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N^4,$O^{3'}$,$O^{5'}$-TRIACETYL-2,2'-ANHYDROCYTIDINE*. A POSTULATED REACTIVE INTERMEDIATE IN A CONVENIENT SYNTHESIS OF 1-β-D-ARABINOFURANOSYLCYTOSINE

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In recent years, one of the most rewarding aspects of nucleoside chemistry has been the study of the pyrimidine anhydronucleosides. The latter, which are normally stable compounds, have often proved to be valuable synthetic intermediates. Although most of the previous work in this field (1) has been concerned with uracil (or thymine) derivatives, the three possible 2-anhydrocytidines (2,3,4) have all been prepared. From this work, it appears that 2,2'-anhydronucleosides are obtained more readily than their 2,3'- and 2,5'-isomers (5), and it also seems probable (2) that anhydrocytidine derivatives are formed more readily than the corresponding anhydrouridines. The present report is concerned with the effect of N^4-acylation on the ease of formation and resultant properties of 2,2'-anhydrocytidine derivatives.

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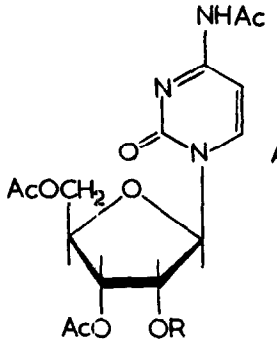
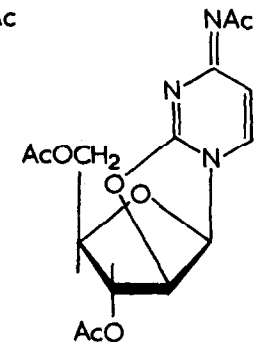
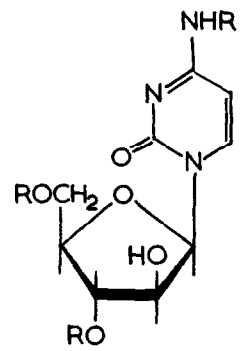
* 2,2'-Anhydrocytidine is an abbreviation for 2,2'-anhydro-1-β-D-arabinofuranosylcytosine.



(I)



(II)



(III)

An equilibrium mixture of $\underline{N}^4,\underline{O}^{3'},\underline{O}^{5'}$-triacetylcytidine (I; R=H) and its $\underline{N}^4,\underline{O}^{2'},\underline{O}^{5'}$-isomer (in the respective proportions of *ca.* 1.5:1) was readily prepared from cytidine, in 64% overall yield, by the orthoester exchange method (6). This material was allowed to react with a slight excess of toluene-*p*-sulphonyl chloride, in anhydrous pyridine solution, until no starting material remained (as indicated by thin-layer chromatography). The products were then concentrated to small volume, dissolved in dichloromethane (1 vol.) and extracted with water (6 x 1 vol.) within a period of 10 minutes. When the combined aqueous extracts were allowed to stand at room temperature, a precipitate of colourless needles, m.p. 213-214°, was obtained.

The formulation of this product as $\underline{N}^4,\underline{O}^{3'},\underline{O}^{5'}$-triacetyl-β-D-arabinofuranosylcytosine (III; R=Ac) is supported by its elemental composition [Found: C, 48.9; H, 5.4; N, 11.2%], and by its thin-layer chromatographic properties (on Merck Kieselgel GF_{254}), which correspond to those of $\underline{N}^4,\underline{O}^{2'}$(and $\underline{O}^{3'}$),$\underline{O}^{5'}$-tri-acetylcytidines. Its ultraviolet absorption spectrum [in 95% ethanol: λ_{max} 248, 300 (logε 4.20, 3.89), λ_{min} 226, 237 mμ (logε 3.76, 3.58)] and 100 Mc/s n.m.r. spectrum [in $(D_3C)_2SO/D_2O$: τ1.85, doublet, assigned to H(6); τ2.61, doublet, assigned to H(5); τ3.80, doublet (J=3.4 c/s), assigned to H(1'); τ4.92, multiplet, assigned to H(3'); τ5.56, multiplet, assigned to H(2'), H(4'), and H(5'); τ7.76, τ7.78, τ7.83, singlets, assigned to methyl protons of acetyl groups] are both in accord with this formulation. The n.m.r. spectrum remained unchanged after the solution had stood at 33° for 24 hr., thus showing that the triacetyl derivative (III; R=Ac) was not susceptible to isomerization by acetyl migration.

When the above product was treated with methanolic ammonia for 24 hr. at 20°, 1-β-D-arabinofuranosylcytosine (III; R=H), m.p. 212-216° (undepressed by admixture with authentic material†) could be isolated in over 90% yield. The identity of the free nucleoside has been confirmed by its elemental analysis [Found: C, 44.6; H, 5.6;

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† We thank Dr. T. Y. Shen (7) for a generous gift of this compound.

N, 17.3. Calc. for $C_9H_{13}N_3O_5$: C, 44.4; H, 5.4; N, 17.3%], specific rotation ($[\alpha]_D^{20}$ = +152°, lit.[2], $[\alpha]_D^{23}$ = +158°), ultraviolet absorption spectrum [in 0.01N hydrochloric acid: λ_{max} 280 (logε 4.13), λ_{min} 240 mμ (logε 3.13)], R_F's in two solvent systems[‡], and paper electrophoretic mobility in 0.1M sodium borate buffer (pH 8.5).

As it seems safe to assume that the tosylation of the equilibrium mixture of triacetylcytidines leads initially to 2'-O-tosyl-N^4,$O^{3'}$,$O^{5'}$- and 3'-O-tosyl-N^4,$O^{2'}$,$O^{5'}$-triacetylcytidines, it is likely that further chemical change occurs when the dichloromethane solution (containing some pyridine) is shaken with water. It is suggested that, during this stage, the 2'-tosylate (I; R=Ts) undergoes cyclization to give the anhydronucleoside (II), which is then hydrolyzed to give the isolated product (III; R=Ac), while the 3'-tosylate remains unchanged in the dichloromethane layer. This proposed mechanism for the formation of the arabinoside (III; R=Ac) requires first that the N^4-acetylcytosine residue should be more nucleophilic than uracil, as 2'-O-tosyl-3',5'-di-O-acetyluridine (8) does not cyclize under these conditions*, and secondly that the anhydronucleoside (II) should be considerably more susceptible to hydrolysis at pH 7 than

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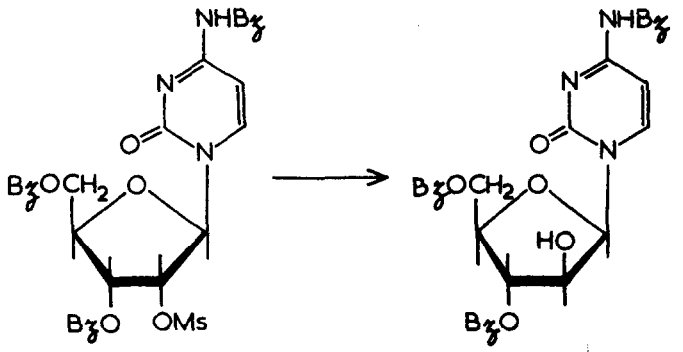
‡ Both Dr. Shen's and our material had R_F's 0.64 and 0.67 in ethanol–M aqueous ammonium acetate (7,3) and isobutyric acid – ammonia(d 0.88) – water (66,1,33), respectively.

* This uridine derivative undergoes less than 5% conversion to 3',5'-di-O-acetyl-2,2-anhydrouridine in pyridine/water (9:1; v./v.) solution, in 2 days at room temperature.

either 2,2'-anhydro-uridine or cytidine. It seems reasonable that $\underline{N}^4$-acylation of 2,2'-anhydrocytidine should make it more liable to nucleophilic attack at C(2), but the apparent instability of (II) is nevertheless surprising.



(IV) (V)

The availability (9) of crystalline 2'-O-methanesulphonyl-$\underline{N}^4$,$\underline{O}^{3'}$,$\underline{O}^{5'}$-tribenzoyl-cytidine (IV) provided us with another opportunity to attempt the isolation of an $\underline{N}^4$-acyl derivative of 2,2'-anhydrocytidine. The tribenzoyl derivative (IV) was found (by thin-layer chromatography) to undergo a slow reaction in aqueous pyridine (containing 10% water) solution at room temperature, to give $\underline{N}^4$,$\underline{O}^{3'}$,$\underline{O}^{5'}$-tribenzoyl-1-β-D-arabinofuranosyl-cytosine (V), which could be isolated as a crystalline solid, m.p. 198-200°. The extent of the conversion of (IV) into (V) was ca. 75% after 11 days, and there was no

indication of the existence of an intermediate. Thus if the reaction proceeds via an anhydronucleoside, its formation must be the rate-determining step, and furthermore it must be extremely susceptible to base-catalyzed hydrolysis. The tribenzoyl derivative (V) was characterized by elemental analysis [Found: C, 64.9; H, 4.7; N, 7.2%], ultraviolet spectroscopy, and its conversion into 1-β-D-arabinofuranosylcytosine (III; R=H). It appears that the mesylate ion undergoes displacement much less readily than the tosylate ion, in this reaction.

There is a possibility that this work will find practical application. In the first place, it has led to a very convenient synthesis of 1-β-D-arabinofuranosylcytosine (III; R=H) (2,7), which has been found to have selective antiviral activity (7), and thus potential use in chemotherapy. Although the yield of crystalline N^4,$O^{3'}$,$O^{5'}$-triacetyl-1-β-D-arabinofuranosylcytosine (III; R=Ac) was only 42%, based on the N^4,$O^{3'}$,$O^{5'}$-triacetylcytidine (I; R=H) content of the equilibrium mixture of isomers, no attempt was made to find the optimum conditions. Furthermore, the practical aspects of all the synthetic steps involved are very straightforward. Finally, the triacetyl compound (III; R=Ac) and the tribenzoyl compound (V) have the correct orientation for the preparation of the 2'-protected derivative of 1-β-D-arabinofuranosylcytosine, required in our oligonucleotide synthesis (10) which, in principle, is not restricted solely to ribonucleotide units.

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1 Despite the numerous papers in this field, we are not aware of the existence of a recent review.

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