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Oxygen-17 NMR of Nucleosides. 2. Hydration and Self-Association of Uridine Derivatives[1,2]

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Abstract: The first use of oxygen-17 NMR spectroscopy to obtain detailed information on water–nucleoside hydrogen-bonding equilibria is described. By monitoring the ^{17}O chemical shifts in water–acetonitrile solvent mixtures of 2′,3′-*O*-isopropylideneuridine (IPU) specifically enriched at either O(4) or O(2) with oxygen-17 by unequivocal chemical synthesis, hydration at the C(4)=O(4) carbonyl group was shown to be significantly higher than at the C(2)=O(2) moiety. Detailed ^{17}O chemical shift measurements on the [4-^{17}O]-IPU as a function of water concentration and temperature enabled equilibrium constants for the hydration process to be calculated. In order to refine these calculations, estimations of the effects of the various competing multiple equilibria were made. Detailed analysis of the ^{17}O chemical shift–water concentration dependence shows the presence of two nucleoside–water hydrogen-bonding equilibria, a 1:1 complex ($K_{1W} = 0.28 \pm 0.03$ at 302 K) with an enthalpy of -5.2 ± 0.5 kcal mol^{-1}, and a 1:2 complex ($K_{1WW} = 0.065 \pm 0.013$ at 302 K) with an enthalpy of -11.0 ± 1.5 kcal mol^{-1}. The ^{17}O data, together with ^{13}C NMR shift measurements, also show a self-association of IPU to form a cyclic dimer. In addition, an estimate has been made of water self-association in water–acetonitrile mixtures.

Proton and carbon-13 NMR spectroscopy has been used extensively in the study of structures and interactions of nucleosides, nucleotides, and their analogues.[6a] One of the major outcomes from such studies has been a definition of the conformational properties and the interrelationships that are important for the structure of polynucleotides. Although the oxygen atom is a critical component of nucleosides and nucleotides, both in structure and in inter- and intramolecular interactions, the potential of ^{17}O NMR in this research has not previously been exploited.[6b]

Until recently, ^{17}O NMR had been relatively difficult to use in biomolecular studies[7-9] primarily because of experimental problems associated with ^{17}O NMR detection, such as low natural abundance (0.037%) and extremely broad lines (up to 1000 Hz or more) due to fast quadrupolar relaxation (spin = $^{5}/_{2}$). These difficulties had limited the range of ^{17}O NMR studies to neat liquids or extremely soluble molecules. The most comprehensive listings of ^{17}O chemical shifts and line widths for organic and inorganic molecules have been reported by Christ et al.[10] and Sugawara et al.[11]

In the present ^{17}O NMR study of nucleic acid constituents, emphasis has been placed on the O(2) and O(4) carbonyl oxygens of uridine as sites for ^{17}O enrichment and NMR measurement. Among factors governing this choice is the importance of these oxygens in intra- and intermolecular hydrogen bonding, particularly of the O(4) oxygen which participates in Watson–Crick hydrogen bonding with adenine.[12] Another important consideration is that the ^{17}O enrichment of carbonyls in uridine is relatively straightforward by known synthetic methods. Finally, the base-ring oxygens of pyrimidines are important metal ion binding sites[13] whose role is yet to be fully understood.

^{17}O NMR hjas been used with success to study various aspects of hydrogen bonding in numerous systems including amides,[14] water,[15-17] substituted acetophenones and benzaldehydes,[18] aldehydes and ketones,[19-21] and various other compounds.[16] As such, ^{17}O NMR has proven to be an extremely useful tool in such hydrogen-bonding studies and may in fact be the most sensitive quantitative measurement of hydrogen bonding, as indicated by the large limiting shift differences (52 ppm) for the hydrogen bonding of water to the carbonyl of acetone[16] and several amides.[14]

The present work focuses primarily on the hydrogen-bonding characteristics of the O(4) and O(2) carbonyl oxygens of uridine and 2′,3′-*O*-isopropylideneuridine (IPU) (see Figure 1). Our initial results, reported in an earlier communication,[1] showed a 32 ppm upfield shift of the O(4) in H_2O relative to the aprotic solvent acetonitrile, while the change in chemical shift between the same two solvents for O(2) was significantly less, 8 ppm. The origin of these effects has been examined in more detail by observing the changes in ^{17}O shift of O(4) as a function of water concentration in water–acetonitrile–IPU mixtures. Although a number of competing equilibria occur in this system, i.e., IPU···IPU, $H_2O\cdots H_2O$, and $H_2O\cdots CH_3CN$, a self-consistent quantitative description of the H_2O–[4-^{17}O]-IPU interaction can be obtained, enabling equilibrium constants and interaction enthalpies to be calculated.

Experimental Section

Synthetic Methods. The general laboratory methods used are described in previous publications.[22] $H_2{}^{17}O$ (54.75% enrichment) was purchased from Mound Laboratory, Monsanto. Ethanol-free, dry $CHCl_3$ was obtained by passage of reagent grade $CHCl_3$ down an alumina

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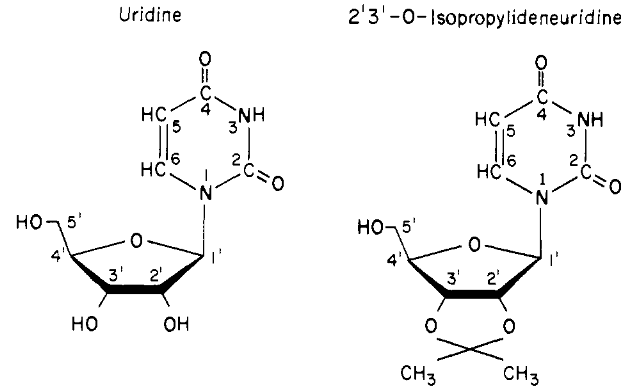

Figure 1. Structure and numbering scheme of uridine and 2′,3′-*O*-isopropylideneuridine.

column and storage over activated 4-Å molecular sieves. Mass spectroscopy was carried out by using chemical ionization on a Bio-Spec quadrupole mass spectrometer (Argonne National Laboratory) or by using electron impact on an AEI MS-50 instrument (Chemistry Department, University of Alberta) at 70 eV.

[4-^{17}O]-2′,3′,5′-Tri-*O*-benzoyluridine. 2′,3′,5′-Tri-*O*-benzoyl-4-chlorouridine[23] (2.83 g; 4.9 mmol) was suspended in dry, ethanol-free $CHCl_3$ (10 mL). $H_2{}^{17}O$ (54.75% enrichment, 0.16 mL) was added and the mixture was stirred in a desiccator for 16 h (total dissolution occurred after ~30 min). The reaction mixture was transferred to a separatory funnel and $CHCl_3$ (40 mL) and saturated aqueous $NaHCO_3$ (50 mL) were added. After separation the organic layer was washed with H_2O (3 × 50 mL) and then evaporated to dryness in vacuo to give a chromatographically pure, stiff white foam of the title compound in quantitative yield.

[4-^{17}O]-Uridine. [4-^{17}O]-2′,3′,5′-Tri-*O*-benzoyluridine (2.397 g; 4.3 mmol) was deblocked with Et_3N-MeOH-H_2O (10:45:45 by volume) overnight at room temperature as described earlier for related compounds.[24] After evaporation of the reaction mixture to dryness, the residue was dissolved in MeOH-H_2O (1:1) and passed down an Amberlite MB-3 resin (H^+, Ac^- form; 4.0 × 16.0 cm). The eluate was evaporated to dryness to yield 3.58 mmol (83%) of product. Crystallization was effected from EtOH-Et_2O. The product was identical by melting point, ^1H NMR, and TLC with unenriched uridine. Mass spectrometry showed 43% enrichment of ^{17}O.

[4-^{17}O]-2′,3′-*O*-Isopropylideneuridine ([4-^{17}O]-IPU). Standard procedures[25] were used to prepare the above compound from the [4-^{17}O]-uridine.

[2-^{17}O]-2′,3′-*O*-Isopropylideneuridine ([2-^{17}O]-IPU). This compound was prepared in essentially quantitative yield by using a similar procedure to that described elsewhere for the preparation of [2-^{18}O]-2′,3′-*O*-isopropylideneuridine.[26] Mass spectrometry of the product showed 54.2% enrichment of ^{17}O.

[2-^{17}O]Uridine. [2-^{17}O]-2′,3′-*O*-Isopropylideneuridine (54.2% enriched) was deblocked with 90% trifluoroacetic acid as described previously.[27] The product was crystallized from EtOH-Et_2O to yield the [2-^{17}O]uridine in essentially quantitative yield. The product was identical by melting point, ^1H NMR, and TLC with unenriched uridine.

[2-^{17}O]-5′-Iodo-5′-deoxy-2′,3′-*O*-isopropylideneuridine. This was prepared from [2-^{17}O]-IPU by using methyltriphenoxyphosphonium iodide as described by Verheyden and Moffatt.[28]

[2-^{17}O]-5′-Deoxy-2′,3′-*O*-isopropylideneuridine. This was prepared from [2-^{17}O]-5′-iodo-5′-deoxy-IPU by catalytic hydrogenation over 10% palladium on charcoal as described previously.[29]

NMR Measurements. Most of the ^{17}O NMR spectra were recorded at 8.14 MHz on a Nicolet TT-14 spectrometer system equipped with a Nicolet multiobserve nuclei accessory (MONA). Acquisition parameters were chosen so as to maximize the observed free induction decay (FID) recorded while the noise and base-line distortion were minimized.[7] Pulses for a 90° spin flip angle (ca. 30 μs) were followed by a delay of 60 to 430 μs before data acquisition. A delay of 0.02 s was used between pulse sequences. Data acquisition for a 10 000 Hz spectral width required 0.051 s, using 1024 points of computer memory. All spectra were processed by using exponential line broadening before Fourier transformation (FT). Several ^{17}O spectra were also measured at 27.1 MHz with a Varian XL-200 spectrometer. The only significant change in experimental data acquisition parameters from that mentioned above was the use of a smaller delay (120 μs) before acquisition.

Both the line widths and chemical shifts were determined by fitting the observed ^{17}O resonances with a Lorentzian curve fitting routine by use of Nicolet 1080 computer software.

All samples of uridine derivatives were contained in sealed 10 mm (o.d.) NMR tubes held coaxially within an 18 mm (o.d.) NMR tube. In most experiments, the outer tube contained acetone-d_6 (Aldrich) that was used for the spectrometer deuterium field lock and as an external ^{17}O chemical shift reference. Proton broad-band decoupling (60 MHz) was used in all experiments reported here. Temperature control was accomplished by flowing filtered air up through the bottom of the NMR probe and around the sample tubes, with control maintained by a Varian V4343 variable temperature controller. Temperatures were monitored by a mercury bulb thermometer with a simulated sample consisting of solvent in the inner tube and acetone in the outer tube. The estimated accuracy of this system was ±1.0 °C. All samples were run without spinning. Due to the natural width of the ^{17}O resonance lines, the resulting decrease in magnetic field homogeneity across the sample was insignificant, amounting to only a few hertz for solvent resonances of about 50-Hz full width at half-maximum (*W*).

The temperature dependence of the H_2O ^{17}O chemical shift has previously been reported.[30] By calibration of the ^{17}O shift difference between acetone and water as a function of temperature and subtraction of the expected shift of water, the absolute shifts of the acetone reference could be determined at the five temperatures studied. Therefore, the acetone natural abundance oxygen resonance could be used as a reference in temperature variation experiments. All ^{17}O shifts reported here are in ppm downfield from H_2O (29 °C).

All H_2O used was doubly distilled. Reagent grade CH_3CN was dried,[31] fractionally distilled, and stored over molecular sieves (Linde 4A) in sealed containers which were stored and transferred in a drybox.

Samples for the IPU-H_2O hydrogen-bonding equilibrium study were evaporated to dryness on a rotary evaporator and placed under vacuum for up to 13 h to assure the removal of water from the sample. The solvent was prepared individually for each mole ratio, by mixing a known volume of H_2O with dry CH_3CN, to a total volume of 10 mL in a volumetric flask. After dissolution, the sample solutions were assayed by UV absorbance to determine the concentration of IPU (extinction coefficient = 10 100).

^{13}C NMR spectra were recorded at 37.7 MHz on a Nicolet NT-150 spectrometer. All spectra were measured with quadrature detection and proton broad-band square wave modulated decoupling. The IPU samples for the ^{13}C studies were natural abundance samples (Aldrich) with no ^{17}O labeling. These IPU samples were contained in a 12- or 15-mm NMR tube containing an antivortex plug, tightly stoppered, and sealed with Parafilm (American Can Co.). This tube was fitted coaxially within a 20 mm NMR tube containing D_2O for the spectrometer lock. A small amount of TSP (sodium 3-(trimethylsilyl)propionate) was dissolved in the D_2O and was used as the external reference (0 ppm) from which all ^{13}C shifts were measured. All ^{13}C measurements were recorded in spinning mode. Sample volumes of 7–12 mL were typically used and 300–1000 acquisitions were normally taken, depending on concentration. A pulse width somewhat less than 90° (≈ 20 μs) and a 50-s delay time between pulses were used. A total spectral width of 8000 Hz was employed, and either 8 or 16 K of computer memory were used. An exponential broadening factor of 3 Hz was employed to enhance the observed signal to noise ratio. The probe temperature could be varied and held constant (±2 °C) for all of the experiments reported here by using the variable-temperature system of the spectrometer.

Results

^{17}O Spectra. At ambient probe temperatures (29 °C) all carbonyl resonances from the uridine samples dissolved in water were very broad (>600 Hz) and difficult to observe. With in-

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Table I. ^{17}O Chemical Shifts[a]

	CH_3CN	H_2O	Me_2SO	CH_3OH
[4-^{17}O]uridine	NS	290 ± 4		
[4-^{17}O]-IPU	333 ± 2 (337 ± 1)	294 ± 6 (299 ± 2)	(335 ± 6)	(317 ± 3)
[2-^{17}O]uridine	NS	240 ± 6		
[2-^{17}O]-IPU	259 ± 2 (259 ± 1)	246 ± 4 (244 ± 2)	(259 ± 4)	(243 ± 4)
[2-^{17}O]-5'-deoxyIPU	263 ± 4			
[2-^{17}O]-5'-deoxy-5'-iodoIPU	263 ± 3			

[a] All shifts measured at 8.14 MHz, except those in parentheses, measured at 27.1 MHz, and at 56 ± 2 °C at concentrations of 0.08 ± 0.03 M. Shifts were measured with respect to carbonyl peak of external acetone-d_6 and corrected to shifts from H_2O (29 °C). NS means sample not sufficiently soluble.

creasing temperature, the resonances sharpened dramatically with an accompanying increase in signal height, allowing comparable signal to noise ratios to be obtained with fewer FID acquisitions. These effects are in agreement with other work.[1] The line width at half-maximum (W) is described by eq 1, where $e^2qQ/\hbar$ is the

$$\frac{1}{T_2} = \frac{1}{125}\left(1 + \frac{\eta^2}{3}\right)\left(\frac{e^2qQ}{\hbar}\right)^2\tau = \pi W \qquad (1)$$

quadrupole coupling constant, η is the assymetry parameter, and τ is the rotational correlation time. If the molecules studied are assumed to be relatively rigid in solution, then in the absence of intermolecular associations, changes in line width with temperature should be due entirely to the temperature dependence of τ.

The average ^{17}O chemical shift values and estimated errors obtained for the enriched uridine compounds in water, acetonitrile, Me_2SO, and methanol are presented in Table I. It should be noted that the presence of the isopropylidene substituent has no significant effect on the carbonyl ^{17}O shifts.

Previous work from this laboratory[1] compared the ^{17}O chemical shifts of the O(2) and the O(4) of IPU in both acetonitrile and water. The O(4) resonance showed a much greater shift change between the two solvents than did the O(2) resonance, indicating that hydrogen bonding of water was preferred at O(4) relative to O(2). Because of these larger shift changes of O(4) (30–35 ppm), most of the detailed water association studies described herein focused on the O(4). The ^{17}O chemical shifts of O(4) as a function of mole ratio (R = $[H_2O]/[IPU]$) and temperature were measured at 8.14 MHz, and these data are represented by the experimental points in Figure 2. Only three of the five temperatures studied (29, 35, 41, 48, and 56 °C) are shown.

In the acetonitrile–water system, the spectra indicate consistent upfield shifts with increasing water concentration, and at any particular mole ratio the shifts increased (moved downfield) with increasing temperature. Both of these observations can be explained in terms of hydrogen bonding to water molecules which causes the carbonyl oxygen resonance to shift upfield. Such an explanation is also consistent with the shifts measured in Me_2SO and methanol (vide infra). The effect on the ^{17}O shift of various carbonyl oxygens due to the change in solvent polarity in going from methanol (dielectric constant ϵ = 32.6), acetonitrile (ϵ = 37.5), or Me_2SO (ϵ = 46.7) to water (ϵ = 80) was calculated by Jallali-Heravi et al.[32] using a "solvation model" and was found to be 1 ppm or less. A more detailed examination of the source of hydrogen-bonding shifts is presented in the Discussion section.

The estimated error in the measured shifts was dependent on solvent and temperature. Factors that increased the line width at half-height (W) increased these errors. Thus for IPU in neat acetonitrile at 56 °C, excellent spectra were obtained that allowed chemical shifts to be measured with an error of no more than ±2 ppm. However for R = 260 (H_2O:CH_3CN ≃ 1:1), the error at 56 °C was estimated at ±5 ppm, and at 29 °C in 100% H_2O, resonances were often difficult to distinguish from base-line roll when the lower field spectrometer was used. Therefore, only chemical shifts measured for R < 200 were used in the detailed analysis of the data.

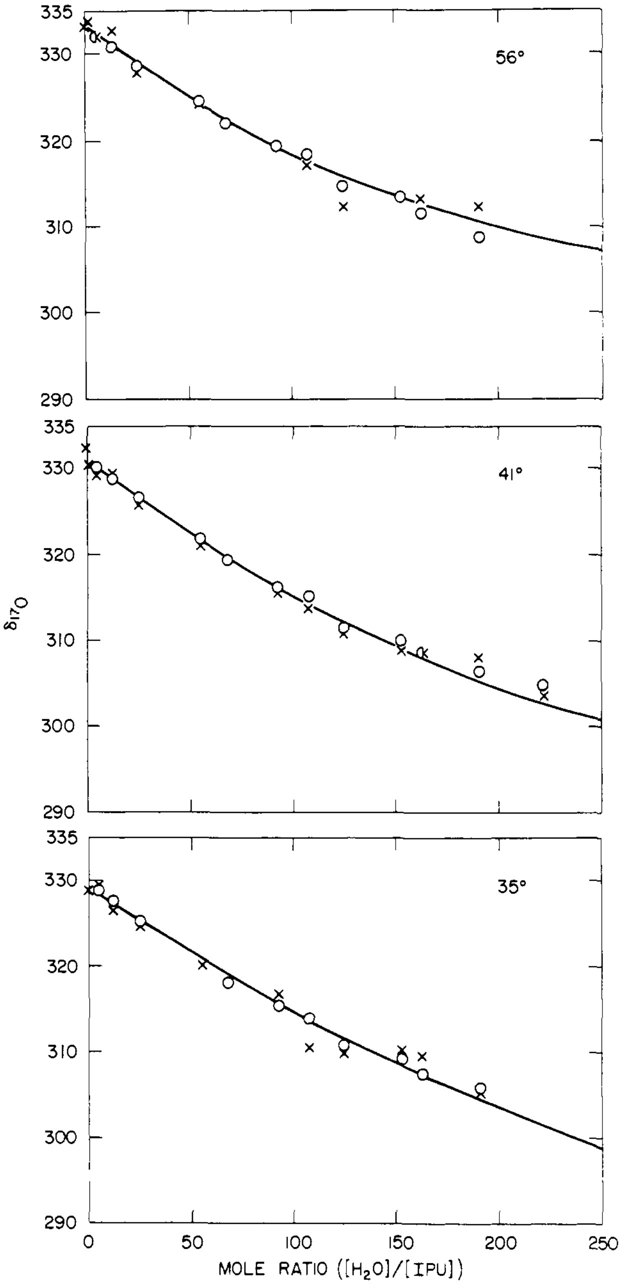

Figure 2. ^{17}O shifts of [4-^{17}O]-IPU in a water–acetonitrile solvent at the indicated temperature. Experimental points are indicated by (x) and points calculated from K_{1W} and K_{1WW} to fit the best fitting curve (—) are indicated by (O).

Measurements were also made of the ^{17}O shifts of the H_2O molecules in the acetonitrile solutions containing [4-^{17}O]-IPU. These shifts, shown in Figure 3, are also indicative of changes in hydrogen bonding, primarily due to H_2O–H_2O and H_2O–CH_3CN interactions. These shifts are also temperature dependent although this dependence is not quite as marked as in the H_2O–[4-^{17}O]-IPU

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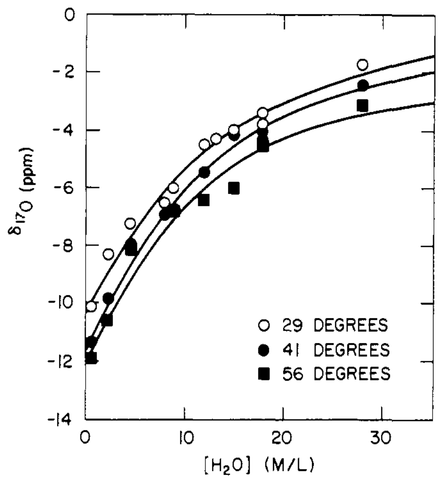

Figure 3. ^{17}O shifts (ppm) of water in the IPU–H_2O–CH_3CN system at the indicated water concentrations at temperatures of 29 °C (○), 41 °C (●), and 56 °C (■).

data. The ^{17}O shift of H_2O at infinite dilution in CH_3CN is estimated at −12 ± 1 ppm, in good agreement with a previous estimate.[16]

The ^{17}O line widths (W) observed for the [4-^{17}O]-IPU and [2-^{17}O]-IPU were essentially identical. Measurements of W ranged from 400 ± 15 Hz at 56 °C in CH_3CN to 1000 ± 100 Hz at 29 °C in water, but detailed examination of the line-width dependence on hydration was not undertaken in this study.

In order to gain a quantitative interpretation of the ^{17}O NMR shifts of the IPU carbonyl oxygens due to hydrogen bonding with water, the contribution of the observed ^{17}O shifts due to IPU self-association was first examined. The three possible cyclic dimers that can be formed by intermolecular self-association of uridine derivatives are shown in Figure 4. The observation of chemical shift changes as a function of sample concentration is a most useful way to study self-association using NMR. Unfortunately, the ^{17}O shifts of both [4-^{17}O]-IPU and [2-^{17}O]-IPU in CH_3CN varied by only about 4 ppm between 0.02 and 0.10 M. Due to the overall scatter of the measurement and an estimated error of ±2 ppm for each measurement, it was not possible to use these data for estimating the amount of self-association. However, it is important to note that the ^{17}O shift changes due to self-association were small relative to the shift changes observed because of interaction with water.

^{13}C Spectra. ^{13}C NMR dilution experiments for IPU in acetonitrile were made in an attempt to obtain more detailed information about these self-association effects. The results of these ^{13}C NMR dilution studies at 37.7 MHz are shown in Figure 5. Although the extent of the observed shift changes for C(2) and C(4) of IPU are rather small (<0.4 ppm), it is apparent that the C(4) shifts markedly more than the C(2). In addition, the C(6) resonance appears to shift as much as C(2). The temperature dependence of the ^{13}C shifts are also shown in Figures 5 and 6. Together these data enable an infinite dilution shift for C(4) (i.e., for completely unassociated IPU) of 6264 Hz (166.1 ppm) from TSP to be extrapolated.

An additional ^{13}C study was made under the conditions used for the ^{17}O NMR experiments previously described on the IPU–H_2O–CH_3CN mixtures. The only major difference in these ^{13}C experiments was the use of unenriched IPU samples and larger sample volumes (7–10 mL rather than 1.2 mL). The results of these experiments at 35 °C for C(2) and C(4) are shown in Figure 7 and are qualitatively very similar to the ^{17}O results. The C(4) shift differences are again significantly larger than for C(2), and both C(2) and C(4) are both much more sensitive to changes in water concentration than to changes in IPU concentration. One distinction from the ^{17}O results is that the experimental error in determining the ^{13}C shifts is not dependent on solvent composition and is dependent only on the spectrometer resolution and the reproducibility of sample temperatures. The estimated error for these ^{13}C shifts, due primarily to the latter constraint, was ±0.7 Hz (~0.02 ppm).

Analysis and Discussion

Previous work from this laboratory demonstrated that the ^{17}O chemical shift for the O(4) of IPU in H_2O is markedly different from the shift measured in CH_3CN (unlike the shift for O(2) which shows little change in the two solvents). That this change is due to a difference in the amount of hydrogen bonding (between O(4) and H_2O molecules) and not due to changes in solvent polarity is based on the following observations: (i) calculations[32] have shown that the effect on the ^{17}O shifts of carbonyl oxygens in going from CH_3OH (dielectric constant, $\epsilon = 32.6$) to H_2O ($\epsilon = 80$) is only of the order of 1 ppm; (ii) the ^{17}O shifts in CH_3CN ($\epsilon = 37.5$) and in Me_2SO ($\epsilon = 46.7$) are essentially the same (see Table I); (iii) a shift change due to solvent polarity would be observed in *both* the O(4) and O(2) shifts; and (iv) an upfield shift (relative to CH_3CN and Me_2SO) is also observed in the protic solvent CH_3OH.[33]

The detailed hydrogen-bonding aspects of this ^{17}O study have centered around the nucleoside derivative IPU. While an X-ray structure has not yet been obtained for IPU, the crystal structure for a similar molecule, 2′,3′-*O*-(methoxymethylene)uridine (MMU), has been determined.[34a] By using an approximate ab initio minimal basis set method, it was determined[34b–d] that the electronic distributions at the C(2) and C(4) carbonyls of uracil, MMU, and uridine were all similar. Thus there is a strong validity to the assumption that interactions at the uracil moiety of IPU will not differ markedly from those for uracil and uridine.

The initial finding that the ^{17}O shifts for O(4) and O(2) in uridine and IPU were nearly identical (see Table I) is further experimental justification for the IPU–uridine comparison. This finding is of interest because of the indications from various sources,[35] including calculated and measured dipole moments[36] and nuclear Overhauser enhancements,[37] that the conformation of IPU is more syn than anti (unlike uridine). Our ^{17}O data for the O(2) carbonyl in particular would indicate that either the isopropylidene does not affect the preferential orientation of the glycosyl torsion angle or more likely that the ^{17}O shift of O(2) is not sensitive to the effects of such a conformational change, although one cannot rule out the possibility that such conformational changes give rise to competing effects on the overall observed ^{17}O chemical shift.

The details of the various factors that influence ^{17}O NMR shifts have been reviewed in various papers.[11,14,18,19,38–41] For carbonyl

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(33) The observation that for O(4) $\delta_{CH_3CN}-\delta_{CH_3OH}$ (20 ppm) is approximately half that for $\delta_{CH_3CN}-\delta_{H_2O}$ (38 ppm) is good evidence that one hydrogen bond forms between O(4) and CH_3OH but two form between O(4) and H_2O molecules. Whether or not this is due to steric reasons is not understood at the present time.

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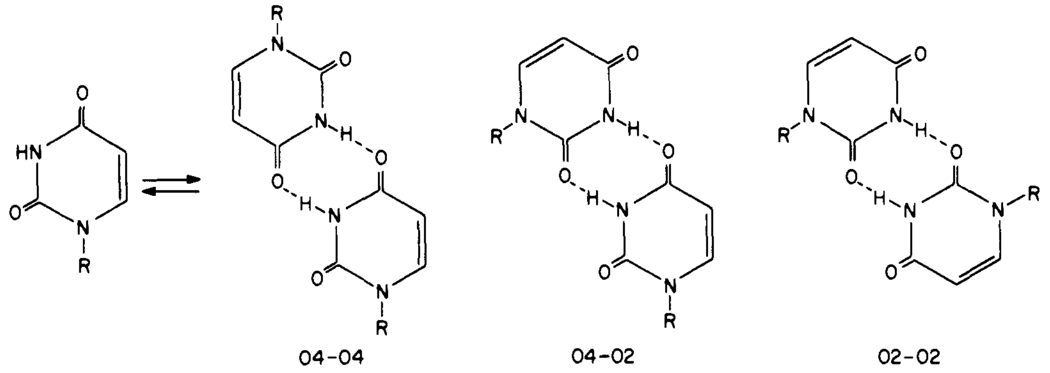


Figure 4. Formation of possible cyclic dimers for uracil derivatives.

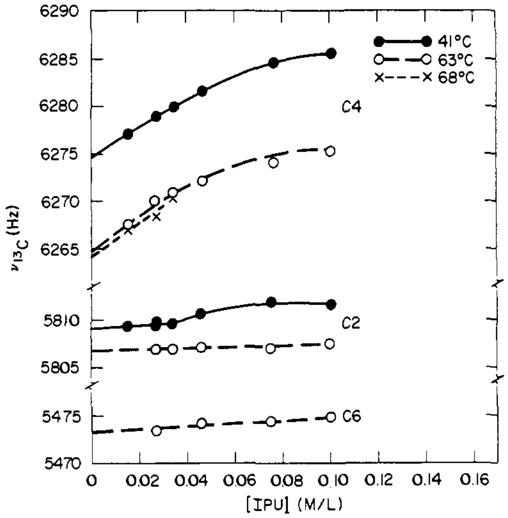


Figure 5. ^{13}C NMR (37.7 MHz) shifts in Hz from TSP of certain downfield carbons of IPU in acetonitrile at the indicated concentrations and temperatures.

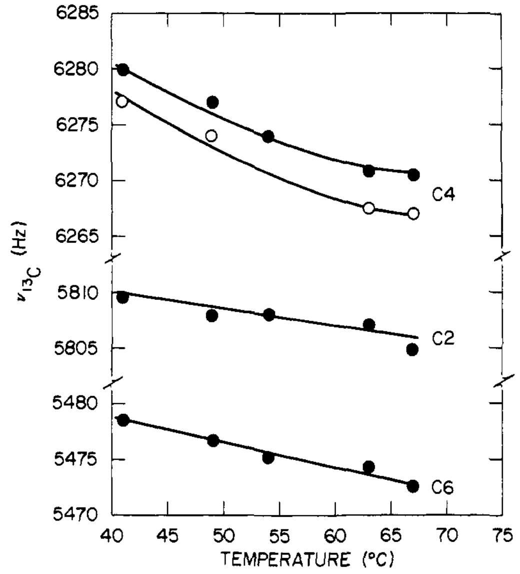


Figure 6. ^{13}C NMR (37.7 MHz) shifts in Hz from TSP of certain downfield carbons of IPU in acetonitrile as a function of temperature at IPU concentration of 0.034 M (●) and 0.015 M (○).

groups, Figgis et al.[41] have shown that the major contribution to the ^{17}O chemical shift is the paramagnetic screening term, σ_p. This term is inversely proportional to the appropriate transition energy (n → π*), and Figgis demonstrated a linear correlation between

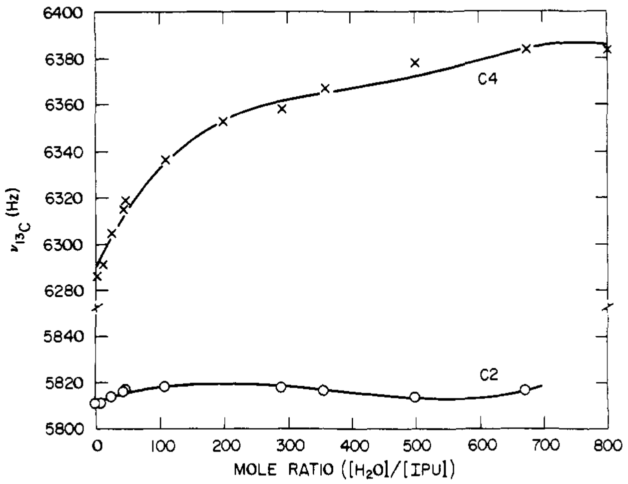
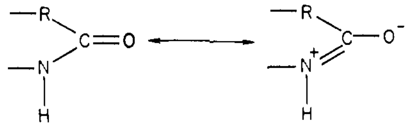


Figure 7. ^{13}C NMR (37.7 MHz) shifts of C(2) and C(4) as a function of mole ratio (R = [H$_2$O]/[IPU]) for 0.083 ± 0.005 M IPU in a mixed acetonitrile-water solvent at 35 ± 2 °C.

^{17}O shift and the inverse of the n → π* transition energy (ΔE), corresponding to the lowest energy optical transition for many carbonyl compounds. These relationships have also been noted in theoretical studies,[42] and numerous empirical correlations have been established relating π bond order and ^{17}O chemical shifts.[8,10,39,43,44]

Of primary importance in the present work is the effect of hydrogen bonding on carbonyl ^{17}O shifts. This topic has been covered in a number of different studies,[14-16,18,19,21] and a relatively detailed analysis of ^{17}O shifts due to hydrogen bonding of amide systems, similar in structure to the uracil carbonyls, particularly O(4), has recently been described.[14]

A qualitative yet useful depiction of carbonyl hydrogen bonding is based on the mesomerism between different resonance forms. For the O(2) and O(4) carbonyls of a uracil base, these can be represented as



I II

where R is N or CH for O(2) and O(4), respectively. Structure II is stabilized by hydrogen bonding of the carbonyl oxygen, so

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that the π electron density in the C=O is decreased and the observed ^{17}O shifts moves upfield.

The quantitative interpretation of the observed ^{17}O shifts due to a hydrogen-bonding equilibrium is a special case of exchange interactions in NMR.[45] The oxygen nuclei have two nonequivalent states, depending on whether they are hydrogen bonded or not, represented by two independent chemical shifts, δ_{free} and δ_{bound}. If exchange is fast, the observed ^{17}O chemical shifts (δ_{obsd}) of a hydrogen-bond acceptor (A) will be the weighted average of these:

$$\delta_{obsd} = \frac{[A]_{free}}{[A]_{total}}\delta_{free} + \frac{[A]_{bound}}{[A]_{total}}\delta_{bound} \qquad (2)$$

where $[A]_{free}$ and $[A]_{bound}$ are the respective molar concentrations of non-hydrogen-bonded and hydrogen-bonded A.

Equation 2 is applicable in the analysis of hydrogen-bonding shifts, for ^{13}C and ^{14}N data as well as for ^{17}O. The accuracy of the calculated values of $[A]_{free}$ or $[A]_{bound}$ will depend on the experimental accuracy in measuring δ_{obsd}, and in the accuracy in the values utilized for δ_{free} and δ_{bound}. One can generally determine δ_{free} by extrapolation to infinite dilution of the observed species. However the determination of δ_{bound} for some of these relatively weak associations is not as straightforward. For hydrogen bonding to IPU, the present calculations have relied primarily on the use of model systems of similar structure where δ_{bound} could be determined experimentally.

The best available data for ^{17}O limiting shift differences (Δ) of carbonyl–H_2O hydrogen-bonding interactions are for acetone–water, where $\Delta = (\delta_{free} - \delta_{bound}) = 52$ or 55 ppm,[16,46] and for formamide (53 ppm), *N*-methylformamide (53 ppm), and *N,N*-dimethylformamide (52 ppm).[14] It is very important to note that these Δ values are almost certainly due to the hydrogen bonding of two water molecules, one to each lone pair of the carbonyl oxygen. Although it is difficult to confirm this result experimentally in solution, the shift differences observed for O(4) of IPU in the protic solvents CH_3OH and H_2O ($\delta_{CH_3CN} - \delta_{CH_3OH} = 20$ ppm and $\delta_{CH_3CN} - \delta_{H_2O} = 38$ ppm; see Table I) are good evidence for this assumption.[33] In addition, the plausibility of this model has been discussed by Del Bene for uracil,[47] by Burgar et al.[14] with respect to the ^{17}O results for amides, and by others with respect to the observed and calculated hydrogen-bonding enthalpies.[48-51] Experimentally, the hydrogen bonding of two proton donors to a carbonyl group has often been observed in crystal structures, including the 9-ethyladenine–1-methyl-5-bromouracil complex,[52] cytosine monohydrate,[53] formamide using IR,[54] and D-tartaric acid.[55]

In order to obtain the desired quantitative results for the hydrogen-bonding equilibrium between water and the O(4) carbonyl group of IPU, the other significant competing equilibria must each be considered. The effects of these must be determined to sufficient accuracy so that the uncertainty in the results determined from these equilibria will not seriously detract from the final results for IPU hydration. These competing interactions are IPU self-association, water self-association, and water association with acetonitrile, and are considered in turn below before the O(4)–H_2O interaction is analyzed.

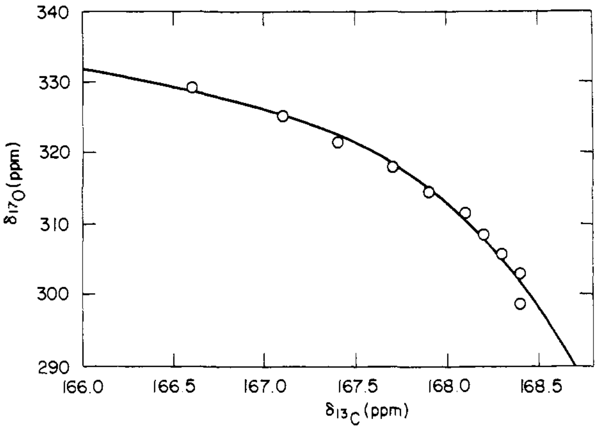

Figure 8. Correlation of ^{13}C with ^{17}O NMR shifts for the O(4) carbonyl group of 0.09 M/L IPU at various mole ratios (*R*). Points are taken from the fitted curves of the respective ^{17}O and ^{13}C experiments at 35 °C for increments of $R = 25$, starting at $R = 0$.

IPU Self-Association. The ^{13}C dilution experiments on IPU in acetonitrile reported here are similar to experiments performed by Iwahashi and Kyogoku[56,57] on various 1-cyclohexyluracil derivatives in chloroform. They found that the C(2) and C(4) carbonyl resonances shifted downfield with increasing concentration due to self-association. These observed shifts were used to estimate equilibrium constants and limiting shift differences for various modes of hydrogen-bonded dimer formation (Figure 4). Their results indicated that both the C(2) and C(4) carbonyls participated significantly in the dimerization interactions, with the ratio of C(4) to C(2) involvement depending on the particular uracil derivative under investigation. (The limiting shift ratio of C(4) to C(2) was approximately two to one for 1-cyclohexyluracil.)

However for IPU, the significantly larger shifts of C(4) relative to C(2), demonstrated in Figures 5 and 6, indicate that the IPU dimer formed through both C(4) carbonyls is the predominant self-associated form of IPU in acetonitrile. This is also the type of dimer most often observed in crystal studies of uracil derivatives.[58] The apparent discrepancy with the results of Iwahashi and Kyogoku[56,57] could be due to the effects of the cyclohexyl substitution or the solvent difference.

In order to obtain the amount of self-association present at a particular concentration, it is necessary to know the limiting shifts for the fully dissociated molecules. From the data in Figures 5 and 6, the ^{13}C shift for C(4) of nonassociated IPU is estimated at 166 ppm. However, the analogous ^{17}O shift for O(4) of nonassociated IPU cannot be obtained directly by measurement of ^{17}O shifts at increasing dilution because of the increasing difficulty of detection of ^{17}O spectral lines at lower concentrations of IPU (vide supra). In order to determine the $^{17}O(4)$ limiting shift for a nonassociated IPU molecule, we have employed an empirical $^{13}C(4)$–$^{17}O(4)$ shift correlation.

The $^{17}O(4)$ and $^{13}C(4)$ chemical shifts at 35 °C, as a function of mole ratio (*R*) of water to IPU, as shown in Figures 2 and 7, respectively, were each fitted by using a five-parameter polynomial least-squares fitting routine to obtain good fits of the data. The $^{13}C(4)$ and $^{17}O(4)$ chemical shifts at increments of 25 mole ratios of H_2O (i.e., $R = 0$, 25, 50, etc.) were then plotted as abscissae and ordinate, respectively (see Figure 8), and a nonlinearity between the ^{17}O and ^{13}C chemical shifts is apparent. Thus, it can be seen from Figure 8 that the ^{13} C shifts become a less sensitive monitor for hydrogen bonding relative to ^{17}O as the hydrogen bonds become saturated (i.e., at the more upfield values of the ^{17}O shifts). This result is not surprising from a purely empirical point of view, due to the carbonyl carbon being one bond farther

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[45] For a general discussion of the effects of chemical exchange on chemical shifts, see: Emsley, J. W.; Feeney, J.; Sutcliffe, L. H. "High Resolution Nuclear Magnetic Resonance"; Pergamon Press: Oxford, 1965; Vol. I, pp 481–485.

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Table II. Self-Association Parameters for 0.09 M [4-^{17}O]-IPU in CH_3CN

temp, °C	29	35	41	48	56
$^{17}O^a$	329.5	330.0	331.0	332.0	333.0
$2[(IPU)_2)]^b$	0.0208	0.0190	0.0156	0.0121	0.0087
K_{11}	2.17	1.88	1.41	1.00	0.66
$K_{11\text{fitted}}{}^c$	2.54	1.83	1.34	0.94	0.64

[a] ^{17}O shifts are the values extrapolated to zero water concentration (within ±0.5 ppm) from the [4-^{17}O]-IPU-H_2O titration data, as in Figure 2. [b] Calculated from ^{17}O shifts, assuming all cyclic dimers are through both C(4) carbonyls using eq 2 with a limiting shift difference ($\delta_{\text{free}} - \delta_{\text{bound}}$) = 26 ppm, and δ_{free} = 335.5 ppm. [c] From points along least-squares fitted line through experimental log K_{11} values in Figure 9.

removed from the actual site of hydrogen bonding relative to the carbonyl oxygen atom. More detailed theoretical studies would be necessary for a complete understanding of this phenomenon. Thus a ^{13}C–^{17}O shift correlation for use in determining the $^{17}O(4)$ chemical shift for a nonassociated IPU molecule (in neat CH_3CN) can be obtained, but it should be noted that the curvature in Figure 8 increases with increasing water concentration. Fortunately, to extrapolate to the non-hydrogen-bonded $^{17}O(4)$ of IPU, only the most linear section of this curve, from R = 0 to 25 mole ratio, must be used. A reasonable estimate for this correlation, 8 ± 1 ^{17}O ppm per ^{13}C ppm, can thus be obtained.

The difference in ^{13}C shift between the infinite dilution shift of C(4) and the shift for 0.09 M IPU in CH_oCN at 41 °C is 0.56 ± 0.07 ppm. The correlation just derived enables the analogous ^{17}O shift to be estimated at 335.5 ± 1 ppm from the ^{17}O shift at 41 °C for 0.09 M [4-^{17}O]-IPU (see Table II). This limiting ^{17}O shift represents δ_{free} in eq 1 (vide supra) when applied to IPU self-association. The temperature-dependent ^{17}O shifts of O(4) for 0.09 M IPU, extrapolated to dry acetonitrile, are reported to within ±0.5 ppm in Table II.

With the use of eq 2, the δ_{free} derived above, and a value for limiting shift differences (Δ) of 26 ppm (see below), the concentrations of IPU dimer and equilibrium constants shown in Table II were derived from equilibrium equations (eq 3 and 4). The

$$2\text{IPU} \overset{K_{11}}{\rightleftharpoons} (\text{IPU})_2 \tag{3}$$

$$K_{11} = \frac{[(\text{IPU})_2]}{([\text{IPU}]_{\text{total}} - 2[(\text{IPU})_2])^2} \tag{4}$$

value of 26 ppm was chosen for Δ, since it is half of the 52 ppm Δ observed for the formation of two carbonyl hydrogen bonds in acetone and for several amides[14] as noted previously. The equilibrium constants, K_{11} in Table II, were used to estimate the enthalpy change of $\Delta H = -10.1$ kcal mol^{-1} for the dimerization equilibrium. An excellent linear least-squares fit (r = 0.999) was obtained if the point at 29 °C is ignored as shown in Figure 9. In Table II the values of $K_{11\text{fitted}}$ were taken from the appropriate temperature points along the fitted line (Figure 9) and judged to be somewhat more accurate than the original values of K_{11}. These values of $K_{11\text{fitted}}$ were used in all subsequent calculations involving K_{11}. The results of other theoretical[59,60] and experimental[56,57,59,61] studies of self-association in uracil derivatives are given in Table III for comparison with the present results. The smaller K_{11} found using CH_3CN as compared to that found earlier for studies of 1-cyclohexyluracil in $CHCl_3$[56,61a,b] as a solvent is understandable due to the increased polarity of the former.

Kyogoku and co-workers[61a,b] have used ^{13}C data directly to obtain self-association constants for certain uracil derivatives; however, in the work described herein we were not able to obtain such parameters due to decreasing values of K_{11} with increasing IPU concentration. This observation and the nonlinear correlation shown in Figure 8 show that the ^{13}C shift is not related to hydrogen bonding in a linear fashion for this experimental system and conclusions based on such a linear correlation may be subject to error.

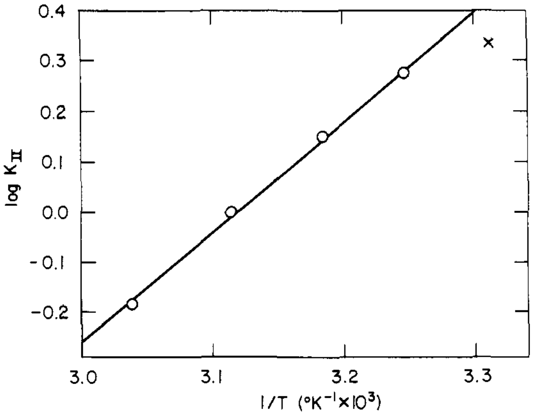

Figure 9. Log of experimental self-association equilibrium constants (K_{11}) vs. the inverse of absolute temperature (T). The line represents a linear least-squares fit to the data points, excluding the point at 29 °C (X). The resulting slope is proportional to the enthalpy change of the self-association as shown in the equation d(log K)/d(1/T) = $-\Delta H/2.3$ R, where R is the universal gas constant.

Hydrogen Bonding of Water. For an adequate quantitative description of the hydrogen-bonding equilibrium between water and a carbonyl group on IPU, it is necessary to obtain a sufficiently accurate description of the self-association of the water itself.

For the present purpose, we have adopted the water hydrogen-bonding model described by W. Luck.[62] An individual H_2O molecule can form two distinct types of hydrogen bond, an "L" bond from a line electron pair on the oxygen and a "P" bond from the hydrogen. Thus each H_2O molecule could form up to four hydrogen bonds (as in ice), two of each type. In pure liquid water the hydrogen-bonding equilibrium could be simply described by

$$\text{L} + \text{P} \overset{K}{\rightleftharpoons} \text{L}\cdots\text{P} \tag{5}$$

$$K = \frac{[\text{L}\cdots\text{P}]}{[\text{L}][\text{P}]} = \frac{[\text{L}\cdots\text{P}]}{[\text{P}]^2} \tag{6}$$

The extent of hydrogen bonding of a water molecule can be probed experimentally by magnetic resonance techniques and, in particular, by ^{17}O NMR. Table IV shows the ^{17}O chemical shifts of water in various hydrogen-bonding environments. If we assume that H_2O in vapor is completely nonassociated, and in ice is completely hydrogen bonded, the resulting shift difference represents the total ^{17}O limiting shift difference for the formation of all four possible hydrogen bonds. However, the ^{17}O shift of ice has not been accurately determined due to experimental difficulties. Accordingly, Luck,[62] using infrared data, estimated that 85–90% of the total possible hydrogen bonds are formed in liquid H_2O at 0 °C. On the basis of such an assumption, the total ^{17}O shift difference related to the formation of two L bonds and two P bonds in water (i.e., difference between H_2O in ice and in vapor) is approximately 43 ppm (see Table IV). The significance of the H_2O infinite dilution shifts of H_2O in acetone or acetonitrile (i.e., no H_2O self-association; see Table IV) is that complexes of the type

$$\text{A}\cdots\text{HOH}\cdots\text{A}$$

[where A represents a hydrogen-bond acceptor (e.g., the carbonyl oxygen of acetone or the nitrile nitrogen of acetonitrile)] in which

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Table III. Comparative Results for Uracil Self-Association

derivative	method	temp, °C	solvent	K, M^{-1}	$-\Delta H$, kcal/mol	ref
1-cyclohexyluracil	IR	25	$CDCl_3$	6.1 ± 0.6	4.3 ± 0.4	61a
1-cyclohexyluracil	^{13}C NMR	27	$CHCl_3$	3.2 ± 0.6		56
1-cyclohexyluracil	^{1}H NMR	30	$CDCl_3$	4.0	5.3	61c
1-methyluracil	field mass spectrometry		vacuum		9.5	59
uracil	quantum mechanical calculations[a]				5.4	60
uracil	atom-atom potentials calculation				8.1	59
IPU	^{17}O NMR[a]	29	CH_3CN	2.5 ± 0.4	10.1 ± 1.2	this work

[a] The results from these methods are specific for the O(4)-O(4) dimer (Figure 4) only.

Table IV. ^{17}O Chemical Shifts of Water in Various Environments

sample	^{17}O chemical shift[a], ppm
ice (0 °C) (extrapolated)	+7
H_2O (0 °C)	+2
H_2O (29 °C)	0
H_2O in CH_3CN (infinite dilution)	−12
H_2O in $(CH_3)_2CO$ (infinite dilution)[16]	−12
H_2O vapor[63]	−36

[a] Shifts reported from H_2O at 29 °C with an average estimated error of ±0.5 ppm.

the water P bonds are saturated but the L bonds will not be formed will predominate. The ^{17}O shift difference between these complexes (−12 ppm, for the infinite dilution shift of H_2O in acetone or acetonitrile—see Table IV) and H_2O vapor (−36 ppm), i.e., 24 ppm, must therefore be due to the formation of the P bonds. If these P bonds are assumed to be independent and additive in nature, the contribution due to each P bond will therefore be approximately 12 ppm. Similarly the shift difference (19 ppm) between ice (7 ppm) and the infinite dilution complexes (−12 ppm) is due to the formation of the L bonds so that each L bond contributes 9.5 ppm to the ^{17}O chemical shift. A similar analysis was previously proposed by Reuben;[16] the primary modification used here is the extrapolation for ice suggested by Luck.[62] Thus for pure H_2O, the observed ^{17}O chemical shift can be described:

$$\delta' = 9.5\frac{[L]_{bound}}{[H_2O]} + 12\frac{[P]_{bound}}{[H_2O]} - 43 \tag{7}$$

where $\delta' = \delta_{obsd} - 7$ ppm, and where the 7 ppm is the chemical shift difference between ice and H_2O at 29 °C (see Table IV).

This description can now be extended to describe the hydrogen-bonding equilibrium in the mixed solvent system actually used in our experiments. If the nitrogen lone pair in CH_3CN is symbolized as "N", the CH_3CN–H_2O interaction can be described by the following equations:

$$N_{free} + P_{free} \rightleftharpoons N\cdots P \tag{8}$$

$$[L]_{free} = [P]_{free} + [N\cdots P] \tag{9}$$

$$= [P]_{free} + ([CH_3CN]_{total} - [N]_{free}) \tag{10}$$

Also, since

$$[L]_{bound} = [L]_{total} - [L]_{free} \tag{11}$$

$$= 2[H_2O]_{total} - [L]_{free} \tag{12}$$

$$= 2[H_2O]_{total} - [P]_{free} - [CH_3CN]_{total} + [N]_{free} \tag{13}$$

and

$$[P]_{bound} = [P]_{total} - [P]_{free} \tag{14}$$

$$= 2[H_2O]_{total} - [P]_{free} \tag{15}$$

substitution for $[L]_{bound}$ and $[P]_{bound}$ permits eq 7 to be rewritten to obtain $[P]_{free}$ (eq 16).

$$\frac{[P]_{free}}{[H_2O]_{total}} = \frac{\frac{9.5}{[H_2O]_{total}}([N]_{free} - [CH_3CN]_{total}) - \delta'}{21.5} \tag{16}$$

Table V. Equilibrium Parameters for $[4\text{-}^{17}O]$-IPU–H_2O Association

temp, °C	K_{IW}, M^{-1} exptl[a]	K_{IW}, M^{-1} fitted[b]	K_{IWW}, M^{-2} exptl[a]	K_{IWW}, M^{-2} fitted[b]
29	0.28	0.28	0.082	0.065
35	0.26	0.24	0.037	0.046
41	0.18	0.20	0.031	0.032
48	0.18	0.17	0.018	0.022
56	0.14	0.14	0.018	0.015
ΔH, kcal/mol	−5.2 ± 0.5		−11.0 ± 1.5	

[a] Calculated by using eq 24 using experimental ^{17}O data for $[4\text{-}^{17}O]$-IPU and H_2O shifts as described in text. [b] Taken from fitted line of log *K* vs. 1/*T* plots in Figure 11.

$[N]_{free}$ can be estimated directly from the ^{14}N shifts of CH_3CN observed as a function of concentration in the CH_3CN–H_2O binary solvent with the equations

$$\delta_{^{14}N} = \delta_{bound}\frac{[N]_{bound}}{[CH_3CN]_{total}} + \delta_{free}\frac{[N]_{free}}{[CH_3CN]_{total}} \tag{17}$$

and

$$[N]_{bound} + [N]_{free} = [CH_3CN]_{total} \tag{18}$$

where $\delta_{^{14}N}$ is the observed ^{14}N shift of CH_3CN, δ_{bound} is the ^{14}N infinite dilution shift for CH_3CN in H_2O, and δ_{free} is the ^{14}N shift for neat CH_3CN.

This analysis of the ^{14}N data assumes that the observed shifts are due primarily to the specific N···P hydrogen bonding rather than other various solvent effects.[64] Lowenstein and Margalit,[65] who performed the ^{14}N experiments, concluded that such an assumption was valid for the acetonitrile–water system, and their data were used for the estimation of $[N]_{free}$ as described above. Their studies and others have indicated CH_3CN to be a relatively weak hydrogen-bond acceptor.[65-67]

The need for absolute accuracy in estimating $[N]_{free}$ is not extremely demanding since this term is a small perturbation with respect to the estimation of $[P]_{free}$, which could itself be considered a first-order perturbation in calculating the equilibrium constant for the IPU–H_2O association.

With use of the assumptions mentioned above, the concentration of non-hydrogen-bonded water protons, $[P]_{free}$, can be determined primarily from the ^{17}O NMR shifts of H_2O in the H_2O–CH_3CN–IPU mixture. These data are shown in Figure 3 for three of the five temperatures studied. Because of the relatively small effects of temperature, relative to the experimental errors involved, the calculated values of $[P]_{free}$ for the 29 and 35 °C experiments, and also the 48 and 56 °C experiments, were averaged together

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(64) Jallai-Heravi, M.; Webb, G. A. *Org. Magn. Reson.* **1980**, *13*, 116–118.

(65) Lowenstein, A.; Margalit, Y. *J. Phys. Chem.* **1965**, *69*, 4152–4156.

(66) CH_3CN has been shown to be a relatively weak proton acceptor in interactions with water and other proton donors. See the following: McTigue, P.; Renowden, P. V. *J. Chem. Soc., Faraday, Trans. 1*, **1975**, 1784–1789. Joesten, M. D.; Schaad, L. J. "Hydrogen Bonding"; Marcel Dekker, New York, 1974; pp 221–226.

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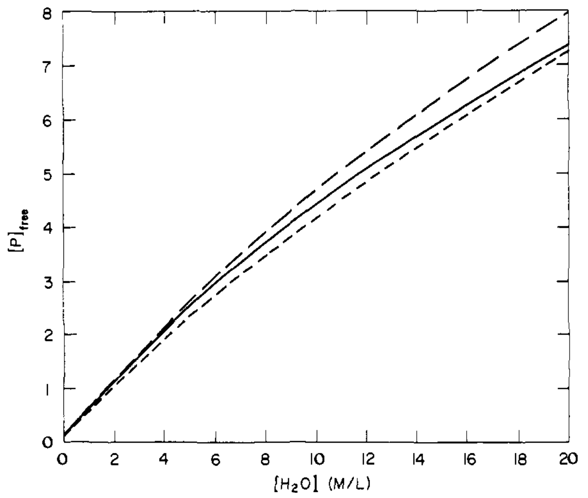

Figure 10. Plots of best fitting curves for concentration of P_{free} bonds calculated vs. total water concentration in the [4-^{17}O]-IPU-H_2O-CH_3CN mixture. Calculations were made from the experimental ^{17}O shifts of H_2O and the previously measured ^{14}N shifts of CH_3CN as described in the text. Least-squares fitting routines using 5-parameter polynomials were used to fit the calculated values of $[P]_{free}$ for the averaged 29 °C and 35 °C points (--), the averaged 48 °C and 56 °C points (— —), and the 41 °C points (—).

to obtain the fitted curves shown in Figure 10. These curves describe $[P]_{free}$ as a function of $[H_2O]$ in the IPU-H_2O-CH_3CN system for the three temperature regimes (29–35, 41, and 48–56 °C), and the values from these curves were used in calculations of equilibrium constants for the [4-^{17}O]-IPU association.

IPU-O(4)-H_2O Association. With the same nomenclature used above for water hydrogen bonding, the IPU-O(4) association with H_2O (assuming a 1:1 interaction) can be written as

$$\text{IPU} + \text{P} \overset{K_{\text{IW}}}{\rightleftharpoons} \text{IPU}\cdots\text{P} \tag{19}$$

which is to be distinguished from

$$\text{IPU} + \text{H}_2\text{O} \rightleftharpoons \text{IPU}\cdots\text{H}_2\text{O} \tag{20}$$

The latter equilibrium (eq 20) describes the interaction of the O(4) only with nonassociated, i.e., monomeric water molecules that would generally be found only in very dilute water mixtures. A more physically realistic description would substitute "oligomeric" water [i.e., $(H_2O)_n$ for H_2O in eq 20] with the stipulation that at least one P bond be free and available in the water polymer. However, using eq 19, such uncertainties in the actual oligomeric state of the H_2O molecules bound to O(4) can be overcome.

With the use of the ^{17}O shifts (δ_{obsd}) of O(4) as a function of R at five temperatures and values of $[P]_{free}$ obtained in Figure 10, calculations were made to obtain the equilibrium constant, K_{IW}, for each temperature by using simultaneous equations below:

$$K_{\text{IW}} = \frac{[\text{IPU}\cdots\text{P}]}{[\text{IPU}]_{\text{free}}[\text{P}]_{\text{free}}} \tag{21}$$

$$\delta_{\text{obsd}} = \frac{2K_{\text{II}}[\text{IPU}]_{\text{free}}^2\Delta}{[\text{IPU}]_{\text{total}}} + \frac{K_{\text{IW}}[\text{IPU}]_{\text{free}}[\text{P}]_{\text{free}}\Delta}{[\text{IPU}]_{\text{total}}} \tag{22}$$

$$[\text{IPU}]_{\text{total}} = [\text{IPU}]_{\text{free}} + 2[(\text{IPU})_2] + [\text{IPU}\cdots\text{P}] \tag{23}$$

The limiting shift difference, Δ, was the value used previously (vide supra) for formation of one hydrogen bond, i.e., 26 ppm. For all five temperatures, these calculations failed to find K_{IW} values that fit the data, regardless of attempts to adjust Δ or $[P]_{free}$. Values calculated for K_{IW} diverged quite strongly at higher water concentrations. Such an observation had been noted previously by Reuben[16] in the acetone–water ^{17}O study. Reuben has suggested a second equilibrium process, namely the interaction of water with two acetone molecules, effective at low water concentrations. Due to the relatively low concentration of IPU in the sample, this type of process is unlikely in the IPU–H_2O–CH_3CN mixtures. However, the hydrogen bonding of two H_2O molecules to the carbonyl is quite likely, i.e.,

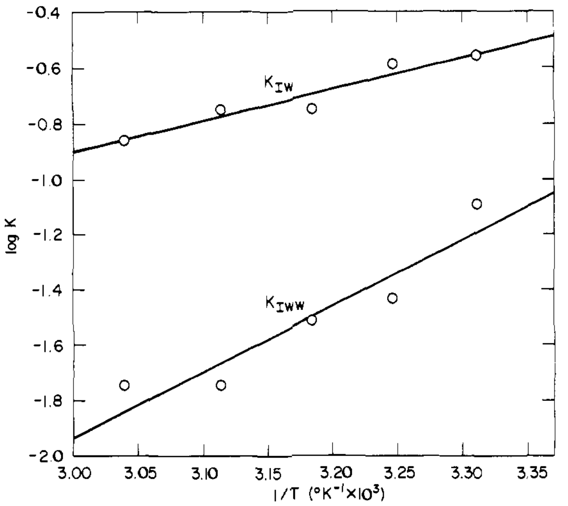

Figure 11. Plot of log K_{IW} and log K_{IWW} vs. the inverse of absolute temperature. The lines were fitted by linear least-squares analysis using all experimental points. The slopes are proportional to the respective hydrogen-bonding interaction enthalpies as determined in the equation $d(\log K)/d(1/T) = -\Delta H/2.3R$, where R is the universal gas constant.

$$\text{IPU} + 2\text{P} \overset{K_{\text{IWW}}}{\rightleftharpoons} \text{IPU}\cdots 2\text{P}$$

and eq 22 and 23 can be adjusted to reflect this second equilibrium (K_{IWW}) as shown in eq 24 and 25.

$$\delta_{\text{obsd}} = \frac{2K_{\text{II}}[\text{IPU}]_{\text{free}}^2\Delta}{[\text{IPU}]_{\text{total}}} + \frac{K_{\text{IW}}[\text{IPU}]_{\text{free}}[\text{P}]_{\text{free}}\Delta}{[\text{IPU}]_{\text{total}}} + \frac{K_{\text{IWW}}[\text{IPU}]_{\text{free}}[\text{P}]_{\text{free}}^2(2\Delta)}{[\text{IPU}]_{\text{total}}} \tag{24}$$

$$[\text{IPU}]_{\text{total}} = [\text{IPU}]_{\text{free}} + 2[(\text{IPU})_2] + [\text{IPU}\cdots\text{P}] + [\text{IPU}\cdots 2\text{P}] \tag{25}$$

When an iterative approach is used in adjusting values of K_{IW}, K_{IWW}, and $[IPU]_{free}$ to fit the observed ^{17}O data, satisfactory fits to the data were obtained for all five temperatures. This can be seen by comparison of the points calculated from these equations and the best fitting curves drawn to fit the experimental points in Figure 2.

The use of 2Δ (52 ppm) as the limiting shift difference for formation of two hydrogen bonds on the carbonyl assumes that the effects of hydrogen bonding on ^{17}O shifts are additive and that the hydrogen bonds are equivalent.[68] This may not be strictly true, as pointed out in models for amides[14] and uracil,[36] but the shift differences for formation of each hydrogen bond probably do not vary by more than the average error in determining experimental ^{17}O shifts (±3 ppm).

The enthalpy changes of both interactions represented by K_{IW} and K_{IWW} were determined from the resepctive slopes of log K vs. $1/T$ plots in Figure 11. The correlation of the K_{IW} points is very good ($r = 0.96$), and the enthalpy determined, -5.2 ± 0.5 kcal/mol, is more accurate than for the individual K_{IW} values. Because of internal consistency, variance in such parameters as Δ or $[P]_{free}$ would translate the fitted line up or down without significantly altering the slope. The correlation for K_{IWW} is $r = 0.94$ and the enthalpy determined was -11.0 ± 1.5 kcal/mol.

Although the dependence of δ_{obsd} on two equilibria is quite clear (vide supra), there are two possible interpretations of the equilibrium process resulting in two hydrogen-bonded water molecules, as shown in eq 26 and 27: one in which the two P bonds are

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[68] Recent nonempirical quantum mechanical calculations of ^{17}O shielding constants have shown nearly identical effects of water hydrogen bonding to each of the carbonyl lone pairs in formamide: Prado, F. R.; Geissner-Prettre, C.; Pullman, A. *Theor. Chim. Acta* **1981**, *59*, 55–69.

$$\mathrm{IPU} + 2\mathrm{P} \underset{}{\overset{K_{\mathrm{IWWA}}}{\rightleftharpoons}} \mathrm{IPU{\cdots}2P} \qquad (26)$$

$$(\mathrm{IPU{\cdots}P}) + \mathrm{P} \underset{}{\overset{K_{\mathrm{IWWB}}}{\rightleftharpoons}} \mathrm{IPU{\cdots}2P} \qquad (27)$$

formed simultaneously (eq 26) and one in which the P bonds are formed sequentially, i.e., the second P bond is formed after the initial formation of an IPU···P complex (eq 27). These two processes cannot be distinguished by chemical shift measurements since δ_{obsd} is only sensitive to the total concentration of IPU···2P, regardless of how formed. If both of these processes are significant, then the K_{IWW} used in eq 24 can be written as

$$K_{\mathrm{IWW}} = K_{\mathrm{IWWA}} + K_{\mathrm{IW}}K_{\mathrm{IWWB}} \qquad (28)$$

Various estimates of the relative contributions of the two terms in eq 28 were utilized in an effort to determine the resulting values of K_{IWWA} and K_{IWWB} as a function of temperature. It was observed that the scatter of points was consistently worse in plots of log K_{IWWB} against $1/T$ compared to plots of log K_{IWWA} against $1/T$. In addition, the estimated enthalpies indicated that K_{IWWA} was the more energetically favorable pathway. In the absence of any more direct experimental evidence, it appeared that K_{IWW} is dominated by K_{IWWA} of the process in eq 26. This is not surprising considering the rapidly exchanging and copperative nature of hydrogen bonds and particularly the tendency for water to aggregate. Thus it is likely that the two water molecules hydrogen bonded to O(4) are themselves part of a water aggregate, of trimer size or larger.

Comparison with Theoretical Methods. The most comprehensive published analysis of hydration models for a uridine derivative is a recently published paper by Del Bene[47] that utilizes ab initio SCF calculations to study hydrogen-bond formation between water and uracil. Many of the conclusions of this study are not directly applicable to our study because they involve hydrogen bonding at the N(1) hydrogen atom, which is shown to be the strongest hydrogen-bonding site for a single water molecule. In uridine nucleosides, this site is precluded due to the glycosidic linkage, so that the energetically preferred hydrogen-bonding sites outlined by Del Bene must be considered with this constraint. Assuming that the remaining electron densities are not significantly perturbed in uridine, relative to uracil, the conclusions of this study can be compared qualitatively to the experimental ^{17}O results. Del Bene's calculations predict two stable H_2O–uracil dimer structures.[47] In one dimer H_2O forms a single hydrogen bond at O(4) (−5.4 kcal/mol) and in the second structure the H_2O is stabilized by two hydrogen bonds ($\simeq$−8.6 kcal/mol), one to the O(4) and another to the proton on N(3). An earlier ab initio study of uracil hydration by Pullman and Perahia[69] reported calculated results qualitatively similar to those of Del Bene[47] with calculated binding energies for a single water bound at O(4) of −4.0 or −7.3 kcal/mol depending on orientation.

Del Bene[47] also calculated electron perturbations caused by water hydrogen bonding at various sites in uracil, in order to estimate the stabilization energies for hydrogen bonding of a second water molecule. The largest increases in electron population occur at O(4), making it an extremely likely site for a second water molecule. On the basis of these theoretical models, it appears that of the two water molecules bonded at the O(4) carbonyl, one has a linear OH···O hydrogen bond that is cis to C(5), while the other water–carbonyl hydrogen bond is nonlinear, is cis to N(3), and is additionally hydrogen bonded via HO···HN3 (see ref 47 for a detailed figure).

Hydrogen Bonding at O(2). The difference in solvent hydrogen bonding ^{17}O shifts between O(4) and O(2) is a very interesting result of this work. One possible explanation for this difference is the formation of an intramolecular hydrogen bond between O(2) and the 5′-OH of IPU. Such hydrogen bonds have been previously deduced for pyrimidines from crystallographic[70] and infrared data.[71] The possibility of these bonds was investigated by observing the ^{17}O spectra of [2-^{17}O]-5′-deoxy- and [2-^{17}O]-5′-iodo-5′-deoxyIPU, in which such intramolecular hydrogen bonds are precluded by the removal of the 5′-OH as a proton donor. These results (Table I) showed only a 4 ± 3 ppm downfield shift for these derivatives with respect to IPU. When a limiting shift difference of 26 ppm is used for the hydrogen bonding of O(2), the observed shift could be interpreted as evidence that approximately 15% of the IPU molecules contain O(2)-5′-OH hydrogen bonds in acetonitrile at 56 °C. Such a result is in qualitative agreement with the interpretation of recent infrared results on IPU in a variety of solvents[71] which estimated the percentage of such intramolecular hydrogen-bonded molecules to be 24% in $CHCl_3$ at room temperature and predicted that in CH_3CN and D_2O this value would be somewhat reduced due to the increased polarity of the solvent. In addition, the lack of temperature dependence for the ^{13}C shift of C(2) is another indication of minimal intramolecular hydrogen bond formation.

Regardless of the exact amount of intramolecular hydrogen bonding at O(2), this contribution is insufficient to explain in itself the observed differences between O(2) and O(4). The theoretical results of Scheiner[72] suggest that a much more likely possibility is that steric effects at O(2), particularly when in the syn conformer, make it impossible for two water molecules to hydrogen bond. Other theoretical studies also suggest that the O(2) may be an intrinsically weaker hydrogen-bond acceptor.[47,73] A preference for O(4) hydrogen bonding was observed by using ^{13}C to study the interactions of uracil derivatives with tripeptides.[74]

Photoelectron studies gave results consistent with CNDO and INDO molecular orbital calculations,[73] indicating definite preference for O(4) hydrogen bonding compared to O(2). A similar theoretical result was found for 1-methylthymine, with increased stabilization energies for O(4) bonding of 1.9 and 0.3 kcal/mol in complexes with acetamide and acetic acid, respectively.[75] From the calculations of Del Bene,[47] little if any significant hydrogen bonding to water occurs at O(2) in uracil.

A brief study of H_2O–[2-^{17}O]-IPU ^{17}O shifts was carried out in an identical fashion to the O(4) study but used 0.022 M [2-^{17}O]-IPU, at three temperatures (29, 41, and 56 °C). The observed signal to noise ratio was significantly less with a consequent increase in experimental error in the ^{17}O shift determinations. However an analysis of the data up to $R \approx 400$ was consistent with the formation of a single O(2)–H_2O hydrogen bond with an estimated enthalpy of formation of −7.9 ± 2 kcal/mol.

π Bond Order. The relationship between π bond order and ^{17}O chemical shift was alluded to previously (vide supra) as one possible contributing factor in observed ^{17}O data. However, the difference in observed limiting shifts for non-hydrogen-bonding O(2) and O(4) (74 ± 5 ppm) cannot be accounted for solely by the difference in calculated π electron densities, which often predict higher π populations on O(2) than on O(4).[34,47] Thus other factors that contribute to σ_p, such as changes in the average value of the inverse cube of the 2p, orbital radius $\langle r^{-3} \rangle$ as suggested by previous studies,[14,44] may be more significant than the $n \rightarrow \pi^*$ transition energy in these molecules.

Conclusions

This research has been the first detailed use of ^{17}O NMR to study hydrogen-bonding interactions of nucleosides, and studies with other substituted uridine derivatives are presently under investigation in our laboratories.[76] The use of ^{17}O NMR techniques has several distinct advantages, namely:

(i) Unlike infrared measurements, specific structural assignments can be made unambiguously, as in uridine, which has two carbonyl groups.

(ii) ^{17}O hydrogen-bonding shifts are relatively sensitive compared to those of other nuclei;[77] and with larger magnetic field

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(75) Remko, M. *Collect. Czech. Chem. Commun.* **1981**, *46*, 957–962.

(76) Schwartz, H. M.; MacCoss, M.; Danyluk, S. S., manuscript in preparation.

strength and improved probe design, better accuracy and sensitivity can presumably be obtained.[78]

(iii) Proton exchange does not create significant problems so that studies in aqueous solution are feasible.

(iv) ^{17}O shifts of carbonyl oxygens are more sensitive to large hydrogen-bonding perturbations than the corresponding carbonyl ^{13}C shifts.

The important specific findings from the present research include the following:

(i) IPU in CH_3CN forms primarily cyclic dimers through the C(4)=O(4) carbonyls of both molecules.

(ii) The hydrogen bonding of water to IPU in CH_3CN occurs mainly through two equilibrium processes. In low concentrations of water, one water molecule hydrogen bonds to O(4) with $K_{1W} = 0.29\ M^{-1}$ at 29 °C and $\Delta H = -5.2$ kcal/mol. At higher concentrations of water, a second process becomes significant in which one water molecule hydrogen bonds to each of the O(4) lone pairs, with $K_{1WW} = 0.061\ M^{-1}$ at 29 °C and $\Delta H = -11.0$ kcal/mol.

(iii) The carbonyl O(2) of IPU in CH_3CN participates relatively weakly in H_2O hydrogen bonding and may be weakly intramolecularly hydrogen bonded to the 5′OH.

Acknowledgment. We thank Professor L. G. Butler for a preprint of his recent work and Rebecca Sheline and James Vaughan for their assistance in various stages of this work. We also thank Dr. Lester Shipman for many useful discussions and Clinton Ainsworth for valuable technical assistance. The support of the Rensselaer Polytechnic Institute Chemistry Department is gratefully acknowledged for the use of the XL-200 spectrometer and the supporting services.

Registry No. [4-^{17}O]-2′,3′,5′-Tri-*O*-benzoyluridine, 86392-68-9; 2′,3′,5′-tri-*O*-benzoyl-4-chlorouridine, 4418-14-8; [4-^{17}O]-uridine, 86392-69-0; [4-^{17}O]-2′,3′-*O*-isopropylideneuridine, 86392-70-3; [2-^{17}O]-2′,3′-*O*-isopropylideneuridine, 86392-71-4; [2-^{17}O]-uridine, 86392-72-5; [2-^{17}O]-5′-iodo-5′-deoxy-2′,3′-*O*-isopropylideneuridine, 86392-73-6; [2-^{17}O]-5′-deoxy-2′,3′-*O*-isopropylideneuridine, 86409-51-0.

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[77] For example, ^{15}N shifts of no more than 8 ppm have been observed for cytidine–guanosine hydrogen bonding: Dyllick-Brensinger, C.; Sullivan, G. R.; Pang, P. P.; Roberts, J. D., *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5580–5582. For derivatives of uridine–adenosine base pairs, limiting shifts of 4.7 and 3.5 ppm for ^{15}N and ^{1}H, respectively, have been reported: Poulter, D.; Livingston, C. L. *Tetrahedron Lett.* **1979**, *755*–758.

[78] Spectra taken on the XL-200 (27.1 MHz for ^{17}O) referred to in Table I could be obtained in about one-tenth of the time required by using the 60-MHz spectrometer. Improvement in both signal to noise and chemical shift accuracy were greatly enhanced as well as enabling larger line widths (i.e., in Me_2SO, $W \sim 1800$ Hz) to be accurately observed.

Communications to the Editor

7-Deoxydaunomycinone, a Catalyst for Achieving a Two-Electron Reduction with a One-Electron Reducing Agent[1]

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Received April 18, 1983

7-Deoxydaunomycinone (**1**) is the product of reductive elimination of daunosamine from the antileukemic drug daunomycin (**2**).[2] The reductive cleavage process leads to reactive intermediates[3] that are proposed to combine with DNA to give **1** covalently bound to DNA.[4,5] Subsequent chemistry of bound **1** as a redox catalyst likely leads to cell death.[6]

Previously we demonstrated that the reducing agent *dl*-bi-(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**3**) reduces **2** to **1** via the transient semiquinone and hydroquinone of **2** and a tautomer of **1**.[3] The reducing agent was 3,5,5-trimethyl-2-oxomorpholin-3-yl (**4**) from bond homolysis of **3**.[7] We now report the anaerobic reduction of **1** to 7-deoxydaunomycinone hydroquinone (**5**) by **4** in one-electron steps and the subsequent reduction of 5,6-dihydro-3,5,5-trimethyl-2-oxazinone (**6**), the product of oxidation of **4**, to 3,5,5-trimethyl-2-oxomorpholine (**7**) by **5** in a two-electron step (Scheme I).

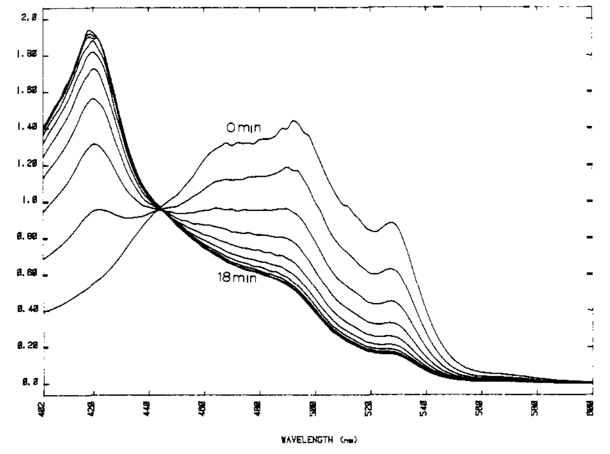
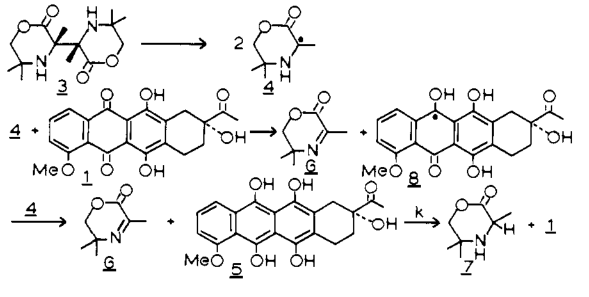


Figure 1. Visible absorption of a rigorously oxygen-degassed methanol solution 1.39 × 10^{-4} M in **1**, 1.39 × 10^{-3} M in **3**, and 2.0 × 10^{-3} M in Trizma buffer at 25.3 ± 0.1 °C as a function of time. Scans were 1 s in duration and occurred every 2 min in the time period 0–18 min.

Scheme I



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[1] This investigation was facilitated by the following support: PHS Grant CA-24665, DHHS; University of Colorado, CCRW faculty fellowship: Developmental Therapeutics Program, NCI; 3M Co.

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