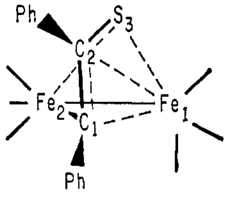

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thioketocarbene through π-complex formation is of interest concerning the nature and mechanism of 1,3-dipolar cycloaddition reactions and can probably be extended to other reactive intermediates of this general type.

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Conformational Analysis of a Minor Nucleoside from Nuclear Magnetic Resonance Data. Pseudouridine

Sir:

We report here a complete analysis of the 100-MHz nmr spectrum of β-pseudouridine (βψ), and the resulting model for the three-dimensional structure. A 60-MHz spectrum has previously appeared,[1] but no detailed analysis was attempted. βψ is a modified nucleoside found in all tRNA sequenced to date.[2,3] It differs from the normal nucleosides in that the uracil is attached to the ribose *via* a C–C glycosyl bond, making the NH group at position one available for hydrogen bonding (Figure 1).

The 100-MHz spectrum was analyzed using the computer program LAOCOON II[4] modified to give spin-tickling information. A simulated spectrum for the 2′–5′ hydrogens is shown above the relevant portion of Figure 1. The assignment was confirmed by double irradiation. The resultant shifts and coupling constants are in Table I. βψ was a product of Calbiochem. Anomeric purity was confirmed by comparison of the nmr data with those for the α anomer.

The data allow three important conclusions to be made regarding (i) rotamer population about the exocyclic $C_{4'}$–$C_{5'}$ bond, (ii) puckering of the ribose ring, and (iii) the sugar–base torsion angle.

(i) The vicinal proton coupling constants are known to depend on the relevant dihedral angles.[5] Thus, $J_{4',5'B}$ and $J_{4',5'C}$ yield information about the relative rotamer populations. The following assumptions are made in the analysis: the *J*'s are rapid time averages weighted over the classical staggered rotamers; the *gauche* and *trans* couplings do not vary among the rotamers; the Karplus equation as modified by Abraham, *et al.*,[6] for carbohydrates applies. Therefore $J_{4',5'B} = 2(P_I + P_{II}) + 10.1P_{III}$, and $J_{4',5'C} = 2(P_I + P_{III}) + 10.1P_{II}$ Hz, where the P_i are fractional populations $\left(\sum_{i=I}^{III} P_i = 1\right)$. The calculated P_i are given in Figure 2. The preferred conformation in aqueous solution is apparently the *gauche–gauche* rotamer in which the 5′-OH lies above the ribose. Although X-ray data are lacking for βψ, those obtained for a wide variety of nucleosides, nucleotides, and polyadenylic acid indicate that this rotamer occurs most frequently in the solid state.[7,8] To the best of our knowledge this is the first experimental evidence for a preferred conformation about the exocyclic $C_{4'}$–$C_{5'}$ bond of a nucleoside in solution.

Table I. Proton Chemical Shifts and Coupling Constants of β-Pseudouridine and Uridine[a]

	Chemical shifts (ppm)[b] βψ 30°	βψ 70°	U 28°		Coupling constants, Hz[b] βψ 30°	βψ 70°	U 28°
H_6	7.660	7.627	7.850[c]	$J_{6,1'}$	0.8	0.8	<0.5
$H_{1'}$	4.674	4.668	5.820	$J_{1',2'}$	5.0	5.2	4.4
$H_{2'}$	4.279	4.260	4.260	$J_{2',3'}$	5.0	5.0	4.6
$H_{3'}$	4.141	4.128	4.150	$J_{3',4'}$	5.2	5.2	4.9
$H_{4'}$	4.009	3.993	4.060	$J_{4',5'B}$	3.2	3.4	
$H_{5'B}$	3.840	3.825	3.825	$J_{4',5'C}$	4.6	4.8	
$H_{5'C}$	3.726	3.719	3.735	$J_{5'B,5'C}$	−12.7	−12.4	

[a] S. Fujiwara and M. Uetsuki in "Recent Developments of Magnetic Resonance in Biological Systems," S. Fujiwara and L. H. Piette, Ed., Hirokawa Publishing Company, Inc., Tokyo, 1968, p 1. [b] The chemical shifts and coupling constants are estimated to be accurate to within 0.003 and 0.1 Hz, respectively. [c] B. J. Blackburn, unpublished results.

(ii) If the ribose ring of βψ existed in any of the conventional puckered or planar conformations, large differences in $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$ would exist, as compiled in Table II. These large differences enable one to use the Karplus equation, despite its known qualifications,[9] to determine the conformation of the ribose ring. Examination of Table II shows that a single conformation predicts neither the near equality of $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$, nor their observed magnitudes. This suggests that either a rapid interconversion exists between the $C_{2'}$-*endo* and $C_{3'}$-*endo* (or $C_{2'}$-*exo* and $C_{3'}$-*exo*) forms, or that the 2′, 3′, 4′ section of the ribose ring is planar. If the latter were true, however, $J_{2',3'}$ should be equal to approximately 9 Hz. Thus, an interconversion must take place, in which the hydroxyl groups at $C_{2'}$ and $C_{3'}$ pass through the eclipsed conformation. The data also exclude the possibility of a simple equilibrium between the 2′-*endo* and 3′-*exo* conformations (since $J_{1',2'} = J_{3',4'}$). The apparent lack of rigidity in the ribose ring in solution must be taken into account in theoretical calculations of polynucleotide

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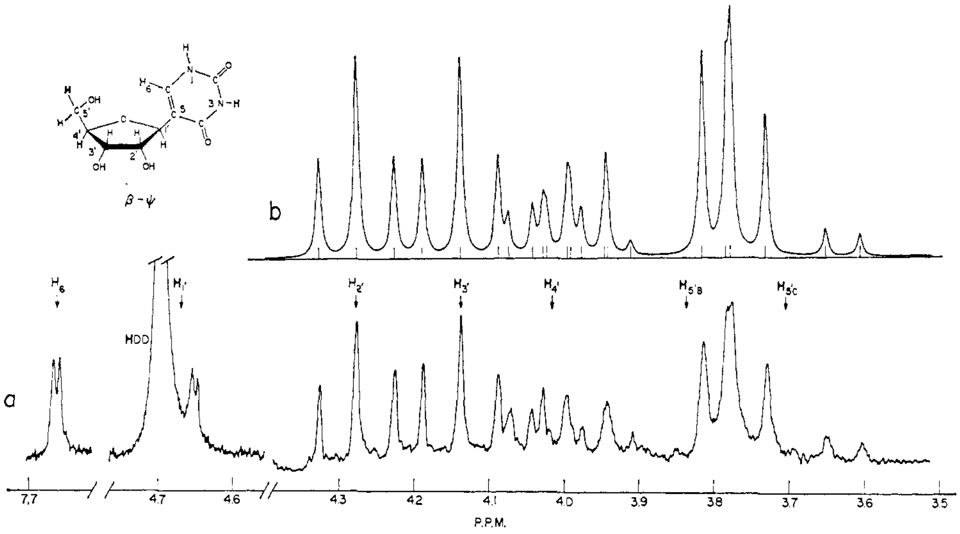

Figure 1. (a) 100-MHz spectrum of βψ in D_2O (pD 6.7, 0.12 *M*, 30°). Chemical shifts are relative to internal 3-(trimethylsilyl)propanesulfonic acid. A second doublet of the quartet due to H_1' is obscured by the HDO resonance. (b) Simulated 100-MHz nmr spectrum of the region due to ribose protons 2′–5′.

conformations; until now a fixed conformation has been assumed.

(iii) The similarity of the 2′ through 5′ chemical shifts for βψ and uridine (U) in Table I suggests that the spatial orientations of the uracils relative to the furanose rings are similar. There is evidence that U exists as the *anti* conformer with H_6 above the ring;[10,11] we infer that βψ has the analogous conformation, in agreement with the interpretation of ORD data.[11]

Table II. Measured Dihedral Angles (φ) and Calculated *J*'s for Various Furanose Ring Conformations

Atom out of plane[a]	$\phi_{1'2'}$, °	$J_{1'2'}$[b]	$\phi_{2'3'}$, °	$J_{2'3'}$[b]	$\phi_{3'4'}$, °	$J_{3'4'}$[b]
C_2'-*endo*	165	9.5	45	4.3	105	0.4
C_3'-*exo*	145	6.7	40	5.0	100	0
H_2', H_3' eclipsed	120	2.3	0	9.0	120	2.3
C_3'-*endo*	105	0.4	45	4.3	165	9.5
C_2'-*exo*	100	0	40	5.0	145	6.7

[a] *endo* means the atom is located on the same side of the plane defined by $C_1'O_1'$ and C_4' as the C_4'–C_5' bond. *exo* means that it is found on the opposite side. [b] In hertz.

The allylic coupling between hydrogens at position 6 and 1′ depends upon the dihedral angle between the planes containing the coupled protons, and thus should be sensitive to the torsional angle between the base and ribose moieties. The constancy of this coupling, the ribose hydrogen couplings, and the ribose hydrogen chemical shifts over a 40° range demonstrates a lack of temperature-induced conformational change. Temperature-independent spectra for three cases have been distinguished;[12] two of these are applicable here. The energy difference between the *syn* and *anti* isomers of nucleosides has been predicted to be large.[13] Thus, a minimal dependence of $J_{6,1'}$ on temperature is expected. In a second case, where energy differences are small, such as rotational isomers of serine,[14] temperature changes do not alter the relative isomer populations appreciably. This applies to the rotamers about the $C_{4'}$–$C_{5'}$ bond, as well as to the ribose ring conformers.

T°C	P_I	P_{II}	P_{III}
30	0.53	0.32	0.15
70	0.47	0.35	0.18

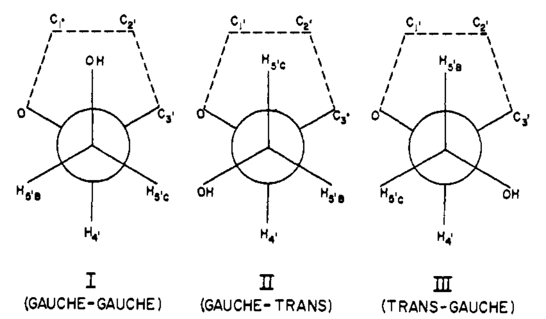

Figure 2. Possible rotational isomers around the C_4'–C_5' bond for βψ. The relative populations of these isomers at 30 and 70° are shown above the structures.

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A New and Simple Template Synthesis of Uninegative, Macrocyclic, Corrin-Type Ligands

Sir:

The utility of the coordination template effect in synthesizing macrocyclic, polydentate ligands is well