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REARRANGEMENT OF 2,2'-ANHYDROURIDINE IN LIQUID HYDROGEN FLUORIDE

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The reaction of nucleosides and nucleotides with liquid hydrogen fluoride (LHF) has been reported to result in cleavage of the nucleosidic N_1-$C_{1'}$ bond[1]. Treatment of 2,2'-anhydro-N_1-(β-D-arabinofuranosyl)uracil (1) with hydrogen fluoride in dioxane at elevated temperatures gives 2'-fluoro-2'-deoxyuridine (2)[2]. Our recent interest in polynucleotides prepared from 2'-halogeno-2'-deoxyuridines[3] led us to explore the reaction of 1 with neat LHF in an attempt to improve the preparation of 2. We have now found that 1 unexpectedly rearranges on treatment with LHF at elevated temperatures to the previously unreported 2,2'-anhydro-N_3-(β-D-arabinofuranosyl)uracil (3a).

Treatment of 1 (2.0 g) with LHF (30 ml) in a monel pressure vessel at 80° for 18 hours was followed by evaporation of the LHF, dilution with water and neutralization of the solution with $CaCO_3$. Thin layer chromatography (Silica Gel GF-254, 4:1 $CHCl_3$-MeOH) of the reaction mixture showed the presence of faster moving major and minor spots in addition to traces of starting material. In subsequent experiments, the fast moving minor component was identified as uracil and the identity of the slowest moving spot as 1 reconfirmed by isolation.

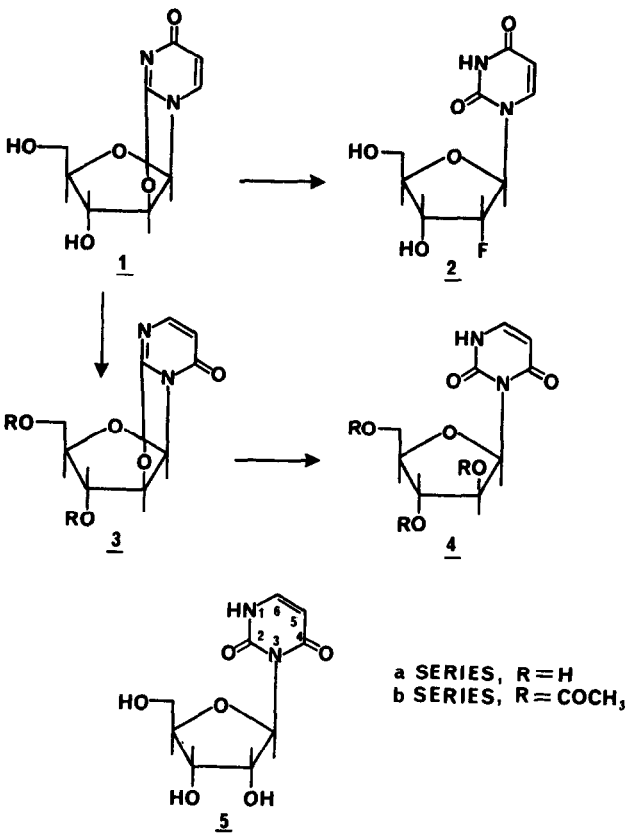
The major product component was isolated by short column chromatography[4] over Silica Gel G (4:1 $CHCl_3$-MeOH as eluant) and crystallized from MeOH-$CHCl_3$ to give 0.9 g of 3a, mp 144-146°. Analysis. Found: C, 47.72; H, 4.36; N, 12.25 ($C_9H_{10}N_2O_5$ requires C, 47.79; H, 4.46; N, 12.38). The UV spectra of 3a differed greatly from that of 1 ($\lambda_{max}^{pH\ 2}$ 249nm, $\lambda_{max}^{pH\ 12}$ 254nm)[5] but is similar to that reported for 2-methoxy-3-methyl-4-pyrimidone ($\lambda_{max}^{H_2O}$ 269nm)[6] indicating that migration of the nucleosidic bond to N_3 had occurred whereas the anhydro linkage remained intact. UV spectra for 3a: $\lambda_{max}^{pH\ 2,7}$ 271nm (ε 6900), $\lambda_{min}^{pH\ 2,7}$ 233nm (ε 900); $\lambda_{max}^{pH\ 12}$ 272nm (ε 6800), $\lambda_{min}^{pH\ 12}$

235nm (ε 1100). UV spectroscopy indicated that 3a was unstable in base. NMR (DMSO-d_6, 36°); δ 7.73 (1H, d, H6); 6.48 (1H, d, H1'); 6.02 (1H, d, H5, J6-5 = 7 cps); 5.82 (1H, d, 3'OH, J3'-3'OH = 4.5 cps, exchanges with D_2O); 5.21 (1H, d, H2', J1'-2' = 5.5 cps); 4.86 (1H, t, 5'OH, J5'A-5'B-5'OH = 5.5 cps, exchanges with D_2O); 4.47 (1H, m, H3'); 4.12 (1, m, H4'); 3.35 (2H, m, H5'A5'B, changes to d with J = 4.5 cps on D_2O addition). Reaction of 3a with Ac_2O/pyridine overnight at room temperature followed by evaporation of the solvent gave the crystalline diacetate 3b, mp 112-115° (from MeOH). NMR (DMSO-d_6, 36°); δ 6.52 (1H, H1', J1'-2'= 5.5 cps); 2.13 and 1.92 (6H, 2 sharp singlets for acetate protons) in addition to the other expected signals.

Treatment of 3a (2.0 g) in 1N NaOH (40 ml) for 30 minutes at room temperature yielded a foam (4a) which was acetylated as above to give a crystalline triacetate (4b), mp 166-167° after recrystallization from EtOH. Analysis. Found: C, 48.83; H, 4.98; N, 7.36 ($C_{15}H_{18}N_2O_9$ requires C, 48.65; H, 4.90; N, 7.56). UV; $\overset{pH\ 2}{\lambda max}$ 263nm (ε 7300), $\overset{pH\ 2}{\lambda min}$ 231nm (ε 1300); $\overset{pH\ 7}{\lambda max}$ 263nm (ε 7300), $\overset{pH\ 7}{\lambda min}$ 231nm (ε 1400); $\overset{pH\ 12}{\lambda max}$ 294nm (ε 10,100); $\overset{pH\ 12}{\lambda min}$ 247nm (ε 500). The large bathochromic shift in basic solution establishes the site of the nucleosidic bond at N_3 of the uracil chromophore[7]. Spectrophotometric pK_a, 9.4. $[\alpha]_D^{25}$ = -37.0° (c = 1.0, $CHCl_3$). NMR (DMSO-d_6, 36°); δ 10.75 (1H, broad s, NH); 7.48 (1H, m, H6); 6.78 (1H, d, H1', J1'-2' = 7.5 cps, indicative of the β-D-arabino configuration); 6.03 - 5.40 (3H, complex m, H2', H3', H5); 4.40 - 4.00 (3H, complex m, H4', H5'A5'B) and 2.12 - 1.86 (9H, three sharp singlets for acetyl protons). On addition of D_2O, the signal at δ 10.75 disappeared and the signal for H6 at δ 7.48 became a sharp doublet with J6-5 = 7.5 cps. Thus D_2O exchange serves to confirm the assignment of the lowest field doublet to H6. The proton for H5, after D_2O addition, could be clearly seen as a sharp doublet at δ 5.65.

The similarity in the UV and NMR spectra of 4b, except for the large coupling constant observed for H1', to that reported for 5, N_3-(β-D-ribofuranosyl)uracil [isouridine][8,9], serves to identify 4b as N_3-(2',3',5'-tri-O-acetyl-β-D-arabinofuranosyl)uracil [tri-O-acetyl-isoarabinouridine]. Thus, the rearrangement product resulting from the treatment of 1 with LHF is conclusively identified as 2,2'-anhydro-N_3-(β-D-arabinofuranosyl)uracil (3a).

It has been reported (in abstract)[10] that treatment of 5',2' (or 3')-di-O-benzoyluridine with polyphosphoric acid at 60° gives, after debenzoylation, a mixture containing uracil, uridine, UMP and the 5'-phosphates of N_1-β-D-arabinofuranosyluracil, N_3-β-D-ribofuranosyluracil and N_3-β-D-arabinofuranosyluracil. The rearrangement of 2,2'-anhydro-N_1-(β-D-arabinofuranosyl)



uracil-6-carboxamide to the N_3 isomer on treatment with HBr in trifluoroacetic acid has recently been reported[11]. Preferential rearrangement to the N_3 isomer in this latter case was ascribed to a considerable steric hindrance at the N_1 position in the glycosyl cleaved intermediate imposed by the 6-carboxamide function. No such preferential steric driving force is present in our rearrangement for intramolecular reglycosylation to occur at N_3.

The application of this novel rearrangement to other anhydronucleosides is currently under investigation in our laboratory and will be reported in detail in the future.

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