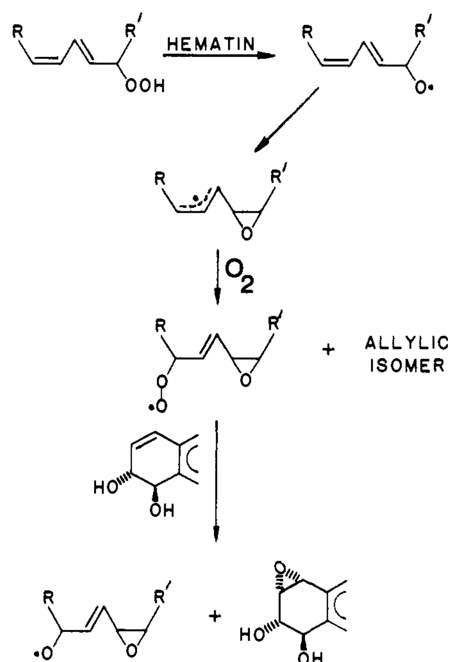
Scheme II



a role in cell transformation by the oxidative activation of xenobiotics to carcinogenic derivatives. This may contribute to the association of dietary fat and carcinogenesis.[22]

Acknowledgment. We are grateful to Dr. Augustine Panthananickal for performing the experiments with polyguanylic acid, Dr. Gregory Reed for starting materials for certain experiments, and Professor A. P. Schaap for a sample of 9,10-bis(carboxyethyl)anthracene. [7-^{14}C]BP-7,8-diol was received from the NCI Chemical Carcinogen Reference Standard Repository, a function of the Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, MD. Financial support was provided by the National Institutes of Health (GM23642).

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^{109}Ag and ^{103}Rh NMR Spectroscopy with Proton Polarization Transfer

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Since the paper of Morris and Freeman[1] first describing a polarization-transfer sequence between two J coupled spins I and S (INEPT sequence), several communications have appeared applying this method for NMR measurements of ^{13}C,[2a] ^{15}N,[2b] and ^{14}N,[2c] (S, ^{1}H; I, observed nucleus). The gain in signal over noise obtained using the INEPT sequence can reach, under optimal experimental conditions, a factor equal to $\gamma S/\gamma I$ for an AX spin system. This enhancement is particularly welcome for NMR observations of isotopes with small γ values. We now report results which show the possibilities offered by the INEPT sequence for direct observation of ^{109}Ag and ^{103}Rh in complexes where these nuclei have a ^{1}H resolved scalar coupling [^{3}J(M,^{1}H)].[3]

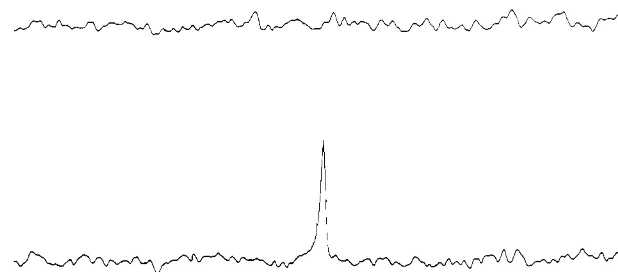
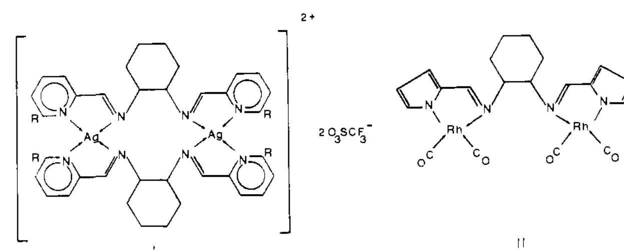


Figure 1. ^{109}Ag spectrum of a 4M solution of $AgNO_3$ in H_2O (1 scan, 90° pulse). Top, with solvent proton irradiation; bottom, normal acquisition.

From a practical point of view NMR experiments on transition-metal nuclei with small γ values suffer from several drawbacks. Firstly, these nuclei often possess a negative γ which implies a negative η (nuclear Overhauser enhancement) when measuring the sample using broadband proton decoupling irradiation; in general the metal center is not directly bonded to hydrogen and the effect of a remote ^{1}H environment will reduce the theoretical enhancement of the metal resonance to a few percent. As the maximum theoretical η value can be quite large ($\eta = -10.7$ for ^{109}Ag), this small operative mechanism often leads to observed η values of ca. -1 and gives a zero nuclear Overhauser effect (the "null signal" problem). Such a phenomenon has been found during measurements of transition-metal salts in protio solvents,[4] and Figure 1 clearly shows this effect on the ^{109}Ag resonance of an $AgNO_3$ solution (4 M in 90:10 (v/v) H_2O/D_2O) when observed with and without broadband ^{1}H decoupling. Secondly, ^{1}H decoupling inescapably produces a slight increase of the sample temperature. Because of the large $\Delta\delta/K$ for many transition-metal nuclei,[5] the comparison of chemical-shift data can be unreliable. Finally, since low-γ spin $^{1}/_{2}$ nuclei sometimes have very large T_1 values (900-1000 s for ^{109}Ag in aqueous solutions of $AgNO_3$[6]) very long relaxation delays and small pulse angles are required.

During the course of a detailed NMR study of complexes of the type [Ag^{I}_2[μ-(R,S)-1,2-[(6-R-2-C_5H_3N)C(H)=N]$_2$cyclohexane]$_2$](O_3SCF_3)$_2$ (I, R = H)[7] and [{$Rh^{I}(CO)_2$}(μ-(R,S)-1,2-[(2-C_4H_3N)C(H)=N]$_2$cyclohexane)] (II) we considered the use of ^{109}Ag and ^{103}Rh NMR spectroscopy to gain more insight into the electronic environment and the structural and dynamic behavior of these complexes in solution. Fortunately they all show



(1) G. A. Morris and R. Freeman, *J. Am. Chem. Soc.*, **101**, 760-762 (1979).

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(3) The γ values for ^{109}Ag, ^{103}Rh (I spins), and ^{1}H (S spin) are -1.2449, -0.8420 and 26.7510×10^{7} rad T^{-1} s^{-1}, respectively. INEPT sensitivity gain is then equal to 21.48 for ^{109}Ag and 31.77 for ^{103}Rh with a corresponding theoretical drop in accumulation time by a factor of 461 for ^{109}Ag and 1000 for ^{103}Rh.

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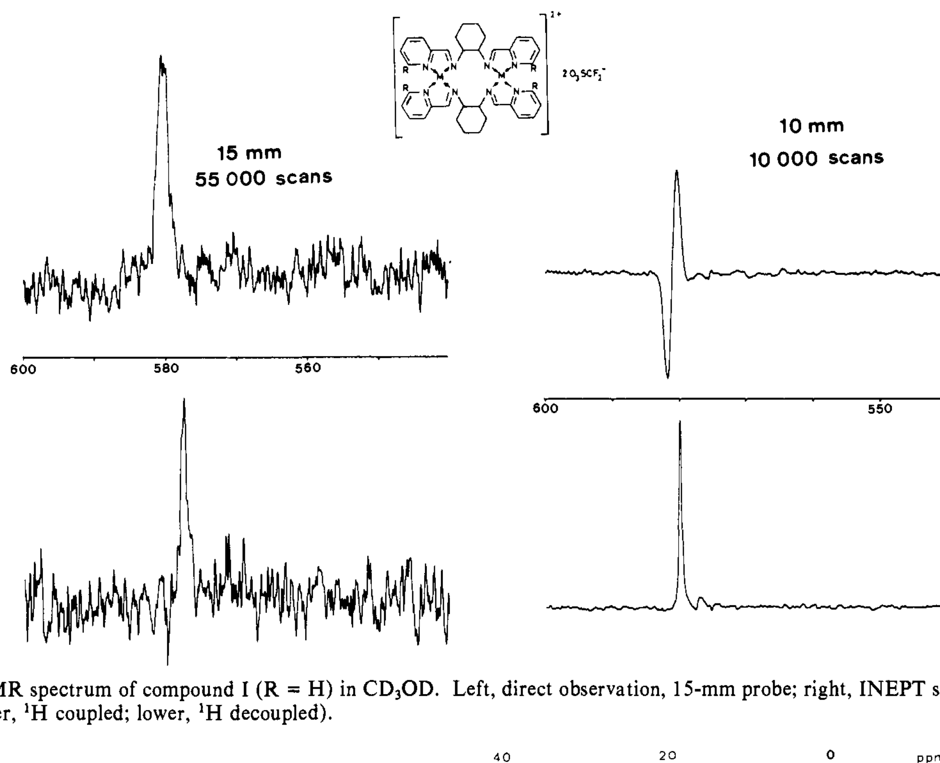

Figure 2. ^{109}Ag NMR spectrum of compound I (R = H) in CD_3OD. Left, direct observation, 15-mm probe; right, INEPT sequence, 10-mm probe, same solution (upper, ^{1}H coupled; lower, ^{1}H decoupled).

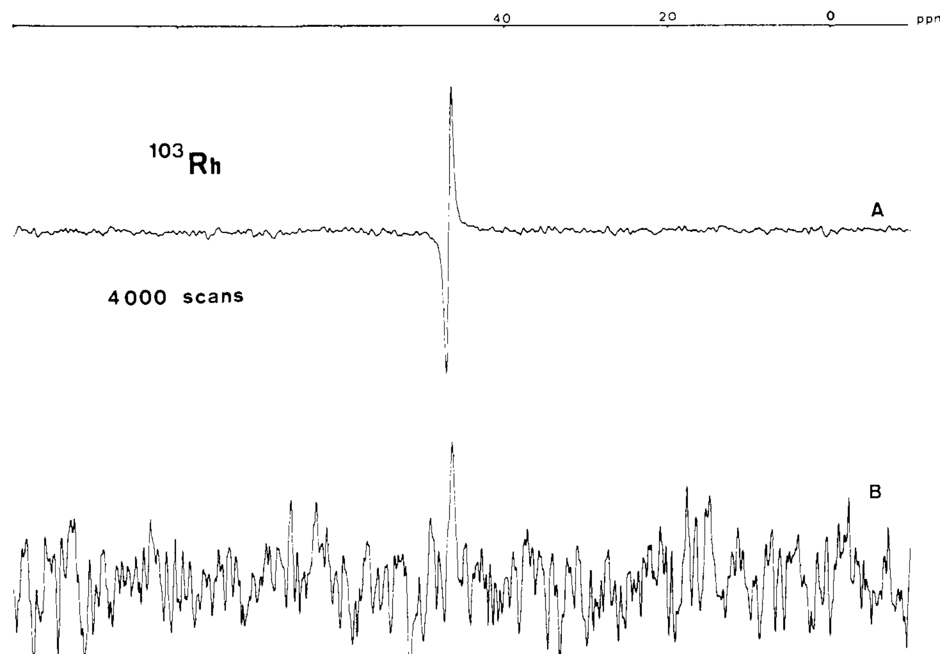

Figure 3. Comparison of normal (B) and INEPT (A) ^{103}Rh spectrum of a 0.1 M solution of complex II in CD_2Cl_2.

at room temperature in their ^{1}H NMR spectra a scalar coupling, ^3J(M,H-imine), in the range 2–10 Hz.[8] Despite these small J values it was tempting to try direct ^{109}Ag and ^{103}Rh NMR experiments by using the INEPT sequence.[9] The successful outcome of this work is illustrated by Figures 2 and 3. Although the spectra speak for themselves, it is worth making the following comments. Firstly since the pulsing rate in the INEPT sequence is no longer governed by the metal T_1, but rather by the proton T_1, the intrinsic experimental time saving, deriving from the absence of long relaxation delays, is appreciable and ranges from a factor 400 for ^{109}Ag to 900 for ^{103}Rh. Secondly, the up–down appearance of the ^{1}H coupled INEPT spectra allows for quite accurate determination of long-range J values: this is particularly useful when the natural line width is increased as encountered with the ^{109}Ag resonance of I that is broadened due to ^{14}N quadrupolar relaxation. A further advantage when accumulating ^{1}H decoupled INEPT spectra is that the duty cycle for the ^{1}H decoupler involves only a low on–off switching rate, and this avoids both a significant temperature gradient in the sample (compare in Figure 3 the slight chemical-shift difference between the ^{1}H-coupled and ^{1}H-decoupled spectra run in the normal mode) and any signal reduction through potential negative nuclear Overhauser enhancement.

The chemical-shift value obtained for the binuclear silver(I) complex I (+580 ppm relative to 2 M aqueous $AgNO_3$) compares

(8) For the anisochronous imine protons in *I* the $^3J(^{107,109}\text{Ag}-^1\text{H-imine})$ is 9.3 Hz (δ 8.87) and 6.3 Hz (δ 8.69). For II the $^3J(^{103}\text{Rh}-^1\text{H-imine})$ is 2.0 Hz (δ 8.00).

(9) All the spectra have been run on a Bruker WM 250 spectrometer. Using a [$90_{H,x}°—\tau—180_{H,x}°;180_{M,x}°—\tau—90_{H,y}°;90_{M,x,x,-x,-x}°$ acquire] or [$90_{H,x}°—\tau—180_{H,x}°;180_{M,x}°—\tau—90_{H,y}°;90_{M,x,x,-x,-x}°—\Delta—$(BB)acquire] sequence, with $\tau = (4J)^{-1}$, $\Delta = (4J)^{-1}$; 10 mm sample tubes; $90_H°$, 35 μs; $90_{Ag}°$, 50 μs; $90_{Rh}°$, 70 μs; a positive δ corresponds to a higher resonance frequency ("deshielding") with respect to the reference frequency.

well with published data for $AgNO_3$ in chelating solvent mixtures.[10] However, the δ^{103}Rh value for II (+46 ppm relative to the accepted Ξ = 3.16-MHz reference) is one of the highest ever reported for a Rh(I) complex,[11] a situation resulting from nitrogen coordination at the rhodium center.[12]

Finally, since polarization-transfer experiments are very easy to set up (no triple irradiation attachment needed as for ^{1}H-{M} INDOR nor precise determination of proton-transition frequencies as in SPI/SPT experiments) we anticipate a widespread use of this technique in metal NMR spectroscopy, since many organometallic complexes show long-range metal proton scalar couplings.[13]

A full paper on the chemistry, NMR parameters, and dynamic behavior of these silver(I) and rhodium(I) complexes will be published shortly.

Acknowledgment. We thank Dr. D. M. Grove and Professor Dr. K. Vrieze for helpful discussions.

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(11) Reference 5a, p 245.
(12) Reference 5b, p 38.
(13) Reference 5a, p 248.

Structural and Dynamic Information about Double-Stranded DNA from Nitrogen-15 NMR Spectroscopy

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Nitrogen-15 NMR resonances have been observed for unfractionated yeast tRNA at natural abundance in solution[2] and ^{15}N-enriched *B. licheniformis* cells.[3] However, no one previously has reported observation of the ^{15}N spectrum of DNA. This communication describes our initial studies of the ^{15}N NMR spectral properties of sonicated native DNA obtained from ^{15}N-enriched *E. coli.*

Lack of any previous ^{15}N NMR experiments with DNA can be attributed to the low sensitivity of natural abundance ^{15}N NMR spectroscopy,[4] which would be aggravated by the extremely broad resonances one might anticipate from a molecule the size of DNA. Our recent ^{31}P and ^{13}C NMR relaxation studies of nucleic acids showed that the nucleic acid backbone is relatively mobile.[5-8] Internal motions in nucleic acids lead to line narrowing with consequent improvement in spectral sensitivity and resolution. In fact Rill et al.[9] have recently obtained a high-field, natural abundance ^{13}C NMR spectrum of DNA in which nearly all peaks are resolved. This background led us to believe the ^{15}N NMR spectrum of DNA could be obtained if the DNA was enriched in ^{15}N.

Consequently, we isolated DNA from *E. coli* cells grown on a medium containing [^{15}N]ammonium chloride enriched 90–95%

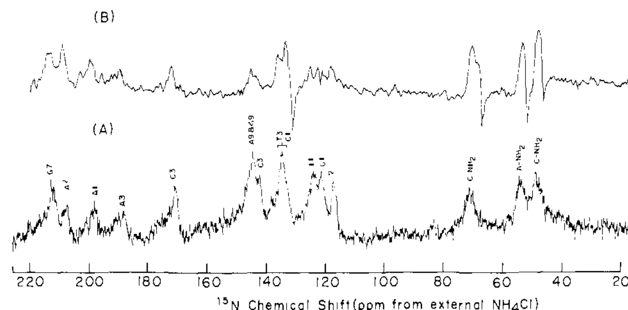

Figure 1. (A) ^{15}N NMR spectrum (30.4 MHz) obtained at 35 ± 2 °C of ^{15}N-labeled DNA (55 mg/mL) in 50 mM sodium cacodylate, pH 7.5, and 50 mM NaCl;[12] 16 412 transients were accumulated with 70° pulses by using an acquisition time of 1.16 s, a pulse recycle time of 4.16 s, and broadband proton decoupling on only during signal acquisition. The Fourier transform spectrum displayed was the result of exponential multiplication equivalent to 4-Hz line broadening. (B) 30.4-MHz spectrum obtained at 45 ± 2 °C of DNA (45 mg/mL) in 50 mM sodium cacodylate, pH 7.5, and 50 mM NaCl;[12] 170 304 transients were accumulated with 90° pulses using an acquisition time of 0.073 s, a pulse recycle time of 0.3 s, and broadband proton decoupling on during acquisition. Exponential multiplication equivalent to 8 Hz was used.

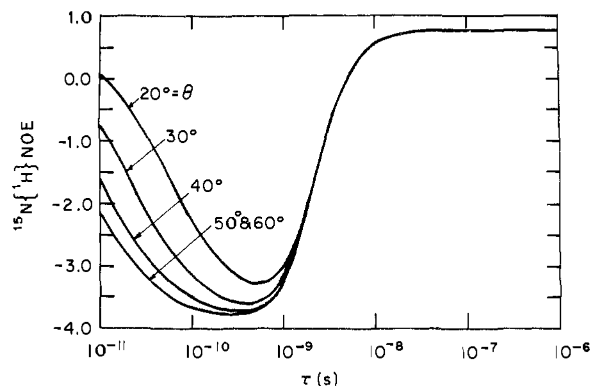

Figure 2. Theoretical dependence of the ^{15}N{^{1}H} NOE for ^{15}N at 30.4 MHz assuming only dipolar relaxation, calculated by using a two-state internal jump model appropriate for rotational jumps about the C1′–N bond, as a function of τ_i the lifetime in either jump state. The calculations are made for a series of angular jump amplitudes θ and assume an isotropic reorientation time of 1 μs.

in ^{15}N.[10] The isolated DNA is therefore enriched with ^{15}N in all positions. The high-purity sample[11] used for the NMR studies was sonicated, yielding a double-stranded DNA sample with a median length of 300 base pairs.[12]

(1) Recipient of Research Career Development Award AM 00291 from NIH. On sabbatical leave from the Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143. Address correspondence to this address.
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(10) DNA was isolated by using a modification of published methods (Marmur, J. *J. Mol. Biol.* **1961**, *3*, 208–218. Berns, K. I.; Thomas, C. A., Jr. *Ibid.* **1965**, *11*, 476–490). Twenty grams (wet weight) of *E. coli* grown on [^{15}N]ammonium chloride were suspended in 100 volumes of (w/v) 0.15 M saline, 0.1 M ethylenediaminetetraacetic acid (EDTA) at pH 8.0, and digested with 250 μg of lysozyme for 20 min. To this, 5% (v/v) of 20% SDS solution was added and swirled for 15 min. Pronase (CalBiochem B grade) was added to a final concentration of 1 mg/mL in 3 increments—0.5 mg/mL was initially added with incubation for 3–3.5 h at 45 °C. Two additional increments of 0.25 mg/mL were added at 2–2.5-h intervals for a total incubation time of 7–8 h. The solution, cooled to room temperature, was extracted with equal volumes of saline–EDTA saturated phenol. Phenol was extracted with diethyl ether. Only fresh white reagent grade phenol was used. DNA was precipitated with 1 volume of iced 2-propranol, spooled onto glass rods, and dissolved in a minimum volume of 0.1X saline–citrate buffer (0.15 M saline, 0.015 M citrate, pH 7.0). RNase A (50 μg/mL) and RNase T_1 (100 units/mL) were added and the solution was incubated at 37 °C for 1 h. Pronase (1 mg/mL) digestion at 45 °C for 2 h was followed with phenol extraction and 2-propanol precipitation. The RNase and pronase steps were repeated, and the solution was doubly extracted with phenol. Any phenol in the DNA solution was extracted with ether, and the DNA was dialyzed against 200 volumes of 0.1 M NaCl and 20 mM cacodylate, pH 8. Any polysaccharides (opalescent material) were removed by ultracentrifugation at 100 000 g for 1 h.
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