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CORRELATION BETWEEN NMR SPECTRAL PARAMETERS OF NUCLEOSIDES AND ITS IMPLICATION TO THE CONFORMATION ABOUT THE GLYCOSYL BOND

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SUMMARY: Analyses of high resolution proton and carbon NMR spectra of a series of guanine nucleosides in DMSO have revealed a near linear correlation between the chemical shift of the $H_{2'}$ atom of the sugar moiety and the vicinal coupling constant $^{3}J_{C4-H1'}$. This unexpected result provides evidence that the variations in the glycosyl torsion angle between nucleosides in solution are less that those which have previously been reported in crystals and it is an experimental basis for analyzing the syn and anti populations from chemical shift and coupling constant data.

Extensive x-ray crystallographic studies on nucleosides and their derivatives have led to the concept of syn and anti conformations about the glycosyl bond as well as to the description of the conformation in terms of a specific glycosyl torsion angle χ (1-4). The conformational nomenclature (5) is illustrated for the case of guanosine (I) in drawings II and III. In crystals, χ is usually in the range of 20-100° or 170-300° (1,2), which are considered to be the syn and anti domains, respectively. Here, we present a systematic study utilizing high-field NMR spectroscopy on the conformation about the glycosyl bond of ten closely related guanine nucleosides in DMSO, in which the conformation about the glycosyl bond has been perturbed, primarily by changing the size of the substituent at the C_8 position of the guanine ring. The spectral results provide an experimental basis for analysis of syn and anti populations in solution. We find that the variations in the glycosyl torsion angle of nucleosides in DMSO are less than that which have been reported for crystals.

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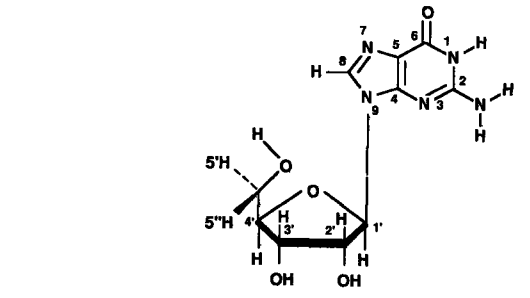

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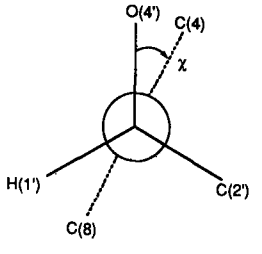

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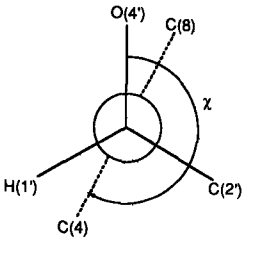

anti

III

MATERIALS AND METHODS

Guanosine, 8-bromoguanosine, 8-thioguanosine, 6-thioguanosine, and 8-aminoguanosine were purchased from Sigma Chemical Co. (St. Louis, MO). All other C8-substituted guanine nucleosides were prepared as previously described (6,7).

NMR measurements were carried out at ambient temperature on a Bruker AM500 NMR spectrometer, operating at 500 MHz for proton or at 126 MHz for carbon. Samples were dissolved in deuterated dimethylsulfoxide (DMSO-d_6). The chemical shifts are reported in ppm by assigning the DMSO signal to 2.50 and 39.5 ppm for proton and carbon, respectively. For carbon-hydrogen coupling constant measurements, the 64K free induction decays were zero-filled to give a digital resolution of 0.18 Hz/point, and processed with Lorentzian to Gaussian filtering using Bruker parameters of -1 Hz and 0.17.

RESULTS AND DISCUSSION

We report a near linear dependence (r=0.97) between the chemical shift of the $H_{2'}$ atom ($\delta H_{2'}$) and the magnitude of the vicinal coupling constant $^3J_{C4-H1'}$ among the guanine nucleosides in DMSO (Figure 1A and Table I). A similar correlation has been found for a limited number of corresponding 2'-deoxyribonucleosides and adenine nucleosides (not shown). The sensitivity of $\delta H_{2'}$, $^3J_{C4-H1'}$ and other vicinal carbon-proton coupling constants to the conformation about the glycosyl bond has previously been reported (8-25), but no study has linked conformational parameters as done here, nor would such a high degree of linearity be expected. It has also been reported that $\delta H_{2'}$ is sensitive to the conformation about the $C_{4'}$-$C_{5'}$ bond (9, 11). We tested the significance of this variable for this specific case by calculating corrections for $\delta H_{2'}$ using a published procedure (9, 11). This procedure

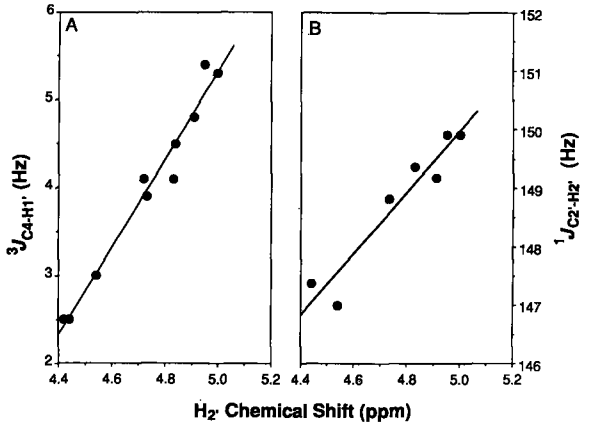

Figure 1. Least-squares fit of the dependence of $\delta H_{2'}$ on (A) $^3J_{C4-H1'}$, and (B) $^1J_{C2'-H2'}$ for guanine nucleosides in DMSO. The nucleosides are listed in Table I.

required determination of the conformer populations about the $C_{4'}-C_{5'}$ bond (gg, gt and tg) for each nucleoside, which in turn were derived from computer simulation of the vicinal J values of the sugar moieties (26-28). However, use of the corrections for $\delta H_{2'}$ did not affect the linear correlation coefficient (r=0.97), and as such are not presented here. The apparent insensitivity is mainly a result of similar conformations about the $C_{4'}-C_{5'}$ bond for this series of compounds. Likewise, the sugar ring conformers indicated relatively small differences in the $C_{3'}$-endo and $C_{2'}$-endo populations. These results indicate that the linear correlation between $\delta H_{2'}$ and $^3J_{C4-H1'}$ is closely related to changes in the conformation about the glycosyl bond.

Table I. $H_{2'}$ Chemical Shifts and Selected Carbon-Hydrogen Coupling Constants of Guanine Nucleosides[a]

Nucleoside	$\delta H_{2'}$	$^3J_{C4-H1'}$	$^3J_{C8-H1'}$	$^1J_{C2'-H2'}$
Guanosine (G)	4.38	2.5	4.5	147.4
6-Thio-G	4.38	2.5	4.2	ND[b]
8-Amino-G	4.53	3.0	5.5	147.0
8-Methoxy-G	4.70	4.1	4.5	ND[b]
8-(Benzyloxy)-G	4.70	3.9	4.2	148.8
8-Oxo-G	4.81	4.1	5.3	149.4
8-(Methylthio)-G	4.83	4.5	4.4	ND[b]
8-Thio-G	4.92	5.4	4.0	149.9
8-Bromo-G	4.95	4.8	4.6	149.2
8-(Methylsulfonyl)-G	4.96	5.3	2.6	149.9

[a] All samples were dissolved in DMSO-d_6. Chemical shifts (δ) are in ppm and coupling constants (J) are in Hz. Concentrations for ^1H measurements were 0.02M or less in order to minimize any dependence of $\delta H_{2'}$ on concentration.
[b] Not determined due to resonance overlap or unresolved couplings.

In principal, $^{3}J_{C8-H1'}$, could also be used in conformational analysis, but it has a much lower linear correlation coefficient for its dependence on $\delta H_{2'}$, (r=0.40) (Table I). The lack of linearity can be accounted for by the variation in the electronegativity of the C_8-substituents. If there was no such effect, all the compounds would have the same Karplus-type dependence between χ and $^{3}J_{C8-H1'}$, and linear relationships would have been observed between $^{3}J_{C8-H1'}$, and both $\delta H_{2'}$, and $^{3}J_{C4-H1'}$. The substituents are too far removed to have much effect on $^{3}J_{C4-H1'}$. This result supports the method of excluding $^{3}J_{C8-H1'}$, in favor of $^{3}J_{C4-H1'}$, for comparative analysis of the conformation about the glycosyl bond of C_8-substituted nucleosides (15,23).

We also found that the one-bond coupling constant $^{1}J_{C2'-H2'}$, of the sugar ring of a limited number of these guanine nucleosides correlates with $\delta H_{2'}$, (r=0.96) (Figure 1B). Further work is needed on a larger number of samples to evaluate the degree of linearity of this and other $^{1}J_{C-H}$ couplings and the factors affecting them. It may be possible to extend analysis of one-bond coupling by using new methodology for isotopic enrichment of nucleosides and their derivatives (29).

The aforementioned linear relationship between chemical shifts and the vicinal coupling constants is best explained by variation in the fraction of syn (n) and anti (1-n) populations according to the following simple model, which assumes a rapid equilibrium between conformations and constant average values of the torsion angle in the anti (χ_{anti}) and syn (χ_{syn}) domains for each set of compounds.

$$^{3}J_{C4-H1'(obs.)} = n \times {}^{3}J_{C4-H1'syn} + (1-n) \times {}^{3}J_{C4-H1'anti} \qquad (1)$$

$$\delta H_{2'(obs.)} = n \times \delta H_{2'syn} + (1-n) \times \delta H_{2'anti} \qquad (2)$$

Thus, the linear relationship between the observed values (obs.) in (1) and (2) is evidence that χ_{anti} and χ_{syn} in solution are similar for this series of compounds. The scatter in points (Figure 1A) can be accounted for in part by the differences in χ_{anti} and χ_{syn}, as well as by small differences in the sugar conformation, and by anisotropic effects from the C_8-substituents, all of which are known to affect $\delta H_{2'}$, (11,23,25). By using DMSO as solvent, stacking interactions which would be expected to differentially affect $^{3}J_{C4-H1'}$, and $\delta H_{2'}$, were prevented. The results should be applicable to aqueous solution in those cases where stacking interactions are minimal.

The results are of significance because they provide an experimental basis for determining the relative syn and anti populations, or estimating the actual populations, from $\delta H_{2'}$, alone (9-11), from $^{3}J_{C4-H1'}$, alone, or from both parameters (Figure 1A). For example, it is apparent from the data that the parent compound G has one of the highest anti populations (or least high syn

population). The issue of whether G is preferentially in the syn or anti form can be addressed by comparison with 8-amino-G. Our analysis shows that 8-amino-G is like several other C_8-amino nucleosides which have a relatively well defined conformation. They preferentially adopt an anti conformation which is stabilized by an intramolecular interaction between the C_8-amino proton and the $O_{5'}$ atom (15,24,30,31), that can only occur with a gg (γ=60°) conformation about the $C_{4'}$-$C_{5'}$ bond (15). The comparisons of $\delta H_{2'}$ and $^3J_{C4-H1'}$ between 8-amino-G and G (Table I) show that G is also preferentially in the anti conformation. This is of interest since analyses of the conformation about the glycosyl bond, particularly for guanine nucleosides and nucleotides, have given conflicting results that depend on the methodology used (11).

In crystals, χ_{anti} and χ_{syn} vary substantially for different nucleosides (vide supra). If this were also the case in solution, there should not be a linear relationship between $\delta_{H2'}$ and $^3J_{C4-H1'}$. This is because $^3J_{C4-H1'}$ exhibits a Karplus-like dependence ($\cos^2$) on χ (18), while $\delta H_{2'}$ is a function of ring currents, diamagnetic susceptibility anisotropies and polarization effects (25), the sum of which have an entirely different dependence on χ. The high degree of correlation of spectral parameters is evidence that the variations in the glycosyl torsion angle between nucleosides in crystals is greater than that observed in solution. This can be explained by crystal packing forces and stacking interactions as well as by inter- and intramolecular hydrogen bonding interactions that are unique to crystals.

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