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SOME PHOSPHATE ESTERS OF CYCLOCYTIDINE AND ARACYTIDINE

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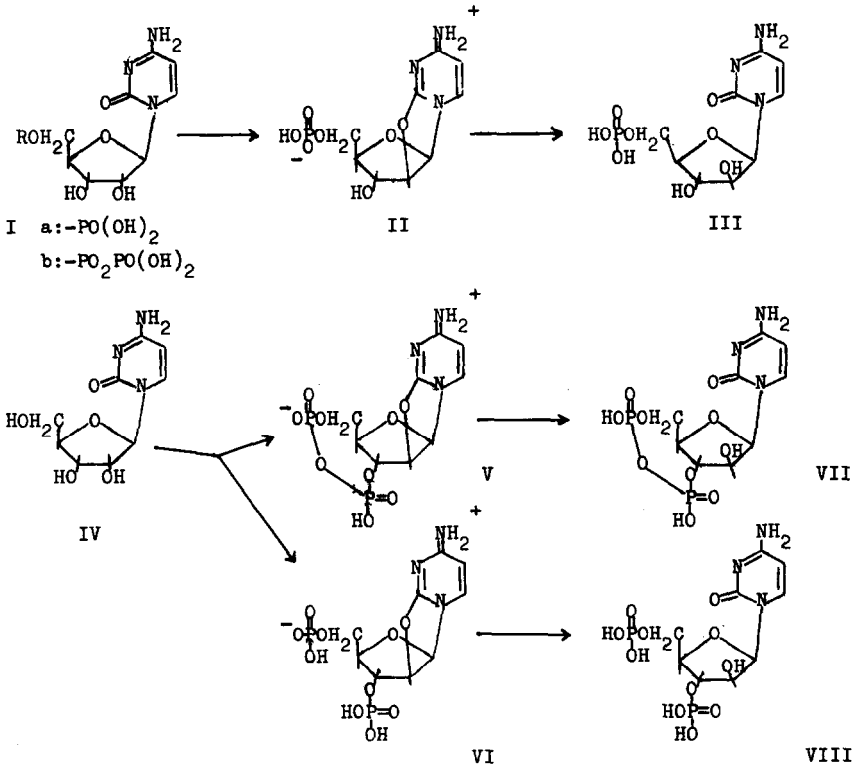
Arabinocytidylic acids, in addition to being a suitable starting material for polimerization, have been under therapeutic interest (1) as the nucleotide of an antiviral and carcinostatic aracytidine (1-β-D-arabinofuranosyl cytosine). But the phosphorylation of arabinonucleoside (2,3) is not an expedient approach for the synthesis of the 3' and 5' phosphate. This paper describes the direct conversion of cytidine, 5'-cytidylic acid into aracytidine-3',5'-diphosphate (VIII) together with its anhydrophosphoric acid ester (VII) and 5'-aracytidylic acid (III), respectively.

Walwick et al (4) synthesized the 3',5'-diphosphate of aracytidine by heating cytidylic acid in polyphosphoric acid and subsequent hydrolysis. O^2,2'-Cyclocytidine-3'-phosphate, a precursor of 3'-aracytidylic acid, was prepared by Nagyvary (5,6), utilizing fully substituted 2',3'-cyclic pyrophosphate, or sulfonic anhydrides, or cyclic trimethylsilyl ester under base catalysis.

We have recently described the conversion of cytidine and N^4-acetylcytidine into O^2,2'-cyclocytidine and N^4,3',5'-triacetylcytidine respectively which are both precursors of aracytidine, under thermal conditions using partially hydrolyzed phosphorus oxychloride in ethyl acetate (7). In this cyclization reaction of cytidine in the presence of ethyl acetate, any phosphorylation of cyclonucleoside did not occure. A related idea proved to be useful for the synthesis of some phosphorylated O^2,2'-cyclocytidine, which could be easily converted to aracytidylic acid, by using cytidylic acid instead. We adapted this method to cyclization of 5'-cytidylic acid. 5'-Cytidylic acid (1 g) was heated for 1 hour at 60° with a partially hydrolyzed phosphorus oxychloride (4.5 ml of $POCl_3$ with 0.8 ml of water) in a small volume of ethyl acetate (4.2 ml). The cyclized product was separated as follows. The aqueous reaction mixture was put on the top of the column of activated charcoal (35 g) and the column was washed with water, followed by elution with MeOH-HCl-H_2O (4:1:2, v/v). To the residue obtained by concentration of the eluate, a large excess of acetone was added to precipitate nucleotide. Separation of cyclized cytidylic acid was achieved on the column of DIAION SA-11B (formate). The combined effluent and washings were concentrated to dryness and precipitation from aqueous solution by addition of acetone gave 0.43 g of O^2,2'-cyclocytidine-5'-phosphate (II). The

structure was established by UV; $\lambda_{max}^{pH\ 1-7}$ 231.5, 262.5 mμ (ε; 8.500, 9.500), 282 mμ (s) (ε; 3.100), $[\alpha]_D^{22}$; -14 (c; 1.0, water), Anal. Calcd for $C_9H_{12}O_7N_3P \cdot \frac{1}{2}H_2O$, C; 34.40, H; 4.17, N; 13.37 %. Found, C; 34.00, H; 3.98, N; 13.40 %. Paper chromatography; Rf, 0.49 (isoPrOH-HCl-H_2O, 34 :8.2 :7.8). Paper electrophoretic movility; 0.42 (relative to 5'-CMP, see Table 1). The hydrolysis of this compound was effected by the addition of 1M triethylammonium bicarbonate at 50° to give 5'-aracytidylic acid (III) in 85 % yield. The separation of the product was achieved on a DIAION SA-11B (formate) column by elution with 0.05 M formic acid. The crystalline sample exhibited the spectral properties characteristic of this system (2,5); NMR spectrum (60 MHz in D_2O, ppm relative to DSS); a doublet of H-6 at 7.95, doublets of H-5 and H-1' centered at 6.10 and 6.21 respectively, UV; $\lambda_{max}^{pH\ 7}$ 274, 213 mμ (ε; 10.700, 10.300). This material was identical by paper chromatography (Table 1) with a sample prepared by Wechter's method (2). The treatment of III with alkaline phosphatase gave rise to aracytidine. By analogy, we applied this method to cyclization of cytidine-5'-diphosphate (Ib), but cyclization occured simultaneously with unexpected dephosphorylation of β-phosphate of Ib to give II. Ib (0.1 g) was heated for 10 min at 70° with partially hydrolyzed phosphorus oxychloride (0.378 ml of



$POCl_3$ with 0.074 ml of H_2O) in ethyl acetate (0.60 ml). Separation and purification was carried out as mentioned above. Yield was 0.035 g. This product was identical with II by all criteria.

When cytidine was heated with a partially hydrolyzed phosphorus oxychloride in the absence of ethyl acetate in its reaction medium, two phosphorylated O^2,2'-cyclocytidine were obtained. The preparation can be very simply performed at any scale ranging from 4.1 mmol to 16.4 mmol of cytidine. 30 equiv. of phosphorus oxychloride were hydrolyzed with 30 equiv. of water in ice-cooled bath for 30 min followed by beeing kept at room temperature for 30 min. To this mixture cytidine (1 g, 4.1 mmol) was added and kept for 14-22 hours at 60°. The aqueous reaction mixture was treated with activated charcoal (35 g) as above mentioned. Isolation of phosphorylated products was achieved by absorption on DIAION SA-11B (formate, 50 ml) column and a linear gradient elution with 0 to 0.1 M formic acid. The first eluted material (fractions 35-45, 18 ml/fraction) and the second (fractions 50-57) were characterized as O^2,2'-cyclocytidine-3',5'-anhydrodiphosphate (V) and O^2,2'-cyclocytidine-3',5'-diphosphate (VI), respectively. The structure of V was established by analysis; Anal. Calcd for $C_9H_{11}O_9N_3P_2$, C; 30.26, H; 3.10, N; 11.76 %. Found, C; 29.87, H; 3.44, N; 11.60 %, ε(P) at 262 mμ (pH 7); 6.100, UV; $\lambda_{max}^{pH\ 1-7}$ 232, 262, 282 (s) mμ (ε; 9.800, 12.000, 3.200), $[\alpha]_D^{22}$; -52.7 (c, 1.0, H_2O) and electrophoretic movility; 0.78 (relative to 5'-CMP). NMR spectrum (60 MHz in D_2O, ppm relative to DSS) was similar to that of VI; a doublet of H-6 at 8.25, two unresolved doublets of H-5 and H-1' centered at 6.73 and 6.81, respectively, a doublet of H-2' at 5.93, a multiplet of H-5' at 4.13. The identification of VI was carried out by comparison of melting point; 190-192°(uncorr.), optical rotation; $[\alpha]_D^{22}$; -36.5 (c, 1.0, H_2O), UV; $\lambda_{max}^{pH\ 1-7}$ 232, 262, 282 (s) mμ (ε; 9.000, 10.400, 3.200) with published data (4). The NMR spectrum of VI taken at 60 MHz in D_2O featured the following resonance (ppm relative to DSS); a doublet of H-6 at 8.10, two unresolved doublets of H-5 and H-1' centered at 6.75 and 6.81, respectively, a tripled of H-2' at 6.00, a multiplet of H-5' at 4.15. The clear separation of the H-2' signal must be due to the deshielding effect of the isourea and phosphate groups. The shift of H-2' and H-5' signal to the low field, comparing with that of O^2,2'-cyclocytidire, must also be due to the effect of phosphate groups. The yields of VI and V were approximately 25 %, 32 % respectively.

V was not hydrolyzed by alkaline phosphatase and spleen phosphodiesterase. This enzymatic stability might be due to the cyclic structure of anhydrophosphate. The hydrolysis of V with N hydrochloric acid at 100° for 2 hours, by which pyrophosphate bond of cytidine-5'-diphosphate (Ib) was almost completely cleaved, did not liberate any inorganic phosphate. This fact also might support the structure of V. The hydrolysis of cyclo bonds of V and VI was achieved by 1 M triethylammonium bicarbonate under the same conditions as in the case of II to give the corresponding phosphate esters of aracytidine, VII and VIII (4), respectively. Electrophoretic mobilities and Rf values of paper chromatography of these compounds were summarized in Table 1.

From a practical point of view, our methods to prepare the phosphate esters of cyclocytidine and aracytidine are more facile and economical than those based on the phosphorylation of protected nucleosides (2,3,5). Thus these nucleotides have become readily available for clinical studies and further chemical synthesis.

TABLE 1

Paper Electrophoresis (PEP) and Paper Chromatography (PPC)

Compound	PEP (migration distance,cm)[a]	PPC (Rf)[b]
5'-cytidylic acid (Ia)	10.5	0.47
5'-aracytidylic acid	10.5	0.58
cytidine-5'-diphosphate (Ib)	13.12	
O^2,2'-cyclocytidine-5'-phosphate (II)	4.41	0.49
5'-aracytidylic acid (III, synthetic)	10.5	0.58
O^2,2'-cyclocytidine-3',5'-anhydrodiphosphate (V)	8.19	0.24
O^2,2'-cyclocytidine-3',5'-diphosphate (VI)	11.7	0.56
aracytidine-3',5'-anhydrodiphosphate (VII)	13.23	0.35
aracytidine-3',5'-diphosphate (VIII)	13.96	0.61

[a]The condition of PEP; 0.05 M phosphate buffer pH 7.5, 700 volt, 50 mA for 2 hours, anodic migration. [b]Solvent; isopropanol: conc HCl: water 34: 8.2: 7.8.

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REFERENCES

(1) C.G.Smith, H.H.Buskirk and W.L.Lummis, J. Med. Chem., 10, 774 (1967)
(2) W.J.Wechter, J. Med. Chem., 10, 762 (1967)
(3) M.P.de Garilhe, Bull. Soc. Chim., 1485 (1968)
(4) E.R.Walwick, W.K.Robwerts and C.A.Dekker, Proc. Chem. Soc., 84 (1959)
(5) J.Nagyvary, J. Am. Chem. Soc., 91, 5409 (1969)
(6) J.Nagyvary and C.M.Tapiero, Tetrahedron Letters, 3481 (1969)
(7) T.Kanai, T.Kojima, O.Maruyama and M.Ichino, Chem. Pharm. Bull., 18, 2569 (1970)