A proton magnetic resonance investigation of the glycosyl torsion angle of uracil nucleosides and nucleotides

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ABSTRACT

The use of line-shape decomposition techniques permitted the small 5-bond ($^{5}J_{51}'$) and 4-bond ($^{4}J_{61}'$) proton-proton coupling constants of a series of uracil nucleosides and nucleotides to be determined accurately. From an analysis of these coupling constants we have determined that the uracil base is in a predominantly anti conformation in aqueous solution and the mean position is not substantially altered by phosphate substitution at the 2', 3', or 5' positions, by changing the furanose stereochemistry from a ribose to a deoxyribose or an arabinose, or by an increase in temperature of 43°C.

INTRODUCTION

The glycosyl torsion angle of uridine and its derivatives in solution has been estimated by circular dichroism (1-6), H(6) chemical shift changes (7-9), H(2') chemical shift changes (10-13), the Nuclear Overhauser Effect (14-15), and by a determination of the long-range coupling between nuclei situated on the base and furanose moieties (13, 16-19).

In basic uridine solutions the resonances of H(5) and H(1') are separated so that they do not obscure each other and thus it is possible to unambiguously determine the 5-bond coupling constant between these nuclei (17). Such splittings are frequently observed in benzylic, unsaturated and bicyclic systems, when an extended planar zig-zag path exists between the nuclei (20-21). This atom arrangement can only occur in uracil nucleosides and nucleotides if the base is in the anti conformation. The dependence of the 5-bond coupling between H(5) and H(1') on the orientation of the intervening bonds (i.e., the glycosyl torsion angle) in pyrimidine nucleosides has recently been calculated.

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using molecular orbital theory (22-23).

To investigate the effect of phosphate substitution and furanose stereochemistry on the glycosyl torsion angle of uridine in aqueous solution, the 5-bond long-range couplings were determined in a series of uracil nucleosides and nucleotides using line-shape decomposition techniques. In addition to measuring $^5J_{51}$, the 4-bond H(6)-H(1') couplings ($^4J_{61}$) were also determined. These investigations were performed both at 37°C and at 80°C to assess the effect of temperature on the orientation of the base relative to the furanose ring.

**EXPERIMENTAL SECTION**

Uracil, uridine, uridine-5'-monophosphate sodium salt (5'-UMP), and 2'-deoxyuridine (dU) were A-grade biochemicals purchased from Calbiochem. Uridine-2'-monophosphate lithium salt (2'-UMP), uridine-3'-monophosphate (3'-UMP), and uracil-$\beta$-D-arabinofuranoside (araU) were obtained from P-L Biochemicals, Inc. The NMR lock compound was hexamethyldisiloxane (HMDS), obtained from K&K Laboratories, Inc.

Solutions containing 0.1 M of the uridine derivative were prepared using double distilled water, treated with Dowex 50 (H$^+$ form, Bio-Rad) and then with Chelex Resin (Bio-Rad). Uracil was then added to give a concentration of 0.1 M and the solution adjusted to pH 12.6 and lyophilized. The samples were redissolved in 99.8% D$_2$O and lyophilized. Lyophilization was repeated once again. They were stored cold and dry until use at which time they were dissolved in 99.8% D$_2$O.

All spectra were taken on a Varian HA-100 NMR spectrometer operated in the frequency sweep internal lock mode. The ambient probe temperature was 37°C; the high temperature runs were performed using the Varian temperature controller. Data acquisition was controlled by an IBM System 7 computer; digitized data were transferred to an IBM 370/195 computer for analysis. A 12-bit analog-to-digital converter provided a resolution of 1 part in 4096; the data point density was 50 points/Hz, the scan width was 50 Hz, and the sweep time was 250 sec. Where necessary to improve signal-to-noise, several scans were averaged; multiple data smoothing using the technique described by Savitsky and Golay (24) was also used to reduce random noise.
RESULTS AND DISCUSSION

The problems encountered in the determination of accurate peak locations and amplitudes from NMR spectra are well known. Even if the conditions for a first-order analysis are met, it is not necessarily true that the appropriate NMR parameters can be determined directly from the spectrum. If there is significant overlap between two peaks, the peak locations can be shifted or even obscured. In such cases spectral decomposition must be performed to obtain accurate peak locations and intensities from overlapping peaks.

The computer program DECOMP (25) was used to analyze the spectra obtained in this investigation. This program uses a least squares method to fit the overlapping resonances utilizing a well separated single peak in the spectrum as a line-shape standard. The references resonance may be fitted to a Lorentzian lineshape or used directly as a digital machine-line shape function. In our studies we used the H(5) and H(6) resonances of uracil as an internal line-shape standard. Both Lorentzian and digital machine line-shape functions were used; both methods gave identical results.

As can be seen from Figure 1, the H(5) resonance of dU is split by two protons which are not magnetically equivalent; the H(6) proton is responsible for the observed splitting of 7.6 Hz while the smaller splitting is due to the proton at H(1'). The apparent value of $^{5}J_{51}$, obtained from the peak splitting is 0.4 Hz, in excellent agreement with the results previously reported by Hruska (17). In several cases, e.g., 2'-UMP + uracil, broad H(5) resonances were observed. In all cases the H(6) resonances showed no splitting whatsoever. In such instances, the use of decomposition techniques are mandatory. Figure 2 shows the experimental spectrum for the H(5) region of 2'-UMP and the theoretical fit using DECOMP. The results of spectral decomposition for the H(5) and H(6) resonances of the compounds studies are presented in Table 1. (The estimated experimental uncertainty, 0.02 Hz, is the average error of the results obtained from fitting each of the two peaks with each of the two standard uracil line-shapes.) For all compounds studied, the values of $^{5}J_{51}$ were identical and independent of temperature within experimental error. Likewise, the values of $^{4}J_{61}$ were found to be identical. Hence,
we conclude that the mean glycosyl torsion angle is not significantly influenced by placement of a phosphate group in the 2', 3', or 5' positions, by furanose stereochemistry, or by changes in temperature.

Giessner-Prettre and Pullman (22-23) have theoretically calculated the values of $^4J_{61}$ and $^5J_{51}$ as a function of glycosyl torsion angle for uridine with the ribofuranase ring in the C(2')-endo and C(3')-endo conformations using a finite perturbation method with either INDO or CNDO approximations. The results obtained by use of these approximations agreed qualitatively although quantitative differences were apparent. The calculations revealed a slight dependence of $^4J_{61}$ and $^5J_{51}$ on ribofuranose ring conformation. In solution the furanose ring exhibits rapid interconversion predominantly between the C(2')-endo and C(3')-endo conformational forms. The values of $^3J_{12}$ for all compounds studied (except araU and dU) are within 0.3 Hz, suggesting a very close similarity in the equilibrium distribution of ribofuranose ring conformers (26-27). Giessner-Prettre and Pullman (22) calculated $^5J_{51}$ and $^4J_{61}$, values of 0.5 and 0.3 Hz, respectively, using the CNDO approximation for uridine whose glycosyl torsion angle was fixed at $\varphi = 250^\circ$ (anti conformation - we have

![Figure 1. Detail of the H(5) region in the NMR spectrum of a solution of deoxyuridine and uracil, pD = 12.6, 37°C. This spectrum was traced by the spectrometer recorder.](image-url)
Figure 2. (A) Detail of the H(5) region in the digitized smoothed NMR spectrum of a solution of 2'-UMP and uracil, pD = 12.6, 37°C. This spectrum was superimposable on a spectrum traced by the spectrometer recorder except for reduced noise due to smoothing. (B) Spectrum of the H(5) region of 2'-UMP and uracil calculated by the program DECOMP, assuming coupling between H(5) and H(1') using the uracil H(5) resonances as internal line-shape standards. A Lorentzian line-shape was assumed. The position and intensity of the resolved contributions are indicated by the vertical bars.

adopted a recently recommended convention (28)) and with the ribofuranose ring in the C(2')-endo conformation. Employing the INDO approximation results in somewhat different values for the same glycosyl torsion angle: $5J_{51'}$, (C(2')-endo) = 0.8 Hz, $5J_{51'}$, (C(3')-endo) = 0.5 Hz, $4J_{61'}$, (C(2')-endo) = -0.1 Hz, $4J_{61'}$, (C(3')-endo) = -0.2 Hz. In the syn conformation ($\chi = 60^\circ$) both approximations yield calculated values of $5J_{51'}$, which are small ($|5J_{51'}| \approx 0.1$ Hz) regardless of the adopted ribofuranose ring conformation. Similarly for $4J_{61'}$, absolute calculated values of 0.3 to 0.7 Hz were obtained. Thus, the only geometry consistent with both the
calculated and observed coupling constants would be one which places the uracil base predominantly in the *anti* conformation. Due to the uncertainty in the values of the calculated coupling constants it is impossible to rule out the existence of a small amount of *syn* conformer. (In order to compare our data with theoretical results we have assumed that ionization of N(3) and substituent changes at C(2') produce negligible electronic effects on the long range coupling between protons of the base and H(1'). This assumption is supported by the observation that 5-bond long-range coupling in mono-substituted benzaldehydes is independent of substituent electronic effects (20) and by calculations (23) which show that $J_{51}$ for 6-methyluridine is very similar to that for uridine.)

**TABLE 1**

Results of spectral decomposition of the H(6) and H(5) regions of several uracil nucleosides and nucleotides

<table>
<thead>
<tr>
<th></th>
<th>$5J_{51}$ (Hz)$^b$</th>
<th>$4J_{61}$ (Hz)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37°C</td>
<td>80°C</td>
</tr>
<tr>
<td>Uridine</td>
<td>0.48</td>
<td>0.46</td>
</tr>
<tr>
<td>2'-UMP</td>
<td>0.50</td>
<td>0.51</td>
</tr>
<tr>
<td>3'-UMP</td>
<td>0.51</td>
<td>0.51</td>
</tr>
<tr>
<td>5'-UMP</td>
<td>0.51</td>
<td>0.50</td>
</tr>
<tr>
<td>dU</td>
<td>0.53</td>
<td>0.50</td>
</tr>
<tr>
<td>AraU</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

$^a$ Determined at pD = 12.6. Estimated uncertainty is ± 0.02 Hz.

$^b$ The signs of the coupling constants could not be determined.

Calculations (29) assuming van der Waal's radii of the atoms in pyrimidine nucleosides have shown that the freedom of rotation about the glycosyl linkage is closely correlated with the type of puckering of the furanose ring. Thus, if phosphate substitution...
were to significantly alter the furanose ring conformation a change in the average value of the glycosyl torsion angle would be expected. The effect of phosphate substitution on nucleoside conformation in the solid state has been examined by Sundaralingam (30). This study vividly illustrates the minimal effect of such substitution on both the furanose ring conformation and the glycosyl torsion angle in the solid state for the compounds uridine, deoxyuridine, 3'-UMP, and 5'-UMP. Potential energy calculations reveal that the anti glycosyl conformer is preferred in uridine and uracil-β-D-arabinofuranoside (29-31). In solution where the conformation of the molecules is not expected to be static, NMR investigations of uridine (26), deoxyuridine (26), 3'-UMP (26), and 5'-UMP (27) have shown that the furanose rings of these compounds is a rapid time-average blend of conformers with phosphate substitution exerting virtually no effect on the equilibrium distribution. Our results complement the crystal studies and the solution NMR investigations. Evidently phosphate substitution does not change the furanose ring conformation or glycosyl torsion angle of pyrimidine nucleosides in solution.

Our results concerning the effect of furanose stereochemistry on the glycosyl torsion angle are in accord with the studies of Fox (16) who observed equal values of $^5J_{F(5)H(1')}$ for 5-fluorouridine and the corresponding arabino- and deoxyribo- derivatives. Both Eyring (5) and Tinoco (6) found from calculations of the optical activity of nucleosides by Kirkwood coupled oscillator theory that a change in furanose stereochemistry at C(2') from ribo- to arabino- would only slightly change the circular dichroism for a fixed torsion angle. Experimentally, however, relatively large differences exist between the circular dichroism of uridine and uracil-β-D-arabinofuranoside which have led these workers to conclude that either the sugar conformation or the glycosyl torsion angle is altered. The results of this study rule out significant changes in torsion angle. Since the changes in furanose ring conformation are likely to be small (26-27) the differences in circular dichroism may be due to one-electron contributions to optical activity not considered by these authors.

CONCLUSION

Proton magnetic resonance evidence has been presented which
indicates that the mean glycosyl torsion angle of uridine in solution is predominantly anti and is not substantially altered by a change in sugar stereochemistry at C(2'), by the attachment of a phosphomonoester group to the 2', 3', or 5' positions, or by changes in temperature.

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