^{1}H NMR STUDIES ON DEUTERIUM - HYDROGEN EXCHANGE AT C-5 IN URIDINES

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During an investigation of the association of nucleic acid derivatives with amino thiols it was observed that the C-5 hydrogen atom in uridine was readily exchangeable in aqueous base (1). In basic D_2O, deuterium was exchanged for the C-5 hydrogen atom and in basic H_2O, hydrogen was exchanged for the C-5 deuterium atom.

The exchange occurred when an equimolar mixture of uridine and various bases in D_2O was held at room temperature (ca. 19°), while no exchange occurred in a 0.4 M uridine/D_2O solution (pH = 5.3) left standing for three months. Using a typical base, 2-mercaptoethylamine (MEA), a new peak appeared after 24 hours in the ^{1}H NMR spectrum (Fig. 1b), midway between the peaks of the C-6 proton doublet (2). Raising the temperature to ca. 60° for 4 days, or allowing the solution to remain at room temperature for 21 days, resulted in the complete disappearance of the C-6 doublet peaks and the new single peak increased in intensity (Fig. 1c). Simultaneously one of the two sets of doublets (H_5,H_1') at ca. δ = 5.9 ppm, also disappeared. The addition of MEA also produced a slight shift of 1-3 Hz in the chemical shifts of H_6 and H_1', while the remainder of the ^{1}H NMR spectrum was unchanged (not shown). Other bases(NaOH, ethylamine and 2-aminoethanol) also produced shifts in the positions of H_6 and H_1'.

Uridine-d_5 was isolated in 95% yield. A mass spectrum of uridine-d_5 gave a parent peak at m/e 249*. Uridine-d_4 and uridine gave parent peaks

* Mass spectra were obtained on an AEI MS 12 mass spectrometer at 100°.

at m/e 248 and m/e 244, respectively.

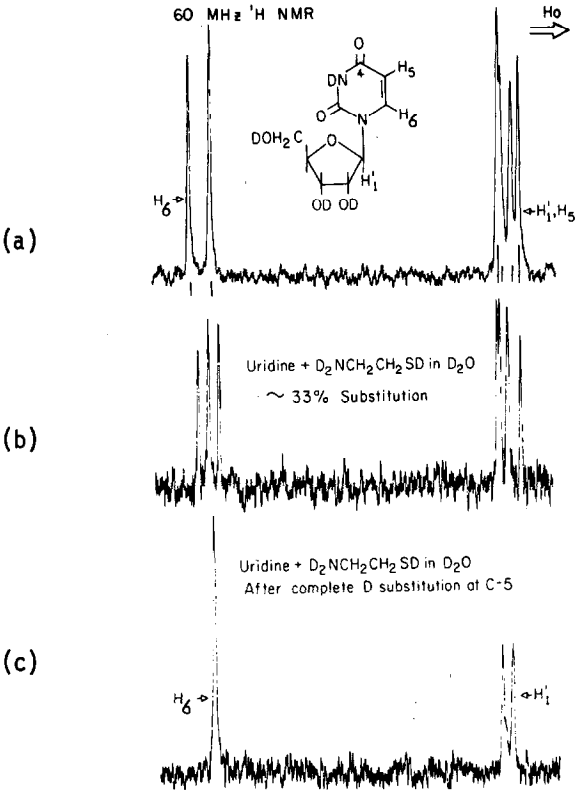


Fig. 1

CHEMICAL SHIFT DATA
(in Hz, downfield from internal standard)*

	H_6	H_5	H_1'
(a)	477.5, 469.5	358.0, 350.0	357.0, 353.0
(b)	474.5, 466.5 471.0	359.0, 351.0	358.0, 354.0
(c)	470.0	---	359.0, 355.0

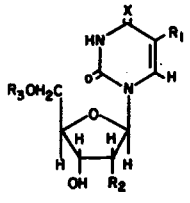
* ^{1}H NMR spectra were obtained on a Varian A-60 NMR spectrometer. Solution concentration varied from 0.1 to 0.4 M in D_2O. Tiers salt, (3-Trimethylsilyl)-propane sulfonic acid sodium salt, was used as an internal standard, δ = 0.00 ppm

In order to determine the scope of this exchange, a number of related nucleophiles and bases were employed and some uridine derivatives were studied (Table 1).

Exchange was found to occur in basic solutions, but not in acidic solutions (MEA·HCl, ethanethiol and 2-mercaptoethanol), hence the reaction was considered to be a base catalyzed exchange. The five compounds studied (Table 1) suggest that a hydrogen atom at C-5 and a carbonyl group at C-4 were required for the exchange to occur. The difficulty of eliminating a methyl carbonium ion accounted for thymidine not incorporating deuterium at C-5.

TABLE 1

Substituted Nucleic Acids



	R_1	R_2	R_3	X	C-5 Deuterium Exchange with MEA in D_2O
CYTIDINE	H	OH	H	NH	No
URIDINE	H	OH	H	O	Yes
THYMIDINE	CH_3	H	H	O	No
DEOXYURIDINE	H	H	H	O	Yes
URIDINE MONOPHOSPHATE	H	OH	PO_3	O	Yes

The probable mechanism for this exchange is the Michael 1,4 addition across the double bond, followed by a keto-enol tautomeric shift and then elimination of the C-5 hydrogen atom and the nucleophile.

The method of very mild selective deuterium substitution should prove useful to molecular biologists working with DNA or polynucleic acids containing the uridine ring systems. Combined with the reverse

exchange reaction this would also be particularly helpful for radioactive tritium labelling and subsequent removal.

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2. The ^{1}H NMR spectrum of uridine has been analyzed (a) Varian Spectra Catalog, Vol 2, #535, Varian Associates, Palo Alto, California 94303 (1963); (b) C. D. Jardetzky and O. Jardetzky, J. Amer. Chem Soc., 82, 222 (1960).