^{13}C N.M.R. Spin–Lattice Relaxation Time Measurements Determining the Major Tautomer of 1-Methylisoguanosine in Solution

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Summary Analysis of the contributions of ^{13}C–^{1}H dipolar interactions to the ^{13}C n.m.r. spin-lattice relaxation times of the quaternary carbons of 1-methylisoguanosine establishes that the predominant tautomer in solution is the 2-keto, 6-amino form.

METHODS commonly used to determine tautomerism in solution rely on comparisons of spectral properties (u.v., i.r., ^{1}H and ^{13}C n.m.r. spectroscopy) of the compound of interest with those of model compounds in which the tautomeric equilibrium is fixed. Here we show that ^{13}C n.m.r. spin-lattice relaxation data can be interpreted to locate the hydrogens in the purine ring of a nucleoside, and thus to define the major tautomer, without reference to data on model compounds. The method is applied to the pharmacologically active marine natural product 1-methylisoguanosine (**1**).[1] Definition of the tautomeric form of (**1**) was necessary for interpretation of its mode of action.[1]

It is established that in many organic molecules the ^{13}C spin-lattice relaxation of quaternary carbons is dominated by ^{13}C–^{1}H dipolar interactions at low magnetic field strength.[2–4] In these cases ^{13}C spin-lattice relaxation times (T_1) of quaternary carbons in molecules of known structure can be estimated by considering contributions from hydrogens which are two bonds removed from each carbon.[3–5] Furthermore, provided a molecule behaves as an isotropic rigid rotor, quantitative analysis of the contributions of ^{13}C–^{1}H dipolar interactions to quaternary carbon T_1 values can be used to determine the number of hydrogens that are two bonds removed from each carbon and thus to establish substitution patterns in covalent structures.[6]

Previous analysis of the ^{13}C spin-lattice relaxation behaviour of quaternary carbons in the purine units of AMP and GMP showed that ^{13}C–^{14}N dipolar interactions also contribute significantly to relaxation.[7] However, provided equation 1 is satisfied,† it is still possible to determine the

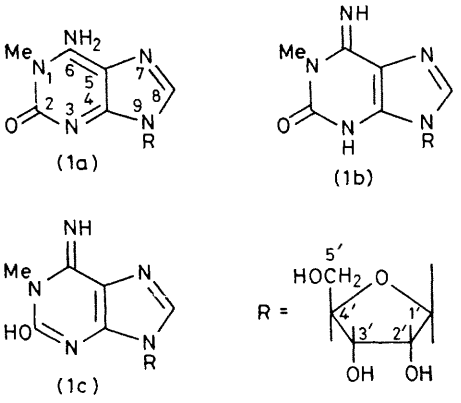
$$(\omega_H + \omega_C)^2 \tau_R^2 << 1 \quad (1)$$

contribution of ^{13}C–^{1}H dipolar interactions (T_1^{CH}) to measured T_1 values by using equation (2), in which η is the

$$T_1^{CH} = 1{\cdot}988/\eta \times T_1^{total} \quad (2)$$

nuclear Overhauser enhancement.

The T_1 values for C-8 of the protonated and deuteriated forms of (**1**) are shorter than those of C-1′ to C-4′ (Table), probably due to internal motion within the ribofuranose ring.[8] Because of this inequality, coupled with the fact that there is only one protonated carbon in the purine ring of (**1**), it cannot be established that (**1**) behaves as an isotropic rigid rotor. However, based on published data for similar molecules,[7,8] this description is probably an acceptable approximation. A τ_R value of 0·31 ns is then obtained from the T_1 for C-8 of the protonated form of (**1**) using equations given previously[2–7] and a C–H bond length of 1·10 Å. Equation (1) is therefore satisfied, and we may use equation (2) to determine T_1^{CH} values (see Table).



Each of the three possible tautomers of 1-methylisoguanosine, (**1a**), (**1b**), and (**1c**), is unique with respect to the number of hydrogens that are two bonds removed from its quaternary carbons. Thus, it should be straightforward to distinguish among them by considering the quaternary carbon T_1 values. Distances from carbons to hydrogens that are two bonds removed and attached to nitrogen or oxygen atoms are typically 2·0—2·1 Å, while those to hydrogens three bonds removed are > 3 Å.[9] With τ_R = 0·31 ns, one hydrogen 2·05 Å away from a carbon would contribute 6·7 s to its T_1 value, whilst a hydrogen 3·1 Å away would contribute 80·2 s. Thus, considering only hydrogens that are two bonds removed, (**1a**) should have one quaternary carbon with T_1 *ca.* 3·3 s (C-6) and three with much longer T_1 values, (**1b**) should have three carbons with T_1 *ca.* 6·7 s (C-2, C-4, and C-6) and one with a long T_1 value, and (**1c**) should have two carbons with T_1 *ca.* 6·7 s (C-2 and C-6) and two with long T_1 values. The observed T_1^{CH} values for the quaternary carbons of the protonated form of (**1**) (Table) are consistent only with the predicted values for (**1a**), indicating that this is the major tautomer in Me_2SO. This conclusion is strengthened by considering contributions to the observed T_1^{CH} values from

TABLE. ^{13}C chemical shifts, spin-lattice relaxation times, and integrated intensities for 1-methylisoguanosine (**1**).

Peak[a]	Assignment[b]	(**1**) (protonated)[c] δ[e]	η[f]	T_1[g]	T_1^{CH}[h]	(**1**) (deuteriated)[d] η	T_1	T_1^{CH}
1	C-2	153·8	1·0	7·7	15·2	0·7	6·4	17·4
2	C-4	152·1	1·0	7·0	13·5	0·6	7·1	22·4
3	C-6	151·5	2·0	2·5	2·5	1·0	6·6	13·3
4	C-8	138·0	1·9	0·16	0·16	2·0	0·17	0·17
5	C-5	108·9	1·1	8·1	14·3	0·5	8·3	33·0
6	C-1′	87·6	1·9	0·22	0·22	1·9	0·20	0·20
7	C-4′	86·0	2·0	0·21	0·21	1·8	0·20	0·20
8	C-2′	72·9	2·0	0·23	0·23	2·1	0·23	0·23
9	C-3′	70·7	2·0	0·26	0·26	2·0	0·24	0·24
10	C-5′	61·8	2·1	0·15	0·15	2·1	0·12	0·12
11	N(1)-Me	30·0	2·2	0·47	0·47	2·2	0·44	0·44

[a] Numbered consecutively from downfield edge of spectrum. [b] Assignment for (**1a**) [based on chemical shifts (ref. 7) and spin-lattice relaxation data]. [c] 0·24 M in $(CD_3)_2SO$ at 32 °C. [d] 0·3 M in $(CD_3)_2SO$ containing *ca.* 5% v/v D_2O at 32 °C. Sample lyophilised from D_2O before dissolution. [e] Chemical shift in p.p.m. from internal Me_4Si. All data were obtained on a Jeol FX-60 spectrometer operating at 15·04 MHz with 10-mm sample tubes. [f] Experimental η obtained by setting the average enhancement of the protonated carbon resonances equal to 1·99. Estimated accuracy is ± 10%. [g] Measured ^{13}C spin–lattice relaxation time (in s). Values for quaternary carbons and protonated carbons were obtained from separate sets of inversion–recovery spectra, using pulse recycle times greater than 4 times the longest T_1 value being measured in each case. Estimated accuracy is ± 10%. [h] Contribution (in s) of ^{13}C–^{1}H dipolar interactions to the observed T_1 value, obtained from equation (2) as described in the text. Estimated accuracy is ± 20%.

† ω_H and ω_C are the resonance frequencies in radians s^{-1} of ^{1}H and ^{13}C, respectively, and τ_R is the rotational correlation time in s.

exchangeable protons only, which are obtained[3,5–7] from the difference between corresponding T_1^{CH} values in the protonated and deuteriated forms of (**1**) after adjusting for the slight difference in effective τ_R values (Table). These values are 208, 37, 3·1, and 26 s for peaks 1, 2, 3, and 5, respectively.

The above conclusions can be drawn without making specific resonance assignments. However, having established that (**1a**) is the major tautomer, assignment of quaternary carbon resonances follows from chemical shift and relaxation data (Table). In principle, ^{13}C spin-lattice relaxation data can be used to estimate the fraction of each tautomer in solution. This requires that quaternary carbon T_1^{CH} values be calculated accurately (taking into account all hydrogens in the molecule). However, as the contributions from ribose protons to relaxation of C-2 and C-4 can be calculated only if the torsion angle about the glycosidic bond is known,[7] we have not attempted to do this. Nevertheless, the fact that the observed contribution of exchangeable protons to relaxation of C-6 is even slightly stronger than predicted for (**1a**), coupled with the very weak contributions from exchangeable protons to relaxation of the other three quaternary carbons, suggests that $> 90\%$ of (**1**) in $(CD_3)_2SO$ is the 2-keto, 6-amino tautomer. This is consistent with u.v. data for (**1**) in aqueous solution,[1,10] which are similar to that for the N(1)H–2-keto, 6-amino tautomer of isoguanosine.[11]

(*Received*, 31*st December* 1979; *Com.* 1336.)

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