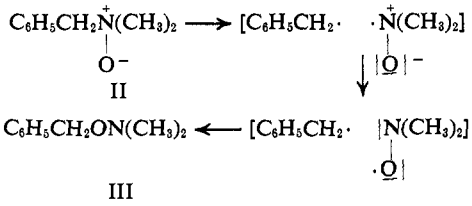
downfield of this location (Figure 1b) toward the upfield position (Figure 1c, d) of an aqueous II solution (Figure 1a). This water shift is directly related to the conversion of II into III. III is less dense than II and insoluble in it. Thus a second phase rapidly forms as rearrangement takes place; III, which does not hydrate, separates above the oxide layer which builds up water from the reacted monohydrate. The upper layer gives a normal pmr absorption spectrum of III since the bulk of the material has undergone nuclear spin relaxation, while the lower layer shows emission as long as conversion remains rapid[12] since most of the III in this layer has just been formed under conditions of nuclear polarization. Thus, if protracted emission is to be observed, care must be taken to position the sample tube so that the detector coil is measuring the lower phase at all times. Although III is the predominant product, some reduction of II occurs, as is evident from the slow appearance of the weak methyl and methylene absorption singlets of N,N-dimethylbenzylamine at δ 2.14 and 3.33 ppm, respectively (Figure 1c and d).

The emission signals from both the methylene and methyl groups of III represent both the migrating and terminus moieties, respectively, involved in the rearrangement process. Since unpaired electron precursors are necessary to build up the abnormal nuclear spin state distributions resulting in pmr emission,[5] emission from both moieties is direct evidence for homolytic bond formation from a radical pair. If thermal homolytic cleavage of the weak CN bond between benzyl and the charged nitrogen of II precedes formation of a



III

CO bond in the electron-redistributed radical pair, a reaction route is provided which accounts for the observed emission. The positive entropy (7.9 ± 2.5 cal/deg) and low enthalpy (34.2 ± 1 kcal) of activation measured[11] for this reaction are appropriate for this type of process. In addition, Closs and Closs have suggested[13] for pairwise-generated radicals that emission will only be evident in recombinations occurring within the cage of initial formation. Thus only the part of the rearrangement which could not be trapped by oxygen[3] would be directly detected by nmr techniques. The oxygen trapping of I[3] and the comparable 60–80% racemization at methylene (CHD) in conversion of II to III[6] would then agree with the amount of escape from the cage.

In addition to furnishing direct evidence for a radical pair in the Meisenheimer rearrangement, the current study is unique in reporting emission from methyl groups which do not directly participate in the migrations. Proton emission from a migrating benzyl methylene and a methyne terminus have been reported in a Stevens rearrangement of a quaternary ammonium reaction intermediate.[14] However the current emission adjacent to the point of bond cleavage is an indication that protons both α and β to migration sites may act as pmr polarization probes for homolytic rearrangement processes.

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(12) After about three-fourths of the crystalline oxide has reacted, 15 min at 130°, and the water peak has shifted three-fourths of the distance toward that shown in Figure 1a, emission changes to weak absorption unless the temperature is increased significantly, *e.g.*, 148–155°.
(13) G. L. Closs and L. E. Closs, *J. Amer. Chem. Soc.*, **91**, 4550 (1969).
(14) A. R. Lepley, *Amer. Chem. Soc., Div. Petrol. Chem.*, **14**, No. 2, C43 (1969).
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Experiments Leading to the Elucidation of the Purine Proton Magnetic Resonance Line Broadening upon Purine Intercalation in Single-Stranded Nucleic Acids

Sir:

When purine is added to aqueous solutions of single-stranded nucleic acids, *e.g.*, dinucleoside monophosphates,[1] or polyuridylic acid (poly U),[2] it has been shown that a purine-intercalated complex is formed which involves sandwiching of a purine molecule between adjacent bases of the dinucleotide or polynucleotide. This complexation has been monitored through the effect of the intercalated purine on the chemical shifts of the base protons of the nucleic acid,[1-5] and by conformational changes in the ribose phosphate backbone reflected by changes in the vicinal coupling constant between the $H_{1'}$ and $H_{2'}$ ribose protons.[3] The purine proton resonances are also appreciably broadened, particularly at low purine/nucleotide ratios where the fraction of incorporated to unbound purine is high.[1,2] The three purine proton resonances are not equally broadened, with the H_6 and H_8 resonances affected to a considerably greater extent than the H_2 resonance. Chan, *et al.*,[1] have proposed that the purine protons experience a strong dipolar field when the purine base is incorporated between the adjacent bases of the dinucleotide segment and the purine proton resonances are broadened by nuclear spin relaxation induced by fluctuations of these local dipolar fields. In particular, it was proposed that the greater part of the dipolar field arises from the $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{5''}$ ribose protons, which are situated around the bend of the "U" on the inner side of the cage when the conformation of the dinucleotide segment corresponds to that for maximum interaction of the nucleic acid bases with the incorporated purine base.

(1) S. I. Chan, B. W. Bangerter, and H. H. Peter, *Proc. Nat. Acad. Sci. U. S.*, **55**, 720 (1966).
(2) B. W. Bangerter and S. I. Chan, *Biopolymers*, **6**, 983 (1968).
(3) S. I. Chan and J. H. Nelson, *J. Amer. Chem. Soc.*, **91**, 168 (1969).
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We wish to report in this communication the results of several experiments which have led to further elucidation of the purine line-broadening mechanism. We have studied the purine line-broadening phenomenon at two different magnetic fields under otherwise identical experimental conditions, have measured the spin–lattice relaxation time (T_1) for the various purine protons, and have observed nuclear Overhauser enhancement of the purine resonances upon strong irradiation of certain ribose protons.

Line-width measurements at two different magnetic fields (or nmr frequencies) for a 0.11 *M* purine solution containing poly U (0.10 *M* in uridine) at 29° showed no magnetic field or frequency dependence of the purine line widths. The observed line widths of the H_6, H_2, and H_8 resonances are, *e.g.*, 16, 6.5, and 11 Hz, respectively, at 220 MHz, which can be compared with line widths of 16, 6, 11 Hz obtained by computer simulation of the observed spectrum at 100 MHz. The absence of an observable field dependence would seem to rule out chemical exchange between chemically shifted purine species as a source of the purine line broadening. Analysis of the line-width data for solutions containing different purine/nucleotide ratios has previously indicated that the chemical exchange between bound and free purine is rapid on the nmr time scale.[5]

The T_1's of the various purine protons have been measured by the progressive saturation method.[6] These results are summarized in Table I and are compared with the T_2's calculated from the observed line widths. In the case of the two purine resonances where T_1 was sufficiently long to be determined in this manner, the spin–lattice relaxation times were found to be extremely short, and, insofar as we were able to ascertain, $T_1 \cong T_2$. This result, we feel, substantiates our contention that the purine resonances are relaxation broadened.

Table I. Observed T_1's and T_2's for the Purine Protons of a 0.11 *M* Purine Solution in the Presence of Poly U (0.10 *M* in Uridine) at 17°

Proton	T_2, sec	T_1, sec[a]
H_6	0.02	Not saturable
H_8	0.032	0.038
H_2	0.064	0.060

[a] Precision of measurements, ±10%.

The nuclear Overhauser effect observed for the purine resonances upon strong irradiation of the ribose protons provides further insight into the mechanism of purine line broadening. The observation of an *intermolecular* nuclear Overhauser effect is important confirmation that the purine protons are magnetically dipolar coupled to the ribose protons of the nucleic acid. Critical to the interpretation of these results is the assignment of the ribose proton resonances. This spectral assignment is shown in Figure 1 and is based on spin-decoupling experiments, intensity correlations, comparison of the spectral parameters with those of 3′-UMP and 5′-UMP, and computer simulation of the spectrum. As shown in Table II, a nuclear Overhauser enhancement of 11% is observed for the purine H_6 resonance when the $H_{5'}$, $H_{5''}$ ribose protons are saturated, and a small enhancement of 6% is observed for the purine H_8 resonance when the $H_{3'}$ ribose proton is strongly irradiated. By contrast, a noticeable nuclear Overhauser enhancement was not observed for any of the purine resonances upon irradiation of the ribose $H_{1'}$, $H_{2'}$, or $H_{4'}$ protons. These observations suggest that the purine base in the purine-intercalated complex is preferentially oriented with either the H_6 or H_8 protons directed at the $H_{3'}$, $H_{5'}$, $H_{5''}$ protons of the ribose moiety.

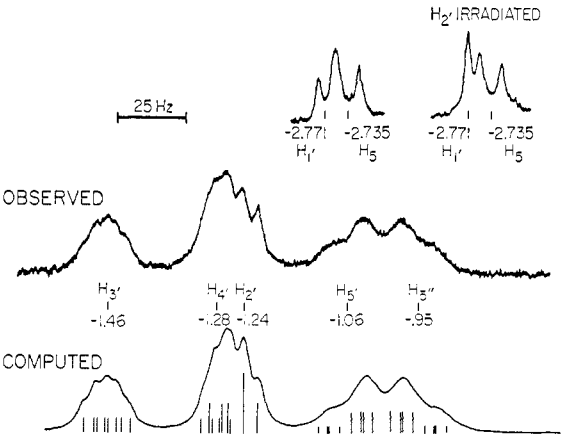

Figure 1. The 220-MHz pmr spectrum of poly U (0.10 *M* in uridine) in the region of the ribose proton resonances. Chemical shifts indicated are in ppm downfield from internal $(CH_3)_4N^+$. The theoretical spectrum was simulated using the following coupling constants (Hz): $|J_{H_{1'}-H_{2'}}| = 5.5$, $|J_{H_{2'}-H_{3'}}| = 5.5$, $|J_{H_{3'}-H_{4'}}| = 4.0$, $|J_{H_{3'}-P}| = 9.0$, $|J_{H_{4'}-H_{5'}} + J_{H_{4'}-H_{5''}}| = 7.5$, $|J_{H_{4'}-H_{5'}} - J_{H_{4'}-H_{5''}}| = 0$, $|J_{H_{5'}-P}| = |J_{H_{5''}-P}| = 4.0$, $|J_{H_{5'}-H_{5''}}| = 12.5$; and line widths of 5 Hz for $H_{2'}$, $H_{3'}$, $H_{4'}$ resonances, and 8 Hz for $H_{5'}$, $H_{5''}$ resonances.

Table II. Nuclear Overhauser Effects Observed for the Purine Proton Resonances upon Strong Irradiation of the Poly U Ribose Protons in a 0.4 *M* Purine Solutions Containing Poly U (0.10 *M* in Uridine)[a]

Ribose proton irradiated	Nuclear Overhauser enhancement observed for purine resonances, % H_6	H_2	H_8
$H_{5'}$, $H_{5''}$	11 ± 3	0	0
$H_{2'}$, $H_{4'}$	0	0	0
$H_{3'}$	0	0	6 ± 3
$H_{1'}$	0	0	0

[a] Experiments were performed on a Varian HR-220 nmr spectrometer operating at a probe temperature of 17°.

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