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REACTIONS OF 5-FLUOROURACIL DERIVATIVES WITH SODIUM DEUTEROXIDE [1]

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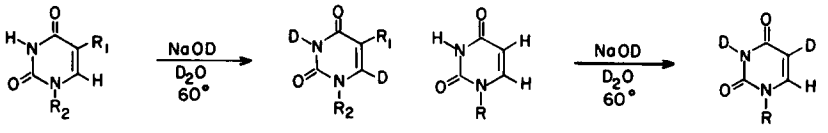
Fox, Miller and Cushley [2] reported that treatment of 1-β-D-arabinofuranosyl-5-fluorouracil (Ara-5-FU) with 0.1N NaOH at 60-70° gave an open-chain ureido derivative formed via a 1,4-addition of the 2'-OH across the α,β-unsaturated ketone of the aglycon followed by cleavage of the 3,4-C-N bond. It was stated [2] that 1-β-D-ribofuranosyl-5-FU (I) and its 2'-deoxy analog (II) did not undergo this reaction "as evidenced by the constancy of their ultraviolet spectra."

Treatment of I, II, 1-methyl-5-FU (III) and 5-FU (IV) with 0.5N NaOD in D_2O (equal to 2 equiv. of alkali) at 60° showed a rapid exchange of H-6 by deuterium. Relative rates of this reaction were determined by proton NMR spectroscopy [3] by the disappearance of the H-6 signal (Fig. 1). Intensities of the C-6 protons were compared with other protons in the molecule which were not replaced, and integrals are the average of 3-4 runs about 1 minute apart. In the case of IV, the reference signal was a known amount of added sodium acetate. NMR parameters have been recorded earlier for these systems [4] and no other change in the NMR spectrum was apparent during the course of the reaction. The products were isolated and gave ultraviolet and mass spectral [5] analyses consistent with their assigned structures.

In contrast, uridine (VII) and 2',3'-O-isopropylideneuridine (VIII) under identical conditions show slow exchange of H-5. The reaction was followed by measurement of the doublet-singlet intensities of the C-6 proton of VII and VIII due to H→D substitution at C-5. Compound VIII has a half-life (t 1/2) = 0.5 days; VII a (t 1/2) = 7 days (Fig. 2). [6] After 5 days, a new singlet slowly appeared in the spectrum of uridine at δ = 8.44 [7] but after 8 days this new peak represented only ∿ 28% of the H-6 intensity. This same singlet also appeared in the spectrum of VIII after essentially complete H-5 exchange had occurred. Preliminary experiments using 0.3N NaOD gave (t 1/2) ∿ 135 hrs for uridine and ∿ 5 hrs for

VIII with no evidence of the new singlet at $\delta = 8.44$. The most likely mechanism for H-5 replacement in uridines is by 1,4-addition across the α,β-unsaturated ketone system [2] involving attack by OD^- [8] or the 5'-hydroxy anion at C-6. [9] Subsequent ketonization [DO-C(H)=C(H)-C(=O)-OR ⇆ -C(=O)-CHD-C(H)-OR] followed by eventual elimination of HOR would yield exclusively 5-deuterated starting material. Anchimeric assistance due to the presence of the isopropylidene group (see Fig. 2) is to be expected on conformational grounds [9,10], if the 5'-hydroxy anion is the nucleophile. This is consistent with the fact that 1-methyluracil in 50% 0.3N $NaOD/D_2O$-DMSO-d_6 shows less than 10% H-5 exchange even after 7 days.



I: R_1 = F; R_2 = β-D-Ribofuranosyl
II: R_1 = F; R_2 = 2'-deoxyribofuranosyl
III: R_1 = F; R_2 = CH_3
IV: R_1 = F; R_2 = H
V: R_1 = CH_3; R_2 = 2'-deoxyribofuranosyl
VI: R_1 = }-S-S-{; R_2 = 2'-deoxyribofuranosyl

VII: R = β-D-ribofuranosyl
VIII: R = 2',3'-O-isopropylidene-ribofuranosyl

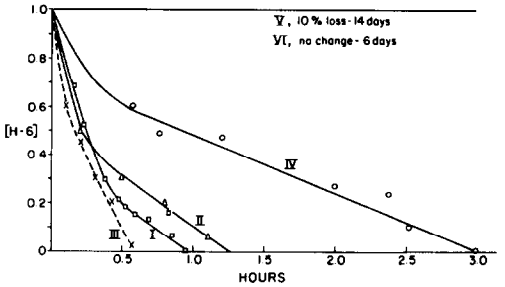


Figure 1

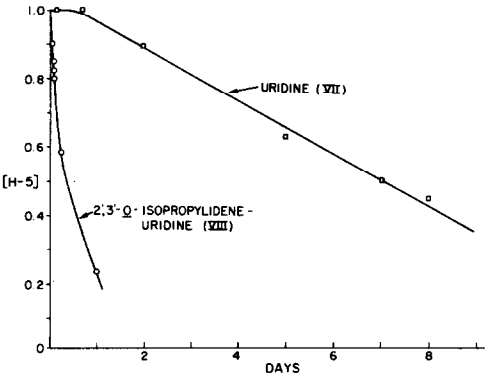
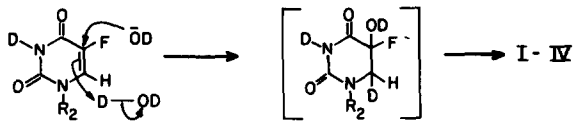


Figure 2

On the other hand, for the 5-fluorouracils (I-IV) a most probable mechanism for H-6 exchange involves an initial attack by OD^- on C-5 followed by abstraction of a deuteron from solvent. Upon removal of DOH from the 5,6-dihydro intermediate, the 6-D products would form. This "C-5 attack" mechanism does not rule out the concomitant occurrence of a normal 1,4-

addition (attack on C-6). Indeed, electronegative substituents at C-5 should enchance both



pathways of nucleophilic addition. However, only attack on C-5 could lead to 6-deuterated products. In support of the proposed "C-5 attack" mechanism, thymidine (V) and the 5-disulfide (VI) [11] with electropositive groups on C-5 undergo attack by alkali extremely slowly (Fig. 1). A systematic study of other 5-halouridines is precluded because these derivatives give new products when treated with base. [9]

Another possible pathway for H-6 exchange in compounds I-IV is direct proton abstraction at C-6. Beak and Bonham [12] have proposed such a mechanism for D/H exchange in 3,5-disubstituted-N-methyl-4-pyridones. The carbanion resulting from base catalyzed proton abstraction at the 2- and 6- positions was stabilized by the adjacent tertiary nitrogen. [12] This mechanism can be excluded in the present case because 5-fluorouracil (IV) — in which stabilization by the 1-N is precluded — does undergo H-6 exchange. In addition, even under neutral conditions (D_2O, 95°) 1-methyl-5-FU (III) also undergoes H-6 exchange (t 1/2 = 3 days). Carbanion generation would surely be unlikely under these conditions.

It should be noted that the rate of 6-H exchange for IV is ~4 times slower than with I-III (Fig. 1). This may be due to the fact that in 0.5N NaOD, IV exists predominantly as the di-anion whereas I-III are in the monoanionic form. It is suggested that, under these alkaline conditions, the monoanionic form is the reactive species.

In conclusion, reaction of 5-fluorouracil derivatives with NaOD-D_2O leads readily to 6-deutero analogs by way of an initial nucleophilic attack of OD^- on C-5. The reaction is base-catalyzed since 1-methyl-5-FU shows no H-6 exchange by deuterium in N DCl (13 days at 65°). This method should be of value for the preparation of selectively-labelled derivatives of these biologically-important compounds.

References

* This paper is considered as "Nucleosides LIII. Transformation of Pyrimidine Nucleosides in Alkaline Media.II."

1. Supported in part by funds from the National Institutes of Health, U.S. Public Health Service, Grant Nos. FR-00356-02 (S.R.L.) and CA 08748 (Sloan-Kettering Institute).

2. J. J. Fox, N. C. Miller and R. J. Cushley, Tetrahedron Letters, 4927 (1967).

3. Determined using a Bruker HFX-3 spectrometer operating at 90 MHz and fitted with a variable temperature probe containing a calibrated thermocouple (Bruker B-ST-100/700 digital accessory). The temperature was constant to $\pm 1^{o}$.

4. R. J. Cushley, I. Wempen and J. J. Fox, J. Am. Chem. Soc., 90, 709 (1968).

5. R. J. Cushley, W. J. McMurray and S. R. Lipsky; to be published.

6. Tritium and deuterium exchange at C-5 of uridylic acid under photolytic conditions has been reported: R. W. Chambers, J. Am. Chem. Soc., 90, 2192 (1968).

7. It is suggested that this resonance signal is due to the vinylic proton of an α,β-unsaturated-5-deuterated-ureido acid [$D_2N-\underset{\underset{O}{\|}}{C}-\underset{R}{N}-CH=CD-COO^-$]. Such a by-product may arise by slower ring-cleavage at the 3,4-positions of the 5,6-dihydro intermediate formed (vide infra) by attack of the 5'-hydroxy anion or OD^- on C-6 followed by elimination of HOD or HOR.

8. S. R. Heller, personal communication. We are indebted to Dr. Heller for making the results of his work available to us prior to publication.

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11. The authors are grateful to Drs. Thomas J. Bardos and Michael P. Kotick for this compound.

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