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AN INTRODUCTION TO NUCLEAR MAGNETIC RESONANCE STUDIES OF NUCLEIC ACIDS

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In addition to the properties of mass and charge possessed by the nuclei of all elements, many nuclei also have magnetic properties. In earlier times the magnetic properties of nuclei were measured by rather difficult and cumbersome atomic- and molecular-beam techniques. In the late forties and early fifties, Block and his collaborators at Stanford as well as Purcell and his collaborators at Harvard discovered the phenomenon of nuclear magnetic resonance (NMR). The discovery of Block and Purcell allows the nuclear magnetism in ordinary bulk samples, such as water and paraffin, to be measured conveniently as experiments in physics. The chemists, however, developed this scientific area with another interest not directly related to nuclear magnetism. It was found that the NMR spectra are influenced by many features of the nuclear environment; therefore, the magnetic properties of the nuclei can be used as probes for the study of molecular structures and interactions involving the nuclei of interest. A general introduction will be given in the following section. The material in that section was selected from four general references which are useful for readers who wish to have a more extensive review of the subject (Bovey, 1969; Hollis, 1972; Carrington *et al.*, 1967; Bloembergen, 1961).

General Introduction

All nuclei with an odd mass number possess the property of a spin; the spin momentum vector is symbolized by $\vec{I}$ and is measured in units of $\hbar$, where $\hbar$ is Planck's constant divided by 2π. The value of the spin, I, is an odd integral multiple of 1/2. Nuclei with an even isotope number may either be spinless (nuclear charge is even) or possess an integral spin (I) with a

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value of 1, 2, 3, etc. The chemist is mostly concerned with the simplest nuclei having a spin of 1/2, such as ^{1}H, ^{13}C, ^{19}F, and ^{31}P, although ^{11}B, ^{15}N, and ^{14}N (spin $I=1$) are also studied.

The possession of both spin and charge confers on a nucleus a magnetic moment $\vec{\mu}_N$ which is proportional to the magnitude of the spin,

$$\vec{\mu}_N = \gamma_N \hbar \vec{I} \quad (1)$$

where γ_N is the *magnetogyric ratio* of the given nucleus and is measured in radian·sec^{-1}·gauss^{-1}. Quantum theory demands that the allowable nuclear spin states are quantized; the component m_I, the nuclear spin quantum number, in any given direction can take up only one set of discrete values which are $+I$, $(I-1)$, ..., $-I$. For the nuclei to have a nuclear spin $=1/2$, m_I may only take the values 1/2 and $-1/2$; for the nuclei to have a nuclear spin $=1$, m_I may take three values, i.e., 1, 0, -1. If a steady magnetic field $\vec{H}$ is applied on the nuclei, there is an interaction between the field and the magnetic moment $\vec{\mu}_N$, which may be represented in terms of a Hamiltonian

$$H = -\vec{\mu}_N \cdot \vec{H} = -\gamma_N \hbar \vec{I} \cdot \vec{H} \quad (2)$$

The energy corresponding to each splitting level when a magnetic field is applied will be

$$E = -\gamma_N \hbar m_I H \quad (3)$$

The selection rule for transitions among the energy levels is that m_I changes by ± 1; therefore

$$\Delta E = \gamma_N \hbar H \quad (4)$$

In order to induce transition between the two nuclear spin levels, an oscillating electromagnetic field must be applied to the system and the frequency ν of the oscillating field must satisfy the resonance condition $h\nu = \Delta E$; therefore

$$\nu = \frac{\gamma_N H}{2\pi} \quad (5)$$

This result clearly implies the following:

(1) For each nucleus (γ_N is a constant), the resonance frequency is directly proportional to the applied field $\vec{H}$.

(2) For a given field, nuclei with a larger γ_N will have larger resonance frequencies. On the other hand, for a fixed fruquency, nuclei with a large γ_N will resonate at smaller magnetic fields.

The absorption of radiation at radio frequency by placing atomic nuclei in a magnetic field usually generates four parameters:

(1) Chemical shifts

When a molecule is placed in a magnetic field H_0, orbital currents are induced in the electron clouds. Therefore each nucleus is, in effect, partially shielded from H_0 by the electrons, and the local magnetic field strength will be

$$H_{loc.} = H_0(1-\sigma) \tag{6}$$

where σ is the so-called screening constant (expressing the reduction of field). Sigma is independent of H_0 but highly dependent upon the chemical structure of the molecule and can be either positive or negative. Therefore the resonance frequencies have to be

$$2\pi\nu = \gamma_N H_{loc.} = \gamma_N H_0(1-\sigma). \tag{7}$$

When an NMR experiment is carried out with a group or a mixture of molecules at a given magnetic field, the signals from the various nuclei are spread out in a spectrum according to their nuclear environments. Since ν is proportional to the applied magnetic field, the spacing between NMR signals corresponding to different types of nuclei is also proportional to magnetic field. Thus, in NMR spectroscopy, in contrast to optical spectroscopy, there is no absolute zero or standard reference. Hence the chemical shift between two sets of nuclei is defined as the difference in their resonance frequencies measured at constant field. It is conveniently expressed in a field independent unit as part per million (ppm) of the constant field or frequency;

$$\delta = \left(\frac{\nu_2-\nu_1}{\nu_1}\right)\times 10^6 \tag{8}$$

where δ is chemical shift in ppm, ν_2 is the measured frequency and ν_1 is the reference frequency. A standard reference substance for proton and carbon-13 spectra, tetramethylsaline (TMS), has been proposed and widely accepted (Tiers, 1958).

(2) Coupling constants

In addition to the lines which had different chemical shifts, the high resolution NMR spectra of many compounds contain patterns which reveal the interactions of neighboring magnetic dipoles. Magnetic nuclei may transmit influence to each other indirectly through the intervening chemical bonds. This interaction occurs by the slight polarizations of the spins and orbital motions of the valence electrons, and the magnitude of the interaction is expressed in terms of a coupling constant J which is not affected by the tumbling of the molecules and is independent of H_0. Usually one neighboring spin (1/2) would split the resonance of a single nucleus into a doublet with

intensity 1:1; and two equivalent neighboring spins (1/2) would split the resonance of a single nucleus into a triplet with intensity 1:2:1. In general, if a nucleus of spin 1/2 has n equivalently coupled neighbors of spin 1/2, its resonance will be split into $n+1$ peaks corresponding to the $n+1$ spin states.

For nuclei whose spin $=1$ (such as ^{14}N and ^{2}H), there are three possible spin states. One such nucleus will split another nucleus into a 1:1:1 triplet, two will give a 1:2:3:2:1 pentad, and so on.

(3) The intensity of the absorption line

In the same molecule, the integrated areas under the resonance signal (or signals) are proportional to the number of nuclei contributing to such a signal (or signals).

(4) The relaxation times

In the previous sections, the behavior of an isolated, spinning nucleus has been examined. When NMR is actually observed in bulk matter, the observed signal represents a large number of identical nuclei. These nuclei may interact among themselves and with their surroundings. Consider an assembly of identical nuclei experiencing the same magnetic field; such an assembly constitutes a magnetically equivalent set. For a spin $=1/2$ nucleus, there are only two magnetic energy levels which correspond to the two alignments of the nuclear magnetic moment, i.e., either along or against the magnetic field. At equilibrium the nuclei are distributed between the two energy levels and the ratio of the number of spins in each level is given as follows

$$\frac{n_+}{n_-} = e^{-(\Delta E/kT)} \qquad (9)$$

where n_+ and n_- are the populations of the upper and lower spin states respectively, ΔE is defined by Eq. (4), k is the Boltzman constant, and T is the absolute temperature. For a given ΔE, the number of lower state spins will always be larger than that of upper state spins. If the system is irradiated at a frequency $\nu=\Delta E/h$, the system absorbs energy from the radiation field with a consequent increase in the n_+/n_- ratio. When the system absorbs sufficient energy to equilize the population of the two states, it is said to be "*saturated.*" A saturated or partially saturated spin system itself will tend to return to a thermal equilibrium when the radiation field is lifted. Two simultaneous processes are involved in the return of a saturated system to equilibrium: (1) The absorbed energy is given up from the spin system to the lattice; this process is called spin-lattice relaxation. A time period is required to accomplish this relaxation and is denoted by T_1, the spin-lattice relaxation time; (2) Redistribution of the absorbed energy among the nuclei

by mutual exchange of nuclei between the higher and lower states; this process is called spin-spin relaxation. T_2 represents the spin-spin relaxation time. These two processes, having different mechanisms, do not necessarily occur at the same rate.

With respect to biochemistry, the principal importance of these four parameters for a NMR measurement is their applicability to the study of the molecular conformation and the intermolecular interactions of molecules of interest, such as nucleosides, nucleotides, etc.

New Developments in the Technology of NMR Spectrometers

The basic requirements and theories of a NMR spectrometer will not be mentioned in this paper. Only two recent advances in technology which are of great importance will be briefly discussed: (1) pulsed NMR and Fourier Transform, (2) supperconducting magnets.

The sensitivity of a NMR spectrometer is relatively poor. The development of pulsed NMR and Fourier Transform provides a new method for increasing the signal-to-noise ratio. The conventional procedure is to sweep through the spectrum rapidly many times in succession, beginning each sweep at exactly the same point in the spectrum and then summing up all the traces (the continuous wave method). The chief drawback of this procedure is the excessive time required to accumulate several hundred scans. To overcome this difficulty, a strong *rf* field is applied to the sample as a pulse which covers the entire frequency range of a NMR spectrum simultaneously in about 100 μsec or less. The free induction decay curve following the original pulse is related to the relaxation times of all signals in the NMR frequency range; therefore, it actually contains all the information of the continuous wave spectrum. In other words, the "continuous wave" NMR spectrum is a function of frequencies and the pulsed NMR is a function of time. Advantage is taken of Fourier Transform to convert the latter spectrum to the former one which we are familiar with. The pulse decays to negligible intensity in a time which depends upon T_2 and is generally of the order of one second. This means 500 scans can be made in the time required to run one ordinary spectrum. In this experiment, the signal-to-noise ratio is increased about 22 times, the square root of 500.

The chemical shift is proportional to the magnetic field strength as mentioned previously. At a low magnetic field strength, the resolution of NMR (especially in proton magnetic resonance) is limited in the investigation on large and complex molecules, because many resonance lines in the spectrum may overlap with each other. This is especially serious for a NMR spectrum which contains some peaks with coupling constant-to-chemical shift ratios

close to unity. In order to obviate these problems, higher magnetic fields were sought. The most significant recent advance toward the goal of obtaining homogenous magnetic fields of great strength was the development of a superconducting magnet. Solenoids which are wound from niobium-alloy wire and maintained at liquid-helium temperature are capable at present of producing fields of the order of 50 kilogauss. For proton magnetic resonance, an NMR spectrometer as high as 360 MHz is now available on the market, which is six times bigger in magnetic field strength (therefore, approximately six times better in resolution) than the first high resolution analytical NMR spectrometer (60 MNz) available about ten years ago.

NMR Studies on Mononucleotides

Mononucleotides are the basic building blocks of nucleic acid. A mononucleotide contains a heterocyclic base, such as adenine, guanine, cytosine, uracil, and thymine, a D-ribose or a D-deoxyribose attached to N(9) of the purines or N(1) of the pyrimidines, and a phosphate group usually attached to the 3′ or 5′ position of the pentose. Fig. 1 shows the structure of the bases and pentoses.

(1) NMR signals assignment of mononucleotides

Though the unsubstituted purine is not a base component of nucleic acid, it is an excellent model for the properties of 6-aminopurine (adenine) and 2-amino-6-oxopurine (guanine), which are bases that appear in nucleic acid. There are three resonance lines in purine spectra for the protons H-2, H-6, and H-8, and the correct assignment of these lines to the appropriate protons was achieved independently by Matsuura and Goto (1963), by Ts'o *et al.* (see Schweizer *et al.*, 1964) and by Bullock and Jardetzky (1964) by selective substitution with deteurium. Therefore this result can be applied in assigning the base protons of adenosine and guanosine. Recently a simple method to distinguish the H-8 and H-2 in adenosine was found by us, which involves measuring the T_1 of these peaks (Kan *et al.*, unpublished); the method will be discussed in a later section. The chemical shift data of the protons (especially the base protons) of these nucleosides are summarized in Table 1.

The assignment of base protons of pyrimidine bases is relatively simple. The assignment of the high-field doublet to H-5 and low-field doublet to H-6 of uracil (or uridine) and cytosine (or cytidine) was first made by the Jardetzkys (Jardetzky, *et al.*, 1960). A singlet (its coupling constant is less than one Hz) in the low field is assigned readily to H-6 and the new peak in

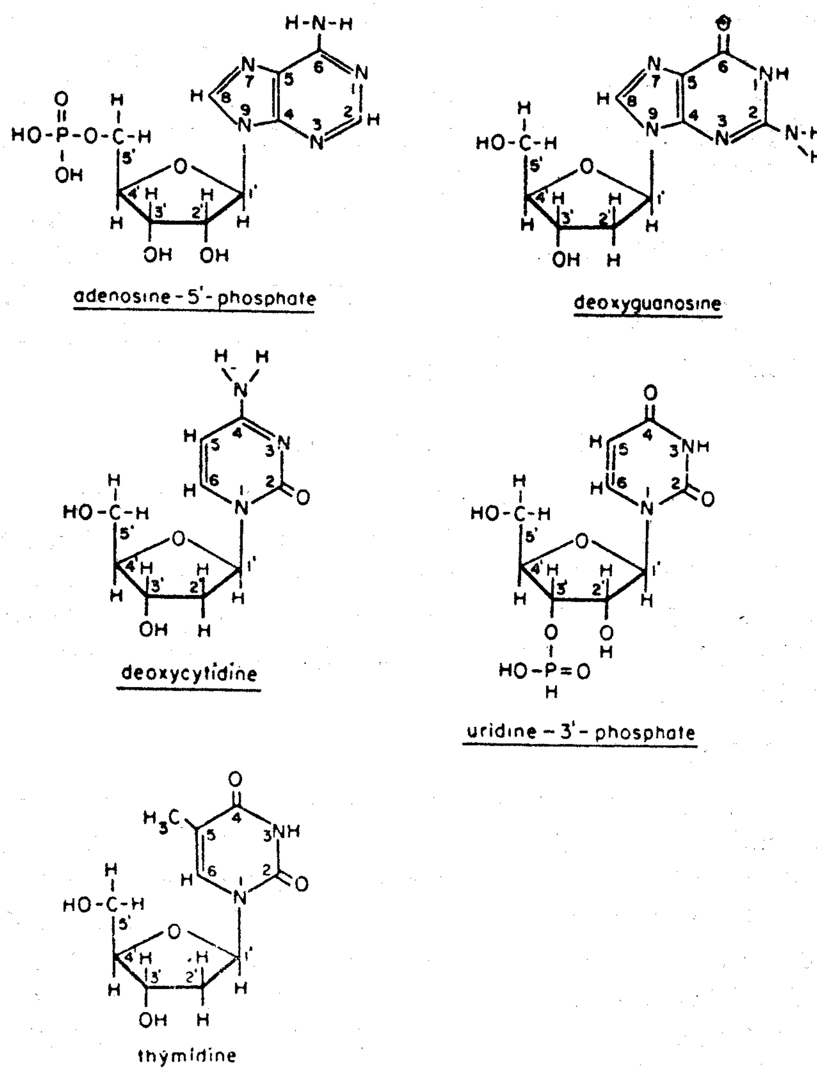

Fig. 1. Structural formula and numbering system for the atoms of the five commonly occurring nucleosides and nucleotides. These compounds are in *anti* conformation.

very high field with intensity three times that of the former one is the 5-methyl group in thymine (or thymidine). The chemical shift data of these compounds are also listed in Table 1.

The assignment of pentose protons of D-ribose and D-deoxyribose was done by Gatin and Davis (1962) in dimethyl sulfoxide medium. The chemical shifts and coupling constants of base, pentose, amino, and imino protons of eight nucleosides are listed in Table 2.

Table 1. *Proton Chemical Shifts for Nucleosides in D_2O at 33–35°C (ppm from TMS capillary) (Ts'o et al., 1969; Smith et al., 1968a)*

	Molal conc.	H(2)	H(8) or H(5)	H(6)	H(1′)	CH_3
Ribosylpurine	0.0 0.1	9.425 0.30	9.16 9.09	9.63 9.48	6.70 6.60	
Ribosyl-6-chloropurine	0.03 0.05	9.26 9.20	9.26 9.20		— 6.66	
1-Methylinosine	0.0 0.1	8.87 8.77	8.80 8.77		6.56 6.48	4.15 4.09
6-Thioinosine	0.03 0.05	8.81 8.79	8.93 8.92		6.59 6.57	
Adenosine	0.0 0.1	8.73 8.48	8.82 8.67		6.50 6.47	
N-6-Methyladenosine	0.0 0.1	8.73 8.31	8.73 8.50		6.49 6.32	3.55 3.31
Inosine	0.0 0.1	8.72 8.64	8.83 8.76		6.62 6.44	
N-6-Methyl-2′-deoxyadenosine	0.0 0.1	8.71 8.34	8.73 8.50		6.90 6.72	3.58 3.36
2′-O-Methyladenosine	0.0 0.1	8.60 8.53	8.76 8.68		6.58 6.50	3.86 3.845
2′-Deoxyadenosine	0.0 0.1	8.69 8.435	8.76 8.585		6.93 6.765	
3′-Deoxyadenosine	0.0 0.1	8.68 8.42	8.76 8.61		6.51 6.35	
N-6-Dimethyladenosine	0.0 0.1	8.63 8.25	8.69 8.48		6.51 6.31	3.89 3 54
Ribosyl-2, 6-diaminopurine	0.03 0.05		8.42 8.40		6.35 6.35	
1-Methylguanosine	0.025		8.42		6.375	3.92
Cytidine	0.0 0.1		6.51 [H(5)] 6.50	8.28 8.275	6.355 6.35	
Uridine	0.0 0.1		6.33 [H(5)] 6.325	8.28 8.275	6.35 6.34	
Thymidine	0.0 0.1			8.075 8.07	6.72 6.70	2.31 2.31
7-Methylinosine	0.0 0.1	8.71 8.60	9.68 (H_2O) 9.67 (H_2O)		6.60 6.51	4.61 4.58

(2) The "*syn*" and "*anti*" conformations of mononucleotides

The base planes of the purines and pyrimidines are rigid; and the furanose ring also has only a limited degree of freedom, such as changing the puckering conformation. Therefore, the conformational state of the nucleoside (or nucleotide) is defined principally by the rotation of these two more or less rigid planes relative to each other about the axis of the C-1′ to N-9 (purine) or N-1 (pyrimidine) bond (Fig. 1). The sugar-base torsion angle, Φ_{CN}, was first defined by Donohue and Trueblood (1960) as "the angle formed by the

Table 2. *Chemical Shifts[a] and Coupling Constants[b] of The Nucleosides in Dimethyl Sulfoxide (Matuura et al., 1963)*

Nucleoside	$H_{1'}$ ±0.01	$H_{2'}$ ±0.01	$H_{3'}$ ±0.01	$H_{4'}$	$H_{5'}$	$OH_{2'}$ ±0.05	$OH_{3'}$ ±0.05	$OH_{5'}$ ±0.05	H_2 ±0.01	H_3 ±0.01	H_4 ±0.01	H_5 ±0.01	NH_2 ±0.05	NH	NH
A	0.55	2.25–2.33	1.83	2.42–2.58	2.75–2.92	J 4.5 1.27	0.97	J 5.4 1.05	−1.68	−1.87			−0.86		
dA	0.09	2.75–3.00	1.92–2.08	2.50–2.67	2.75–3.00		1.12	J 5.6 1.16	−1.70	−1.87			−0.87		
G	0.71	2.25–2.33	2.00	2.53	2.75–3.00	1.25–1.42	J 5.7 1.02	1.25–1.33		−1.52			−0.06	−4.33–−4.42	
dG	0.31	2.75–3.08	2.08–2.17	2.58–2.75	2.75–3.08		1,17–1.25	1.42–1.50		−1.50			−0.05	−4.17–−4.50	
C	0.70	2.25–2.58	2.25–2.58	2.25–2.58	2.67–2.92						J 7.3 0.38	J 7.3 −1.7	−0.17–−0.33		
dC	0.39	4.22	2.18	2.60	2.75–2.92						J 7.9 0.22	J 7.9 −1.83		−2.25–−2.33	−3.42–−3.50
U	0.70	2.42–2.67	2.42–2.67	2.42–2.67	2.75–3.08	1.33–1.58	1.08–1.25	1.33–1.58			J 8.2 0.82	J 8.2 −1.42		−4.92	−4.67
T	0.31	4.40	2.17–2.25	2.58–2.83	2.75–3.08		1.31	1.53	4.72			−1.21		−4.72	

a) All shifts in ppm measured from aromatic toluene peak. The aromatic toluene peak was measured to be 6.86 ppm below the peak of a tetramethylsilane internal standard.

b) J in Hz ±0.5 Hz.

trace of the plane of base with the projection of the C-1′ to O-1′ bond of the furanose ring when viewed along C-1′ to N bond. This angle will be taken as zero when the furaose-ring oxygen atom is antiplanar to C-2 of the pyrimidine or purine ring and positive angles will be taken as those measured in a clockwise direction when viewing from C-1′ to N." Donohue and Trueblood further concluded that there are two ranges of Φ_{CN} for the nucleosides (or nucleotides), ca. −30° for the *anti* conformation and ca. +150° for the *syn* conformation. This torsion angle, Φ_{CN}, is a very important parameter in defining the conformation of dinucleotides and of polynucleotides.

There are five approaches to determine the Φ_{CN} of nucleosides (or nucleotides) in solution by PMR study. The first approach, which is based on the work of Cushley, Watanabe, and Fox (1967), involves the assignment of anomeric structures for the pentofuranosyl and pyranosyl pyrimidine nucleosides by PMR. These authors have observed the anisotropic effect of the double bond in the pyrimidine aglycon on the chemical shifts of the acetyls substituted at the C-2′ position of the sugars. This anisotropic field effect can be clearly illustrated by comparison of the chemical shift of the acetyl substitutes before and after the catalytic hydrogenation of the 5, 6 double bond in the pyrimidine base. With 2-substituted pentofuranosyl nucleosides, hydrogenation of the double bond causes the chemical shift of the acetyl group to move downfield. In order to explain why the anisotropic effect of the 5, 6 double bond has an effect on the 2′-acetyl substitution, the pyrimidine nucleoside has to assume an anti conformation.

The second approach is based on the comparative study of 6-substituted pyrimidine nucleosides *vs.* 5-substituted compounds and the parent molecules (Dugas *et al.*; Schweizer *et al.*, 1973). In 6-oxocytidine, 6-oxouridine (1-β-D-ribofuranosyl barituric acid) and β-cyanuric acid, there is an α-keto group at both the 2- and 6-position; therefore, the protons on the ribose ring, especially the H-2′ and H-3′ must experience the magnetic anisotropy of the keto group. In contrast, H-2′ and H-3′ protons of a 5-substituted pyrimidine nucleoside do not show this deshielding effect. For instance, it was found that the H-2′ and H-3′ of 6-oxocytidine are deshielded by 0.55 and 0.14 ppm respectively, as compared with that of 5-methylcytidine. Fortunately, on the other hand, it was found that the chemical shifts of H-2′, H-3′, and H-4′ protons of 6-methylcytidine are practically the same as those of 6-oxocytidine. Therefore, these results lead to the conclusion that 5-methylcytidine prefers an *anti* conformation and 6-methylcytidine prefers a *syn* conformation. A summary of the conformational states of eleven pyrimidine nucleosides determined by this method are shown in Table 3.

Table 3. *Proton Chemical Shifts, ppm and The Preference of Syn and Anti conformation, for Various Pyrimidine Nucleosides (Cushley et al., 1967)*

Nucleoside	H-6 (or CH_2)	H-5 (or CH_2)	H-1′	H-2′	H-3′	H-4′	H-5′, 5″	Syn, anti preference
Cytidine	8.25 8.20	6.46 6.47	6.33 6.32	4.65 4.64	4.65 4.64	4.65 4.64	4.32 4.30	Anti
5-Methylcytidine	8.18	2.44	6.40	4.71	4.71	4.71	4.40	Anti
6-Methylcytidine	2.85 2.83	6.31 6.34	6.15 6.16	5.30 5.29	4.86 4.85	4.48 4.50	4.31 4.31	Syn
6-Oxocytidine			6.61 6.63	5.16 5.19	4.80 4.81	4.42 4.42	4.27, 4.13 4.31, 4.15	Anti
Uridine	8.34	6.33	6.36	4.81	4.69	4.62	4.42, 4.22	Anti
5-Methyluridine	8.15 8.09	2.35 2.35	6.36 6.35	4.69 4.70	4.69 4.70	4.69 4.70	4.34 4.30	Anti
6-Methyluridine	2.86 2.82	6.19 6.20	6.10 6.14	5.27 5.26	4.85 4.81	4.46 4.47	4.30 4.28	Syn
6-Oxouridine			6.57	5.10	4.85	4.48	4.20	Anti
6-Azauridine		8.05 8.06	6.56 6.54	5.03 5.02	4.82 4.78	4.56 4.55	4.31, 4.11 4.29, 4.10	Anti
5-Bromouridine	8.80 8.70		6.30 6.29	4.69 4.71	4.69 4.71	4.69 4.71	4.44, 4.28 4.40, 4.26	Anti
4-Thiouridine	8.23	7.01	6.33	4.74	4.74	4.74	4.36	Anti
Showdomycin		7.14 7.16	5.23 5.22	4.60 4.58	4.60 4.58	4.60 4.58	4.24 4.22	Anti
1-β-D-Ribofuranosyl-quinazoline-2, 4-dione			6.55 6.55	4.93 4.99	4.54 4.58	4.22 *e*	4.10 4.09	Syn

The third approach is based on the affinity of paramagnetic ions (Mn^{2+}, Cu^{2+}, etc.) to the phosphate groups (Chan *et al.*, 1969). Paramagnetic ions have a broadening effect on the proton resonance through a T_2 relaxation mechanism and this effect is extremely dependent on distance. Therefore, a comparative study of the broadening effect on the NMR resonance lines before and after the addition of paramagnetic metal ion will contain information about the conformation of nucleotides. This method can be applied on both purine and pyrimidine nucleotides. In 5′-AMP, for example, the H-8 proton signal is more broadened than that of H-2 after Mn^{2+} (in trace amount) is added. The only situation that makes the H-8 on 5′-AMP closer to the 5′-phosphate group than that of H-2 is for the 5′-AMP to be in an anti conformation. In a pyrimidine nucleotide, the H-6 proton will be more affected by adding Mn^{2+} if this pyrimidine nucleotide is in an *anti* conformation. Such an effect has been observed.

The fourth approach involves measuring the T_1 of nucleosides (or nucleotides), which has been done by us (Kan and Ts'o, unpublished). The protons

in nucleic acid are mainly relaxed by the dipole-dipole mechanism. Therefore, the base proton close to the furanyl ring can be more readily relaxed than the base proton which is not influenced by the sugar ring. The T_1 of H-8 in a 5′-AMP molecule is much shorter (easily relaxed) than H-2. On the contrary, the T_1 of H-8 and H-2 in 9-ethyladenine, a compound which lacks a bulky sugar group, are almost equal to each other. Using T_1 measurements to determine the conformation of Φ_{CN} is an improvement over the addition of paramagnetic metal ions because the latter method can only be applied to nucleotides, it contaminates the sample, and the addition of a probe may disturb the conformation; the former one does not have these drawbacks.

The last approach is based on the Nuclear Overhauser Effect (Hart *et al.*, 1969), which is observed during nuclear magnetic double resonance experiments and allows the detection of a nuclear-nuclear mutual relaxation process. One can observe an enhancement of absorption intensity of one member of a pair of spacially proximate nuclei as an effect of irradiating the other. This field is still in its infancy, and it is difficult to obtain precise measurements in intensity. In principle, the measurement of T_1 will be more effective in taking advantage of the relaxation mechanism.

In summary, we can draw a rather conclusive picture of the purine and pyrimidine nucleosides (or nucleotides) which must spend a considerable time in the *anti* conformation in solution except when a bulkly group is substituted on the 6-position of a pyrimidine base or the 8-position of a purine base (such as 8-bromoadenosine).

(3) The conformation of pentose

The pentose sugar ring and the phosphate group constitute the backbone of nucleic acid; hence the study of the conformation of the pentose is another important aspect to nucleic acid research.

In general, the conformation of the furanose ring has four atoms of the ring approximately co-planar, with the fifth, either C-2′ or C-3′, displaced from this plane by 0.5 to 0.6 Å. The out-of-plane atom may be displaced either on the same side of the C-5′ atom, designated as the "*endo*" conformation or the opposite side of the C-5′ atom, designated as the "*exo*" conformation. There are four general conformations, i.e., C-2′-endo, C-3′-endo, C-2′-exo, and C-3′-exo.

The first NMR approach to this problem was done by C. Jardetzky (1960); it was based on experimental measurements of the vicinal coupling constants and Karplus relationship between the coupling constant and the dihedral angle of a H-C-C′-H′ system (Karplus, 1959, 1963). Though the application of the Karplus analysis to sugar conformation is subject to the influence of other

effects, such as substituent perturbations, C–C′ bond length, etc. (Lemieux *et al.*, 1962; Lemieux *et al.*, 1965), it is generally recognized that the following equation holds

$$J_{HH'} = J_0 \cos^2\phi - 0.28\ \text{Hz} \tag{10}$$

where $J_{HH'}$ is the vicinal coupling constant, ϕ is the dihedral angle between the H–C–C′ and the C–C′–H planes in the fragment of H–C–C′–H′. For the carbohydrate system, several J_0 values have been used (Jardetzky, 1960; Jardetzky, 1961, 1962; Abraham *et al.*, 1962; Blackburn *et al.*, 1970).

Recently the coupling constants of the protons in the furanose ring became known. These J values for five compounds are listed in Table 4. Included in Table 4 also are the computed dihedral angles (ϕ) from the Karplus equation (Eq. (10)) based on J_0=9.27 Hz for ϕ below 90° and J_0=10.36 Hz for ϕ above 90°. For each observed coupling constant, $J_{HH'}$, two possible dihedral angles ϕ and ϕ' can be computed from Eq. (10); one is below 90° and the other is above 90°. The choice between these two ϕ and ϕ' values as the real dihedral angle depends on the knowledge of the stereochemistry of the bonds involved. One major problem is that of $J_{2''-3'}$ (trans) of 3′-dAMP (2′-deoxyadenosine-3′-monophosphate), where both 55° and 125° are possible. The value of 55° is preferred tentatively because of the comparison with the same angle adopted for dApdA (Fang *et al.*, 1971).

In comparing the computed ϕ values for β-pseudouridine, uridine, 5′-AMP, and 5′-dAMP, it is interesting to note that they are similar to each other but different from the ϕ values computed for the five idealized furanose conformations also listed in Table 4. However, the experimental ϕ values for these four nucleosides (and nucleotides) are quite close to the ϕ values obtained from the average of the C-2′ endo and C-3′ endo conformations, or to a lesser extent perhaps, to the ϕ values obtained from the average of the C-2′ exo and C-3′ exo conformations (Table 4). This comparison suggests that the furanose conformation of these four nucleosides and nucleotides is not frozen in a given conformation but exists in a dynamic equilibrium among several forms. The observed J values and the calculated ϕ values are therefore "time-averaged," i.e., their magnitudes depend on the residence time in these various conformational states. The equilibrium must be rapid in terms of the NMR time scale. The line width is reasonably narrow, and there is only one set of chemical shifts and coupling constants for each compound. The most plausible equilibrium is that of a rapid C-2′ endo to C-3′ endo interconversion.

If the furanose conformation is indeed in a state of rapid equilibrium, factors which may perturb such an equilibrium should be properly assessed. The coupling constants of these nucleosides (or nucleotides) are not sensitive

Table 4. *The Observed Coupling Constants (Hz) and The Computed Dihedral Angle (ϕ) for The Furanose Ring Protons in Nucleosides and Nucleotides in D_2O (Blackburn et al., 1970; Fang et al., 1971)*

	$J_{1'-2'}$ (trans)	$J_{1'-2'}$ (cis, deoxyribose)	$J_{2''-3'}$ (trans, deoxyribose)	$J_{2'-3'}$ (cis)	$J_{3'-4'}$ (trans)
β-Pseudouridine	5.0			5.0	5.0
ϕ	135°			40°	135°
Uridine	4.4			5.3	5.5
ϕ	133°			40°	138°
3′-UMP	4.3			5.3	4.3
ϕ	133°			40°	132°
5′-UMP	4.8			5.3	4.0
ϕ	134°			40°	131°
5′-AMP	5.6			4.8	3.8
ϕ	140°			50°	130°
3′-dAMP	8.1	6.0	2.9	6.3	Not determined
ϕ	155°	35°	55° (125°)	35°	
5′-dAMP	6.9	6.6	3.8	5.8	Not determined
ϕ	145°	35°	130°	40°	
C-2′-endo	9.5	4.3	0.3	4.3	0.4
ϕ	165°	45°	75°	45°	105°
C-3′-endo		7.4	0		0.03
ϕ	145°	25°	80°	40°	100°
$H_{2'}$, $H_{3'}$ eclipsed	2.3	9.0	2.3	9.0	2.3
ϕ	120°	0°	120°	0°	120°
C-3′-endo	0.4	8.4	9.5	4.3	9.5
ϕ	105°	15°	165°	45°	165°
C-2′-exo	0.03	8.0	9.5	4.3	6.6
ϕ	100°	20°	165°	45°	145°
Average:					
C-2′-endo + C-3′-endo					
ϕ	135°	30°	120°	45°	135°
Average:					
C-2′-exo + C-3′-exo					
ϕ	120°	22°	122°	42°	122°

to temperature because there is only a 0.2 Hz change in the range of 70–80°C. This means that the enthalpy difference between two conformational states is small. Similarly, a solvent change from D_2O to DMSO-d_6 exerts no effect on the $J_{1'-2'}$ value of 5′-AMP.

Very recently, carbon-13 NMR has been used as a tool to study the

dihedral angles of P–O–C–H and P–O–C–C′. A representative example of this kind work was done by Smith *et al.* (Lapper *et al.*, 1973a, 1973b). Besides the H-H′ coupling constant, ^{1}H–^{31}P and ^{13}C–^{31}P coupling constants have also been analyzed in order to obtain the dihedral angles in 2′,3′-cyclic and 3′,5′-cyclic nucleotides. These cyclic nucleotides have an advantage in that the sugar ring conformation is more rigidly fixed. Table 5 is a summary of the dihedral angles between C–2′ to O–2′ and C–3′ to O–3′ bonds of 2′,3′-cyclic nucleotides (Lapper *et al.*, 1973b). A comparison of the data in Table 5 to the predicted data from rigid conformation will reveal that 2′,3′-cyclic nucleotides show slight preferences in their furanose ring conformational equilibria which are base dependent in normal temperature. Pyrimidine bases prefer 3′-endo (2′-exo) conformation and purine bases prefer 2′-endo (3′-exo) conformation. On the other hand, the three bond (P–O–C–C′) $J_{^{13}C-^{31}P}$ for a series of 3′,5′-cyclic nucleotides indicate that all members of the series have similar conformations; i.e., the phosphate ring is locked in the chair conformation (Lapper *et al.*, 1973a).

Table 5. *Dihedral Angles of C–2′ to O–2′ and C–3′ to O–3′ Bonds (Lapper et al., 1973b)*

Nucleotide	Calculated from Proton-Phosphorus and Carbon-Phosphorus Vicinal Coupling Constants						$\alpha(O_{2'}C_{2'})$	$\alpha(O_{3'}C_{3'})$
	Dihedral angles from ^{1}H–^{31}P coupling constants			Dihedral angles from ^{13}C–^{31}P coupling constants				
	$\theta(O_{2'}C_{2'})$	$\theta(O_{3'}C_{3'})$	Sum	$\gamma(O_{2'}C_{2'})$	$\gamma(O_{3'}C_{3'})$	Sum		
2′,3′-UMP	122	136	258	157	124	281	297	260
2′,3′-CMP	121	137	258	153	122	275	274	256
2′,3′-AMP	133	124	257	134	137	271	267	261
2′,3′-GMP	128	130	258	141	132	273	269	262

NMR Studies on Dinucleoside Monophosphates and Oligonucleotides

(1) Dinucleoside monophosphates

A unit of 3′–5′ phosphodiester linkage backbone contains a linkage of the phosphate group of a 5′-nucleotide to the O–3′ position of an adjacent uncleoside, i.e., a dinucleoside monophosphate. The backbone of a nucleic acid is formed by repeating this 3′–5′ phosphodiester linkage with all types of bases attached to this backbone. Therefore, the next step in the study of nucleic acid should be the investigation of dinucleoside monophosphates.

The possible combination of four nucleotides (A, C, G, and U) in the dimer with a 3′–5′ linkage is sixteen. For deoxyribofuranosyl sugars there will be another 16 dimers. Instead of dealing with all these dimers, we chose four dimers, namely, $rA_{3'}p_{5'}rA$ (rAprA), $dA_{3'}p_{5'}dA$ (dApdA), $dA_{3'}p_{5'}rA$ (dAprA),

and $rA_{3'}p_{5'}dA$ (rApdA), which not only have been studied extensively in our laboratory, but also are the representative models for study on the dimer conformation.

(a) Assignments and the conformation of the glycosyl bond

The first task in PMR study is the spectral assignments, which is not a difficult problem for a heterodimer, but can be challenging for a homodimer, especially the purine compounds, such as rAprA (Schweizer *et al.*, 1964; Ts'o *et al.*, 1969). Fortunately, the H-8 of the purine are exchangeable with deuterium in D_2O at high temperature. This procedure provides a simple means by which to distinguish the H-8 protons from the H-2 protons. Another very useful procedure, as mentioned earlier (Chan *et al.*, 1969), exists for separating the H-8 and H-1′ of the 5′-residue (-pN) from the H-8 and H-1′ protons of the 3′-residue (Np-). The H-8 proton of -pN and H-1′ of Np- are closer to the phosphate group in the dimer, so that these two resonance peaks are more readily broadened by added Mn^{2+}. This effect also indicates that the dimer is in an anti-anti conformation. In addition, spin-lattice relaxation measurements revealed that the two H-8 protons in the dimer have shorter T_1 values than that of the H-2's. Assuming the T_1 is relaxed by the dipole-dipole interaction, this result confirms that the dimer is in an anti-anti conformation.

Since the conformation is established, the assignment of H-2 protons can be done by examining the CPK model of the observed dimer (for example, rAprA). The H-2 proton in the rAp- residue is more exposed to adenine in the -prA residue than the H-2 of -prA to the adenine in rAp- residue; hence the more upfield one of the two H-2 resonances is assigned to the H-2 of rAp- because it is more shielded. This assignment is based on the knowledge about the diamagnetic effect of the ring current of the aromatic adenine exerted on the atom above or below the base plane. This will be discussed in the following paragraph. Recently, Kondo and Danyluk (1972) have synthesized rA*prA and rAprA*, where rA* is a total deuterated adenosine. Since deuterium will

Table 6. *Chemical Shifts of The Base and H-1′ Protons of Adenine Dinucleoside Monophosphates at 4°C (in D_2O, pD=7.4, ppm from capillary of TMS) (Kondo et al., 1972a)*

Dinucleotied	($A_{3'}$p) Residue			($p_{5'}$A) Residue		
	H-8	H-2	H-1′	H-8	H-2	H-1′
rAprA	8.59	8.29	6.20	8.585	8.48	6.31
rApdA	8.595	8.38	6.16	8.645	8.47	6.685
dApdA	8.375	8.25	6.47	8.715	8.445	6.635
dAprA	8.38	8.28	6.47	8.71	8.38	6.25

not be seen in PMR, only the residue with protons in these two dimers can be detected. Their efforts gave an unambiguous assignment of rAprA dimer. Their assignment of base and H-1′ protons entirely agree with Ts'o's earlier work (Schweizer *et al.*, 1964; Ts'o *et al.*, 1969). In addition, all the sugar protons in rAprA were also assigned by these two compounds (rA*prA and rAprA*). The chemical shifts of base and H-1′ protons of some selected adenosine dimer at 4°C in D_2O are listed in Table 6.

(b) Base-base stacking

As mentioned in an earlier section, the local electron cloud will generate a local magnetic field which shields the protons that are close to the electron cloud. The π-electrons in nucleoside bases are the source of the shielding center. If a proton is being shielded, its chemical shift will be moved toward the TMS reference (upfield) from its original position. Hence, in the analysis of the chemical shift data for the study of the conformation of base-base interaction, one useful procedure is calculation of the "dimerization shift ($\Delta\delta_D$)". This term is defined as the difference between the chemical shift values of the -pN or Np- in the dimer and the chemical shifts of the corresponding 5′ and 3′ mononucleotides ($\Delta\delta_D=\delta_{Np}-\delta_{NpN}$, or $\delta_{pN}-\delta_{NpN}$) at the same temperature. The $\Delta\delta_D$ of base protons and the H-1′ proton of the compounds in Table 6 are listed in Table 7 (at 4°C).

Giessner-Prettre and Pullman (1970) have calculated the magnetic isoshielding contours of various bases from the theoretical consideration of the ring current effect. The calculation of two bases parallel to each other with a distance of 3.4 Å was adopted for mapping the $\Delta\delta_D$ values. A typical result of base-base interactions of rAprA and dApdA is shown as a schematic diagram (Fig. 2) (Kondo *et al.*, 1972a). This diagram is constructed from their $\Delta\delta_D$ values (Table 7).

Recent study on the conformation dynamics of dimers (Kan *et al.*) showed that the bases of the dimers are not in fixed positions in solution. On the

Table 7. *Dimerization Shifts, $\Delta\delta_D$, of The Base and H-1′ Protons of Adenosine Dinucleoside Monophosphates at 4°C (in D_2O, pD 7.4) (Kondo et al., 1972a)*

Dinucleotide	($A_{3'}$p) Residue			($p_{5'}$A) Residue		
	H-8	H-2	H-1′	H-8	H-2	H-1′
rAprA	0.155	0.315	0.285	0.285	0.11	0.19
rApdA	0.15	0.225	0.325	0.23	0.15	0.225
dApdA	0.325	0.31	0.42	0.16	0.175	0.275
dAprA	0.32	0.28	0.41	0.16	0.21	0.25

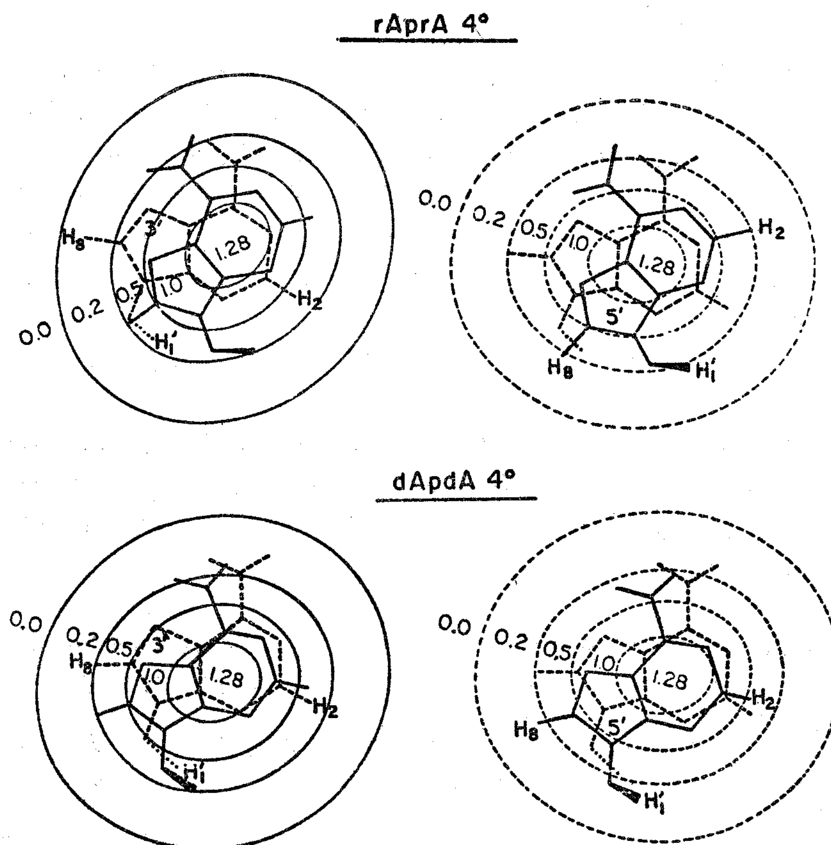

Fig. 2. Schematic presentation of the conformational model of rAprA (on top) and dApdA (on bottom) as viewed in the 5′ (solid line) to 3′ (broken line) direction. The dimensions of the bases and the magnetic isoshielding zones (elliptical shape curve) are from the work of Giessner-Prettre and Pullman (1970). The bases are parallel to each other with a distance of 3.4Å. The H-1′ protons are out of the plane.

contrary, the bases rotate parallel to each other in a fast motion in comparison to NMR time scale. The magnitude of the angle between the principal geometrical axes of the two bases is dependent on temperature. At low temperature, this angle is small, and the bases are staying in the stacking conformation; at high temperature, the angle becomes large and the bases begin to rotate more and more. Such a rotation may constitute the unstacked state. Therefore, the conformation diagram in Fig. 2 is the time-averaged, population-averaged position of the bases at 4°C. Not only do the bases rotate rather rapidly, but the handedness of the screw of the backbone in a dimer is also in a rapid equilibrium between right- and left-handedness at moderate temperature (Tazawa *et al.*, 1972).

(c) The conformation of furanoses in dApdA

Our laboratory has made the spectral assignment of H-1′, H-2′, and H-2″ of both the dAp- and -pdA portions of dApdA (Fang *et al.*, 1971). The four coupling constants, $J_{1'-2''(cis)}$, $J_{1'-2'(trans)}$, $J_{2'-3'(cis)}$, and $J_{2''-3'(trans)}$ of the furanose of both the dAp- and -pdA portion of dApdA, 3′-AMP and 5′-AMP were determined by first order analysis. In Table 8, one can find that the coupling constants between the dAp- and -pdA portion of dApdA are similar to those of 3′-dAMP and 5′-dAMP respectively, while the coupling constants of 3′-dAMP and 5′-dAMP are not the same. Through the application of the Karplus equation, four dihedral angles, which are listed in Table 9 were calculated. In conclusion, the furanose conformation of 3′-AMP and dAp- in dApdA is C-2′-endo (envelop) or C-2′-endo C-3′-exo (twisted), while the furanose for 5′-AMP and -pdA in dApdA are in a rapid equilibrium between C-2′-endo and C-3′-endo.

Table 8. *The Coupling Constants of Mono- and Dideoxynucleoside Monophosphates at 20°C (in Hz) (Fang et al., 1971)*

	$J_{1'-2'(cis)}$		$J_{1'-2'(trans)}$		$J_{2'-3'(cis)}$		$J_{2''-3'(trans)}$	
	3′	5′	3′	5′	3′	5′	3′	5′
dApdA	5.6	6.6	8.8	6.7	5.5	6.7	2.2	4.0
dAprA	5.7		8.7		5.5		2.0	
3′-dAMP	6.0		8.1		6.3		2.9	
5′-dAMP		6.6		6.9		5.8		3.8

Table 9. *Calculated Dihedral Angles for dApdA, 5′-AMP, and 3′-AMP and for The Four Furanose Conformations (in degree) (Abraham et al., 1962; Smith et al., 1968a)*

	$\phi_{1'-2''}$ (cis), deg	$\phi_{1'-2'}$ (trans), deg	$\phi_{2'-3'}$ (cis), deg	$\phi_{2''-3'}$ (trans), deg
dAp-(3′)	40	160	40	60 (120)
pdA-(5′)	30	145	30	130 (50)
5′-dAMP	35	145	40	130 (50)
3′-dAMP	35	155	35	55 (125)
C-2′-endo-C-3′-exo	45	165	60	60
C-2′-endo	45	165	45	75
C-3′-exo	15	135	45	75
C-3′-endo	15	105	45	165

(d) The comparison of rAprA *vs.* dApdA (ribosyl nucleosides *vs.* deoxyribosyl nucleosides)

The only structural difference between ribosyl nucleosides and 2'-deoxynucleosides is that the 2'-OH group is substituted by an *H* atom. This difference is of great biological significance, i.e., the difference between DNA and RNA. In the study on the conformation of the rA dimer *vs.* that of the dA dimer, their difference in base-base stacking has been compared by using the following compounds: rAprA, rApdA, dAprA, and dApdA (Kondo *et al.*, 1972a). Their dimerization shift data (Table 7) indicated that the order of the extent of base-base stacking is dApdA~dAprA>rAprA~rApdA. The measurements of coupling constants $J_{1'-2'}$ of these four compounds at various temperatures should now be discussed (Table 10).

The data in Table 10 indicate that the decrease in $J_{1'-2' \text{ or } 2''}$ values of the ribosyl dimer *vs.* that of the ribosyl monomer is related to the compression of the furanose ring due to stacking. This compression can be released at elevated temperature or in a destacking solvent such as DMSO-d_6. In dApdA and dAprA, the $J_{1'-2' \text{ or } 2''}$ values of all four furanoses are similar to those of the mononucleotides, as well as being temperature independent and solvent independent. This observation implies that the furanoses in these dimers are not compressed in stacking of the bases; perhaps this is the reason why the bases in dApdA and dAprA can have a large extent of overlap. In rAprA and rApdA, the $J_{1'-2' \text{ or } 2''}$ values of the three riboses in these dimers are significantly smaller than those of the mononucleotides and are temperature and solvent dependent. This result implies that the riboses in these dimers are being compressed in stacking. Thus, the 2'-OH group in these riboses may provide a steric hindrance in forcing the bases to have a less extensive overlap of bases in rApdA and rAprA. This conclusion is in accord with the diagrams shown in Fig. 2. In summary, the $\Delta\delta_D$ and $J_{1'-2' \text{ or } 2''}$ data on these four adenine dinucleoside monophosphates indicate that the influence of the

Table 10. *Temperature and Solvent Effect on The Coupling Constant of The H-1' Protons of Adenine-Dinucleoside Monophosphates (in* D_2O*, pD 7.4, in Hz) (Kondo et al., 1972a)*

Temp (°C)	rAprA		rApdA		dAprA		dApdA	
	$J(3')$	$J(5')$	$J(3')$	$J(5')$	$J(3')$	$J(5')$	$J(3')$	$J(5')$
5	2.5	2.0	3.5	5.6	8.8, 4.8	6.0	8.8, 5.6	6.5
30	3.2	3.5	4.0	6.0	8.0, 5.5	5.7	8.5, 5.5	6.4
60	4.5	4.1	4.5	6.3	8.1, 6.0	5.3	7.9, 6.0	6.7
Me_2SO-d_6 (30°)	6.8	5.2			7.5, 5.2	5.2	7.9, 5.7	6.1

2′-OH group of the ribose on conformation of the ribose-containing dimers is exerted through a steric hindrance of this group, especially when it is located in the 3′-residue.

(2) Oligonucleotides

The discussion on the dinucleoside monophosphates in the above section has prepared the foundation for the study of oligonucleotides, a study which is still in its infancy. From the standpoint of conformational analyses, we can envisage at least three distinct changes when the dimer is lengthened to trimer or oligomer: (a) The presence of more than one phosphate in a trimer introduced the factor of electrostatic interaction between the negative charge groups, (b) Now an interior residue is generated for the first time which is less accessible by solvent, and (c) Additional restriction to the conformational equilibrium is imposed on the trimer.

A comparative study of 3′-IMP, 5′-IMP, IpI, pIpI, IpIpI, and pIpIpI by PMR has been made in our laboratory (Tazawa *et al.*, 1972). The following conclusions can be drawn from this study: (1) a possible existence of a distant neighboring field effect between two terminal residues in the trimer, (2) the absence of right-handedness to left-handedness interconversion in the trimer, (3) a reduction of the rotational freedom of the nucleosidyl unit in the interior residue in the trimer, and (4) a possible asymmetry in the geometrical relationship among residues in the trimer.

Recently, the long-distant shielding effect has been extensively studied through the analysis of $\Delta\delta_D$ and $\Delta\delta_T$ (the trimerization shifts) of TpT, TpdA, dApT, TpTpdA, dApTpT, and poly T (Kan *et al.*). Since the thymine base lacks both ring current shielding and stacking ability, any shielding effect on the terminal thymine in the trimers as compared with TpT must come from the dA base on the other end. After carefully comparing all the possible conformations, we concluded (a) the long-distant shielding effect does exist, as large as 0.1 ppm in magnitude for TpTpdA and dApTpT, (b) the anti-anti-anti right-handed form is the major conformation of the trimers.

NMR Studies on Polynucleotides and Nucleic Acid (Transfer RNA)

PMR studies on the single-stranded homopolynucleotides present no technical problem and have been conducted extensively (Jardetzky, 1964; Alderfer *et al.*, 1971; McDonald *et al.*, 1964; McTaque *et al.*, 1964; Smith *et al.*, 1968b; Bangerter *et al.*, 1968). A more recent paper (Alderfer *et al.*) from our laboratory on the PMR study on poly dA, poly rA, poly 2′-*O*-methyl A and poly 2′-*O*-ethyl A confirm the basic conclusion about the comparative investigation on dApdA and rAprA described above. The substituents (from OH

to OCH_2CH_3) at the 2'-carbon of the polynucleotide backbone constitute a steric hindrance to the base-base overlap in the polymer; the base-base stacking in return causes the change of the furanose conformation because of this hindrance. Recently, poly A and poly U have also been investigated by carbon-13 NMR (Komokoski *et al.*, 1972; Mantsch *et al.*, 1972). The ^{13}C NMR spectra of poly A and poly U have narrow spectral lines; all these resonances were assigned properly by comparison with the mononucleotides at high temperature.

As far as the double-stranded nucleic acid is concerned, so far no NMR study can be made on this class of macromolecules. Simply no resonance can be observed from them! The general explanation is that the relaxation of the excited states of the nuclei from these molecules is so slow that the system is saturated rapidly and will not absorb any more input energy; thus no spectral line is found. This phenomenon is related to the rate of change of the environment of the nuclei (or the rate of motion of the molecule or part of the molecule). The faster the rate of motion, the easier for the relaxation to take place. Currently, symmetrical microhelices 6 to 10 bases long of both ribosyl and deoxyribosyl types are being synthesized in our laboratory. Hopefully, the conformation of these microhelices can be studied by PMR or ^{13}CMR.

Some important advances recently have been made in the NMR study on transfer RNA (or tRNA). tRNA contains 75–85 nucleotides and has a molecular weight about 25,000–30,000. In a tRNA spectrum, there are at least 100 base proton resonances crowded in the region about 8 ppm downfield from 2,2-dimethyl-2-silapentane-5-sulfonate (DDS), and another 27 base proton and 76 H-1' proton resonances in the region about 6 ppm downfield from DSS. Obviously, it is hard to draw any precise structural information form this overcrowded NMR spectrum. Recent studies in other types of tRNA spectra have succeeded at least partially in obtaining valuable information about its conformation. These studies concern (1) the proton resonances involved in a NH–N hydrogen bond in H_2O as studied by Kearns, Schulman and their collaborators (Kearns *et al.*, 1971a; Kearns *et al.*, 1971b; Wong *et al.*, 1972; Lightfoot *et al.*, 1973; Shulman *et al.*, 1973a; Shulman *et al.*, 1973b), and (2) the methyl proton resonances of the modified bases in tRNA which is currently under investigation in our laboratory (Kan *et al.*, unpublished).

In the Watson-Crick scheme of base pairing (Watson *et al.*, 1953), adenine specifically forms hydrogen bonds with thymine (or uracil in RNA); and cytosine with guanine in the DNA double helix structure. There is only one NH–N hydrogen bond in each base pair. This NH proton can be exchanged with solvent and, therefore, cannot be seen in D_2O. However, the rate of exchange is sufficiently slow because of the restriction in hydrogen bonding

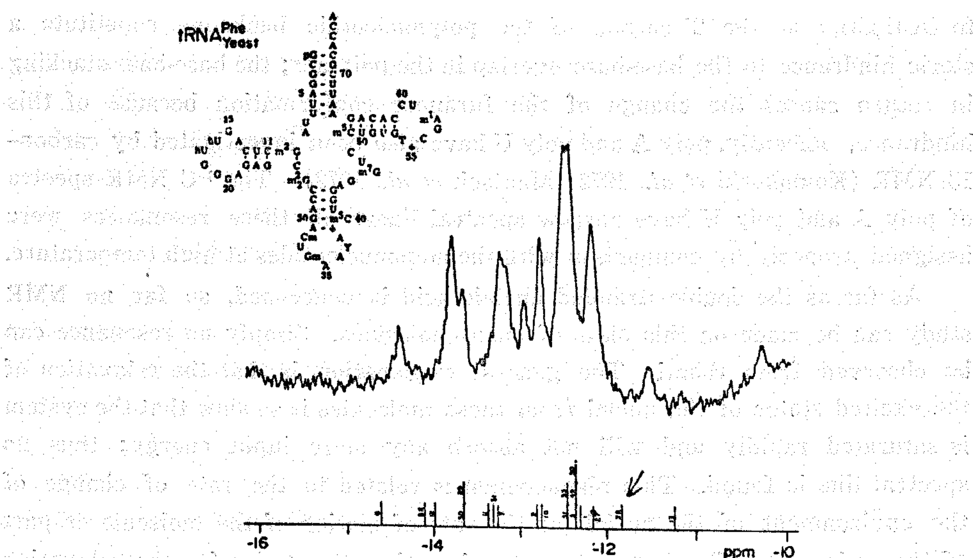

Fig. 3. The 300 MHz PMR spectrum of yeast $tRNA^{Phe}$ at 7.0 in 0.01 Mg^{2+}–0.1 M NaCl–(tRNA) about 50 mg/ml, and temperature about 35°C. The cloverleaf form of the primary sequence is shown as well as the calculated spectrum and its assignments to particular base pairs.

so that it can be detected in H_2O solution at the region of 11 to 15 ppm downfield from DSS. Fig. 3 demonstrates these proton resonance signals of $tRNA^{Phe}_{yeast}$ recorded by a 300 MHz NMR spectrometer. From this NMR spectrum one can get the integration of areas under these peaks which gives the number of base pairs in the $tRNA^{Phe}_{yeast}$ molecule, since each base pair contributes one proton resonance in this region. The number turns out to be 20±2 base pairs, which is very close to that proposed in the cloverleaf structure of $tRNA^{Phe}_{yeast}$ (Fig. 3). Also, the melting temperature of tRNA can be determined by NMR. The hydrogen bonds in the molecule are broken when the double helix begins to melt at elevated temperature. The signals of NH–N simply disappear from −11 to −15 ppm region on the NMR spectrum due to the exchange with the solvent, H_2O. The advantage of this NMR study is that not only the melting temperature of the whole molecule can be determined, but also the T_m's of the individual double helical stems in the tRNA can be determined once the NH–N resonances are assigned properly to these short stems. The intrinsic spectral positions of NH–N of the A:U pair and G:C pair in water are at −14.8 and −13.7 ppm from DSS respectively. The shielding effect of the neighboring base moves these resonances upfield. For instance, in $tRNA^{Phe}_{yeast}$, G:C at 28 is extensively shielded by A at 29, therefore, the NH–N signal from G:C at 28 is moved to upfield (Fig. 3). Supposedly, one can calculate all the chemical shift positions of NH–N resonances of a tRNA based on its

secondary structure, and then compare them to the observed spectrum. Based on this type of approach, hopefully, the tertiary structure of tRNA in solution can also be determined. However, the theoretical calculation of the chemical shifts is difficult to do because of the lack of model compounds for comparison. Some success has been made by the study on the fragments, however.

Our efforts on the NMR study of the methyl groups in tRNA molecules also is concerned with the determination of its tertiary structure. We try to compare the high field region of NMR specturm of an intact $tRNA^{Phe}_{yeast}$ to its fragments which are obtained by partial cleavage by T_1 ribonuclease. The advantage of using the fragments is that they will reveal the close neighboring effect of the methyl groups. Therefore, the difference between the same methyl group of the fragment to whole tRNA must come from the influence of the tertiary structure.

In conclusion, over the last ten years nuclear magnetic resonance has been developed to be a most powerful tool in the study of molecular structure, conformation, and interaction. In fact, the power and ability of NMR in studying molecules in solution have been compared to those of X-ray in studying molecules in solid state. We hope, in this brief introduction on the NMR study on nucleic acid, that we have stimulated the interest of the readers in this important field.

Literature Cited

ABRAHAM, R. J., D. HALL, L. HOUGH, and K. A. MCLAUCHLAN. 1962. J. Chem. Soc. London, 3699.

ALDERFER, J. C. and S. L. SMITH. 1971. J. Amer. Chem. Soc. **93**: 7305.

ALDERFER, J. L., I. TAZAWA, S. TAZAWA, and P. O. P. TS'O. Submitted to Biochemistry.

BANGERTER, B. W. and S. I. CHAN. 1968. Biopolymers **6**: 983.

BLACKBURN, B. J., A. A. GREY, and I. C. P. SMITH. 1970. Can. J. Chem. **48**: 2866.

BLOEMBERGEN, N. 1961. "Nuclear Magnetic Relaxation," W. A. Benjamin, Inc. New York.

BOVEY, A. 1969. "Nuclear Magnetic Resonance Spectroscopy," Chapters 1 and 2, Academic Press, New York.

BULLOCK, F. J. and O. JARDETZKY. 1964. J. Org. Chem. **29**: 1988.

CARRINGTON, A. and A. D. MCLACHLAN. 1967. "Introduction to Magnetic Resonance with Applications to Chemistry and Chemical Physics," Harper & Row, New York.

CHAN, S. I. and J. H. NELSON. 1969. J. Amer. Chem. Soc. **91**: 168.

CUSHLEY, R. J., K. A. WATANABE, and J. J. FOX. 1967. J. Amer. Chem. Soc. **89**: 394.

DONOHUE, J. and K. N. TRUEBLOOD. 1960. J. Mol. Biol. **2**: 263.

DUGAS, H., B. J. BLACKBURN, R. K. ROBBINS, R. DESLAURIESS, and I. C. P. SMITH. In preparation.

FANG, K. N., N. K. KONDO, P. S. MILLER, and P. O. P. TS'O. 1971. J. Amer. Chem. Soc. **93**: 6647.

FARRAR, T. C. and E. D. BECKER. 1971. "Pulse and Fourier Transform NMR Introduction to Theory and Methods," Academic Press, New York, New York.

FERGUSON, R. C. and W. D. PHILIPS. 1967. Science **157**: 257.

GATLIN, L. and J. C. DAVIS, JR. 1962. J. Amer. Chem. Soc. **84**: 4464.

GIESSNER-PRETTRE, C. and B. PULLMAN. 1970. J. Theor. Biol. **27**: 81 and 341.
HART, P. A. and J. P. DAVIS. 1969. J. Amer. Chem. Soc. **91**: 513; Biochem. Biophys. Res. Commun. **34**: 733.
HOLLIS, D. P. 1972. "Methods in Pharmacology," Volume 2, p. 191. Appleton-Century-Crofts Educational Division, Meredith Corporation, New York.
JARDETZKY, C. D. 1960. J. Amer. Chem. Soc. **82**: 229.
JARDETZKY, C. D. 1961. J. Amer. Chem. Soc. **83**: 2919; 1962. J. Amer. Chem. Soc. **84**: 62.
JARDETZKY, C. D. and O. JARDETZKY. 1960. J. Amer. Chem. Soc. **82**: 222.
JARDETZKY, O. 1964. Biopolymers Symposia **1**: 501.
KAN, L. S. and P. O. P. TS'O. Unpublished data.
KAN, L. S., J. C. BARRETT, P. S. MILLER, and P. O. P. TS'O. Biopolymers. In press.
KAN, L. S., J. C. BARRETT, and P. O. P. TS'O. Biopolymers. In press.
KAN, L. S., P. O. P. TS'O, A. MAELICKE, F. V. D. HAAR, and F. CRAMER. Unpublished data.
KARPLUS, M. 1959. J. Chem. Phys. **30**: 11; 1963. J. Amer. Chem. Soc. **85**: 2870.
KEARNS, D. R., D. J. PATEL, and R. G. SHULMAN. 1971a. Nature **229**: 338.
KEARNS, D. R., D. PATEL, R. G. SHULMAN, and T. YAMANE. 1971b. J. Mol. Biol. **61**: 265.
KOMOKOSKI, R. A. and A. ALLERHAND. 1972. Proc Natl. Acad. Sci. USA **69**: 1804.
KONDO, N. S., K. N. FANG, P. S. MILLER, and P. O. P. TS'O. 1972a. Biochem. **11**: 1991.
KONDO, N. S. and S. S. DANYLUK. 1972b. J. Amer. Chem. Soc. **94**: 5121.
LAPPER, R. D., H. H. MANTSCH, and I. C. P. SMITH. 1973a. J. Amer. Chem. Soc. **95**: 2878.
LAPPER, R. D. and I. C. P. SMITH. 1973b. J. Amer. Chem. Soc. **95**: 2880.
LEMIEUX, R. V., J. D. STEVENS, and R. R. FRASER. 1962. Can. J. Chem. **40**: 1955.
LEMIEUX, R. V. and J. D. STEVENS. 1965. Can. J. Chem. **44**: 249.
LIGHTFOOT, D. R., K. L. WONG, D. R. KEARNS, B. R. REID, and R. G. SHULMAN. 1973. J. Mol. Biol. **78**: 71.
MANTSCH, H. H. and I. C. P. SMITH. 1972. Biochem. Biophys. Res. Commun. **46**: 808.
MATSUURA, S. and T. GOTO. 1963. Tetrahedron Letters, no. 22, 1499.
MCDONALD, C. C., W. D. PHILLIPS, and S. PENMAN. 1964. Science **144**: 1234.
MCTAQUE, J. P., V. ROSS and J. H. GIBBS. 1964. Biopolymers **2**: 163.
NELSON, F. A. and H. E. WEAVER. 1964. Science **146**: 223.
SCHWEIZER, M. P., S. I. CHAN, G. K. HELMKAMP, and P. O. P. TS'O. 1964. J. Amer. Chem. Soc. **86**: 696.
SCHWEIZER, M. P., E. B. BANTA, J. T. WITKOWSKI, and R. K. ROBBINS. 1973. J. Amer. Chem. Soc. **95**: 3770.
SHULMAN, R. G., C. W. HILBERS, D. R. KEARNS, B. R. REID, and Y. P. WONG. 1973a. J. Mol. Biol. **78**: 57.
SHULMAN, R. G., C. W. HILBERS, Y. P. WONG, K. L. WONG, D. R. LIGHTFOOT, B. R. REID, and D. R. KEARNS. 1973b. Proc. Natl. Acad. Sci. USA **70**: 2042.
SMITH, M. and C. D. JARDETZKT. 1968a. J. Mol. Spectrosc. **28**: 70.
SMITH, I. C. P., T. YAMANE, and R. G. SHULMAN. 1968b. Science **159**: 1360.
TAZAWA, S., I. TAZAWA, J. L. ALDERFER, and P. O. P. TS'O. 1972. Biochemistry **11**: 3544.
TIERS, G. V. D. 1958. J. Phys. Chem. **62**: 1151.
TS'O, P. O. P. 1970. "Fine Structure of Proteins and Nucleic Acids," Vol. 4, Chapter 2, Marcel Dekker, Inc., New York.
TS'O, P. O. P., N. S. KONDO, M. P. SCHWEIZER, and D. P. HOLLIS. 1969. Biochemistry **8**: 997.
WATSON, J. D. and F. H. C. CRICK. 1953. Nature **171**: 737, 964.
WONG, Y. P., D. R. KEARNS, B. R. REID, and R. G. SHULMAN. 1972. J. Mol. Biol. **72**: 725.