Determination of Molecular Geometry by Quantitative Application of the Nuclear Overhauser Effect

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Abstract: A theory for the quantitative interpretation of nuclear Overhauser effects (NOE) in multispin systems is presented and applications to the determination of molecular configurations in solution are discussed. The NOE between several of the protons of 2′,3′-isopropylidene-3,5′-cycloguanosine have been measured and are interpreted to illustrate the use of the theory to measure relative interproton distances. The computed distances were within 5–10% of the distances measured on molecular models constructed using bond lengths and angles known by X-ray diffraction for a similar compound, 3,5′-cycloadenosine iodide. The experimental and theoretical problems of using the NOE for conformation studies of molecules in solution are discussed.

Nuclear magnetic double resonance methods have found wide application in the study of molecules in solution, including studies of relaxation mechanisms and chemical exchange, and as an aid in analyzing complex spectra. Double resonance effects and their applications have been reviewed by Hoffmann and Forsen,[2a] and by McFarlane.[2b]

Among these effects is the nuclear Overhauser effect (NOE), which is the change in the integrated intensity of one resonance in an nmr spectrum when a strong radiofrequency field is applied at the frequency of a second resonance in the spectrum. In spin systems which relax primarily through dipole–dipole interactions, such as all-proton systems, the magnitude of the Overhauser effect has a $1/r^6$ dependence on the spatial separation of the observed and irradiated spins. Because of this, the NOE has also found application in studies of organic molecular conformation and structure.[3–18]

Previously, only qualitative application of this structure tool has been made, but because of its well-defined spatial dependence, the Overhauser effect may also be used quantitatively to determine relative internuclear distances.

In this paper, the relation between internuclear distances and the observed change in line intensity during an NOE experiment is presented and the conditions on its validity are considered. The simple two-spin case is presented first and then generalized to the case of N spins which relax by intramolecular dipole–dipole interactions and intermolecular mechanisms. This treatment applies directly to most cases of organic chemical interest since it will, with few exceptions, be valid for all-proton systems. The inclusion of other relaxation mechanisms, such as the scalar and quadrupole mechanisms, is discussed. Factors important in obtaining reproducible experimental results, such as the strength and accurate positioning of the irradiating rf field, are then considered in some detail. Finally, the technique is illustrated by determining the ratios of several internuclear distances in 2′,3′-isopropylidene-3,5′-cycloguanosine (**1**) (see Figure 5) and comparing them with values based on Zussman's[19] X-ray study of the very similar compound, 2′,3′-isopropylidene-3,5′-cycloadenosine iodide (**2**).

Theory

Consider an Overhauser experiment in which the spin i is irradiated while the resonance of spin d is detected. Such an experiment consists of applying a strong rf field of amplitude H_2 at a frequency ω near ω_i, where ω_i is the Larmor frequency of spin i, and observing the indirect effect of the strong rf field on spin d by sweeping a weak rf field of amplitude H_1 through the resonance at ω_d. With the z axis chosen in the direction of the static magnetic field and H_1 too weak to cause saturation of spin d, the area of the observed signal is proportional to $\langle I_{zd}\rangle$,[20] where $\gamma_d\hbar\langle I_{zd}\rangle$ is the expectation value of the magnetization in the z direction due to spins d. It is the change produced in $\langle I_{zd}\rangle$ by the H_2 field that is of interest.

Throughout this paper, it will be assumed that the extreme narrowing condition is met. Under the three additional conditions (1) the relaxation of the observed spin, d, by the irradiated spin, i, occurs exclusively by a dipole–dipole mechanism, (2) H_2 is

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(1) (a) Department of Chemistry; (b) School of Pharmacy.

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sufficiently strong to completely saturate the resonance of i, so that $\langle I_{zi}\rangle = 0$, and (3) H_1 is sufficiently weak so that it causes no change in the population of the states of spin d, Abragam[21] gives the relation

$$\langle I_{zd}\rangle = I_{0d} + (1/2)I_{0i}S_d(S_d + 1)/S_i(S_i + 1) \qquad (1)$$

where S_k is the total spin quantum number of nucleus k and I_{0k} is the equilibrium value of $\langle I_{zk}\rangle$ in the absence of all irradiating fields. I_{0k} is thus proportional to the area of the nmr line of spin k in the absence of the strong rf field. Using the relation $I_{0i}/I_{0d} = \gamma_i S_i(S_i + 1)/\gamma_d S_d(S_d + 1)$, where γ_k is the gyromagnetic ratio of spins k, $\langle I_{zd}\rangle$ can be written as

$$\langle I_{zd}\rangle = I_{0d}(1 + \gamma_i/2\gamma_d)$$

In terms of the fractional change in intensity of line d due to saturation of spin $i, f_i{}^d$, this becomes

$$f_i{}^d = \frac{\langle I_{zd}\rangle - I_{0d}}{I_{0d}} = \gamma_i/2\gamma_d \qquad (2)$$

If i and d are the same chemical species, eq 1 shows a 50% enhancement in the intensity of d when i is saturated. It also shows that, if only two spins are present, no information on geometry is available from the experiment. Both these calculations are altered when interactions between three or more spins are involved. If a molecule contains more than two spins, concrete information on its geometry can be extracted, provided the number of intramolecular NOE experiments at least equals the number of parameters to be determined.

The procedure used by Abragam to obtain eq 1 can be applied to a system of N spins with the result that

$$f_i{}^d = (1/2)\frac{\gamma_i \rho_i{}^d}{\gamma_d P_d} - (1/2)\sum_{k \neq i,d} \frac{\gamma_k \rho_k{}^d f_i{}^k}{\gamma_d P_d} \qquad (3)$$

with $\rho_j{}^k$ = the contribution of spin j to the relaxation of spin k

$$P_d = \sum_{j=1}^{N} \rho_j{}^d + \rho_{\text{other}}{}^d$$

and $\rho_{\text{other}}{}^d$ = the intermolecular contribution to the relaxation of d. A more detailed justification of eq 3 is given in the Appendix. The notation used here is similar to that of Solomen[22] and Solomen and Bloembergen,[23] and is connected to that of Abragam by

$$\rho_j{}^i = 1/T_1{}^{ii}, \qquad \sigma_j{}^i = 1/T_1{}^{ij}$$

In addition to extreme narrowing and conditions 2 and 3 discussed in connection with eq 1, eq 3 also requires the following four conditions. (1) The spins relax entirely by intramolecular dipole–dipole interactions and intermolecular interactions. (2) The irradiated line is sufficiently far from the observed line so that $(\omega_i - \omega_d)^2 T_{2d}{}^2 \gg \gamma_d{}^2 H_2{}^2 T_{2d} P_d{}^{-1}$, where T_{2d} is the transverse relaxation time of spin d. This condition assures that the intensity of the observed line will not be altered by direct saturation at the high H_2 strengths used. This is the usual condition for nonsaturation[24] with $P_d{}^{-1}$ serving in place of T_1, the longitudinal relaxation time of spin d. (3) Any contributions to the relaxation rate due to correlated motions of the interacting spins is small. Numerous calculations based on the semiclassical density matrix theory have been carried out on three- and four-spin systems, and in all cases, the additional terms arising from correlated motions of the spins have, in fact, been negligible in the extreme narrowing case.[25–32] (4) For any spin which is J coupled to the observed spin, the magnitude of the coupling constant must be much less than the chemical shift difference between it and the observed spin. This "weak-coupling" condition also applies to J couplings with the irradiated spin. In such weakly coupled cases, the populations of the states of one spin are relatively independent of the populations of the states of the other spin, except insofar as they are connected by some relaxation mechanism. Under these circumstances, the only effect of the coupling is to distribute the intensity of the resonance among the lines of the resulting multiplet, thus requiring integration of the multiplet in order to obtain the intensity. However, even with a singlet, it is advisable to use the area rather than the amplitude as measure of intensity because the presence of the strong rf field and/or unresolved J couplings may alter the width of the line and thus destroy the proportionality between intensity and amplitude. This qualification of the statement about peak height measurements made by Hart and Davis[18a] is necessary for rigorously quantitative NOE studies. Nevertheless, since peak height measurements are so much easier, it is acceptable to use them for semiquantitative studies as long as it is proven that areas and amplitudes correlate well in the given case.

In the presence of J coupling, the spin functions in a multispin system become mixed and the energy levels are changed. The extent of this mixing becomes large as the chemical shift difference approaches or becomes less than the coupling constant. In such tightly coupled spin systems, strong irradiation of one transition may affect the populations of all levels in the system. These cases cannot be properly handled by the simple treatment given here, but rather require a full density matrix treatment.[33–35]

Because both sides of eq 3 are divided by P_d, which is the sum of all contributions to the relaxation of spin d, any relaxation mechanism of d in addition to its interaction with i tends to decrease the value of $f_i{}^d$. Since a given spin will usually interact strongly with only a few other spins, it will nearly always be the case that either $\rho_k{}^d \ll P_d$ or $f_i{}^k$ will be small for most values of k. This results in the second term in eq 3 being small so that $f_i{}^d$ will normally satisfy the inequalities $0 \leq f_i{}^d \leq \gamma_i/2\gamma_d$. This is confirmed by the many cases already noted.[3–19] It is possible, how-

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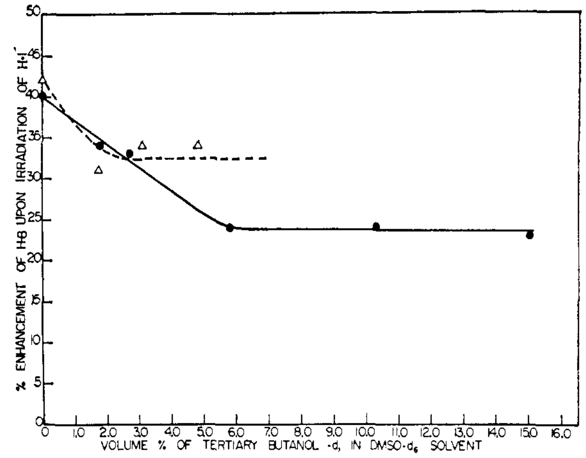

Figure 1. Dependence of the enhancement $f_8{}^{1'}$ on concentration of *t*-butyl alcohol-d_1: △, 2′,3′-isopropylidene-3,5′-cycloguanosine (**1**); ●, 3,5′-cycloguanosine (**3**). The rf field strength was 2.78 mG for all experiments depicted here.

ever, for the second term in eq 3 to be large and, in some cases, its absolute magnitude may exceed that of the first term. It is then possible to have $f_i^d > \gamma_i/2\gamma_d$ or $f_i^d < 0$. A negative value of f_i^d is most likely to occur if a third spin is closer to both the irradiated spin and the observed spin than they are to each other. Such a case has, in fact, been reported by Bell and Saunders.[36] Their explanation of this phenomenon is essentially a special case of the theory presented here.

Another interesting consequence of eq 3 is that the effect of saturating more than one resonance at the same time is approximately the sum of the effects obtained by saturating each separately. This additivity will be almost exact if the quantities in the sum in eq 3 are each small.

In the only triple resonance Overhauser experiment attempted in this study, this additivity was confirmed. H-1′ of the guanosine cyclonucleoside was observed while saturating the H-8 and H-2′ resonances individually, and then simultaneously. The results were $f_{8'}{}^{1'} = 0.25$, $f_{2'}{}^{1'} = 0.13$, and $f_{8',2'}{}^{1'} = 0.39$, in reasonable agreement with the expected additivity. These enhancements have not been corrected for the replacement of 19% of the H-8 protons with deuterium as this correction does not affect their additivity.

The inclusion of other relaxation mechanisms in eq 3 is straightforward. First consider relaxation mechanisms of the observed spin, *d*, which do not depend upon an interaction with other spins. This would include the quadrupole, spin–rotation, and anisotropic chemical shift mechanisms, for example.

The contribution of each such mechanism to the relaxation of *d* is simply added into P_d, and does not enter the problem in any other way. As far as the Overhauser experiment is concerned, the only effect of these terms is to decrease the fractional enhancements observed.

If the additional relaxation mechanism involves interaction with other spins in the system, then a term $\Sigma_k \rho'{}_k{}^d$ is added to P_d, where $\rho'{}_k{}^d$ is the contribution of spin *k* to the relaxation of *d* through this additional mechanism. In addition, eq 3 must be rewritten as

$$f_i^d = \frac{\gamma_i}{\gamma_d}\frac{(\rho_i{}^d/2 + \xi\rho'{}_i{}^d)}{P_d} - \sum_{k\neq i,d}\frac{\gamma_k(\rho_i{}^d/2 + \xi\rho'{}_k{}^d)f_i^k}{\gamma_d P_d} \qquad (4)$$

where the constant ξ depends upon the type of relaxation mechanism involved. Abragam has given values for ξ;[37] if the mechanism involves a bilinear coupling (the dipole–dipole interaction being of this type), two quantum transitions (*e.g.*, $|\alpha\alpha\rangle \rightarrow |\beta\beta\rangle$) are allowed, and this leads to the value $\xi = +{}^1/_2$. If the mechanism involves a scalar coupling which is modulated by chemical exchange, only zero quantum transitions (*e.g.*, $|\alpha\beta\rangle \rightarrow |\beta\alpha\rangle$) are allowed, and $\xi = -1$. If the modulation of the scalar coupling results from rapid relaxation of the spin *k* instead of chemical exchange, and if the relaxation of *k* results from an interaction which has a white frequency spectrum (*e.g.*, if *k* experiences quadrupole relaxation), then $\xi = -{}^1/_2$. However, of the latter two mechanisms, only the exchange modulated scalar mechanism is likely to be of importance[22,23] in liquid systems of nuclear spins. There are no bilinear mechanisms effective in liquids other than the dipole–dipole interaction, and the terms $\rho_j{}^k$ arising from scalar coupling are, in practice, usually far too small to have any effect. Bell and Saunders[36] suggested scalar relaxation might be important in the system they studied, but substitution of typical values into Abragam's equations[38] shows that unless $J \sim \delta$ (a case for which the equations are not valid), scalar relaxation will not be an important contribution to the Overhauser effect. Thus, provided $J \ll \delta$, NOE experiments can routinely be done between *J*-coupled spins and the enhancement expected in such cases will be the same as that which would arise if *J* were zero.

For intramolecular relaxation through the dipole–dipole interaction, the ρ's in eq 3 are given by[39]

$$\rho_j{}^k = \rho_k{}^j = \hbar^2\gamma_j{}^4\tau_c\langle r_{kj}{}^{-6}\rangle \qquad (5)$$

where $\langle r_{kz}{}^{-6}\rangle$ is the mean of the reciprocal sixth power[40] of the distance between *k* and *j*, and τ_c is the correlation time for the dipole interaction. If *j* and *k* are different nuclear species, the factor $\gamma_j{}^4$ in eq 5 is replaced with $\gamma_j{}^2\gamma_k{}^2$.

If the tumbling of the molecule is isotropic and any internal motions are slow, τ_c will be the same for all intramolecular interactions and, upon substituting (5) into (3), the constant $\hbar^2\tau_c$ can be removed from the expression. Further assuming that all spins have the same gyromagnetic ratio

$$f_i^d = \frac{1}{2}\frac{\langle r_{id}{}^{-6}\rangle}{R_d} - \frac{1}{2}\sum_{k\neq d,i}\frac{\langle r_{dk}{}^{-6}\rangle f_i^k}{R_d} \qquad (6)$$

where $R_d = \Sigma_k\langle r_{dk}{}^{-6}\rangle + a_d = P_d/\gamma^4\hbar^2\tau_c$ and $a_d = \rho_{\text{other}}{}^d/\hbar^2\gamma^4\tau_c$. Equation 6 is a convenient form for calculating the effect expected for a given molecular geometry, as all the f_j^k can be estimated by computing just the first term of eq 6 for each possible pair of indices, *i* and *d*, and then improving these values iteratively by substituting them back into (6).

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(36) R. A. Bell and J. K. Saunders, *Can. J. Chem.*, 46, 3421 (1968).

(37) Reference 21, p 312.

(38) Reference 21, p 311, eq 127.

(39) Reference 21, p 295.

(40) The third power size dependence mentioned by Hart and Davis[18a] occurs only when the interaction between two spins is intermolecular and the interspin distance is determined by diffusion.

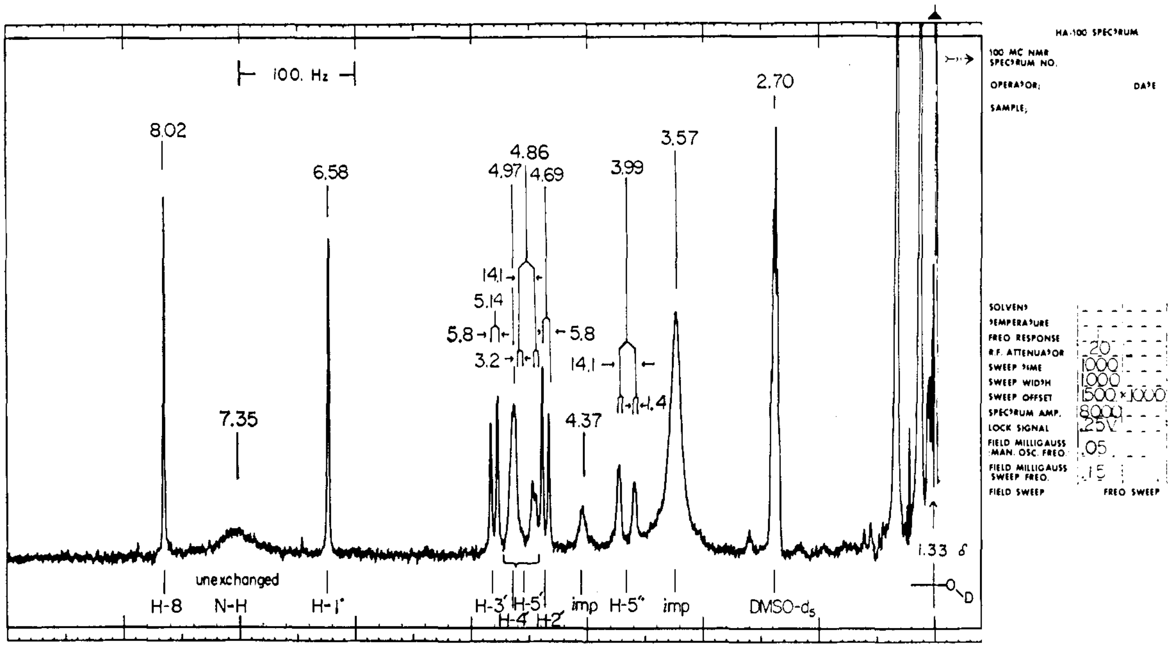


Figure 2. Spectrum (100 MHz) of a 0.25 *M* sample of 2',3'-isopropylidene-3,5'-cycloguanosine (**1**) in DMSO-d_6 containing 1.8% *t*-butyl alcohol-d_1 v/v. Chemical shifts are in parts per million from TMS, and coupling constants in hertz. Actual parameters for the 4',5',5'' system, which is tightly coupled, are given in the text. Line widths ranged from approximately 0.8 Hz for H-8 to approximately 2.4 Hz for the H-4', H-5', and H-5'' protons. The field homogeneity was maintained at 0.4 Hz or better.

In cases where the Overhauser experiment can be carried out between a sufficient number of spin pairs, eq 6 can be inverted to calculate relative values of the $\langle r_{ij}^{-6}\rangle$ from the observed Overhauser effects. If the molecule is rigid, this leads directly to relative values of the r_{ij}. In carrying out the inversion, it is more convenient to write eq 6 in the form

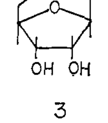
$$a_d f_i^d + \sum_{j\neq d}\langle r_{dj}^{-6}\rangle(f_i^d + (^1/_2)f_i^j) = 0 \qquad (7)$$

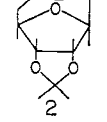
Substituting actual experimental enhancements for the f_i^j, the equations are solved simultaneously for the $\langle r_{ij}^{-6}\rangle$. Notice that the term in parentheses involves the enhancements of all lines j in the spectrum when i is irradiated. However, some knowledge of the molecular structure is generally available before beginning the analysis, and this permits us to drop many of the terms in the sum because $\langle r_{dj}^{-6}\rangle$ will be very small for all but a few spins, namely those closest to d. Also note that the quantity f_i^i which arises in one term of the sum will be $f_i^i = -1$, as the intensity of the irradiated line is zero.

Before proceeding to illustrate the application of this procedure, it will be useful to consider several experimental factors important in obtaining reliable experimental values for the f_j^k. Instrumental considerations can be found in the review by Hoffmann and Forsen,[2a] and references therein.

First, as each additional relaxation path available to the spins reduces the magnitude of the observed Overhauser effect, the maximum effect consistent with the molecular geometry will be observed when intermolecular relaxation is negligible. For this reason, intermolecular relaxation should be minimized by use of low solute concentrations, solvents composed of atoms of low magnetic moment (CCl_4, CS_2, deuterated solvents, etc.), and by careful removal of dissolved oxygen. Nonetheless, the concentration of solute required to obtain good spectra, plus the internal reference usually required for field locking, will usually make it impossible to eliminate intermolecular effects entirely, so they must be specifically included in any calculations based on the observed effects.

The importance of eliminating sources of intermolecular relaxation is indicated in Figure 1, where the per cent enhancement observed in H-8 upon irradiation of H-1' is plotted against the percentage of *t*-butyl alcohol-d_1 in samples of **1** and 3,5'-cycloxanthosine (**3**). Both H-1' and H-8 are well-isolated singlets in these molecules (see Figure 2). A rapid decrease in the per cent enhancement as the concentration of *t*-butyl alcohol-d_1 increases is apparent. The leveling of the curve at higher percentages of the internal lock sample is not typical behavior, but probably reflects strong preferential solvation of the nucleosides by dimethyl sulfoxide (DMSO). This would cause the local concentration of *t*-butyl alcohol-d_1 in the region around the nucleoside molecules to be much lower than the concentration in the bulk solvent phase and to vary only slowly with it. This opinion is supported by the observations that (a) both the xanthosine cyclonucleoside **3** and the guanosine cyclonucleoside **1** are relatively insoluble in *t*-butyl alcohol; (b) **3** is noticeably more soluble than **1** in DMSO-*t*-butyl alcohol mixtures, as would be expected from the differences in behavior shown in Figure 1, which indicate that *t*-butyl alcohol competes more effectively with DMSO for





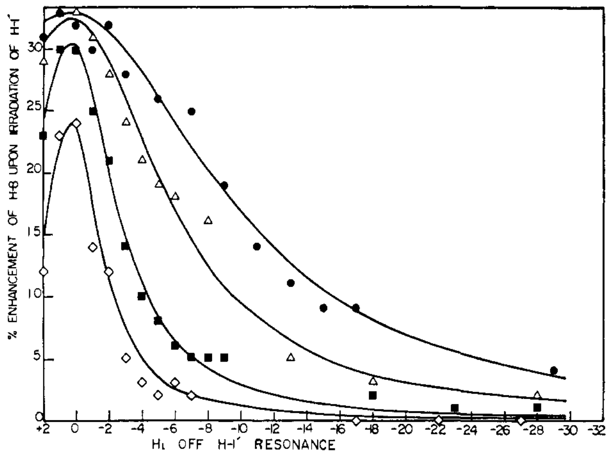


Figure 3. Dependence of the observed enhancement $f_8{}^{1'}$ on the distance of the irradiating field from the center of the saturated resonance: ●, 2.78 mG H_2; △, 1.86 mG H_2; ■, 0.94 mG H_2; ◇, 0.5 mG H_2. The sample was 0.25 *M* 2′,3′-isopropylidene-3,5′-cycloguanosine (**1**) in DMSO-d_6 containing 1.8% *t*-butyl alcohol-d_1, v/v.

association with **3** than for association with **1**; and (c) in the regions where the curves flatten out, addition of *t*-butyl alcohol to the sample causes rapid decrease in the solubility of the nucleoside.

Another important experimental factor is the strength and position of the H_2 field. This is most conveniently considered for the case of a system in which the observed spins *d* interact strongly only with the irradiated spins *i*. The observed value of $f_i{}^d$ is proportional to $\langle I_{zi}\rangle - I_{0i}$. But if the resonance of *i* is a singlet, then $\langle I_{zi}\rangle - I_{0i}$ will be a Lorentzian function of the frequency of the irradiating field, and $f_i{}^d$ will also be a Lorentzian function of ω. The Lorentzian will be centered on ω_i and have a half-height width of $(1 + \gamma_i{}^2H_2{}^2T_{1i}T_{2i})^{1/2}/\pi T_{2i}$.

The observed dependence of $f_i{}^d$ on ω and H_2 is shown in Figure 3 where the per cent enhancement of H-8 is plotted against $\omega - \omega_{\mathrm{H1'}}$ for four values of H_2. The lines through the points are Lorentzians obtained by calculating curves for various values of the width and visually selecting the one which gave the best fit.

It can be seen in the figure that the enhancement *vs.* frequency curve becomes quite flat on top at high powers. This makes it advantageous to operate at a power in excess of that required to saturate *i* (for $\omega = \omega_i$), because the effects of small instabilities or error in H_2 field or frequency settings will be minimized. Also taking into account the possibility of saturating neighboring resonances, H_2 should be selected as large as is consistent with the inequalities

$$|2\pi\delta|^2 \gg \gamma^2H_2{}^2 \gg T_{2i}{}^{-1}T_{1i}{}^{-1}$$

where δ is the chemical shift difference between *i* and the nearest other resonance. This may be operationally defined as operating at as high an rf field strength as possible while remaining in the flat region of the curve in Figure 4. This curve should decrease again at higher powers than those shown in the figure.[35]

Experimental Section

3,5′-Cycloxanthosine (**3**) was prepared according to Hampton and Nichol[41] and 2′,3′-isopropylidene-3,5′-cycloguanosine (**1**) according to Holmes and Robins.[42]

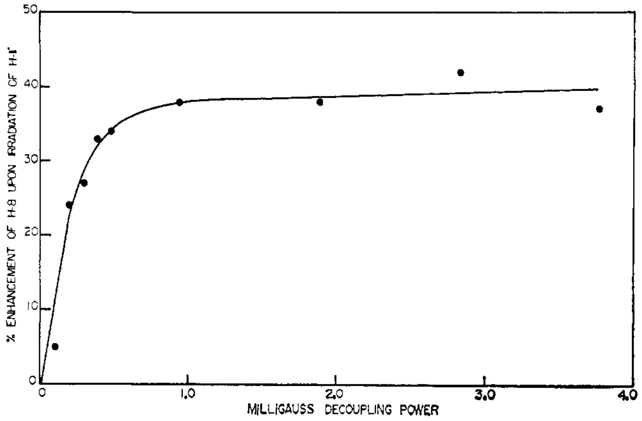


Figure 4. Relation between enhancement of H-8 in 2′,3′-isopropylidene-3,5′-cycloguanosine (**1**) upon irradiation of H-1′ at various power levels. The sample was a 0.25 *M* solution in DMSO-d_6 in a coaxial assembly.

For samples in which intermolecular relaxation by the lock sample was to be avoided, a coaxial assembly was employed consisting of an inner tube (Wilmad No. 516-I) fit snugly and concentrically into a standard precision nmr tube (Wilmad No. 507-pp) by means of two Teflon shims appropriately placed between the cylinders. Hexamethyldisilizane, placed in the annular space, served as an external lock sample.

Internal standard samples were prepared in Wilmad No. 507-pp tubes. The lock sample was *t*-butyl alcohol-d_1 (Merck, 98% isotopic purity), as it has optimum solubility and chemical shift characteristics. The volume percentages quoted for concentrations of the internal lock material are based on volumes before mixing.

After recrystallization of **1** from D_2O and a single lyophilization of **3** from D_2O, each compound was dissolved in dimethyl sulfoxide (Merck, 99.5% isotopic purity) with or without internal lock material, as required, and degassed by at least three freeze–pump–thaw cycles at less than 10^{-5} Torr before being sealed off. All cyclonucleoside solutions were 0.25 *M*.

A 100-MHz spectrum of cycloguanosine is shown in Figure 2, along with the assignments, apparent coupling constants, and chemical shifts. The two 5′ protons and the 4′ proton constitute an ABC system with the approximate parameters ν_A 97 Hz, J_{AB} = ±3.0 Hz; ν_B 100 Hz, J_{BC} = ±2.4 Hz; ν_C 0 cps, J_{AC} = +14.4 Hz. Calculations of a spectrum using these parameters and a line width of 2.4 Hz reproduced the positions and all structural features of the envelope, but there were some small differences between the observed and calculated spectra with respect to the relative amplitudes in the downfield group. The large value of J_{AC} would seem to indicate that these are the 5′ protons while B is the 4′ proton. Decoupling the upfield resonance (C) gave an AB quartet downfield which was in reasonable agreement with these parameters. Because the lines are broad and overlap considerably, including some overlap with the adjacent H-2′ and H-3′ resonances, further improvement of these values would be difficult.

The resonances at δ 4.37 and 3.57 are impurities, at least one of them probably being water. A portion of the same batch on which the spectrum was taken was recrystallized from anhydrous ethanol. Following this treatment, the impurity lines were barely detectable in the nmr spectrum. Recrystallization of another portion from D_2O and subsequent drying at 100° for 120 hr under 5 Torr of pressure completely eliminated the line at δ 4.37 and greatly reduced the intensity of the line at δ 3.57. A high-resolution mass spectrum at a sample pressure of 10^{-5} Torr and 35° showed a large peak at *m/e* 18 indicating the presence of an unusual amount of H_2O, probably incorporated as a nucleoside hydrate.

Experiments were performed on a Varian HA-100 nmr spectrometer run in the frequency sweep mode. Experiments done to show the effects of internal lock material and frequency and strength of the irradiating field were run as described by Hart and Davis[18a] using a 1000-Hz sweep width and a 2-Hz/sec sweep rate. Enhancements to be used in the calculations were measured in the same manner except that the resonance was recorded with a 50-Hz sweep

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(41) A. Hampton and A. W. Nichol, *J. Org. Chem.*, **32**, 1688 (1967).

(42) R. E. Holmes and R. K. Robins, *ibid.*, **28**, 3483 (1963).

width and 1.0-Hz/sec rate, and the areas were measured with a planimeter. Ten pairs of reference and Overhauser traces were taken for each different experiment and each trace was integrated twice and averaged to give the area used in computing an enhancement for the particular experiment. Planimeter integrations were thought to give better accuracy and precision than the standard nmr capacitor integrator.

Multiple resonance experiments were facilitated by a bank of four Wein bridge oscillators, individually amplitude and frequency tunable over the range of 1-4 kHz. Frequencies were measured using a Transistor Specialties, Inc. Model 361-R universal counter.

Illegitimate negative effects[43] and erratic results due to overloading part of the Model 4354A internal reference stabilized controller were avoided by moving the spectrum amplitude control to a point between the second and third stages of the 4354A, allowing attenuation of the excessive signals often generated in multiple resonance experiments before overloading of the 60-db amplifier and/or the audio phase sensitive detector could occur.

Calibration of the voltage output of the decoupling oscillators in terms of milligauss was made by measuring Bloch-Siegert shifts as described by Johnson.[44]

The probe temperature during all experiments was 31 ± 1°.

Application to Cycloguanosine

The quantitative use of the nuclear Overhauser effect in conformation studies will be illustrated by determining the relative distances between H-8, H-1′, and H-2′ in compound **1** (see Figure 5). The guanosine cyclonucleoside is a particularly good model because the resonances of protons 1′, 2′, 3′, and 8 are well separated in the spectrum (see Figure 2) making experiments between several spin pairs possible, and because the molecule is fairly rigid, thus minimizing any complications that might arise from internal motions. Further, the ratios of intermolecular distances obtained can be checked by comparison with values based on Zussman's[19] X-ray study of **2**, which is expected to be very similar to **1**.[45]

The relative distances are obtained by writing eq 7 for the four experiments: irradiate H-8 while observing H-1′ and H-2′, and irradiate H-1′ while observing H-2′ and H-8. In the notation of eq 7, these experiments correspond to $(i, d) = (8, 1'), (8, 2'), (1', 2')$, and $(1', 8)$. These four equations are then solved simultaneously for $\langle r_{1'8}{}^{-6}\rangle$ and $\langle r_{2'8}{}^{-6}\rangle$ in terms of $\langle r_{1'2'}{}^{-6}\rangle$. The values of the experimental enhancements are given in Table I. Substituting these values into eq 7, the equations to be solved are

$$0.36a_8 - 0.41\langle r_{2'8}{}^{-6}\rangle - 0.14\langle r_{1'8}{}^{-6}\rangle = 0 \qquad (8)$$

$$- 0.50\langle r_{2'8}{}^{-6}\rangle + 0.05\langle r_{1'8}{}^{-6}\rangle = 0 \qquad (9)$$

$$0.10a_{1'} - 0.40\langle r_{1'2'}{}^{-6}\rangle + 0.10\langle r_{1'8}{}^{-6}\rangle = 0 \qquad (10)$$

$$0.32a_{1'} + 0.32\langle r_{1'2'}{}^{-6}\rangle - 0.18\langle r_{1'8}{}^{-6}\rangle = 0 \qquad (11)$$

In writing eq 8–11, it has been assumed that $\langle r_{j8}{}^{-6}\rangle$ is negligible for $j \neq 1'$ or $2'$, and that $\langle r_{j1'}{}^{-6}\rangle$ is negligible for $j \neq 2', 3'$, or 8. It can be seen from models of this molecule that the separations of the nuclei whose interactions are assumed negligible here are sufficiently large in all physically allowed conformations to make this assumption reasonable. Interactions which are ignored but are not entirely negligible will be partly compensated by the presence of the terms a_i in the equations.

Table I. Fractional Enhancements Observed in 2′,3′-Isopropylidene-3,5′-cycloguanosine[a]

	Observe		
Irradiate	H-2′	H-1′	H-8
H-3′		0.05 ± 0.04	0.00
H-2′		0.10 ± 0.05	0.00
H-1′	0.10[b]		0.36 ± 0.02
H-8	0.00	0.32[c]	

[a] Experiments reported here done in external lock mode with a coaxial sample tube. Uncertainties listed are standard deviations. [b] This value is only approximate as 2′ lies too close to part of the 4′,5′ resonances for accurate determination of its intensity. [c] The experimental value is 27 ± 0.04. However, H-8 is exchanged to the extent of 19% (as determined by integration of the H-8 and H-1′ resonance), and the value in the table is corrected for this.

This can be seen by considering the effect of the interaction of H-1′ and H-4′ (assumed negligible above) on the experiment in which H-8 is irradiated and H-1′ observed. This interaction enters the problem through the term with $j = 4'$ in eq 7, and thus contributes the quantity $\langle r_{1'4'}{}^{-6}\rangle(f_8{}^{1'} + {}^1/_2 f_8{}^{4'})$ to eq 8. Using the experimental fact that $f_8{}^{4'} = 0$, this term reduces to $\langle r_{1'4'}{}^{-6}\rangle f_8{}^{1'} = 0.36\langle r_{1'4'}{}^{-6}\rangle$, which is of exactly the same form as the first term in eq 8. Thus, if $\langle r_{1'4'}{}^{-6}\rangle$ is not actually negligible, the only effect of ignoring it will be to cause the calculated value of "a_8" to correspond to $a_8 + \langle r_{1'4'}{}^{-6}\rangle$. Provided we have no intention of using a_8 in any further analysis, this loss of definition will not matter.

Equations 8 and 9 are now solved for a_8 in terms of $\langle r_{1'8}{}^{-6}\rangle$, and eq 10 and 11 for $a_{1'}$ in terms of the same quantity. The result is $a_8 = 0.28\langle r_{1'8}{}^{-6}\rangle$ and $a_{1'} = 0.25\langle r_{1'8}{}^{-6}\rangle$. The similarity of these values is expected considering the close proximity of H-1′ and H-8 in the molecule. Substituting these expressions for a_8 and $a_{1'}$ back into eq 8–11 and solving, we obtain $r_{1'8} = 0.83r_{1'2'}$ and $r_{2'8} = 1.29r_{1'2'}$.

In order to compare these ratios with the X-ray study of **2**, a model of **1** (Framework Molecular Models, Prentice-Hall, Inc.) was carefully constructed using the bond lengths and angles given by Zussman.[19] The X-ray study did not locate hydrogens, so these were positioned using a C–H bond length of 1.07 Å and the usual tetrahedral fasteners (ribose protons) or trigonal fasteners (H-8). The structure of the molecule, including important bond lengths, is shown in Figure 5. The glycosidic bond and the purine base are coplanar. The comparison is made by measuring the model and using this value to calculate $r_{1'8}$ and $r_{2'8}$ from the experimental ratios. The values of $r_{1'8}$ and $r_{2'8}$ are then compared

Table II. Comparisons of Internuclear Distances in Models with Calculated Results

	$\theta = 0°$ [a]		$\theta = 35°$ [a]	
Distance	Measured on model, Å	Calcd from exptl ratios, Å	Measured on model, Å	Calcd from exptl ratios, Å
$r_{1'2'}$	2.8		2.9	3.1
$r_{1'8'}$	2.5	2.4	2.6	
$r_{2'8}$	3.7	3.6	4.6	4.0

[a] θ is the angle between the glycosidic bond and its projection on the plane of the base. In the second case, the plane of the base is tilted away from the ribose.

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(43) B. M. Fung, *J. Amer. Chem. Soc.*, 90, 219 (1968).

(44) L. Johnson, Varian Associates Technical Information Bulletin, Summer, 1965, p 7.

(45) Unfortunately, the X-ray analysis of compound 1 is not available. The differences in structure between 1 and 2 should be minor in terms of the gross geometry of the two molecules.

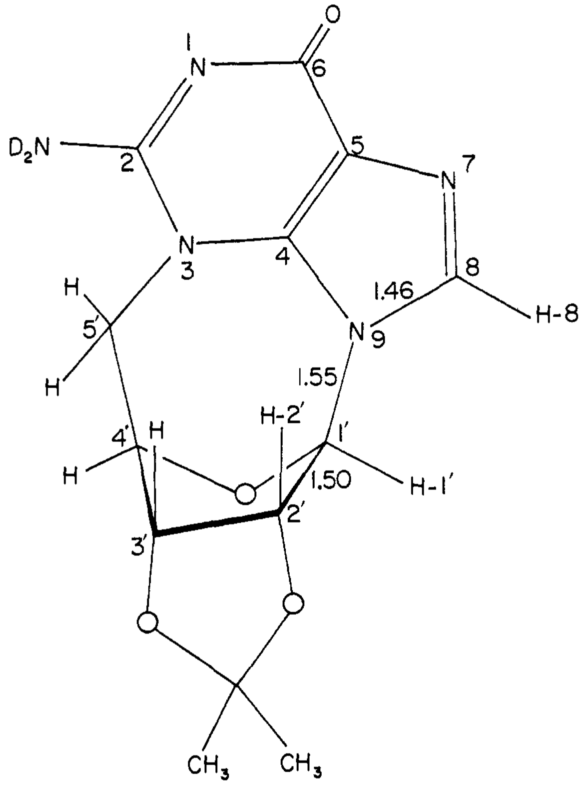

Figure 5. 2',3'-Isopropylidene-3,5'-cycloguanosine (1). Indicated distances are in ångströms.

with the corresponding distances in the model. The results appear in columns one and two of Table II.

In order to obtain some idea of how strongly the experimental distance ratios would limit the conformation of the molecule, calculations were made on a model that differed from the one discussed above only in that the purine plane formed a 35° angle with the glycosidic bond. The data are recorded in columns three and four of Table II. The values in the table, in which $r_{1'8}$ is used as the reference distance, are the best fit possible within the limits set by the bond lengths used and the cyclic structure of the molecule. It can be seen that the Overhauser data would eliminate angles of this magnitude from consideration.

Discussion

The connection between the Overhauser effect and internuclear distances described in this paper allows the ratios of these distances to be determined for a molecule in solution. In the event that the basic structure of a molecule severely limits the number of conformations it may assume, as in the present case, a reasonable set of values for bond lengths combined with the experimental distance ratios will allow the conformation-dependent internal angles to be determined uniquely.

The accuracy in such a determination is difficult to estimate and will vary from case to case, as the sensitivity of the ratios of internuclear distances to changes in an internal angle are very dependent on the molecular structure intervening between the nuclei. As an example, it can be seen in Figure 5 that the ratios used in our example are not as sensitive to the angle between the base and the glycosidic bond as might be desired because the distance of H-8 from the axis of rotation is quite small. All ten measurements that comprised each of the nuclear Overhauser enhancements reported in Table I fell within 2σ of the average of the ten determinations of the particular interaction. Standard deviations in the experimental values are included in Table I and are generally ±0.03 or an experimental error of about ±10% of the larger observed enhancements. Besides random error, there are also several sources of systematic error which cannot be estimated easily. These include nonlinearity in the instrument, particularly at the high power levels required, errors in phase settings, and errors in determining the base line used in the integrations. Each of these errors would tend to reduce the signal intensity, but some compensation will occur because only ratios of intensities are used.

The most serious limitation on the application of this method in conformation studies is the requirement that several nuclei between which the experiment can be done must be situated in such a way that their separation depends upon the internal coordinate of interest. In order for the experiments to be possible, the resonances of the nuclei must be well separated in the spectrum. Nonetheless, we feel that the accuracy and range of applicability of the method are sufficient to make its potential utility in conformation studies very high, particularly when the simplicity of the experiment and calculation is considered.

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Appendix. The Multispin Case. Justification of Eq 3

In a many nuclear spin system, we assume that the relaxation Hamiltonian can be written generally as

$$\mathcal{H}'(t) = \sum_{i>j} \mathcal{H}_{ij}^{\mathrm{dd}} + \sum_{i} \mathcal{H}_{i}^{z} \qquad \text{(A1)}$$

where $\mathcal{H}_{ij}^{\mathrm{dd}}$ is the dipole–dipole coupling of spins i and j and $\mathcal{H}_i^z$ represents other relaxation mechanisms of spin i, *e.g.*, intermolecular dipole–dipole, quadrupole, spin rotation, etc.

Using the density matrix equations of ref 21, Chapter VIII, it can be shown that the equation of motion of $\langle I_{zd}\rangle$ for spin d is

$$\frac{\mathrm{d}\langle I_{zd}\rangle}{\mathrm{d}t} = -\sum_{j\neq d}[\rho_j^d(\langle I_{zd}\rangle - I_0^d) + \sigma_j^d(\langle I_{zj}\rangle - I_{0j})] - \rho_{\mathrm{other}}^d(\langle I_{zd}\rangle - I_{0d}) \qquad \text{(A2)}$$

providing that cross correlations between terms of eq A1 can be neglected (see text). In eq A2, ρ_j^d and σ_j^d are due to $\mathcal{H}_{jd}^{\mathrm{dd}}$, the dipole–dipole coupling of spin d with spin j, and are calculated as in ref 21. ρ_{other}^d is due to $\mathcal{H}_d^z$, the other relaxation mechanisms of spin d. Equations similar to A2 apply to the other spins j. If we assume that one of the other spins j is saturated (call it $i \neq d$) so $\langle I_{zi}\rangle = 0$ and solve eq A2 in the steady-state approximation, defining

$$P_d = \rho_{\text{other}}{}^d + \sum_j \rho_j{}^d$$

we get

$$\mathrm{d}\langle I_{zd}\rangle/\mathrm{d}t = 0 = P_d(\langle I_{zd}\rangle - I_{0d}) - \sigma_i{}^d I_{0i} + \sum_j{}''\sigma_j{}^d(\langle I_{zj}\rangle - I_{0j}) \quad \text{(A3)}$$

or where the double prime sum means $j \neq i, d$

$$_i{}^d = \frac{\langle I_{zd}\rangle - I_{0d}}{I_{0d}} = \frac{\sigma_i{}^d I_{0i}}{P_d I_{0d}} - \frac{1}{P_d}\sum_j{}''\sigma_j{}^d \frac{I_{0j}}{I_{0d}}\,\frac{\langle I_{zj}\rangle - I_{0j}}{I_{0j}} \quad \text{(A4)}$$

with

$$I_{0j}/I_{0d} = S_j(S_j + 1)\gamma_j/S_d(S_d + 1)\gamma_d$$

and

$$\rho_j{}^d/\sigma_j{}^d = 2S_j(S_j + 1)/S_d(S_d + 1)$$

and $f_i{}^i = (\langle I_{zj}\rangle - I_{0j})/I_{0j}$, the enhancement of *j* when *i* is saturated, we get

$$f_i^d = \gamma_i\rho_i{}^d/2\gamma_d P_d - (1/2P_d)\sum_k{}''\gamma_k\rho_k{}^d f_i{}^k/\gamma_d \quad \text{(A5)}$$

The second term on the right-hand side of eq A5 is thus the indirect polarization of *d* through the other spins *j* when *i* is saturated.

The Thermodynamics of the Association of Tri-*n*-butylammonium Cation with a Series of Alcohols and Ethers in *o*-Dichlorobenzene[1]

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Abstract: The conductances of dilute solutions of tri-*n*-butylammonium picrate in *o*-dichlorobenzene with added methanol, *n*-propyl alcohol, isopropyl alcohol, *t*-butyl alcohol, tetrahydrofuran, diethyl ether, and triphenylphosphine oxide have been measured at 0, 25, 35, and 45°. Cation–ligand association constants, K_L, for each ligand have been calculated from these data at each temperature. Values of enthalpy and entropy changes for cation–ligand association have been obtained from these results. The affinities of the alcohols for the cation as measured by values of K_L at 25° differ only by 50% but are in the order MeOH > *i*-PrOH ~ *n*-PrOH > *t*-BuOH. The exothermicity ($\Delta H°$) for the association reactions are in the reverse order, that for *t*-BuOH being almost 2 kcal more negative than that for MeOH. A second molecule of alcohol adds to the cation–alcohol complexes as the alcohol concentrations increase. The ethers and triphenylphosphine oxide do not add a second molecule to the cation–ligand complex.

Equilibrium constants, K_L, for the formation of cation–ligand complexes, eq 1, in medium and low

$$BH^+ + L \rightleftarrows B\text{–}H^+\cdots L \qquad K_L = [BH^+L]/[BH^+][L] \quad (1)$$

dielectric solvents, have been determined at 25° in a number of laboratories.[2,3] Information concerning the factors which govern the extent of reactions such as eq 1 is important in developing our understanding of specific ion–solvent interaction and all the phenomena in which this latter is involved.

We have now begun a program to determine the enthalpy and entropy changes that accompany reactions of this type. This, our first report under this program, is concerned with the measurements of values of K_L, eq 1, at 45, 35, 25, and 0° for the cation tri-*n*-butylammonium (Bu_3NH^+) as BH^+, with methanol, *n*-propyl alcohol, isopropyl alcohol, *t*-butyl alcohol, tetrahydrofuran, diethyl ether, and triphenylphosphine oxide as L. We believe the thermodynamic data obtained from these measurements are among the first of their kind to be reported. The only comparable measurements of which we are aware are those of Kebarle and Searles[4] on the thermodynamics of the gas-phase association of NH_4^+ with NH_3.

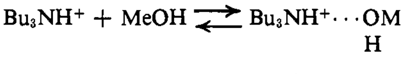
Experimental Section

Our experimental approach is based on the following: a salt, typically tri-*n*-butylammonium picrate, in *o*-dichlorobenzene solvent, exists principally as the ion pair, Bu_3NH^+, Pi^-, in equilibrium with a small fraction present as the free ions, able to conduct an electric current

$$Bu_3NH^+, Pi^- \rightleftarrows Bu_3NH^+ + Pi^- \quad (2)$$

$$K_0 = [Bu_3NH^+][Pi^-]/[Bu_3NH^+,Pi^-]$$

K_0 has been found[3a] to be 2.9 × 10^{-10} at 25°. Addition of a reagent such as methanol, which forms a 1:1 complex with Bu_3NH^+,



$$Bu_3NH^+ + MeOH \rightleftarrows Bu_3NH^+\cdots\underset{\displaystyle H}{O}Me \quad (3)$$

results in an increased free ion concentration, due to mass action, thus an increased electrical conductivity. Measurements of the conductances of the salt solution as a function of the ligand concentration furnish the raw data from which values of the cation–ligand association constants may be obtained by suitable treatment.[3a,c]

In the experiments reported here, all solutions were made up by weight. A salt solution, typically 2–3 × 10^{-4} mol/l., was placed in the conductance cell and thermally equilibrated in the bath. The

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[1] This work has been supported in part by Grant No. GP-6949 from the National Science Foundation.

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