate buffer, pH 9.2), and the resulting two spots in each case were in complete agreement with those of authentic CMP (V) and ethanolamine phosphate (IV), when examined under ultraviolet rays and by color reaction.

That the isomer (VI) of (III) was not formed is evident from the findings described under (ii) and (iv).

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* Ratio of the migration distance of the sample divided by that of CMP.