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ARABINONUCLEOTIDES III. THE CONVERSION OF CYTIDYLIC ACID INTO ARACYTIDINE-3' PHOSPHATE AT LOW TEMPERATURE

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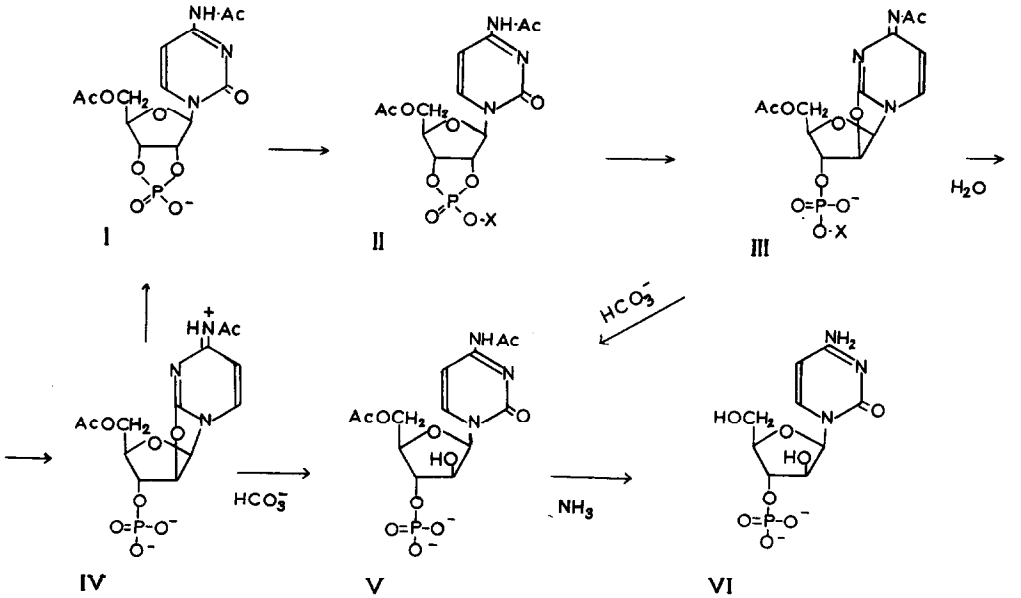
The considerable toxicity and metabolic instability of 1-β-D-arabinofuranosyl-cytosine (1) prompted us to search for improved versions of this antiviral and carcinostatic agent. A number of modifications can be based on aracytidine-3' phosphate (2) which, in addition to being itself of therapeutic interest, is a suitable starting material for polymerization. Since the phosphorylation of arabinonucleosides (2,3) is not an expedient approach for the synthesis of the 3' phosphates, we have attempted the conversion of the ribose moiety of cytidylic acid into the corresponding arabinoside. The first such conversion by Walwick et al. (4) consisted in heating cytidylic acid in polyphosphoric acid where the reactive intermediate probably involved the 2',3'-cyclic pyrophosphate, and the end product was the 3',5' diphosphate of 1-β-D-arabinofuranosyl-cytosine.

This type of reaction could be made more efficient by improving both the quality of the leaving group and the nucleophilicity of the base, i.e., by utilizing fully substituted 2',3'-cyclic pyrophosphates, or sulfonic anhydrides under base catalysis, and suitably protecting the N^4 amino group. Two examples are described below which lead to the formation of a monophosphorylated product, aracytidine-3' phosphate.

1. One mmole of 5'-O,N^4-diacetylcytidine-2',3'-cyclic phosphate (5) tri-n-butylammonium salt, was dissolved in a mixture of 20 ml of anhydrous dioxane and 2 ml of tri-n-butylamine; 1.2 to 1.6 equivalents of an activating agent were added under cooling. As activating agents, diphenyl phosphorochloridate, methanesulfonyl chloride and p-toluenesulfonyl chloride were employed, of which the sulfonyl chlorides were found to be preferable. The rearrangement (scheme 1) was allowed to go to an extent of 50 per cent which took about 15 hours at 20° or 2 hours at 50°. Further prolongation proved to be more of a disadvantage because of the concomitant for-

mation of fluorescent by-products. The use of dimethylformamide instead of dioxane increased the rate of the reaction but also led to the formation of increased amounts of the fluorescent contaminant. The hydrolysis of the unstable O^2,2'-cyclocytidine intermediate III was effected by the addition of 5 ml 1 M triethylammonium bicarbonate directly to the reaction mixture. In water alone the initial hydrolysis product (IV) reverted to the starting material, as a consequence of the neighboring group interaction on part of the 3' phosphate dianion. The separation of the starting material (I) from 5'-O,N^4-diacetylaracytidine-3' phosphate was achieved on a DEAE (bicarbonate) column. The acetyl groups were hydrolyzed with methanolic ammonia, and the resulting aracytidine-3' phosphate (VI) was further purified by absorption on a Dowex 1-X2 (formate) column and elution with dilute formic acid. The yield was 45% as determined by UV.

The freeze-dried crystalline sample (155 mg) exhibited the characteristic spectral properties of this system (2,6). The NMR spectrum taken at 100 MHz in D_2O at pD 7 featured the following doublets (δ ppm, J Hz, relative to acetone): H-6, 5.65(8); H-5, 3.83(8); H-1', 4.03(3). The ORD characteristics in 0.1 M Na_2HPO_4, pH 7.8 ([M] at c=9.3 x 10^{-5} M) were: peak at 288 mμ +15,900°; broad trough centered at 240 mμ -18,800°; crossover at 272 mμ. The UV in water exhi-



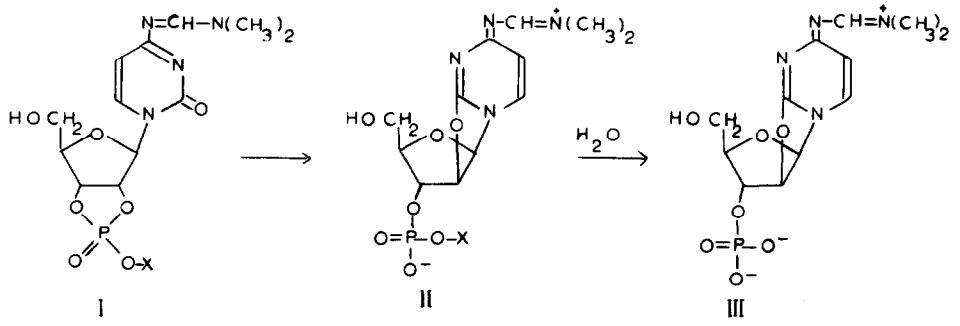
X: diphenylphosphoryl
mesyl
tosyl

Scheme 1

bited λ_{max} 275 mμ (ε 10,600), 211 (10,400) and in 0.1 N HCl λ_{max} 279 (13,700), 212 (9,700). After drying 16 hours at 0.02 mm over P_2O_5, the compound gave correct C, H, N, P analyses for the formula $C_9H_{14}N_3O_8P.\frac{1}{2}H_2O$. A recrystallized sample sintered between 180 and 190°. Treatment with alkaline phosphatase produced 1-β-D-arabinosylcytosine, which was undistinguishable from the commercial sample on paper chromatograms in several solvent systems and on electrophoresis in boric acid.

2. Since an electron donating protecting group is obviously preferable to the acetyl in this reaction, the N^4-dimethylaminomethylene derivative of 2'(3') cytidylic acid was prepared by the general method of Zemlicka (7) in quantitative yield. One mmole of N^4-dimethylaminomethylene-cytidine-2'(3') phosphate was dissolved in a mixture of 30 ml DMF and 3 ml tri-n-butylamine, and 2.5 equivalents of p-toluenesulfonyl chloride were added under cooling. The initially formed mixed anhydride (I, scheme 2) rearranged instantaneously, and hydrolysis with ice (II→III) gave N^4-dimethylaminomethylene-O^2,2'-cyclocytidine-3' phosphate in 70 to 80 per cent yield. This compound is relatively stable in water at neutral pH and it can be isolated by electrophoresis at pH 5.5 (λ_{max} 325 mμ). It is partially hydrolyzed on freeze-drying or at higher ionic strength, therefore the reaction mixture was hydrolyzed by dilute KOH prior to separation on Dowex 1-X2 resin (formate, 1.5 X 40 cm). Aracytidine-3' phosphate emerged as the third peak on gradient elution with formic acid (3 l. H_2O-3 l. 0.03 M), following the isomeric cytidylic acids. Yields were consistently above 60% based on cytidylic acid.

Scheme 2



The most important feature of this work is that is provides the first low temperature conversion of the ribose moiety to the arabinose moiety in a nucleotide. The reversible nature of such conversion has also been recognized, which must have been similarly operational under the conditions of Walwick et al. (4). From a practical point of view, our second method to prepare aracytidine-3' phosphate is more facile and economical than those based on the phosphorylation of the arabinosides (2,3). Thus this nucleotide has become readily available for clinical studies and further chemical syntheses.

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