

Table I. Hyperfine Splittings Calculated by INDO¹³

CCCC dihedral angle, deg	C-C-C angle, deg	Energy, au	$a^{13}\text{C}$, G	$a^{1}\text{H}$, G
41.69	114.0	-34.26398439	74.73	27.25
38.13	115.0	-34.26920943	64.47	27.95
34.18	116.0	-34.27427814	59.96	28.67
29.67	117.0	-34.27919785	52.16	29.42
24.28	118.0	-34.28397676	44.04	30.20
17.20	119.0	-34.28862434	35.50	31.02
0.0	120.0	-34.29315149	26.90	31.89

were calculated for 1° increments in the bond angle. The INDO calculation predicts a planar radical but the deformation energy is predicted to be small. The bond angle corresponding to the observed ¹³C hfs is 117.8°.

The geometry of the *tert*-butyl radical was also determined by *ab initio* molecular orbital calculations using the STO-3G basis.^{13,14} Because of computer time considerations, the methyl groups were constrained to be tetrahedral, and the radical was constrained to have C₃ symmetry. The optimum values for the CC and CH bond lengths, the CCC bond angles, and the HCCC dihedral angles¹⁵ are 1.526 Å, 1.087 Å, 117.6°, and 14.8°, respectively.

The ~117.5° bond angle indicated by our work is outside the error limits indicated by Pauling for his relationship if we assume $E(\text{M}) - E(\text{X}) = 0$. However, it is probably incorrect to take the electronegativity of the methyl group equal to that of carbon. In fact, one might use the measurement of the bond angle in the *tert*-butyl radical as a measure of the electronegativity of the methyl group. A calculation using Pauling's equations and our bond angle yields an electronegativity of 2.36 for methyl relative to 2.5 for carbon. Substitution of carbon for hydrogen at the β position would be expected to make the radical more nearly tetrahedral; thus *tert*-butyl would be expected to be the most nearly planar of all tertiary alkyl radicals.

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(15) The CCCH dihedral angle refers to the angle with respect to the CCC plane which places the CH bond anti to the CC bond and on the side of the reference plane opposite to the out-of-plane carbon atom.

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A Carbon-13 and Hydrogen-1 Nuclear Magnetic Resonance Study of the Conformations of 3',5'- and 2',3'-Cyclic Nucleotides. A Demonstration of the Angular Dependence of Three-Bond Spin-Spin Couplings between Carbon and Phosphorus

Sir:

It has been demonstrated recently that the ¹³C nuclear magnetic resonance spectra of nucleotides¹⁻³ and polynucleotides^{2,3} contain three-bond couplings between ¹³C and ³¹P which vary from 1 to 10 Hz in magnitude. It has been suggested that these couplings are sensitive to the dihedral angles between the planes ³¹P-O-C and O-C-¹³C in these compounds, and therefore are useful for conformational analyses.^{2,3} To test this hypothesis we have studied the ¹³C and ¹H nmr spectra of the 3',5'- and 2',3'-cyclic nucleotides of uridine (U), cytidine (C), adenosine (A), and guanosine (G), and also of thymidine 3',5'-cyclic phosphate and *N*⁶,*O*^{2'}-dibutyryl adenosine 3',5'-cyclic phosphate. Our data confirm the angular dependence of the ³J_{POCC}, and yield values for the trans and gauche couplings in these compounds of 8 and 2 Hz, respectively.

X-Ray studies on single crystals of adenosine 3',5'-phosphate⁴ and adenosine 3',5'-phosphonate⁵ have indicated that the ribose and phosphate rings of these compounds are rigid, and that the ribose ring has the twist conformation 3'-endo,4'-exo (³T₄)⁵ or 4'-exo,3'-endo (*T*³).⁴ Molecular models indicate that, in contrast to the uncyclized 3' and 5' nucleotides,⁶⁻⁹ the ribose rings of these compounds are incapable of converting between various puckered forms. They thus serve as excellent models for the calibration of the proposed angular dependence. In the ³T₄ conformation⁵ the phosphate is trans relative to C₂' and gauche relative to C₄'.

¹³C resonances were assigned by comparison with earlier data on nucleosides and nucleotides.^{2,3} The ³¹P splittings are evident on the resonances of carbons 2', 3', 4', and 5'. In the six 3',5'-cyclic nucleotides studied, the couplings P-C₂' are 8.0 ± 0.3 Hz, which gives a value for the trans coupling. On the other hand, the P-C₄' couplings are 4.6 ± 0.2 Hz. Since the gauche coupling takes place *via* two routes (PO-C₅'C₄' and POC₃'C₄') we can only estimate the single path gauche coupling to be approximately 2 Hz. It is also interesting to note that the ²J_{POC} couplings to carbons 3' (4.2 ± 0.5 Hz) and 5' (7.2 ± 0.2 Hz) are different, but essentially constant throughout the series. Although they are not expected to be angular dependent, these couplings do reflect the different environments or

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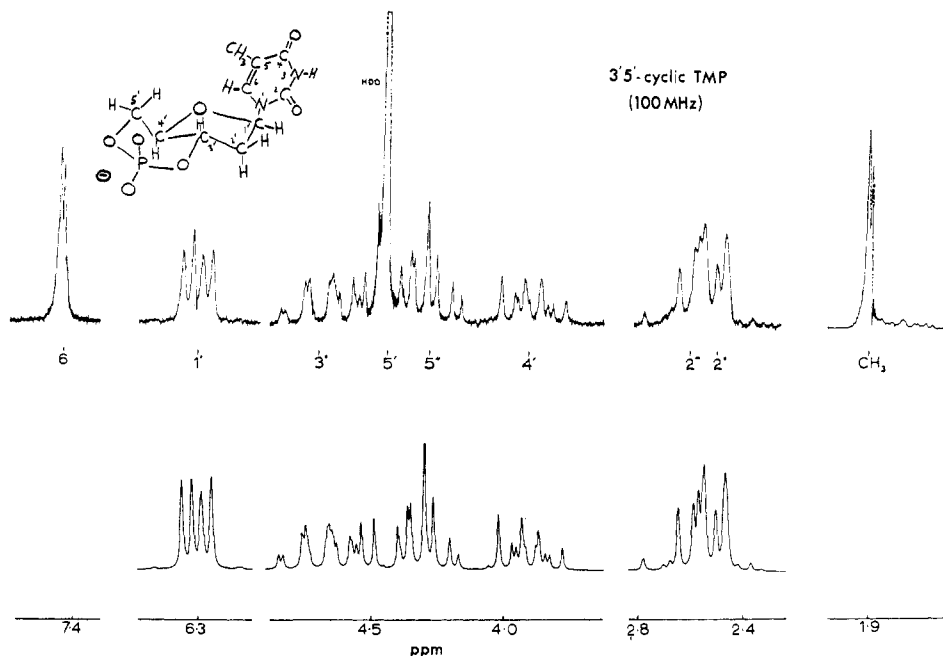


Figure 1. ^1H nmr spectrum (Varian XL-100) of a 0.2 M solution of thymidine 3',5'-cyclic phosphate in deuterium oxide, pD 6.8, 50°. Beneath the spectrum is a computer simulation using the parameters derived by an iterative analysis using LAOCOON III.

bond angles due to ring strain at carbons 3' and 5'. In all the noncyclic nucleotides studied to date,¹⁻⁸ these 2J have been found to be essentially constant and equal to 5.0 ± 0.6 Hz.

A confirmation that the 3',5'-cyclic nucleotides are rigidly fixed in the 3T_4 conformation comes from analysis of the ^1H nmr spectra of thymidine 3',5'-cyclic phosphate, Figure 1, adenosine 3',5'-cyclic phosphate,¹⁰ and $N^6,O^{2'}$ -dibutyryladenosine 3',5'-cyclic phosphate.¹⁰ It has been demonstrated that the $^1\text{H}-^1\text{H}^{6-9}$ and $^31\text{P}-^1\text{H}^{6,11-18}$ couplings may be used to ascertain the conformations of such compounds. Taking J_t and J_g values of 10.1 and 2.1 Hz for $^1\text{H}-^1\text{H}^{7-9}$ and 21 and 2 Hz for $^1\text{H}-^31\text{P}$ couplings^{11,12} we find the couplings predicted for the 3T_4 conformation from the molecular model are approximately in accord with experimental values. However, discrepancies of up to 1 Hz are evident. It is probable that the angles and bond lengths in these molecules vary from those predicted by molecular models for pure sp^3 carbon atoms, and that substituent effects modify individual couplings differently. Such variances in the couplings are not sufficient, however, for the data to be inconsistent with the 3T_4 conformation, and we can rely on the J_t and J_g values determined from these compounds for the $^31\text{P}-^13\text{C}$ three-bond couplings within small error limits.

In an extension of this study we have analyzed the ^{13}C and ^1H nmr spectra of the 2',3'-cyclic nucleotides. X-Ray studies have shown that in the solid state uridine 2',3'- O,O -cyclophosphorothioate¹⁴ and cytidine 2',3'-cyclic phosphate¹⁵ have the four carbon atoms of the

ribose ring in a plane, with the ribose ether oxygen puckered exo. Molecular models suggest that the 2',3'-cyclic phosphates may have considerable conformational flexibility. This is confirmed by both the ^{13}C and ^1H nmr data. The different couplings between P and $\text{H}_{2'}$ and $\text{H}_{3'}$ ($J_{\text{P}-\text{H}_{2'}} = 6.9$ (U), 6.5 (C), 10.6 (A), and 8.9 (G) Hz and $J_{\text{P}-\text{H}_{3'}} = 11.5$ (U), 12.1 (C), 7.6 (A), and 9.5 (G) Hz) and between P and $\text{C}_{1'}$ and $\text{C}_{4'}$ ($J_{\text{P}-\text{C}_{1'}} = 6.8$ (U), 6.3 (C), 3.8 (A), and 4.8 (G) Hz, and $J_{\text{P}-\text{C}_{4'}} = 2.5$ (U), 2.2 (C), 4.3 (A), and 3.5 (G) Hz) demonstrate that the four carbon atoms cannot be coplanar, but must be in a conformational equilibrium between ring-puckered forms. The pyrimidine cyclic nucleotides demonstrate a preference in this equilibrium for the 3'-endo pucker of the ribose ring, while the purine cyclic nucleotides have a preference (less) for the 2'-endo pucker. It is worthy of note in this case that due to the greater sensitivity of the $^31\text{P}-^1\text{H}$ coupling constants, relative to those between $^1\text{H}-^1\text{H}$ or $^31\text{P}-^13\text{C}$, to dihedral angle they are better suited for conformational analysis of nucleotides.

The present data on $^31\text{P}-^1\text{H}$ couplings in both the 2',3', and 3',5'-cyclic phosphates allow us to check the validity of the J values used previously in conformational analyses of nucleosides and nucleotides using $^1\text{H}-^1\text{H}$ couplings.⁶⁻⁹ We find that the values used earlier were justified.

This study demonstrates the usefulness of a multinuclear magnetic resonance approach to conformational analysis, and confirms the dihedral angle dependence of $^31\text{P}-^13\text{C}$ couplings in phosphates.

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