

FOURIER-TRANSFORMED  $^{13}\text{C}$  NMR SPECTRA OF POLYURIDYLIC ACID,  
URIDINE, AND RELATED NUCLEOTIDES - THE USE OF  $^{31}\text{POC}^{13}\text{C}$  COUPLINGS  
FOR CONFORMATIONAL ANALYSIS

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ABSTRACT

The  $^{13}\text{C}$  magnetic resonance spectra of polyuridylic acid, uridine, and related nucleotides in aqueous solution are reported. Earlier assignments of the 2' and 3' resonances are corrected. Conformational information is derived from the chemical shifts and from the magnitudes of  $^{31}\text{POC}^{13}\text{C}$  couplings. These couplings are shown to be particularly useful. The polynucleotide backbone is found to have distinct conformational preferences.

Nuclear magnetic resonance of  $^{13}\text{C}$  (CMR) is now a common tool in organic chemistry. Many correlations of substituent and conformational effects with  $^{13}\text{C}$  chemical shifts and  $^{13}\text{C}$ - $^1\text{H}$  coupling constants have been made; these are amply described in two recent reviews (1,2). Much less has been done in applying the technique to large biological molecules. Recently the CMR spectra of gramicidin S-A (3), a decapeptide antibiotic, and the enzymes ribonuclease A (4,5) and lysozyme (6) have been described. The resonances of the monomeric amino acids (7,8), nucleosides (9,10), and 5'-nucleotides (11) have been reported.

We describe here the CMR spectrum of polyuridylic acid (poly U) of molecular weight 130,000, and comparison with the high resolution spectra of uridine (U), uridine-2'-phosphate (2'-UMP), uridine-3'-phosphate (3'-UMP), and uridine-5'-phosphate (5'-UMP). Earlier assignments of the 2' and 3' resonances of U (9,10) and 5'-UMP (11)

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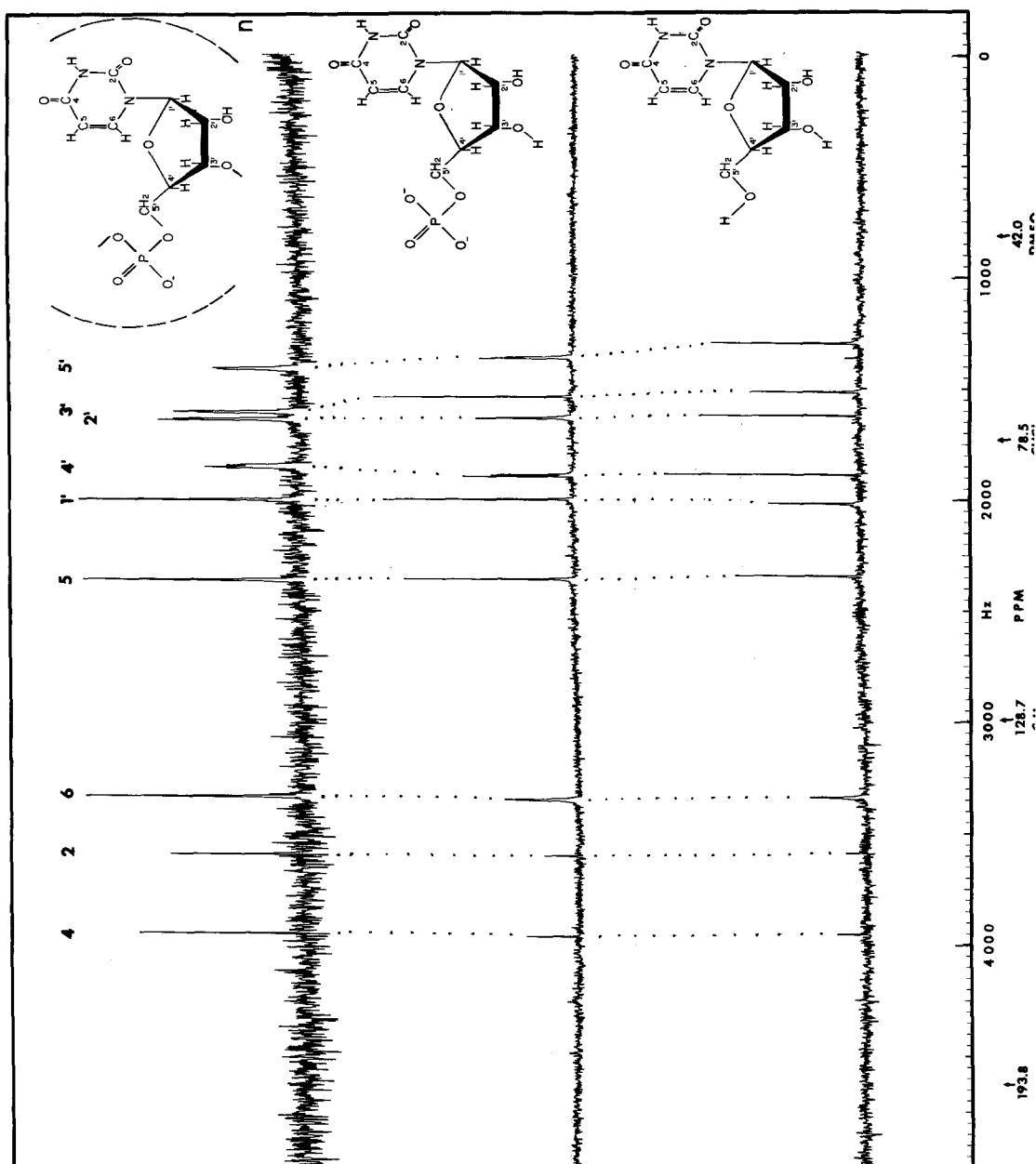


Figure 1. CMR spectra of 0.2 M solutions of poly U (40,000 accumulations), 5'-UMP (12,000 accumulations), and U (12,000 accumulations) in  $D_2O$ ,  $pD 7.8 \pm 0.6$ ,  $37^\circ C$ . Increasing positive values indicate increasing frequency (decreasing field).

are corrected. Aspects of the conformation of poly U are inferred from the  $^{13}\text{C}$  chemical shifts and the  $^{31}\text{POC}^{13}\text{C}$  coupling constants.

#### EXPERIMENTAL

Spectra were obtained on a Varian XL-100-15 spectrometer, operating in Fourier transform mode with proton noise decoupling. Data were accumulated in a Varian 620i computer using either 5000, 2500, or 1000 Hz sweep widths in 4096 points (resolution of 2.4, 1.2, and 0.6 Hz respectively). Data acquisition and pulse delay times were 0.4 and 0.8 sec. (recycle time 1.2 sec.) in the first case, 0.8 and 0.8 sec. in the second case, and 2.0 and 0 sec in the third case. Chemical shifts were relative to external  $\text{C}_6\text{H}_6$ , and converted to the TMS scale using  $\delta_{\text{C}}^{\text{C}_6\text{H}_6} = 128.65$  ppm. Some representative spectra are shown in Figure 1, and the data are summarized in Figure 2 and Table 1.

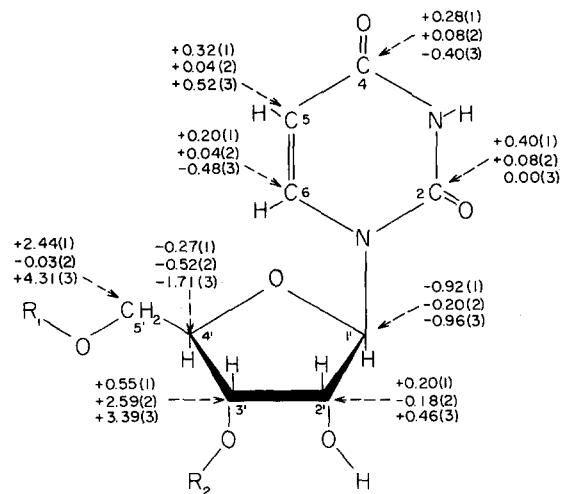


Figure 2. Differences between the chemical shifts of the carbon atoms in uridine and the corresponding carbons in (1) 5'-UMP,  $R_1 = \text{p}$ ,  $R_2 = \text{H}$ ; (2) 3'-UMP,  $R_1 = \text{H}$ ,  $R_2 = \text{p}$ ; (3) poly U,  $R_1 = R_2 = \text{oligo U}$ . A positive difference indicates that the resonance lies to higher frequency (lower field) than the corresponding resonance in uridine.

TABLE 1  
 $^{13}\text{C}$  Chemical Shifts and  $^{31}\text{P}$  -  $^{13}\text{C}$  Coupling Constants of Aqueous Solutions  
 of Polyuridylic Acid and Related Compounds.<sup>a</sup>

COMPOUND	$\delta_{\text{C}}^{\text{TMS}}$	$\text{C}_4$	$\text{C}_2$	$\text{C}_6$	$\text{C}_5$	$\text{C}_1$	$\text{C}_{4'}$	$\text{C}_{2'}$	$\text{C}_{3'}$	$\text{C}_{5'}$
URIDINE	167.33	152.82	142.96	103.42	90.54	85.37	74.84	70.55	61.92	
5'-UMP	167.61	153.22	143.16	103.74	89.63	85.11 <sup>c</sup>	75.04	71.10	64.37 <sup>b</sup>	
3'-UMP	167.41	152.90	143.00	103.46	90.34	84.86 <sup>d</sup>	74.65 <sup>e</sup>	73.14 <sup>b</sup>	61.88	
2'-UMP	167.73	153.22	143.96	103.66	90.32 <sup>f</sup>	85.33	76.51 <sup>b</sup>	70.96 <sup>g</sup>	62.32	
poly-U	166.93	152.82	142.49	103.93	89.59	83.66 <sup>h</sup>	75.30 <sup>i</sup>	73.95 <sup>b</sup>	66.24 <sup>b</sup>	

a) Chemical shifts are expressed in parts per million relative to external TMS. Positive values indicate increasing frequency (decreasing field). The shifts and coupling constants are estimated to be accurate to  $\pm 1 \text{ Hz}$ ,  $\pm 0.04 \text{ ppm}$ .

b) Doublets,  $2\text{J}_{31\text{PO}13\text{C}} = 4.5 \text{ Hz}$ . f) Doublet,  $3\text{J}_{31\text{POC}_2, 13\text{C}_1} = 9 \text{ Hz}$ .  
 c) Doublet,  $3\text{J}_{31\text{POC}_5, 13\text{C}_4} = 8.5 \text{ Hz}$ . g) Doublet,  $3\text{J}_{31\text{POC}_2, 13\text{C}_3} = 3 \text{ Hz}$ .  
 d) Doublet,  $3\text{J}_{31\text{POC}_3, 13\text{C}_4} = 6 \text{ Hz}$ . h) Quartet,  $3\text{J}_{31\text{POC}_5, 13\text{C}_4} = 7 \text{ Hz}$ ;  $3\text{J}_{31\text{POC}_3, 13\text{C}_4} = 3 \text{ Hz}$ .  
 e) Doublet,  $3\text{J}_{31\text{POC}_3, 13\text{C}_2} = 2.5 \text{ Hz}$ . i) Doublet,  $3\text{J}_{31\text{POC}_3, 13\text{C}_2} = 5 \text{ Hz}$ .

Sources of chemicals were: Raylo (uridine); Sigma (5'-UMP, Na salt, and 3'-UMP, Li salt); PL-Biochemicals (2'-UMP, Li salt); Miles (poly U, NH<sub>4</sub> salt); and Calbiochem (poly U, K salt).

Resonances were assigned by comparison of the data for the various derivatives, and by consideration of literature data (9-14). For U (9,10) and 5'-UMP (11) our assignments are in accord with the literature values, with the exception of the 2' and 3' resonances whose assignments must be reversed.

#### RESULTS AND DISCUSSION

Appreciable chemical shifts can be observed on phosphorylation of the various hydroxyl groups of uridine. For the 5' carbon, which is unequivocally assignable due to the known shielding effect in hydroxymethyl groups (15), phosphorylation deshields the <sup>13</sup>C resonance by 2.44 ppm (Table 1, Figure 2). A similar effect is expected in comparing the 3' resonances of U and 3'-UMP; we assign the resonance at 70.55 ppm in uridine to the 3' carbon because it is shifted by +2.59 ppm on phosphorylation at 3', whereas the resonance at 74.84 is nearly unaffected. A similar corroborative argument can be made from the data for 2'-UMP (Table 1).

Couplings between <sup>13</sup>C and <sup>31</sup>P are manifest in the spectra (Figure 1, Table 1). As was noted by Dorman and Roberts (11), the <sup>31</sup>P coupling in 5'-UMP is greater at the 4' than at the 5' carbon. By analogy with the behaviour of three bond couplings for <sup>31</sup>POC<sup>1</sup>H and <sup>1</sup>HCC<sup>1</sup>H, we might expect the <sup>31</sup>POC<sup>13</sup>C couplings to depend on dihedral angle in a manner similar to the Karplus relationship (16). On the other hand, we have found the two bond <sup>31</sup>PO<sup>13</sup>C couplings of phosphomonoesters such as those of nucleotides to be centered around 5 Hz (17). The presence of a 4.5 Hz <sup>31</sup>P-<sup>13</sup>C coupling on the resonance of 3'-UMP at 73.14 ppm confirms the relative assignments of C<sub>2</sub>, and C<sub>3</sub>. Further confirmation comes from a similar consideration of the data for 2'-UMP (Table 1).

Figure 2 presents the changes in chemical shifts experienced by the various carbon atoms in the uridine moiety in 5'-UMP, 3'-UMP and poly U. Combination of the effects in 5'-UMP and 3'-UMP gives an estimate of the hypothetical shift for the pUp repeating unit of poly U. Deviations from additivity are taken as indications of secondary or tertiary structure effects. They occur for C<sub>2</sub>, C<sub>4</sub>, C<sub>6</sub>, C<sub>4</sub>, and C<sub>5</sub>. Upfield shifts of carbonyls are indicative of weaker hydrogen bonds in the polymer than in the monomers (18) - the effect is greater for C<sub>4</sub> (0.76 ppm) than for C<sub>2</sub> (0.48 ppm). The shielding effect on C<sub>6</sub> suggests that this position is less acidic in poly U than in the monomer. The large shifts at C<sub>4</sub>, and C<sub>5</sub>, may be related to an alteration in the position of the phosphate group relative to the ribose ring. Such an alteration is also suggested by the  $^3J_{^3\text{P}-^1\text{C}_5}$ , and comparable PMR data (19).

The magnitudes of the three bond  $^{13}\text{C}$ - $^{31}\text{P}$  couplings,  $^3J_{^3\text{P}-^1\text{C}}$ , listed in Table 1 provide considerable insight into molecular conformation. In 5'-UMP,  $^3J_{^3\text{P}-^1\text{C}_4}$ , is 8.5 Hz, whereas in 3'-UMP  $^3J_{^3\text{P}-^1\text{C}_4}$ , is 6 Hz and  $^3J_{^3\text{P}-^1\text{C}_2}$ , is 2.5 Hz. In 2'-UMP,  $^3J_{^3\text{P}-^1\text{C}_1}$  is 9 Hz, and  $^3J_{^3\text{P}-^1\text{C}_3}$ , is 3 Hz. These couplings are obviously sensitive to the relative populations of the rotamers about the corresponding CO bonds. By analogy with  $^1\text{H}$ - $^1\text{H}$ , and  $^1\text{H}$ - $^{31}\text{P}$  couplings, we would expect large couplings (approximately 10 Hz) for trans rotamers, and small couplings (approximately 1 Hz) for gauche rotamers. Thus, a preference is indicated in 5'-UMP for the 4'-trans rotamer, in 2'-UMP for the 1'-trans rotamer and in 3'-UMP (somewhat less) for the 4'-trans rotamer. The corresponding data for poly U imply that the 5'-phosphate has a preference for the 4'-trans rotamer, and the 3'-phosphate has a preference for the 2'-trans rotamer. Consideration of the approximate data available for the corresponding  $^3J_{^3\text{P}-^1\text{H}}$  couplings in poly U (20), yields a similar conclusion. Our conclusions and assignments are consistent

with the corresponding  $^{13}\text{C}$  and  $^1\text{H}$  data for uridine-2',3'-cyclic phosphate, uridine-3',5'-cyclic phosphate, uridine-5'-diphosphate, uridine-5'-triphosphate, uridylyl-3',5'-uridine, deoxyuridine, and deoxyuridine-5'-phosphate, which will be reported subsequently (17).

The relaxation times of the  $^{13}\text{C}$  resonances can provide further information about molecular conformation. With our present experimental arrangement we cannot measure  $T_1$  directly, but we can draw qualitative conclusions about relative  $T_1$ 's from the relative amplitudes of resonances due to carbons with the same expected Overhauser enhancement. For example, with uridine in  $\text{D}_2\text{O}$  carbon 2 relaxes more slowly than carbon 4, and carbon 5 more slowly than carbon 6. This cannot be due only to relative mobilities or different nuclear Overhauser effects, and is apparently related to interaction with solvent. We have found large differences in the relative relaxation rates of the carbon atoms of uridine in going from  $\text{D}_2\text{O}$  to  $\text{H}_2\text{O}$  or dimethyl sulfoxide- $d_6$ . It is significant that in poly U the relaxation times of all carbon atoms, including the carbonyls, become much shorter than the corresponding values in the monomers. This is due to the relative immobility of all carbon atoms in the polymer, and the consequent long correlation time for molecular reorientation (21).

#### CONCLUSIONS

Previous assignments of the 2' and 3'  $^{13}\text{C}$  resonances of ribonucleosides (9,10) and ribonucleotides (11) are incorrect, and should be reversed. Three bond  $^{13}\text{C}$ - $^{31}\text{P}$  couplings are sensitive to dihedral angle and may be used to determine the conformations of nucleotides and polynucleotides. Polyuridylic acid manifests conformational preferences in aqueous solution, favouring the 4'-trans rotamer for the 5'-phosphate and the 2'-trans rotamer for the 3'-phosphate.  $^{13}\text{C}$  NMR should provide a powerful tool for analysis of nucleic acid conformation.

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