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^{17}O NMR SPECTROSCOPY OF NUCLEOSIDE DERIVATIVES; BONDING CHARACTERISTICS OF PYRIMIDINE CARBONYLS

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Summary Various uridine derivatives have been selectively enriched (≈ 50%) at the O4 and O2 oxygens, and the ^{17}O chemical shifts and linewidths measured. The chemical shifts, which are primarily dependent on π-bond-order and hydrogen bonding, show effects that are selective for the O2 and O4 oxygens. In particular, significantly more H-bonding to D_2O and higher π bond order are found for the O4 oxygen.

There is a great deal of interest in the π bonding and electron donor properties of pyrimidine carbonyl groups stemming in large part from their involvement in biologically important hydrogen bonding interactions,[1] metal ion complexation,[2] and tautomeric equiliria.[3] Semi-empirical and ab initio molecular orbital calculations[4] generally suggest a higher ground state π bond order and lower ionization potential for C4 carbonyl as compared with a C2 carbonyl of uracil (see Figure 1 for numbering scheme). Direct experimental confirmation of these theoretical results is difficult to achieve, although NMR[5] and infrared measurements[6] provide indirect evidence of a greater H-bonding acceptor ability for the C4 carbonyl. In prinicple, the carbonyl π character could be established by appropriate ^{17}O NMR studies,[7] but in practice the measurement of ^{17}O spectra for biological molecules is complicated by line-broadening and poor signal-to-noise. These problems have now been partially resolved for nucleosides by a combination of ^{17}O enrichment and decreased solvent viscosity. In this communication we report the first ^{17}O NMR studies on a series of pyrimidine derivatives.

Uridine (Urd), isopropylideneuridine (ipUrd), and N3-methyluridine (N3-methyl Urd) were selectively enriched in ^{17}O at O2 and O4 positions by procedures described elsewhere.[8] In all instances, the 8.15 MHz ^{17}O spectra measured in D_2O at approximately 35°C consist of a single broad peak with a linewidth in the range of 800-1200 Hz, Figure 1A and Table 1. The linewidth decreases markedly at higher temperatures or in solvents of lower viscosity, Figure 1B,C.[7,9] A detailed comparison of linewidth-temperature dependence for the O2 and O4 signals with the water signal[8] confirms that the line broadening is of quadrupolar origin and is not due to tautomeric equilibria (see below) or intramolecular conformational processes, i.e., syn-anti reorientation.

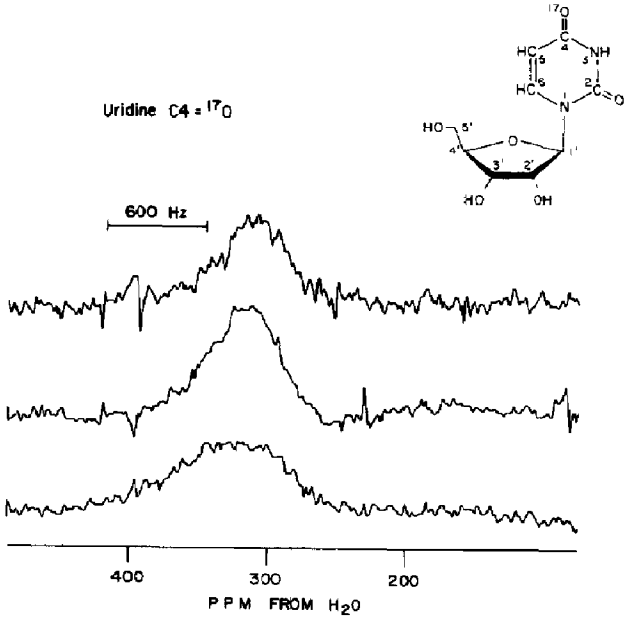

Figure 1. ^{17}O NMR spectra of [4-^{17}O]-uridine - D_2O at various temperatures: (A) 47.4°C, 40,000 acquisitions, $\Gamma_{1/2}$ = 760 Hz; (B) 62.3°C, 30,000 acquisitions, $\Gamma_{1/2}$ = 520Hz; (C) 73.2°C, 20,000 acquisitions, $\Gamma_{1/2}$ = 520 Hz.

Two chemical shift ranges are found for the uridine carbonyls, Table 1. On the basis of ^{17}O enrichment, the signal at 305 ppm downfield from H_2O is attributable to C4=O, while the second at 248 ppm is due to C2=O. These shifts lie midway between those of carbonyl and alcohol ^{17}O shifts,[7] suggesting a potential contribution from keto-enol tautomerism. This possibility is ruled out by the shift data for the N3-methylUrd derivative, Table 1. Both the O2 and O4 carbonyl resonances for the methylated compound, where the enol form is precluded, are virtually identical with values for the parent compound. We conclude that there is an overwhelming preference for the diketo form of Urd in aqueous solution. This finding is not unexpected, since previous infrared work indicated a favored diketo form for N1-methyl uracil in the gas phase and in CCl_4.[6c] The preference for the diketo form is thus relatively impervious to changes in state and solvent, a property of considerable relevance to biological recognition processes.

Apart from linewidth changes, both carbonyls show no variation in shift (within experimental error) over the temperature range covered, 30°-75°C. In contrast, the transition from a proton donor (D_2O) to an aprotic solvent (CH_3CN) causes a 32 ppm downfield shift of O4 and a smaller shift change (8 ppm) in O2, Table 1. This behavior is clearly indicative of preferential H-bonding at O4, with only minimal interaction at O2. While the result for O4 is not surprising and is consistent with theoretical expectations,[4] the minimal effect on the O2 shift is in disagreement with ^{13}C shift data which show a roughly comparable involvement of the uracil C4 and C2 carbonyls in self-association and H-bonding interactions with adenine derivatives.[5c] The discrepancy could arise, however, because the ^{17}O measurements were made at elevated temperatures, 50-70°C, where formation of weak H-bonds would be much less favored.

Table 1
^{17}O Chemical Shifts[a] in D_2O

	O_2	O_4
Uridine	248 ± 7	305 ± 7
Isopropylidine-uridine	255 ± 9 (263 ± 4)[b]	304 ± 5 (336 ± 4)[b]
N3-methyluridine	253 ± 9	313 ± 6

[a]Shifts were measured from the internal D_2O resonance and are reported as chemical shifts downfield from H_2O ($\delta_{H_2O} - \delta_{D_2O} = 3$ ppm)

[b]Shifts in parentheses were measured in acetonitrile and referenced to external D_2O.

An indication of relative π bond character for C4=O and C2=O can be obtained from the data in Table 1. In acetonitrile solutions, where shift contributions from inter- and intramolecular interactions are not significant, the observed ^{17}O resonance is expected to correlate linearly with C=O π bond order.[7] Assuming a shift difference of ~ 600 ppm between pure C=O and >C-OH bonds,[7] a rough estimate of 0.5 can be made for the π bond order in uridine carbonyls. Moreover, the greater downfield shift of O4 compared to O2 indicates a somewhat greater π bond order at the former carbonyl. This experimentally observed trend is in satisfying agreement with the calculated order.[4f]

The present results show that ^{17}O NMR measurements can provide useful information for nucleoside derivatives under appropriate conditions. Of particular interest is the potential for monitoring selective H-bonding interactions, as, for example, between complementary nucleoside bases.

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