Nuclear Magnetic Resonance Studies of 5′-Ribo- and Deoxyribonucleotide Structures in Solution†

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ABSTRACT: An extensive proton nuclear magnetic resonance (nmr) study is reported for all common purine and pyrimidine 5′-ribo- and deoxyribonucleotides at 220 MHz. Spectra for these nucleotides were measured in D_2O solutions at 20 ± 2° and signal assignments made with the aid of selected ^1H-^1H and ^{31}P-^1H decoupling experiments. Complete sets of accurate chemical shifts and coupling constants were derived for each nucleotide by iterative procedures and yielded close agreement between observed and calculated spectra. The nmr parameters have been utilized in a quantitative evaluation of several key nucleotide conformational features including equilibrium conformations of ribose and deoxyribose rings, preferred exocyclic group orientations, and base–ribose ring orientation. A quantitative conformational analysis was made for the D-ribose and D-deoxyribose rings of all the 5′-riboncleotides following procedures analogous to those developed recently by Altona and Sundaralingam. The analysis is based on an assumption of a rapidly equilibrating mixture between N type [C(3′)-endo, C(2′)-exo] and S type [C(2′)-endo, C(3′)-exo] conformers, N ⇄ S. With the aid of graphical plots quantitative estimates were made of pseudorotational angle, *P*, degree of pucker, Υ, and ring conformer populations. The results show that the pseudorotational parameters (*P*, Υ) do not vary significantly between nucleotides and are generally within ranges found in the crystalline state. An S-type conformation is favored in both ribo- and deoxyribonucleotides at 20° with the equilibrium lying somewhat more in favor of the S conformer in the latter, *i.e.*, 70:30 *vs.* 60:40. Possible effects of electronegativity change upon pseudorotational parameters were explored and it was concluded that no particular advantage is gained by using adjusted Karplus expressions for individual ring molecular fragments at the present accuracy of measured coupling constants. A conformational analysis was also made of rotamer populations about exocyclic C(4′)–C(5′) and C(5′)–O(5′) bonds. In each instance rapid interconversion was assumed between three classical staggered rotamers, gg, gt, and tg. Numerical analysis of the nmr data led to the result that the gg rotamer is markedly preferred about both C(4′)–C(5′) and C(5′)–O(5′) bonds in all the 5′-nucleotides studied. This preference is somewhat greater in ribonucleotides and appears to be correlated with furanose ring conformation. The exocyclic side chain accordingly exists predominantly in an all-trans bonding arrangement with the phosphate group directed over the ribose ring of the nucleotide. Taken together, these results point to a surprising constancy of ribose–phosphate conformational features throughout the entire series of 5′-nucleotides. Moreover, comparison with crystallographic results shows that solution structures differ only slightly from those in the crystalline state. Although the results are apparently consistent with recent hypothesis of a "rigid" nucleotidyl structural unit, it is important to note that nucleotide structures in solution are actually a dynamical average of a number of rapidly interconverting conformers. Finally, sizable deshieldings observed for base protons of 5′-ribo- and deoxyribonucleotides relative to corresponding 2′ and 3′ derivatives and the surprisingly large deshieldings of ribose ring protons in purine relative to pyrimidine 5′-nucleotides are both compatible with a preferred anti orientation of base and ribose rings about the glycosidic bond in these nucleotides. The ribose proton deshieldings in purine nucleotides are qualitatively accounted for by the effect of "in-plane" purine ring diamagnetic anisotropy. It is concluded that 5′-nucleotides exist in a dynamic equilibrium between two preferred ranges of anti conformations, *i.e.*, anti-N ⇌ anti-S.

The structure, conformations, and interactions of purine and pyrimidine nucleosides and 5′-ribo- and deoxyribonucleotides have been the subject of extensive investigations in recent years (Sundaralingam, 1969, 1973; Saenger, 1973; Ts'o, 1970; Hruska, 1973; Arnott, 1970). Interest in these molecules stems from their importance as the simplest constituents of naturally occurring ribonucleic and deoxyribonucleic acids and the possibility that simple monomers may exhibit some of the structural features which exist in naturally occurring nucleic acids. For example, it has been suggested that the furanose ring exists in the C(3′)-endo conformation (Arnott *et al.*, 1972) in different RNA crystal forms but in B-DNA and A-DNA the C(3′)-exo and C(3′)-endo conformations are preferred (Arnott and Hukins, 1972).

Most of the quantitative structural data for 5′-nucleotides have been derived from X-ray diffraction experiments in the solid state. These reveal a number of interesting conformational details for functional groups of the nucleotides. For example, a majority of purine and pyrimidine 5′-nucleotides favor an anti (Donohue and Trueblood, 1960; Haschemeyer and Rich, 1967) conformation of base ring with respect to the sugar ring. It has also been observed (Sundaralingam, 1969) that the D-ribose and D-deoxyribose rings exhibit a nonplanar, puckered, conformation in which either the C(2′) or C(3′) atom is furthest from the plane of the other atoms of the ribose ring, *i.e.*, C(4′)–O(1′)–C(1′).

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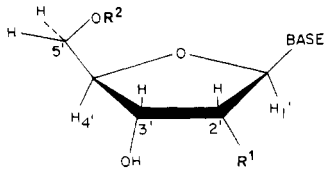


FIGURE 1: Numbering scheme for ribose and 2′-deoxyribose ring. R^1 = OH = ribonucleoside; R^1 = H = deoxyribonucleoside = H(2″); R^2 = H = nucleoside; $R^2 = (PO_3)^-$ = nucleotide.

Atoms lying on the same side of the plane as C(5′) are designated endo and those on the opposite side as exo. The majority of crystal structures of mononucleosides and mononucleotides show that the furanose ring exists in either the C(2′)-endo or C(3′)-endo conformations (Sundaralingam, 1969). Finally, the third major functional group, *i.e.*, the exocyclic group, exists predominantly in a gauche-gauche conformation about the C(4′)–C(5′) bond (Sundaralingam, 1969) with the O(5′) atom projecting over the furanose ring. These conformational features recur in a number of 5′-nucleotides in the solid state and have led to the proposal of a "rigid" nucleotidyl unit (Yathindra and Sundaralingam, 1973).

Rather less quantitative structural data have been reported for 5′-nucleotides in solution, with most of the available information derived mainly from nuclear magnetic resonance (nmr) measurements. Investigations of concentration and pH effects (Danyluk and Hruska, 1968; Schweizer *et al.*, 1968; Broom *et al.*, 1967) and nuclear Overhauser enhancements (Tran-Dinh Son *et al.*, 1972a) have confirmed the existence of an anti conformation for AMP-5′ and GMP-5′ in solution and have provided limited information for other conformational features of these molecules. Analyses have also been reported for the ^1H spectra of AMP-5′ at 100 MHz (Feldman and Agarwal, 1968, partial analysis; Fujiwara and Uetsuki, 1968, more complete analysis) and of UMP-5′ at 100 MHz (Fujiwara and Uetsuki, 1968; Kreishman and Chan, 1971). More recently the proton magnetic resonance spectra of UMP-5′ in aqueous solution have been analyzed at 100 and 220 MHz, the spectra checked by computer simulation, and the nmr parameters discussed in terms of overall molecular conformation (Wood *et al.*, 1973a,b). It is clearly desirable to extend such measurements to a complete series of 5′-ribo- and deoxyribonucleotides in order to establish not only the conformational features of these molecules in solution, but also to assess the influence of substituent change at the 2′ position upon nucleotide conformation. The further possibility of deriving quantitative conformational data for the furanose ring by pseudorotational analysis procedures developed recently by Altona and Sundaralingam (1972, 1973) also permits comparison between crystal and solution structures.

A systematic study of the proton nmr spectra at 220 MHz for all important 5′-ribo- and deoxyribonucleotides has been carried out and is reported in this work. Measurements were made in D_2O solution under conditions where intermolecular effects (aggregation) are not expected to be a significant factor. Furthermore, multiplet separations at 220 MHz are sufficient to permit unambiguous assignment of all observed signals (except for exocyclic methylenes of several nucleotides). Parameters obtained from iterative analysis of the spectra were then used to deduce conformational data for each nucleotide. In particular, the conformations of the D-ribose and D-deoxyribose rings were analyzed in terms of a conformational equilibrium (Altona and Sundaralingam, 1972, 1973) between N and S type[1] conformers, *i.e.*, N ⇄ S. All of the data taken together provide a conformational model for each 5′-nucleotide and permit an assessment of the conformations of the ribose and deoxyribose rings and of change of state on the conformational properties of nucleotides.

Experimental Section

Materials. All of the nucleotides were purchased from Sigma Chemical Co. and were used without further purification; 99.8% D_2O, obtained from U.S.A.E.C., was used for initial lyophilization of the samples. For nmr measurements lyophilized samples were dissolved in 100.0% D_2O purchased from Diaprep.

Preparation of Samples. Commercial samples of nucleotides contain significant amounts of bound H_2O, and exchangeable acidic, base-ring, and hydroxyl protons. Since these contribute to a residual HDO peak, which in some cases is large enough to overlap key ribose proton multiplets, it is essential to minimize the HDO peak. Weighed amounts of nucleotides were, accordingly, lyophilized 4–5 times from 99.8% D_2O and the final lyophilized sample was dissolved in 100% D_2O.

Measurements of Spectra. The 220-MHz proton magnetic resonance spectra of 0.1 M solutions of the nucleotides were measured on a Varian HR 220-MHz spectrometer operating at a probe temperature of 20 ± 2°. Spectra were calibrated by the audio side-band method, using a Hewlett-Packard 4204A oscillator calibrated in turn with a Hewlett-Packard 5245L electronic counter. TSP[2] (3-trimethylsilylpropionate-*2,3,3,3-d*$_4$ sodium salt) was used as an internal reference standard and measured line positions are accurate to 0.002 ppm. Signal assignments were aided by ^{31}P decoupling experiments using a Rhode and Schwartz KV5D frequency generator operating at appropriate decoupling frequencies (89 MHz). Spectral assignments were followed by simulation of each spectrum using the 6-spin nmr simulation program for the Varian 620i computer. Parameters were then further refined using NMREN and NMRIT iterative programs.

Results

Analysis of Spectra. Figure 1 summarizes the numbering system of the ribose and deoxyribose ring followed in this work. The methine protons of the furanose ring are numbered according to the carbon atom to which they are attached, *e.g.*, H(1′), H(2′) etc. In the 2′ position of the deoxyribose ring (R^1 = H) the two methylene protons are labeled H(2′) and H(2″) such that R^1 = H(2″) in Figure 1. In order to demonstrate similarities and differences between ribose and deoxyribose purine and pyrimidine nucleotide spectra detailed analyses are presented for AMP-5′, UMP-5′, dAMP-5′, and TMP-5′.

Pyrimidine 5′-Ribonucleotides. The 220-MHz proton spectrum of the ribose protons of a 0.1 M UMP-5′ solution in D_2O at 20 ± 2° is shown in Figure 2. As with 220-MHz spectra for uridine and similar compounds, the multiplet

[1] Type N conformers are defined as those which include all conformations occupying the northern half of a pseudorotational cycle. Included in this category are all [C(2′)-exo, C(3′)-endo] conformations. A type S conformer defines all conformers in the southern half of the pseudorotational circle which includes all the [C(2′)-endo, C(3′)-exo] conformers (Altona and Sundaralingam, 1972).

[2] Abbreviation used is: TSP, 3-trimethylsilylpropionate-*2,3,3,3-d*$_4$ sodium salt.

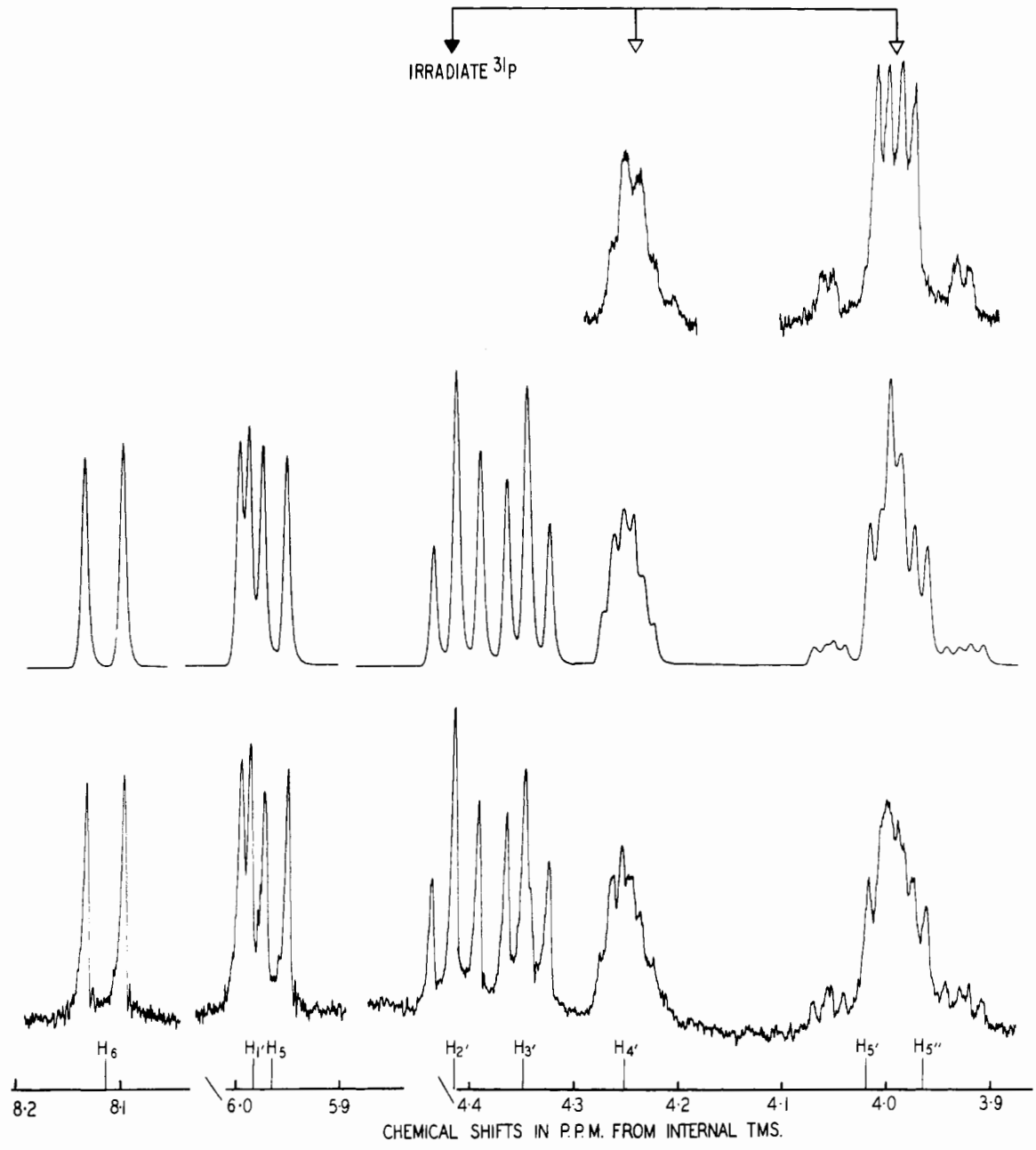


FIGURE 2: Lower spectrum, 220-MHz proton magnetic resonance spectrum of 0.1 M UMP-5′ in D_2O at 20°; middle spectrum, computer-simulated spectrum; upper spectrum, ^{31}P decoupled, 220-MHz pmr spectrum of UMP-5′.

patterns for UMP-5′ are pseudo first order and, in conjunction with chemical shifts, permit an assignment of signals in the spectrum. Differences between the uridine (Schleich *et al.*, 1972) and UMP-5′ spectra (Wood *et al.*, 1973b) arise mainly in the splitting patterns of the upfield C(4′) and C(5′) proton signals and are due to coupling of the C(5′) protons with ^{31}P of the exocyclic phosphate group of the latter. Irradiation at the correct ^{31}P resonance frequency collapses the C(5′) proton multiplet to a readily analyzable eight-line AB pattern of an ABX spin system yielding H(4′)–H(5′) and H(4′)–H(5″) couplings of 3.8 and 5.2 Hz, respectively. ^{31}P spin-decoupling experiments also simplified the complex multiplet at 4.25 ppm due to the C(4′) proton and yielded $^4J(^{31}P,H(4')) = 1.7$ Hz. Furthermore, the ^{31}P spin-decoupled spectrum permits determination of a reliable value of 4.1 Hz for the H(3′)–H(4′) coupling.

Following the assignment, an initial set of nmr parameters (δ,J) was obtained by simulation of the complete undecoupled UMP-5′ spectrum with a Varian Spectroscan simulation program. The set of parameters giving the best visual fit of observed and simulated spectra was then used as input into a full iterative analysis. Excellent agreement was noted between simulation and iteration δ and J values. A comparison of observed and calculated spectra is shown in Figure 2, and a listing of final parameters (iteration) is given in Tables I and II. Procedures similar to those described above were followed in the analysis of CMP-5′ and final δ and J values are also summarized in Tables I and II.

Purine 5′-Ribonucleotides. Figure 3 shows the 220-MHz proton resonance spectrum for the ribose protons of AMP-5′ in 0.1 M D_2O solution. The assignment of signals in Figure 3 was made on the basis of splitting pattern and chemical shift considerations and is in agreement with an earlier 100-MHz assignment (Feldman and Agarwal, 1968; Fujiwara and Uetsuki, 1968). All of the proton–proton spin coupling constants can be determined at 220 MHz; also,

TABLE I: Chemical Shifts of 5′-Mononucleotides in Aqueous Solution.[a]

Nucleotide			H(1′)	H(2′)	H(2″)	H(3′)	H(4′)	H(5′)	H(5″)
Purines	H(8)	H(2)							
AMP-5′	8.554	8.117	6.044	4.772		4.463	4.350	4.012[b]	4.012
GMP-5′	8.204		5.925	4.759		4.501	4.334	4.016[b]	4.016
dAMP-5′	8.499	8.117	6.431	2.809	2.586	4.727	4.263	3.945[b]	3.945
dGMP-5′	8.179		6.292	2.789	2.530	4.729	4.223	3.946[b]	3.946
Pyrimidines	H(6)	$H(5)CH_3$							
UMP-5′	8.108	5.972	5.981	4.413		4.340	4.254	4.017	3.961
CMP-5′	8.127	6.136	6.011	4.361		4.342	4.245	4.039	3.989
TMP-5′	7.894	(1.918)	6.327	2.410	2.320	4.572	4.159	3.990[b]	3.990
dCMP-5′	8.044	6.113	6.327	2.380	2.319	4.545	4.145	3.945[b]	3.945
dUMP-5′	8.054	5.936	6.318	2.388	2.348	4.562	4.154	3.945[b]	3.945

[a] Proton chemical shifts measured from TSP as internal reference in 0.10 M aqueous solutions at 20 ± 2° to an accuracy of ±0.002 ppm. [b] Signals are coalesced at 220 MHz.

TABLE II: Spin-Coupling Constants of 5′-Mononucleotides in D_2O Solution.[a]

	Coupling Constant (Hz)										
Nucleotide	1′,2′	1′,2″	2,′2″	2′,3′	2″,3′	3′,4′	4′,5′	4′,5″	5′,5″	4′,P	5′,P	5″,P
AMP-5′	5.9			5.0		3.6	3.2[b]	3.2[b]	c	1.0	4.3[d]	4.3[d]
GMP-5′	6.0			5.0		3.7	3.4[b]	3.4[b]	c	~1.0	4.7[d]	4.7[d]
UMP-5′	5.1			4.8		4.1	2.3	2.8	−11.8	1.7	3.8	5.2
CMP-5′	4.4			4.5		4.6	2.5	2.9	−12.0	~1.0	3.8	4.9
dAMP-5′	7.3	6.2	−14.0	6.1	3.4	3.0	3.7[b]	3.7[b]	c	0.5	4.6[d]	4.6[d]
dGMP-5′	7.5	6.5	−13.4	6.0	3.4	3.2	3.5[b]	3.5[b]	c	1.0	4.5[d]	4.5[d]
TMP-5′	7.6	6.2	−14.0	6.6	2.6	3.0	3.7[b]	3.7[b]	c	1.7	4.7[d]	4.7[d]
dCMP-5′	7.0	6.3	−14.1	6.0	4.0	3.2	3.5	4.0	−12.0	0.6	4.5	5.6
dUMP-5′	7.5	6.5	−14.0	6.5	3.0	3.0	3.5[b]	3.5[b]	c	1.3	4.7[d]	4.7[d]

[a] Solution 0.10 M at 20 ± 2°. [b] $J = (J_{4',5'} + J_{4',5''})/2$. [c] Due to near magnetic equivalence of two $C_{5'}$ protons at 220 MHz individual spin-coupling constants could not be determined. [d] $J = (J_{5',P} + J_{5''P})/2$.

with the aid of ^{31}P spin-decoupling experiments a measurement of proton–phosphorus spin-coupling constants is feasible. In particular, ^{31}P spin-decoupling experiments show that ^{31}P is coupled not only to the C(5′) protons but also to the C(4′) proton with the four-bond long-range coupling for the latter being equal to 1.0 Hz.

As a result of near magnetic equivalence of the two C(5′) protons individual values for H(4′)–H(5′), H(4′)–H(5″), ^{31}P–H(5′), ^{31}P–H(5″) coupling constants cannot be determined; only their sum (*i.e.*, $J_{4'5'} + J_{4'5''}$ and $J_{5'P} + J_{5''P}$) can be extracted from the spectrum. Final values for the nmr parameters of AMP-5′ were derived by procedures described in the previous section and are listed in Tables I and II. Comparison of the present AMP-5′ data with an earlier partial set[3] of couplings shows good agreement (±0.2 Hz) for $J_{2'3'}$ and $J_{3'4'}$ but a substantially higher value (±0.6 Hz) for $J_{1'2'}$ in the current set.

The 220-MHz GMP-5′ spectrum in D_2O solution is similar to the spectrum for AMP-5′ (except for the base ring proton region). The assignment and final calculated values of chemical shifts and spin-coupling constants are given in Tables I and II. Comparison of the data in Tables I and II shows that the major difference between the purine and pyrimidine 5′-ribonucleotides is a near equivalence of C(5′) proton shifts in purines and a substantial nonequivalence in pyrimidine derivatives as well as the fact that the chemical shifts of the furanose ring protons are further downfield for the purine compared to the pyrimidine derivatives.

Purine 5′-Deoxyribonucleotides. Substitution of a hydrogen for the C(2′) hydroxyl group (Figure 1) produces marked changes in the sugar ring proton region of the dAMP-5′ spectrum, as is evident in Figure 4. Features of note are the upfield location of both C(2′) proton signals and their large chemical shift nonequivalence. Additionally, the multiplet patterns for the C(2′) protons permit determination of values for relevant couplings to H(1′) and H(3′). The H(1′) signal at ~6.45 ppm appears as a pseudotriplet of width 13.5 Hz and analysis of the C(2′) proton signals as an ABMX spin system leads to unequal couplings of 7.3 and 6.2 Hz between H(1′) and the two C(2′) protons. The analysis also shows unequal couplings of 3.4 and 6.1 Hz between the C(3′) proton and the two C(2′) protons. In each instance, the C(2′) proton signal at lower field shows the larger coupling, *i.e.*, 7.3 and 6.1 Hz.

A direct assignment of the 2′ and 2″ proton multiplets to individual protons is not possible from shift differences alone. However, a reasonable indirect assignment can be made from a consideration of coupling constant magnitudes expected for likely furanose ring conformations. In a planar

[3] Values were reported only for $J_{1'2'}$, $J_{2'3'}$, $J_{3'4'}$ in the earlier work (Feldman and Agarwal, 1968; Fujiwara and Uetsuki, 1968).

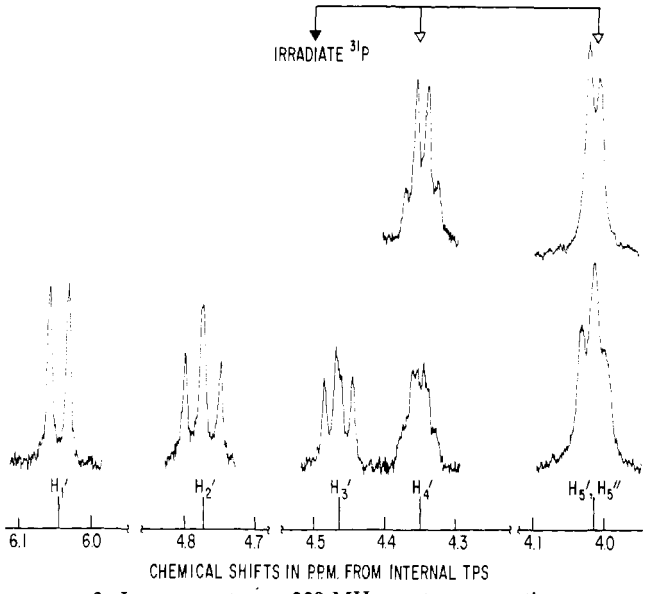


FIGURE 3: Lower spectrum, 220-MHz proton magnetic resonance spectrum of 0.1 M AMP-5′ in D_2O at 20°; upper spectrum, ^{31}P-decoupled 220-MHz spectrum of AMP-5′.

deoxyribose ring, couplings of ~9–10 Hz and ~1–3 Hz would be expected for $J_{1'2''}$, $J_{2'3'}$, and $J_{1'2'}$, $J_{2''3'}$ respectively, corresponding to dihedral angles of 0 and 120°; a small value would also be expected for $J_{3'4'}$ with a dihedral angle of 120°. Of the various twist conformations possible for the ribose ring, only a twist to an approximate C(2′)-endo form results in a favorable situation for relatively large magnitudes of both $J_{1'2'}$ and $J_{1'2''}$, one large and one smaller coupling for $J_{2'3'}$ ($J_{2''3'}$), and a $J_{3'4'}$ value of ~3–4 Hz. Furthermore, in the C(2′)-endo conformation the C(3′) proton exhibits the larger coupling to the "cis" proton, *i.e.*, H(2′), and a smaller coupling to the "trans" proton, *i.e.*, H(2″). From this line of reasoning, the upfield signal of the methylene group can be assigned to H(2″) and the downfield signal to H(2′); the assignment of $J_{1'2'}$ and $J_{1'2''}$ couplings also follows directly. The present result confirms an assignment reported earlier (Fang *et al.*, 1971) based on the shielding effect of the neighboring 3′-OH group upon H(2″). The values of coupling constants derived by Fang *et al.* (1971) for a 0.05 M solution of dAMP-5′ in D_2O (pD 6–7) at 20°, considering the coupling between the C(1′), C(3′), and two C(2′) protons as an ABMX spin system, were $J_{1'2'}$ = 6.9 Hz, $J_{1'2''}$ = 6.6 Hz, $J_{2'3'}$ = 5.8 Hz, and $J_{2''3'}$ = 3.8 Hz. The values differ somewhat from the iterated results in the present study.

Decoupling of the phosphorus resonance simplified the multiplet patterns for C(4′) and C(5′) protons (upper spectrum in Figure 4). However, individual values of $J_{5'(5'')\,^{31}P}$ cannot be determined unambiguously and an average value of 4.6 Hz is given in Table II. For the C(4′) proton, a line narrowing observed on decoupling, upper spectrum in Figure 4, indicates a coupling in the range 0.2–0.5 Hz. Final δ and J values summarized in Tables I and II, respectively, were obtained by simulation-iteration; an indication of the excellent fit between calculated and observed spectra is shown by the middle spectrum in Figure 4. The spectrum for dGMP-5′ was similar to that for dAMP-5′ and the assignment and analysis followed identical procedures; final parameters are listed in Tables I and II.

Pyrimidine 5′-Deoxyribonucleotides. Figure 5 shows the 220-MHz proton magnetic resonance spectrum for the deoxyribose ring protons of a 0.1 M TMP-5′ solution in D_2O. Apart from slight shift differences for the C(2′) protons the spectra of dUMP-5′ and dCMP-5′ are basically the same as for TMP-5′. Spectral assignments and analyses followed similar procedures to those for purine derivatives. ^{31}P spin-decoupling experiments again permitted determination of $J_{^{31}P\text{-}H(4')}$ couplings ranging from 0.6 Hz in dCMP-5′ to a relatively large coupling of 1.7 Hz in TMP-5′. Final calculated δ and J values are listed in Tables I and II and a com-

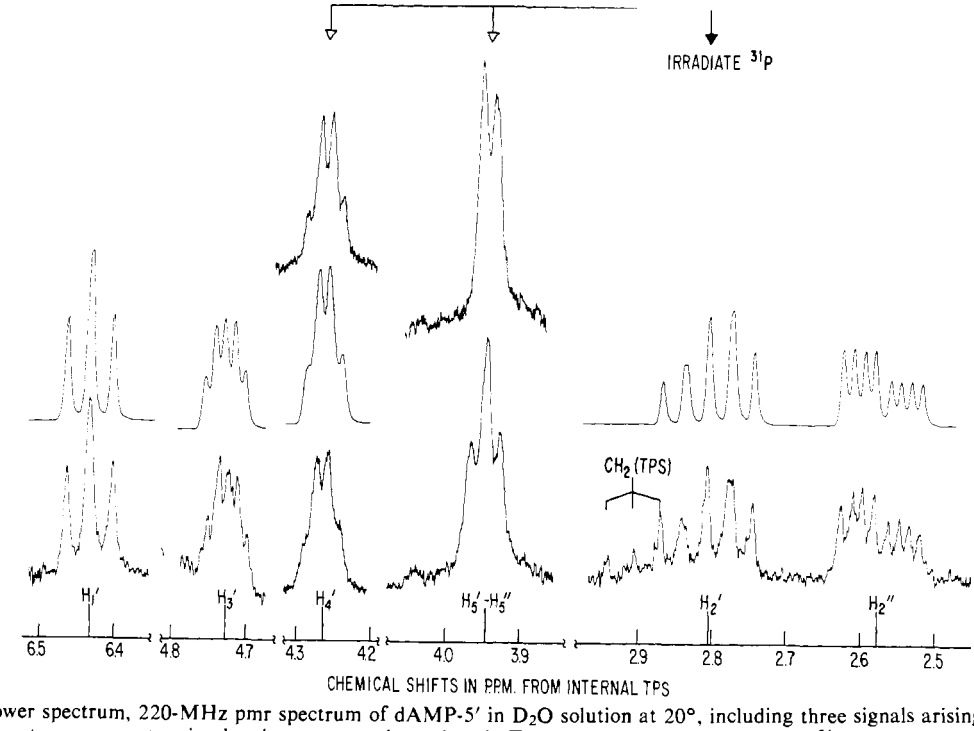


FIGURE 4: Lower spectrum, 220-MHz pmr spectrum of dAMP-5′ in D_2O solution at 20°, including three signals arising from the CH_2 group in TPS; middle spectrum, computer-simulated spectrum using values in Tables I and II; upper spectrum, ^{31}P-decoupled 220-MHz pmr spectrum of dAMP-5′.

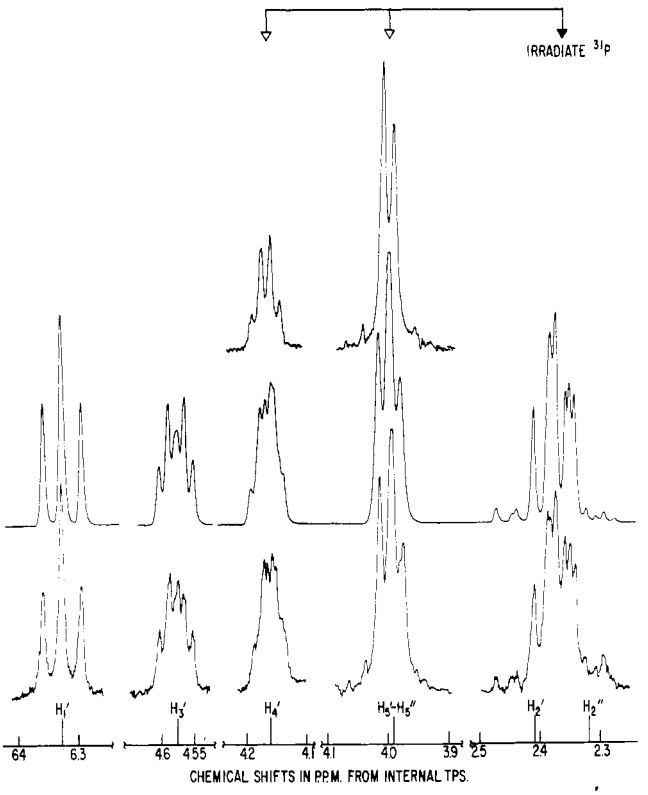


FIGURE 5: Lower spectrum, 220-MHz pmr spectrum of 0.1 M TMP-5′ in D_2O at 20°; middle spectrum, computer simulated spectrum; upper spectrum, ^{31}P-decoupled, 220-MHz pmr spectrum of TMP-5′.

parison of calculated and observed spectra of TMP-5′ is illustrated in Figure 5.

Several features are worth noting from the data in Tables I and II. The furanose ring proton couplings are basically the same in purine and pyrimidine deoxyribonucleotides, apart from $J_{2''3'}$ where some variability (outside experimental error) is evident. A similar behavior holds for the ribonucleotide couplings; however, the ribonucleotide couplings differ significantly from corresponding deoxyribonucleotide J values. It would appear that substitution in the 2′ position by a hydrogen atom is the major factor affecting furanose ring couplings. These observations have implications for conformational deductions of ribose rings and deoxyribose rings (*cf.* Discussion).

Comparison of chemical shifts is complicated by the known effects of concentration, pH differences, etc., upon nucleotides (Danyluk and Hruska, 1968; Schweizer *et al.*, 1968; Broom *et al.*, 1967) particularly for purine derivatives. Within the limitations of such effects, it can be noted that in contrast to the behavior of purine 5′-ribonucleotides and purine and pyrimidine 5′-deoxyribonucleotides, the C(5′) proton signals of pyrimidine ribonucleotides show a sizable chemical shift nonequivalence. Other chemical shift differences are apparent when comparison is made between C(2′) protons in purine and pyrimidine deoxyribonucleotides. Here $\delta(2') - \delta(2'')$ is much larger in purines (0.223 ppm for dAMP-5′) than in pyrimidines (0.061 ppm for dCMP-5′). Both C(2′) protons are also at lower field in purine derivatives, a possible reflection of greater purine-ring deshielding effects compared to pyrimidines.

As expected, replacement of a C(2′) hydroxyl group by a hydrogen atom produces substantial upfield shifts of the 2′ proton (~2.0 ppm) in all compounds studied, presumably because of a decrease in inductive deshielding influences of the oxygen atom. Accompanying this are sizable deshieldings of H(1′) (~0.30–0.40 ppm) and H(3′) (~0.20–0.30 ppm) protons in both purine and pyrimidine derivatives. Small upfield shifts ~0.10 ppm are also noted for H(4′). Similar, though less marked, trends were observed in earlier AMP-5′–dAMP-5′ studies (Fang *et al.*, 1971) and attempts have been made to interpret the changes in terms of various inter- and intramolecular interactions (*cf.* Discussion).

Discussion

A nucleotidyl unit can be considered in terms of three key structural features, *i.e.*, the ribose ring conformation, exocyclic group orientation, and base–ribose ring orientation. Information for each of these features can be derived from appropriate chemical shift and coupling constant data listed in Tables I and II and then integrated into a complete conformational model for the nucleotide according to steps outlined in following sections.

1. D-Ribose Ring Conformations

In principle, it would appear possible to establish the complete conformation of a ribose ring from a knowledge of all the vicinal proton coupling constants. Such a straightforward approach is not feasible for nucleotides for a number of reasons. First, the available evidence overwhelmingly indicates that the furanose ring does not possess a unique rigid structure in solution but is in a dynamical equilibrium between at least two favored puckered conformations, a type N conformer and type S conformer.[1] If the interconversion rate between conformers is sufficiently rapid then the observed couplings represent weighted averages of couplings in individual conformers (two or more). A determination of the conformer equilibrium, therefore, requires knowledge of the number of allowed conformations and of the couplings in specific conformers, information which is not readily available. Finally, a further complicating factor is the ultimate reliance on approximate vicinal proton coupling constant–torsion angle correlations essential for conformational analysis of the ribose ring.

Despite the above problems, several empirical and semiempirical approaches for relating coupling constants to ribosyl conformational properties have been reported (Hruska, 1973). Of these, the method outlined in detail recently by Altona and Sundaralingam (1972, 1973) has considerable merit. Essentially, the method assumes that a conformation of a five-membered ring can be described in terms of two pseudorotational parameters: P, the angle of pseudorotation, and τ_m, the degree of pucker. Combining structural input from extensive crystallographic measurements with empirical vicinal coupling constant correlations it is then feasible to calculate values for pseudorotational parameters from proton–proton spin coupling constants. Steps for carrying out such calculations for 5′-ribo- and deoxyribonucleotides are outlined in the following sections.

Pseudorotational Parameters—Crystalline State. For purposes of comparison of ribose and deoxyribose rings in solution and crystalline states, it is necessary to compile average values for $\bar{P}$ and $\bar{\tau}_m$ from crystallographic data. Values were calculated for N and S type conformations of both ribose and deoxyribose rings from data reported earlier (Altona and Sundaralingam, 1972, 1973) and the results are given in Table III. The compilation extends earlier work (Altona and Sundaralingam, 1973) to include τ_m along with average errors for these parameters in order to determine the reliability of comparisons for purine and py-

TABLE III: Average Values of Pseudorotational Parameters Calculated from X-Ray Data.[a]

	Purine		Pyrimidine	
	Ribose	Deoxyribose	Ribose	Deoxyribose
Type N				
No. of analyses	8	1	10	3
$\bar{P}$	9.8 (±4.5)	*b*	10.1 (±3.4)	*b*
$\bar{\Upsilon}$	38.4 (±3.1)	*b*	38.6 (±1.4)	34.9 (±2.2)
Type S				
No. of analyses	15	3	9	7[c]
$\bar{P}$	157.7 (±7.1)	180 (±10)	165.7 (±6.8)	162.8 (±11.3)
$\bar{\Upsilon}$	40.1 (±2.2)	35.5 (±2.1)	38.3 (±3.0)	38.5 (±1.6)

[a] Altona and Sundaralingam (1972). [b] Too few data to give meaningful average. [c] Molecule 60 (Altona and Sundaralingam, 1972) omitted from calculation of average.

rimidine ribo- and deoxyribonucleotides.

Several trends are apparent from Table III. For both the N and S type conformations $\bar{P}_N$, ${}^N\bar{\Upsilon}_m$ and $\bar{P}_S$, ${}^S\Upsilon_m$ are similar in purine and pyrimidine ribonucleotides. For pyrimidine derivatives in type S conformations where a sufficient number of analyses are available, $\bar{P}_S$ and ${}^S\bar{\Upsilon}_m$ are essentially the same in ribo- and deoxyribose derivatives, whereas for purine derivatives in type S conformations the limited data indicate that $\bar{P}_S$ (ribose) < $\bar{P}_S$ (deoxyribose) and $\bar{\Upsilon}_m$ (ribose) > $\bar{\Upsilon}_m$ (deoxyribose). Small differences are also evident between pseudorotational parameters for type N pyrimidine ribo- and deoxyribonucleotides. Although the differences in average values of P and Υ_m are greater than the difference in average error, too few analyses of purine deoxyribonucleotides in either the N or S type of conformations and of pyrimidine deoxyribonucleotides with N type conformations are available at present to validate fully such differences.

Altona and Sundaralingam Analysis—5′-Ribonucleotides. Following assumptions and procedures described in Altona and Sundaralingam (1972, 1973) and using $\bar{\phi}$ values calculated by the indirect method (Altona and Sundaralingam, 1973) it is possible to derive eq 1-5, where X_N = mole

$$J_{1'2'} = X_N J_{89°} + (1 - X_N) J_{158°} \quad (1)$$

$$J_{1'2''} = X_N J_{-32°} + (1 - X_N) J_{39°} \quad (2)$$

$$J_{2'3'} = X_N J_{43°} + (1 - X_N) J_{-39°} \quad (3)$$

$$J_{2''3'} = X_N J_{166°} + (1 - X_N) J_{80°} \quad (4)$$

$$J_{3'4'} = X_N J_{-158°} + (1 - X_N) J_{-96°} \quad (5)$$

fraction of conformer N, and $(1 - X_N) = X_S$. Since $J_{43°} \simeq J_{-39°}$ and $J_{89} \simeq J_{96} = 0$, it follows that $J_{2'3'}$ and $(J_{1'2'} + J_{3'4'})$ are predicted to be nearly constant. Inspection of the 5′-ribonucleotide data in Table II shows this to be the case with $J_{2'3'}$ having values close to the mean of 4.8 ± 0.2 Hz and $(J_{1'2'} + J_{3'4'})$ being nearly constant at 9.3 ± 0.3 Hz. The present mean value of $J_{2'3'}$ differs only slightly (0.3 Hz) from that reported by Altona and Sundaralingam (1973); $(J_{1'2'} + J_{3'4'})$, however, is substantially lower, *i.e.*, 9.3 *vs.* 10.1. This difference leads to an adjustment of constants in the Karplus expressions (Karplus, 1959) derived by Altona and Sundaralingam (1973) to values of A = 9.8 Hz and B = −0.9 Hz. These values give rise to significant changes in the Karplus curve in the region $90 \leq \phi \leq 180$. In particular, the difference in J values for ϕ_{158} of 0.8 Hz has a sizable impact on preferred furanose ring conformations.

Pseudorotational Parameters (Solution Calculations). Pseudorotational parameters were calculated for each molecule using the average value of $J_{2'3'}$ = 4.8 Hz and the parameters derived for the Karplus relation from nucleotide measurements in this work (*i.e.*, A = 9.8 and B = −0.9). Owing to the difficulty of framing a convenient algebraic expression for the determination of the pseudorotational parameters, values of Υ, P, $\phi_{1'2'}$, $\phi_{2'3'}$, $J_{1'2'}$, $J_{3'4'}$, and $(J_{1'2'} + J_{3'4'})$ were computed for Υ_m varying in integral steps from 25 to 50 using one set of Karplus parameters for coupling in each molecular fragment. Curves were drawn of the relevant parameters (P, Υ, $J_{1'2'}$, and $J_3'4'$) against computed values of $(J_{1'2'} + J_{3'4'})$ for both N and S types of conformers so that the correct value of each parameter could be interpolated for each set of observed $(J_{1'2'} + J_{3'4'})$. In order to facilitate subsequent discussion of the effect of observed error in J on pseudorotational parameters and to highlight differences between ribose and deoxyribose rings, each curve of P, Υ, $J_{1'2'}$, and $J_{3'4'}$ against $(J_{1'2'} + J_{3'4'})$ was generated for different values of $J_{2'3'}$ ranging from 4.1 Hz (less than the lowest value yet observed for ribose rings) up to 6.1 Hz (the average value observed for deoxyribose rings in this work). The curves shown in Figure 6 are of general usefulness for the analysis of observed ribose ring coupling constants in terms of pseudorotational parameters.

Figure 6a and b show that the sum of $(J_{1'2'} + J_{3'4'})$ varies from 9 to 11 Hz for the range of pseudorotational angles found in the solid state, *i.e.*, P_N (0–36) and P_S (144–180). The set of curves for different $J_{2'3'}$ values also shows that both P_N and P_S vary by approximately 1° for a variation in $J_{2'3'}$ of 0.1 Hz at constant $(J_{1'2'} + J_{3'4'})$. As the error in $J_{2'3'}$ in this work is ±0.1 Hz, interpolated values of P_N and P_S are rounded to the nearest integer. In Figure 6c and d, the variation of Υ_m exhibits similar behavior for N and S conformations except that the curve for ${}^S\Upsilon_m$ is displaced by 2–3° compared to that for ${}^N\Upsilon_m$ at similar values of $(J_{1'2'} + J_{3'4'})$. That the displacement arises as a consequence of the analysis rather than reflecting real conformational differences will be discussed later. The set of curves for different $J_{2'3'}$ values shows that both ${}^N\Upsilon_m$ and ${}^S\Upsilon_m$ vary by ±0.5° for a variation in $J_{2'3'}$ of ±0.1 Hz at constant $(J_{1'2'} + J_{3'4'})$ and by ±0.7° for a variation of ±0.2 Hz in $(J_{1'2'} + J_{3'4'})$ at con-

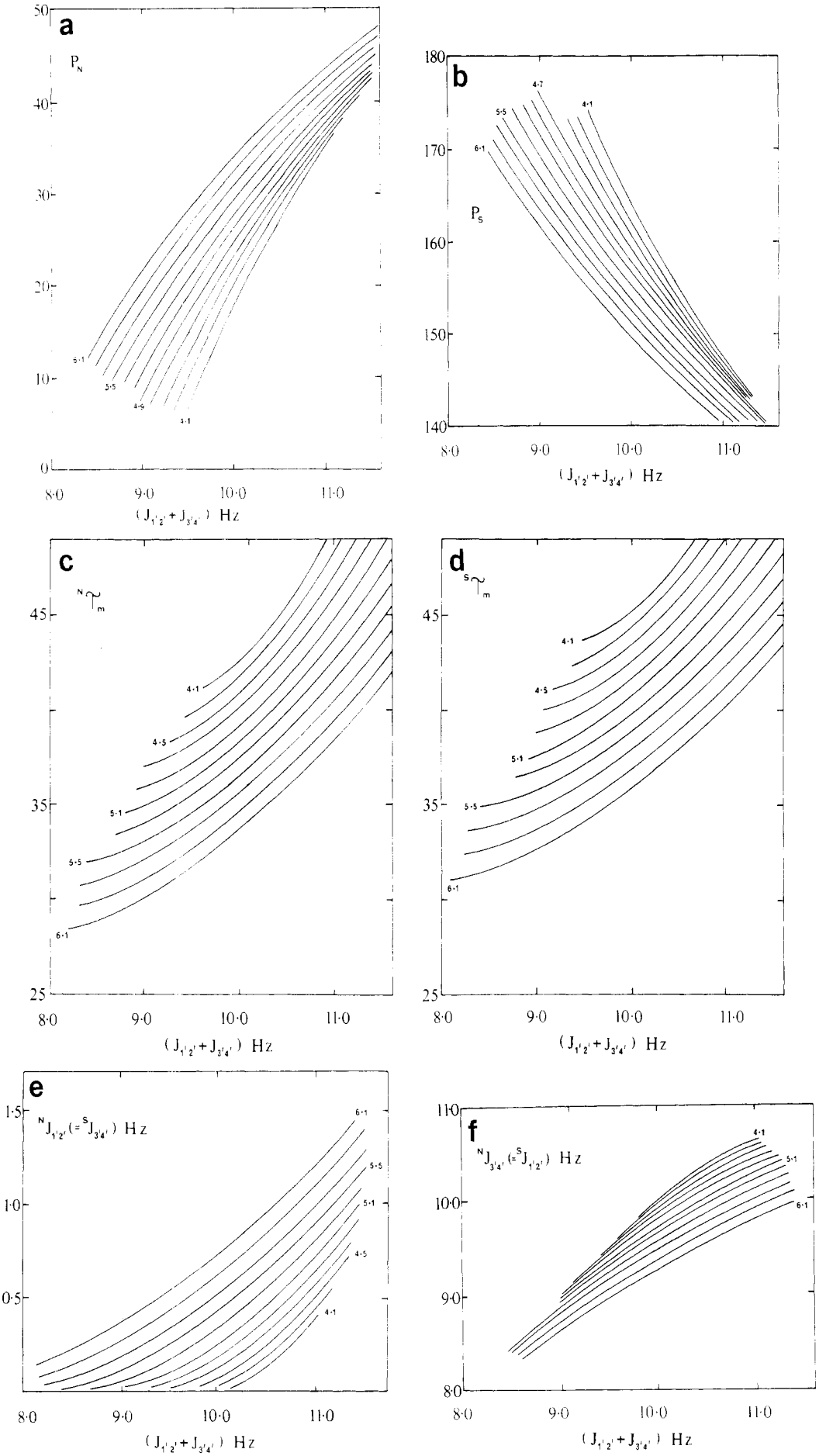


FIGURE 6: Curves generated by computing parameters of the pseudorotational analysis of furanose rings for different values of $J_{2'3'}$ (varying from 4.1 to 6.1 Hz) using one Karplus relation for each molecular fragment, *i.e.*, $J(HH) = 9.8 \cos^2 \phi - 0.9 \cos \phi$. Computations were made using the method and equations given by Altona and Sundaralingam (1973). (a) Variation of P_N with $(J_{1'2'} + J_{3'4'})$; (b) variation of P_N with $(J_{1'2'} + J_{3'4'})$; (c) variation of $^N\Upsilon_m$ with $(J_{1'2'} + J_{3'4'})$; (d) variation of $^S\Upsilon_m$ with $(J_{1'2'} + J_{3'4'})$; (e) variation of $^NJ_{1'2'}$ $(= {}^SJ_{3'4'})$ with $(J_{1'2'} + J_{3'4'})$; (f) variation of $^SJ_{1'2'}$ $(= {}^NJ_{3'4'})$ with $(J_{1'2'} + J_{3'4'})$.

TABLE IV: Pseudorotational Parameters and Equilibrium Compositions for 5′-Ribonucleotides in Aqueous Solution (A = 9.8, B = −0.9; $\bar{J}_{2',3'}$ = 4.8 Hz).

Nucleotide	$(J_{1',2'} + J_{3',4'})$	P_N	${}^N\Upsilon_m$	P_S	${}^S\Upsilon_m$	${}^NJ_{1',2'} = {}^SJ_{3',4'}$	${}^NJ_{3',4'} = {}^SJ_{1',2'}$	%N	K_{eq}	K_{eq}[g]
AMP-5′	9.5	17	37.2	165	39.9	0.05	9.45	38	1.63	1.64
GMP-5′	9.7	19	37.9	163	40.5	0.1	9.6	38	1.63	1.62
UMP-5′	9.2	12	36.3	170	39.2	0	9.2	45	1.22	1.24
CMP-5′	8.9	6	35.8	175	38.7	0	8.9	51	0.96	0.96
AMP-5′[a]	9.1	9	36.1	173	39.0	0	9.1	42	1.38	1.40
AMP-5′[b]	9.5	16	37.2	166	39.9	0.05	9.45	47	1.13	1.11
AMP-5′[c]	9.4	14	36.9	168	39.6	0.05	9.35	40	1.50	1.48
UMP-5[d]	8.8	Does not fit								1.20
Poly(U)[d]	9.5	16	37.2	166	39.9	0.05	9.45	42	1.38	1.38
UMP-5′[e]	9.2	11	36.3	171	39.2	0	9.2	42	1.38	1.36
UMP-5′[f]	8.9	6	35.8	175	38.7	0	8.9	44	1.27	1.28

[a] 0.02 M D_2O solution at 27° (Feldman and Agarwal, 1968). [b] 0.5% D_2O solution at 28° (Fujiwara and Uetsuki, 1968). [c] Ts'o (1970). [d] Kreishman and Chan (1971). [e] 0.1 M D_2O solution, pD = 8.0, 30° (Wood *et al.*, 1973a). [f] 0.2 M D_2O, pD = 7.8, 30° (Smith *et al.*, 1973). [g] Calculated using eq 10.

stant $J_{2'3'}$ = 4.8 Hz. Thus differences of Υ_m of ±1° are expected within the experimental accuracy of the proton spin-coupling constants.

From Figure 6e, it is evident that the curves for $J_{1'2'}$ for N conformations (*i.e.*, ${}^NJ_{1'2'}$) and $J_{3'4'}$ for S conformations (*i.e.*, ${}^SJ_{3'4'}$) exhibit the same trend with respect to variation of $(J_{1'2'} + J_{3'4'})$. A similar pattern is observed for ${}^NJ_{3'4'}$ and ${}^SJ_{1'2'}$ in Figure 6f. This behavior results from a symmetry in N and S conformations as expressed by calculated $\bar{\phi}_{1'2'}$ and $\bar{\phi}_{3'4'}$ values (Altona and Sundaralingam, 1973) and the use of a single Karplus curve for both molecular fragments.

Values of P_N, ${}^N\Upsilon_m$, P_S, ${}^S\Upsilon_m$, ${}^NJ_{1'2'}$ (= ${}^SJ_{3'4'}$) and ${}^NJ_{3'4'}$ (= ${}^SJ_{1'2'}$) were interpolated from appropriate curves in Figure 6 using $(J_{1'2'} + J_{3'4'})_{obsd}$ for each nucleotide and an average value of $\bar{J}_{2'3'}$ = 4.8 Hz for all 5′-ribonucleotides. The pseudorotational parameters for each nucleotide are summarized in Table IV and are discussed separately in following sections.

Since values of ${}^NJ_{1'2'}$ (= ${}^SJ_{3'4'}$) and ${}^NJ_{3'4'}$ (= ${}^SJ_{1'2'}$) are assumed to be those for pure N and S conformers while observed values of $J_{1'2'}$ and $J_{3'4'}$ are weighted averages of J values for pure conformers, the relative proportions of each conformer can be calculated from

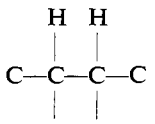
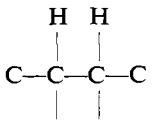
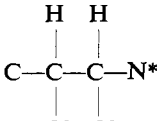
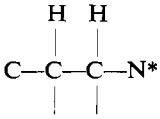
$$J_{obsd} = X_N{}^NJ + (1 - X_N)^SJ \qquad (6)$$

where X_N is the fraction of N conformer and $(1 - X_N) = X_S$ is the fraction of S conformer (Altona and Sundaralingam, 1973). The results of these calculations are also listed in Table IV.

Degree of Pucker, Υ_m. The parameters in Table IV show a slightly lower degree of pucker in the N conformer of purine and pyrimidine 5′-nucleotides, a tendency also evident in ${}^N\Upsilon_m$ and ${}^S\Upsilon_m$ values calculated from nmr data of other workers (Altona and Sundaralingam, 1972, 1973). On the other hand, no apparent variation of Υ_m occurs for either the N or S conformers in going from purine to pyrimidine nucleotides. This would suggest that base-ring characteristics have little influence upon ribose ring puckering in pure conformers, at least in the family of molecules studied in this work.

The numerical data in Table IV also indicate that ${}^N\Upsilon_m - {}^S\Upsilon_m$ for each nucleotide varies between 2.5 and 2.9 depending on the value of $(J_{1'2'} + J_{3'4'})$, and that $(J_{1'2'} + J_{3'4'})$ is dominated by ${}^SJ_{1'2'}$ for S conformations and ${}^NJ_{3'4'}$ for N-type conformations. In the calculation of pseudorotational parameters of the furanose ring, one composite Karplus relation was used for proton couplings in each molecular fragment C(1′)-C(2′), C(2′)-C(3′), and C(3′)-C(4′). However, the coupling across C(1′)-C(2′) is influenced to

TABLE V: Molecular Fragments for Vicinal Interproton Couplings in Ribose and Deoxyribose Rings.

Fragment	Diagram[a]	Vicinal Interproton Coupling: Ribose Ring	Vicinal Interproton Coupling: Deoxyribose Ring
I		$J_{2',3'}$	
		$J_{3',4'}$	$J_{3',4'}$
II			$J_{2',3'}$
			$J_{2'',3'}$
III		$J_{1',2'}$	
IV			$J_{1',2'}$
			$J_{1',2''}$

[a] N* represents the attachment of the furanose ring to the N atom of the purine or pyrimidine base.

the extent of ~0.5 Hz (decrease) by electronegativity effects (Karplus, 1963; Booth, 1965; Sternhell, 1969), compared to coupling in the other molecular fragments which have only one oxygen atom bonded to each carbon atom (see Table V). An estimate of the E_n effect on ${}^{N}\Upsilon_m$ and ${}^{S}\Upsilon_m$ can be made by recalculating the degree of pucker using a composite Karplus equation and a $J_{1'2'}$ value adjusted by +0.5 Hz. Since the pseudorotational parameters in S conformations are dominated by ${}^{S}J_{1'2'}$, an increase in $J_{1'2'}$ of about 0.5 Hz corresponds to an increase in Υ_m of approximately 2°, Figure 6c, assuming an average $J_{2'3'}$ of 4.8 Hz and $(J_{1'2'} + J_{3'4'})$ of 9.3 Hz. Thus the differences in ${}^{S}\Upsilon_m$ and ${}^{N}\Upsilon_m$ in Table IV are a consequence of assumptions made in the analysis rather than a measure of conformational differences for each nucleotide.

Comparison of solution ${}^{N}\Upsilon_m$ and ${}^{S}\Upsilon_m$ values with those in the solid state (Table III) shows little difference in puckering in the two states implying that solution and crystal intermolecular forces evidently have a similar effect upon ribose ring pucker in ribonucleotides.

Pseudorotational Angle, P. The ranges of pseudorotational angles (P_N, 6–19 and P_S, 163–175) for the 5′-ribonucleotides in solution are close to those for nucleosides and nucleotides in the crystalline state. Experimental errors of ±0.1 Hz in $J_{2'3'}$ and ±0.2 Hz in $(J_{1'2'} + J_{3'4'})$ lead to expected variations of ±1 and ±2°, respectively, in values P_N and P_S, Figure 6a and b. Thus differences in pseudorotational angles greater than ±3° are likely to be significant. For molecules measured in this work, the results in Table IV show that average values of P_N and P_S differ for purine and pyrimidine ribonucleotides by amounts outside experimental and computational limits.[4] The value of $\bar{P}_N$ (purine) = 18 (±1) is the same as that expected for a C(3′)-endo conformation (P_N = 18), whereas $\bar{P}_N$ (pyrimidine) = 9 (±3) reflects a conformation between C(3′)-endo and C(2′)-exo values. Similarly for S conformers a $\bar{P}_S$ value of 164 ± 1 (purines) is close to that expected for a C(2′)-endo conformation whereas for pyrimidine derivatives the $\bar{P}_S$ value reflects conformations between C(2′)-endo and C(3′)-exo. The origin of differences in pseudorotational angles between purine and pyrimidine 5′-ribonucleotides in solution is obscure but could arise from variations in electronic charge distribution or steric hindrance.

From the data in Tables III and IV, it would appear that differences in pseudorotational angles between purines and pyrimidines are not as marked in the solid state as in solution. Also, except for the increase in $\bar{P}_N$ (purines) a change of state produces no real variation of pseudorotational angles in other conformers. It may be noted that P_N and P_S values calculated using coupling constant data reported previously (summarized in Table IV) are substantially different from those obtained with the present nmr data, emphasizing the need for accurate coupling constant values as input for conformational analyses.

Equilibrium Populations. The present calculations show a favored S conformation for all of the 5′-ribonucleotides. The % N conformer for AMP-5′ is close to the value reported by Altona and Sundaralingam (1973) but the present UMP-5′ results show a favored S conformation while the earlier AS value shows a slight preference for the N type form. This discrepancy arises because of differences in coupling constants used in the analysis, *e.g.*, $J_{1'2'}$ = 5.1 ± 0.1 Hz in the present work and 4.6–4.8 Hz in earlier studies. Agreement of the pseudorotational parameters and equilibrium populations calculated with the present set of couplings is satisfactory when compared with those determined using the most reliable set published recently by Wood *et al.* (1973a,b).

An alternative less involved procedure for calculating equilibrium populations from observed $J_{1'2'}$ and $J_{3'4'}$ couplings has been reported previously (Davies and Danyluk, 1972). If assumptions analogous to those of the Altona and Sundaralingam method are made, then the observed vicinal couplings are related to the relative proportion of conformers by

$$J_{1'2'} = 9.3(1 - X_N) = 9.3X_S \quad (7)$$

$$J_{2'3'} = 4.6X_N + 5.3(1 - X_N) \quad (8)$$

$$J_{3'4'} = 9.3X_N \quad (9)$$

Equations 7–9 can be solved, using observed coupling constants,[5] to obtain equilibrium populations. It is found that equilibrium populations calculated from $J_{1'2'}$ and $J_{3'4'}$ are approximately the same for individual nucleotides but both differ appreciably from those obtained with $J_{2'3'}$. The latter value is less reliable, however, because the expected variation in $J_{2'3'}$ between pure N and pure S conformers is substantially less (~0.7 Hz) than the variation in the other couplings (~9.3 Hz). The equilibrium constant (K_{eq}) can be calculated directly from the observed values of $J_{1'2'}$ and $J_{3'4'}$ according to the approximate eq 7 and 9

$$K_{eq} = X_S/X_N = J_{1'2'}/J_{3'4'} \quad (10)$$

Values of K_{eq} calculated according to eq 10 compare very favorably with those obtained by the full pseudorotational analysis, Table IV. Hence a rapid determination of K_{eq} can be made directly from $J_{1'2'}$ and $J_{3'4'}$ values without involving the full pseudorotational analysis for each nucleotide.

2. *Deoxyribose Ring Conformations*

A furanose ring conformational analysis is also possible for deoxyribonucleotides providing criteria and assumptions discussed for ribonucleotides are fulfilled. A survey of the limited crystallographic data available for deoxyribonucleotides (Altona and Sundaralingam, 1972) shows that furanose ring conformations can in fact be described in terms of appropriate pseudorotational parameters. Moreover, a summary of average values of pseudorotational parameters for the deoxyribose ring, Table III, reveals similarities to values for ribonucleotides, at least in the case of the pyrimidine derivatives where a sufficient number of analyses are available for S type conformations. Criteria for carrying out a pseudorotational analysis from nmr data in solution are also fulfilled. Thus observed values of $J_{2'3'}$ and $(J_{1'2'} + J_{3'4'})$, Table II, are approximately constant for the set of 5′-deoxyribonucleotides studied. However, it should be noted that $J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$ values vary markedly between corresponding ribo- and deoxyribonucleotides. Part of this difference can be accounted for by an E_n change at C(2′) (Karplus, 1963; Booth, 1965; Sternhell, 1969; Kotowycz and Lemieux, 1973). The remainder is likely to be due to con-

[4] Adjustment of $J_{1'2'}$ for E_n effects will alter absolute magnitudes of P_N and P_S but will not change the relative trends for purine and pyrimidine ribonucleotides.

[5] Caution must be exercised to ensure that $J_{1'2'}$ and $J_{3'4'}$ values are determined by proper spectral analysis in cases where virtual coupling effects may be present.

formational differences and/or changes in equilibrium compositions, *e.g.*, $\bar{J}_{3'4'}$ for the 5'-deoxyribonucleotides is 3.1 Hz compared to 4.0 Hz for the 5'-ribonucleotides even though the molecular fragment, C(3')-C(4'), is the same in both ring systems, Table V. Before proceeding with a conformational analysis it is useful to consider possible effects of substituent electronegativity changes on the vicinal proton spin-coupling constants in different molecular fragments of the deoxyribose ring.

Electronegativity (E_n) Effects and Karplus Parameters. Substitution of a 2'-hydroxyl of a ribose ring by a hydrogen atom is expected to affect mainly those couplings in molecular fragments containing the C(2') atom, *i.e.*, fragments I and III of ribose rings become fragments II and IV for deoxyribose rings, Table V. Specifically, substitution of the hydroxyl group by a more electropositive hydrogen atom is expected to produce an increase in $J_{1'2'}$ and $J_{2'3'}$. In Table II, it can be seen that $\bar{J}_{2'3'}$ is 6.3 Hz for 5'-deoxyribonucleotides and 4.8 Hz for 5'-ribonucleotides under the same solution conditions. Thus electronegativity effects account for at least part of the coupling constant differences in ribo- and deoxyribonucleotides. However, the effect of E_n decreases rapidly with the number of intervening bonds and since the bonding arrangements and attached groups in fragment I are the same for ribose and deoxyribose rings, it follows that any differences in $J_{3'4'}$ reflect conformational differences in the two rings.

An estimate of the contribution to $J_{1'2'}$ and $J_{2'3'}$ arising from E_n changes between ribose and deoxyribose rings can be made from a consideration of proton coupling constant values in model 3',5'-cyclic mononucleotides. Blackburn *et al.* (1973) concluded from ^1H-^1H and ^{31}P-^1H coupling constant data for TMP-3',5' and AMP-3',5' that phosphate and furanose ring structures are rigid in both molecules and consistent with C(4')-exo and C(3')-endo-C(4')-exo furanose conformations, respectively. The differences in furanose ring conformations are inferred largely from differences in $J_{1'2'}$ and $J_{2'3'}$ observed for the cyclic deoxy- and ribonucleotides. Alternatively, it is at least as likely that these two couplings reflect changes in substituent E_n at the 2' position, perhaps to a greater extent than conformational variations. For example, the rather close agreement between the sets of couplings $J_{4'5'(5'')}$ (4.7 ± 0.1 and 10.6 ± 0.1 Hz); $J_{^{31}P\,5'(5'')}$ (20.9 ± 0.5 and 2.0 ± 0.3 Hz), $J_{^{31}P\,3'}$ (1.8 ± 0.2 Hz) and $J_{3'4'}$ (9.0 ± 0.2 Hz) for the two fused rings can be equally taken as a strong indication of ring conformational similarities.[6]

A sensitive criterion for conformational differences is the magnitude of couplings between atoms in approximately anti-periplanar arrangements; here a small change in dihedral angle produces a relatively large coupling constant variation. For 3',5'-cyclic mononucleotides the pertinent couplings are $J_{^{31}P\,5'}$, $J_{4'5''}$, and $J_{3'4'}$ and values for these couplings are nearly constant in AMP-3',5' and TMP-3',5' (Blackburn *et al.*, 1973) suggesting that furanose and phosphate ring conformations do not vary much in these nucleotides. However, the magnitudes of $J_{1'2'}$ and $J_{2'3'}$ differ appreciably between AMP-3',5' (0.7 and 5.3 Hz, respectively) and TMP-3',5' (2.4 and 8.0 Hz) indicating that the effect of substitution of a 2'-OH group by the more electropositive H atom increases ^3J(HH) in molecular fragments containing the CH_2 group; the magnitude of the E_n effect is in turn dependent upon dihedral angles between coupled protons (Williamson, 1963; Williams and Bhacca, 1964). Furthermore, comparison of the results for dAMP-3',5' ($J_{1'2'}$ = 2.8 Hz) and UMP-3',5' ($J_{1'2'}$ < 1 Hz) (Davies and Danyluk, 1972) with data for AMP-3',5' ($J_{1'2'}$ = 0.7 Hz) and TMP-3',5' ($J_{1'2'}$ = 2.4 Hz) (Blackburn *et al.*, 1973) shows that differences in $J_{1'2'}$ between a purine and pyrimidine derivative are substantially smaller than between a ribose and deoxyribose derivative. For the 5'-ribo- and deoxyribonucleotides the electronegativity effect may contribute up to ~1.5 Hz in both $J_{1'2'}$ and $J_{2'3'}$, Table II. Although it is obviously desirable to have a Karplus expression for individual molecular fragments I–IV, there are currently insufficient data to derive quantitative expressions. Nevertheless, it is instructive to carry out Altona and Sundaralingam type analyses for a number of cases using adjusted Karplus expressions.

D-*Deoxyribose Ring.* Pseudorotational parameters and equilibrium compositions were calculated by the same procedures used for ribonucleotides. In an attempt to assess the effect of individual correlations for different molecular fragments, three cases were considered for fragments I, II, and IV.

CASE A. Pseudorotational parameters and equilibrium compositions were calculated using one composite Karplus curve for fragments I, II, and IV derived from observed mean values of $J_{2'3'}$ = 6.3 Hz and $(J_{1'2'} + J_{3'4'})$ = 10.5 Hz. The values of the constants in the Karplus relation $J = A\cos^2\phi + B\cos\phi + C$ are $A = 11.7$, $B = -0.4$, $C = 0$.

CASE B. A second set of parameters was calculated utilizing the Karplus curve derived from the ribose ring (A = 9.8, $B = -0.9$, $C = 0$) for coupling in fragment I and the Karplus curve derived for the deoxyribose ring ($A = 11.7$, $B = -0.4$, $C = 0$) for couplings in fragments II and IV.

Because $J_{2'3'}$ values for 5'-deoxyribonucleotides show a greater spread than in ribonucleotides, pseudorotational parameters were also calculated using individual $J_{2'3'}$ for each deoxyribonucleotide and the two vicinal correlations for ribose and deoxyribose rings.

CASE C. In the third case, the ribose ring correlation (A = 9.8, $B = -0.9$, $C = 0$) was used for fragment I and an adjusted Karplus correlation increased by 1 Hz at each dihedral angle was used for couplings in fragments II and IV.

Despite these approximations, it is possible to compare relative values for pseudorotational parameters and equilibrium compositions of 5'-deoxyribonucleotides within a series and with the 5'-ribonucleotides using a consistent analytical method. Pseudorotational parameters were derived from calculated curves similar to those for 5'-ribonucleotides and the results are summarized in Table VI. A negligible variation occurs in pseudorotational parameters and equilibrium compositions calculated with either individual or $\bar{J}_{2'3'}$ values in case B and parameters derived for $\bar{J}_{2'3'}$ values will therefore be used for comparisons with those calculated for case A and case C.

Degree of Pucker, Υ_m. In all cases the magnitudes of Υ_m, Table VI, vary only slightly between purine and pyrimidine deoxyribonucleotides in both the N and S conformations. The small differences in $^N\Upsilon_m$ and $^S\Upsilon_m$ calculated for each case are attributable mainly to the type of correlation used and the fact that $J_{1'2'}$ dominates the calculation of parameters for the S conformer while $J_{3'4'}$ dominates N conformer calculations.

[6] Comparison of $J_{1'2'}$ for purine and pyrimidine derivatives shows that any inductive effect on $J_{1'2'}$ arising from base-ring differences is nearly negligible. Note also that rigid structures proposed in solution are similar to those reported in the solid state, AMP-3',5' (Watenpaugh *et al.*, 1968), UMP-3',5' (Coulter, 1968, 1969, 1970).

A comparison of deoxyribonucleotide Υ values, Table VI, with those for corresponding ribonucleotides, Table IV, shows that, within approximations of the analyses, the degree of puckering is essentially the same in both systems for the N and S conformations and that puckering of the furanose ring in solution is not changed significantly from that in the solid state, Table III.

Pseudorotational Angle, P. The different methods of analysis have only a minor effect on P values of the S conformation but give rise to considerable differences in P_N values, *e.g.*, $\bar{P}_N$ (case A) = 17 (±2) and $\bar{P}_N$ (case C) = 20 (±2) to $\bar{P}_N$ (case B) = 31 (±2). The former values are closer to those found for nucleosides and nucleotides in the solid state, Table III, and if the trends established for other parameters, *i.e.*, P_N, ${}^N\Upsilon_m$, P_S, and ${}^S\Upsilon_m$ for ribonucleotides and ${}^S\Upsilon_m$ and P_S for deoxyribonucleotides do not change in going from solid to solution states, then procedures followed for case A or C give reliable sets of final parameters. Since application of case A is relatively straightforward it is the preferable approach for pseudorotational analyses of deoxyribose rings.

Equilibrium Compositions. Equilibrium conformer populations were calculated with eq 6 for all three cases separately and the values are listed in Table VI. Interpolated ${}^NJ_{1'2'}$, ${}^NJ_{3'4'}$, ${}^SJ_{1'2'}$ and ${}^SJ_{3'4'}$ magnitudes now depend on the vicinal correlation used; thus, for case A, the common correlation leads to ${}^NJ_{1'2'} = {}^SJ_{3'4'}$ and ${}^NJ_{3'4'} = {}^SJ_{1'2'}$ analogous to the situation for ribonucleotides. For cases B and C, ${}^NJ_{1'2'} \neq {}^SJ_{3'4'}$ and ${}^NJ_{3'4'} \neq {}^SJ_{1'2'}$ since different Karplus relationships were used for coupling across C(1′)-C(2′) and C(3′)-C(4′). However, the final calculated conformer populations are essentially the same in each case presumably reflecting the fact that ${}^NJ_{1'2'} \ll {}^SJ_{1'2'}$ (also ${}^NJ_{3'4'} \gg {}^SJ_{3'4'}$). The equilibrium compositions are apparently independent of base type with the S form favored ~70:30 in both purine and pyrimidine 5′-deoxyribonucleotides. A further result of interest is the greater preference for an S conformation in deoxyribonucleotides when compared with respective ribonucleotides (60:40). The difference is outside the error limits of the analytical procedures and it is concluded that conformational differences exist between furanose rings of ribo- and deoxyribonucleotides in solution. The direct method of calculating furanose ring conformer populations noted for ribonucleotides can also be extended to deoxyribonucleotides. In this case five vicinal proton coupling constants are available for calculation of equilibrium compositions, but in practice two of the coupling constants in eq 1-5, *i.e.*, $J_{2'3'}$ and $J_{1'2''}$, do not vary by more than 1 Hz between "pure" N and S conformers and hence cannot serve as reliable parameters. Equilibrium calculations were made for each of the three cases using $J_{1'2'}$, $J_{2''3'}$, $J_{3'4'}$ and the appropriate Karplus relation for each molecular fragment. It was found that the calculated magnitudes again vary only slightly across the series of deoxyribonucleotides and the average values are close to those obtained by the full pseudorotational analysis, Table VI. For case A the equilibrium constant can be calculated using eq 10 and the values are within 2-3% of those calculated by the full pseudorotational analysis (Table VI). Since the correlations and parameters used in case A yield acceptable values both of pseudorotational parameters and equilibrium compositions and in view of the somewhat simpler analytical procedures, it is concluded that approach A is preferable for conformational analysis of deoxyribose rings.

TABLE VI: Pseudorotational Parameters and Equilibrium Compositions for the Deoxyribonuleotides in Aqueous Solution Assuming an Average $J_{2',3'}$ = 6.3 Hz.

Nucleotide	P_N	${}^N\Upsilon_m$	P_S	${}^S\Upsilon_m$	${}^NJ_{1',2'}$	${}^SJ_{1',2'}$	${}^NJ_{3',4'}$	${}^SJ_{3',4'}$	% N	K_{eq}	K_{eq}[a]
Case A, A = 11.7, B = −0.4											
TMP-5′	19	38	164	41	0.05	10.55	10.55	0.02	28	2.57	2.53
dCMP-5′	14	37	179	40	0	10.2	10.2	0	31	2.22	2.19
dUMP-5′	17	38	165	41	0.05	10.45	10.45	0.05	28	2.57	2.50
dAMP-5′	14	37	168	40	0	10.3	10.3	0	29	2.44	2.43
dGMP-5′	20	38	162	41	0.05	10.65	10.65	0.05	30	2.33	2.34
Mean	17 (±2)	38 (±1)	166 (±2)	41 (±1)					29 (±1)		
Case B, $J_{1'2'}$ and $J_{2'3'}$, A = 11.7, B = −0.4; $J_{3'4'}$, A = 9.8, B = −0.9											
TMP-5′	33	43	164	41	0.35	10.55	10.25	0.05	29		
dCMP-5′	28	41	170	40	0.2	10.2	10.0	0	32		
dUMP-5′	32	42	165	41	0.3	10.45	10.2	0.05	29		
dAMP-5′	29	41	168	40	0.25	10.3	10.05	0	30		
dGMP-5′	34	44	162	41	0.4	10.6	10.3	0.1	30		
Mean	31 (±2)	42 (±1)	166 (±2)	41 (±1)					30 (±1)		
Case C, $J_{1'2'}$ and $J_{2'3'}$, A = 9.8, B = −0.9, C = 1.0; $J_{3'4'}$, A = 9.8, B = −0.9											
TMP-5′	22	36	160	38	1.2	10.4	9.4	0.2	30		
dCMP-5′	16	35	166	37	1.1	10.1	9.1	0.1	34		
dUMP-5′	21	36	162	38	1.2	10.3	9.3	0.2	31		
dAMP-5′	18	35	165	37	1.1	10.2	9.2	0.1	32		
dGMP-5′	24	36	159	38	1.3	10.4	9.4	0.2	32		
Mean	20 (±2)	36 (±1)	162 (±2)	38 (±1)					32 (±1)		

[a] Calculated from eq 10.

TABLE VII: Calculated Populations of the Three Classical Rotamers of the Exocyclic Phosphate Group.

Nucleotide	C(4′)–(5′) Bond[a]			C(5′)–O(5′) Bond[b]			$^4J_{P,4'}$
	ρ_I	ρ_{II}[c]	ρ_{III}[c]	ρ_{IV}	ρ_V[c]	ρ_{VI}[c]	
AMP-5′	0.76	*d*	*d*	0.74	*d*	*d*	1.0
GMP-5′	0.72	*d*	*d*	0.72	*d*	*d*	1.0
UMP-5′	0.89	0.08	0.03	0.72	0.18	0.10	1.7
CMP-5′	0.86	0.09	0.05	0.73	0.17	0.10	1.0
dAMP-5′	0.66	*d*	*d*	0.71	*d*	*d*	~0.5
dGMP-5′	0.70	*d*	*d*	0.72	*d*	*d*	1.0
dUMP-5′	0.70	*d*	*d*	0.70	*d*	*d*	1.3
dCMP-5′	0.65	0.20	0.15	0.66	0.20	0.14	0.6
TMP-5′	0.66	*d*	*d*	0.70	*d*	*d*	1.7
UMP-5′[e]	0.65	*f*	*f*	0.77	*f*	*f*	*j*
GMP-5′[g]				0.72	*h*	*h*	*j*
IMP-5′[g]				0.72	*h*	*h*	*j*
UMP-5′[i]	0.85	0.10	0.05	0.75	0.18	0.07	1.8
UMP-5′[j]	0.87	0.07	0.06	0.74	0.17	0.09	2.2

[a] ρ_I, ρ_{II}, ρ_{III} (the mole fractions of each staggered rotamer of the C(4′)–C(5′) bond) were calculated from the following expressions: $\rho_I = [(J_t + J_g) - (J_{4'5'B} + J_{4'5'C})]/(J_t - J_g)$, $\rho_{II} = (J_{4'5'B} - J_g)/(J_t - J_g)$, and $\rho_{III} = (J_{4'5'C} - J_g)/(J_t - J_g)$. [b] ρ_{IV}, ρ_V, ρ_{VI} (the mole fractions of each staggered rotamer of the C(5′)–O(5′) bond) were calculated from the following expressions: $\rho_{IV} = [(J_t + J_g) - (J_{31PB} + J_{31PC})]/(J_t - J_g)$, $\rho_V = (J_{31PB} - J_g)/(J_t - J_g)$, and $\rho_{VI} = (J_{31PC} - J_g)/(J_t - J_g)$. [c] Values of both ρ_{II} and ρ_{III} and of ρ_V and ρ_{VI} were calculated according to the assignment of the C(5′) methylene protons (Davies and Rabczenko, 1974) which is opposite to that published previously (Remin and Shugar, 1972). [d] Unique values of either $J_{4',5'}$ and $J_{4',5''}$ or $J_{5',P}$ and $J_{5'',P}$ cannot be determined at 220 MHz. [e] Kreishman and Chan (1971). [f] Only average values of $J_{4'5'}$, $J_{4\,5''}$, $J_{5'P}$, and $J_{5''P}$ given. [g] Tran-Dinh Son *et al.* (1972b). [h] Individual values of $J_{5'P}$ and $J_{5''P}$ not given. [i] Wood *et al.* (1973b). [j] Not observed.

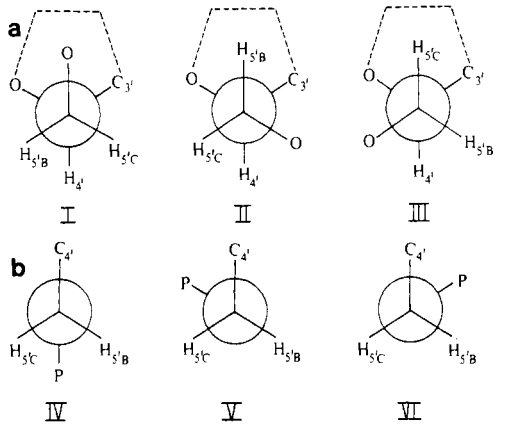


FIGURE 7: (a) Classical staggered rotamers for the C(4′)–C(5′) bond: I, gauche-gauche; II, trans-gauche; III, gauche-trans. (b) Classical staggered rotamers for the C(5′)–O(5′) bond: IV, gauche-gauche; V, trans-gauche; VI, gauche-trans.

3. Conformations of Exocyclic CH_2OR Groups (R = phosphate)

A considerable body of information is already available concerning the conformational properties of exocyclic groups in the crystalline state. Crystallographic measurements establish that the torsional angle ψ for rotation about the C(4′)–(5′) bond shows three distinct ranges of 35–65, 160–190, and 295–325° (Sundaralingam, 1969; Altona and Sundaralingam, 1972). All crystal structures of the 5′-nucleotides and most of the X-ray analyses of DNA and RNA have ψ in the range 35–60° (Sundaralingam, 1969; Altona and Sundaralingam, 1972). A torsional angle of 60° corresponds to a conformation in which C(5′)–O(5′) is anti-periplanar to the C(4′)–H(4′) bond so that the C(4′)–H(4′) bond bisects the angle between the two C(5′) protons, *i.e.*, H(4′) exists in a gauche-gauche relationship to the two C(5′) protons. The torsional angle, ϕ, for rotation about C(5′)–O(5′), spans a range of angles between 128 and 271°, though the majority of known structures of 5′-nucleotides have ϕ values of approximately 180°, *i.e.*, an average of ~175° (Sundaralingam, 1969; Altona and Sundaralingam, 1972). Molecular models show that ϕ angles of polynucleotides, especially those with helical structures, are limited to the range 160–200°. In this conformation, the P–O(5′) bond is anti-periplanar to the C(5′)–C(4′) bond and also bisects the angle between the two C(5′) protons, *i.e.*, the phosphorus atom exists in a gauche-gauche relationship to the two C(5′) protons. Because intermolecular forces in the crystal state are likely to differ in kind and magnitude from those in solution, it is necessary to supplement the crystallographic data with conformational data in solution, particularly in those cases where rotational isomerism is present. The present nmr parameters have been analyzed with this in mind.

C(4′)–C(5′) Bond. Previous analyses (Hruska *et al.*, 1970; Grey *et al.*, 1971; Blackburn *et al.*, 1970) of $J_{4'5'}$ and $J_{4'5''}$ couplings for the exocyclic group of several nucleosides have established the existence of rapid rotation (on an nmr time scale) about the C(4′)–C(5′) bond. The coupling constants can be interpreted in terms of three classical staggered rotamers shown in Figure 7 with a gauche-gauche conformation preferred in all of the nucleotides.

An analogous treatment of $J_{4'5'}$ and $J_{4'5''}$ data can be made for nucleotides studied in the present work.[7,8] Since

[7] Observed proton signals which are labeled H(5′) and H(5″) refer to downfield and upfield signals, respectively. H(5′B) and H(5′C) of Figure 7 correspond to the individual methylene protons.

[8] An attempt to rationalize a C(5′) proton assignment (Remin and Shugar, 1972) on the basis of selective phosphate shielding effects is open to serious question (Davis and Rabczenko, 1974).

vicinal coupling data are not available for the pure rotamers estimates of J_g = 2.04 Hz and J_t = 11.72 Hz were made from the appropriate Karplus relation discussed by Blackburn *et al.* (1970).[9] Using the coupling constant data from Table II rotamer populations were calculated for all of the 5′-nucleotides and are summarized in Table VII. Within the limitations of the present analysis, it is evident from the computational results that there is a marked preference for a gg rotamer about C(4′)-C(5′) in all of the 5′-ribo- and deoxyribonucleotides, *i.e.*, ρ_I varies from 0.65 to 0.89. This preferred conformation is particularly noticeable in pyrimidine 5′-nucleotides where more than 80% of the rotamers are in a gg conformation.

Given the approximate nature of the Karplus relation, the calculation of absolute rotamer populations must be viewed with some caution.[10] Relative comparisons for closely related series of compounds are more realistic and in this regard the uniformity in rotamer populations in solution is quite surprising, particularly in view of the diversity of base rings encompassed in the series of molecules. As noted previously virtually all 5′-nucleotides exist in the crystalline state in a gg conformation about C(4′)-C(5′) (Altona and Sundaralingam, 1972). Recent conformational energy calculations are in accord with the crystallographic results and point to an AMP-5′ conformation in which base and ribose rings are anti and the C(4′)-C(5′) bond orientation is gg as being the most favored (Yathindra and Sundaralingam, 1973; Olson, 1973). The present nmr results are thus in line with X-ray and theoretical structures, and the overall picture which emerges is one of an apparently highly stable anti (glycosidic bond), gg ($C_{4'}$-$C_{5'}$) conformation for the majority of 5′ ribo- and deoxyribonucleotides (*cf.* later section).

Hruska (1973) has previously found a correlation between ($J_{4'5'} + J_{4'5''}$) and either $J_{1'2'}$ or $J_{3'4'}$ for a series of nucleosides, suggesting that conformational features of the exocyclic carbinol group are influenced by the ribose ring conformation. Recent computations of conformational properties for exocyclic carbinol groups and furanose ring conformations further suggest a destabilization of the gg conformer in a C(2′)-endo pucker relative to a C(3′)-endo pucker of the furanose ring (Pullman, 1973). In this connection the present 5′-ribo- and deoxyribonucleotides show that as the S conformer population increases (~C(2′)-endo), *i.e.*, in going from ribonucleotides to the deoxyribonucleotides, the gg conformer (I) population decreases. The data, however, are not sufficiently accurate to show up any significant conformer population differences attributable to base-ring effects. Finally, the preferred gg conformer (I) is a feature which recurs in various nucleosides (Remin and Shugar, 1972), nucleoside 2′,3′-cyclic monophosphates (Lapper and Smith, 1973), and some pyrimidine 5′-ribonucleotides (Hruska *et al.*, 1973; Wood *et al.*, 1973b) in aqueous solution as well as in the majority of nucleosides and nucleotides in the solid state (Altona and Sundaralingam, 1972).

C(5′)-O(5′) Bond. In principle a calculation of rotamer populations about C(5′)-O(5′) can be made from ^{31}P-H(5′) and ^{31}P-H(5″) coupling constants by procedures analogous to those used for C(4′)-C(5′). If a threefold potential is assumed for the C(5′)-O(5′) bond, then observed J values, in the limit of rapid rotation, are given by the weighted average of couplings in the three staggered rotamers (IV, V, VI) shown in Figure 7. As individual values of J_g and J_t for each conformer are not known estimates can be made from a Karplus relation for the vicinal proton-phosphorus coupling constant in the fragment H-C(5′)-O(5′)-^{31}P. The data available for establishing such a correlation are much more limited than for proton-proton couplings. However, recent values for H-C-O-P couplings for a number of 3′,5′-cyclic nucleotides combined with data for other model compounds (Blackburn *et al.*, 1973; Davies and Danyluk, 1972) leads to a dependence of the form

$$^3J(\mathrm{HCOP}) = 16.3 \cos^2 \phi - 4.6 \cos \phi \quad (11)$$

where ϕ is the dihedral angle between the HCO and OCP planes. Although (11) is approximate it represents a useful starting point and gives values of 1.8 and 20.9 Hz for J_g and J_t, respectively. Relative rotamer populations were then calculated from observed J(HCOP) magnitudes and are summarized in Table VII. In a number of instances, the near equivalence in chemical shift of H(5′) and H(5″) prevented determination of specific values for the two phosphorus-proton couplings, and a population could only be calculated for the gg rotamer. If the expected effect of the orientation of the electronegative substituent is accounted for as previously[10] (*i.e.*, a decrease of 1 Hz in ^3J(HCOP) values in which the electronegative phosphorus atom is anti-periplanar to the coupling path), the effect on the calculated gg population (ρ_{IV}) is only about 1% for the J values observed for these molecules.

From the results in Table VII, it is apparent that the gg conformation is overwhelmingly preferred in all of the 5′ ribo- and deoxyribonucleotides. Where individual coupling constants have been determined, the calculations also show a greater preference for one conformer, *e.g.*, gt (~20%), over the other conformer, *e.g.*, tg (~10%). In the gg conformation, the phosphorus atom bisects the angle between the two C(5′) protons when observed along the C(5′)-O(5′) bond and the P-O(5′) bond is thus anti-periplanar to the C(5′)-C(4′) bond, *i.e.*, ϕ is approximately 180°. This preferred gg solution conformation is very similar to the conformations observed about the C(5′)-O(5′) bond of 5′-nucleotides in the crystalline state where ϕ falls in a narrow range about 180° (Sundaralingam, 1969; Rubin *et al.*, 1972).

Long-Range Couplings: H(4′)-C(4′)-C(5′)-O(5′)-P. The magnitudes of long-range (>3 bonds) homo- and heteronuclear couplings often provide useful information about the arrangements of bonds along the coupling path. In general, long-range couplings have a high degree of stereospecificity with the largest couplings observed for planar "M" or "W" type orientations of intervening bonds (Barfield and Chakrabarti, 1969). Hall and coworkers (1972a-c) have re-

[9] In a following paper, it is shown that substitution of a hydroxyl group of a nucleoside by a phosphate group in either the 2′- or 3′-mononucleotides has no significant effect upon the neighboring proton vicinal coupling, *i.e.*, $J_{2'3'}$. Any difference in electronegativities between the two groups is apparently not sufficient to produce a coupling constant change in $J_{2'3'}$. If this is the case, then an initial reasonable assumption can be made that substitution of a 5′ OH by a PO_4 group has no significant effect on $J_{4'5'}$ and $J_{4'5''}$ values.

[10] For example, it is known that the orientation of an adjacent electronegative substitunt can markedly affect a vicinal coupling constant (Karplus, 1963; Kotowycz and Lemieux, 1973), and it therefore might be expected that the magnitudes of $J_I - J_{III}$ for pure conformers will reflect this influence. An estimate of this effect is difficult, but assuming that J is reduced by ~1 Hz when an oxygen atom is anti-periplanar to a proton involved in a coupling interaction, then calculation shows that ρ_I is reduced by a factor of approximately 0.75, *i.e.*, the gauche-gauche conformer is less preferred on such an adjusted analysis than the amounts listed in Table IX.

TABLE VIII: Chemical Shift Differences (ppm) between Base Ring Protons of 2′, 3′- and 5′-Nucleotides.[a]

		Chemical Shift Differences (ppm)[b]	
Purine nucleotides		H_8	H_2
AMP-5′	$\Delta\delta(5',2')$	0.228	0.0
	$\Delta\delta(5',3')$	0.217	−0.034
GMP-5′	$\Delta\delta(5',2')$	0.214	
	$\Delta\delta(5',3')$	0.300	
dAMP-5′	$\Delta\delta(5',2')$	0.173	0.0
	$\Delta\delta(5',3')$	0.162	−0.034
dGMP-5′	$\Delta\delta(5',2')$	0.189	
	$\Delta\delta(5',3')$	0.275	
Pyrimidine nucleotides		H_6	H_5
UMP-5′	$\Delta\delta(5',2')$	0.268	0.082
	$\Delta\delta(5',3')$	0.218	0.082
CMP-5′	$\Delta\delta(5',2')$	0.351	0.105
	$\Delta\delta(5',3')$	0.201	0.055
dUMP-5′	$\Delta\delta(5',2')$	0.214	0.046
	$\Delta\delta(5',3')$	0.164	0.046
dCMP-5′	$\Delta\delta(5',2')$	0.268	0.082
	$\Delta\delta(5',3')$	0.128	0.032

[a] $\Delta\delta(5',2')$ = δ(5′-nucleotide) − δ(2′-nucleotide) and $\Delta\delta(5',3')$ = δ(5′- nucleotide) − δ(3′-nucleotide). [b] Negative sign denotes an upfield shift.

ported four-bond long-range coupling constants in the H-C-C-O-P fragment for the same all-trans (W) conformation. A similar long-range coupling has been reported for several nucleotides with a coupling path along the C(4′)-C(5′) and C(5′)-O(5′) bonds (Hruska *et al.*, 1973; Wood *et al.*, 1973b; Sarma *et al.*, 1973). Moreover, in the latter work it was observed that, as the gg conformer about the C(4′)-C(5′) and C(5′)-O(5′) bonds decreased, $^4J_{^{31}P\,H(4')}$ decreased.

For the series of 5′-nucleotides studied in this work, a measurable long-range coupling was observed in only one case,[11] the four-bond heteronuclear coupling $^4J(H_{4'}\text{-}C_{4'}\text{-}C_{5'}\text{-}O\text{-}^{31}P)$. Here the magnitudes range from 0.5 Hz (dAMP-5′) to 1.7 Hz (CMP-5′) with nucleotides having the lowest gg populations about C(4′)-C(5′) and C(5′)-O(5′) bonds generally exhibiting the smallest values of $^4J_{^{31}P,H(4')}$ as observed previously (Davies and Danyluk, 1972; Sarma *et al.*, 1973). Since it has already been established that gg conformations are strongly preferred around the C(4′)-C(5′) and C(5′)-O(5′) bonds, the resulting preferred time-averaged structure along the H(4′)-C(4′)-C(5′)-O(5′)-P chain is, therefore, one in which the backbone bonds are essentially all trans. The phosphorus and H(4′) atoms are thus linked by a planar "M" type arrangement and a sizable coupling between the two is possible. Deviations from this conformation would result in a decrease of $^4J_{^{31}P,H(4')}$ as observed.

In general the conformational features for the exocyclic groups of nucleotides in aqueous solution, as derived from nmr data, parallel closely those which exist for these molecules in the crystalline state. Evidently solvent forces are less important in determining the overall conformation along H(4′)-C(4′)-C(5′)-O(5′)-P than are intramolecular factors.

Additional features revealed by the results are the small but real preference for the gg conformer about C(4′)-C(5′) and C(5′)-O(5′) of 5′-ribonucleotides as compared with 5′-deoxyribonucleotides, a behavior previously observed (Wood *et al.*, 1973a) for several other nucleotides. A loose correlation of these features is also noted with S conformer preference. It is tempting to suggest tht each of these is related in some way to the base-ring orientation about ϕ_{CN}, but more work is necessary to substantiate this possibility.

4. *Conformation about the Glycosidic Bond*

A large body of crystallographic data for nucleosides and nucleotides clearly establishes that the torsional angle, χ_{CN}, defining the orientation of a base ring with respect to the ribose ring falls into two relatively narrow ranges designated as syn and anti conformations (Donohue and Trueblood, 1960; Haschemeyer and Rich, 1967; Sundaralingam, 1969). For β anomers it has further been found that a correlation exists between χ_{CN} and the furanose ring conformation in nucleosides and nucleotides (Haschemeyer and Rich, 1967; Altona and Sundaralingam, 1972). Thus, for β-purine and β-pyrimidine derivatives in N conformations, χ_{CN} ranges from about −1 to 44°, while for S conformations χ_{CN} has values in the range 36–73° though for the β-purine derivatives two further ranges of χ_N (110–123 and −149 to −103°) are also observed (Altona and Sundaralingam, 1972).

Analogous quantitative information for nucleosides and nucleotides in solution is not yet available although a number of nmr observations can be interpreted in terms of a syn ⇌ anti equilibrium. Initial nmr measurements of selective pH-induced shift changes in proton spectra of purine and selected pyrimidine derivatives were attributed to a preferred anti conformation (Ts'o, 1970; Danyluk and Hruska, 1968; Schweizer *et al.*, 1968). Nuclear Overhauser experiments have shown that a syn conformation makes a significant contribution to the equilibrium of a number of pyrimidine nucleosides (Noggle and Schirmer, 1971; Schirmer *et al.*, 1970, 1972; Hart and Davis, 1971). Further substantiating this result is the observation of long-range coupling constants between the base-ring H(5) and the sugar-ring H(1′) nuclei (Hruska, 1971). A recent extensive application of NOE measurements for a number of guanine mononucleotides (Tran-Dinh Son *et al.*, 1972a) has indicated a small energy difference between syn and anti conformers. A small energy difference between syn and anti conformations has also been found from potential energy calculations of various nucleosides (Berthod and Pullman, 1971a,b; Lakshminarayanan and Sasisekharan, 1969a,b; Jordan, 1973).

Further supporting evidence for a syn-anti equilibrium and the interrelationship between this equilibrium and conformational features of the furanose ring and exocyclic group is provided by the present results on the 5′-nucleotides when compared to the results of the corresponding 2′- and 3′-nucleotides (Davies and Danyluk, 1974). Inspection of Table VIII reveals a number of interesting trends in shift differences, $\Delta\delta$, between 5′- and 3′(2′)-mononucleotides. For example, the chemical shift of H(2) is approximately constant in AMP-5′, AMP-3′, and AMP′2′ while H(8) of AMP-5′ is shifted to lower field by ~0.23 ppm relative to the 2′ and 3′ isomers. A similar behavior is found for H(8) of dAMP-5′. In both instances, the shift differences can be

[11] Decoupling experiments designed to establish limits for the magnitudes of four-bond and five-bond couplings, *e.g.*, irradiation of H(3′) while observing H(1′), etc., showed that in all cases except $^4J(H_{4'}\text{-}^{31}P)$, the couplings were less than 0.2 Hz.

TABLE IX: Chemical Shift Differences (ppm) between Furanose Ring Protons of Purine and Pyrimidine 5′-Nucleotides and 5′-Deoxyribonucleotides.

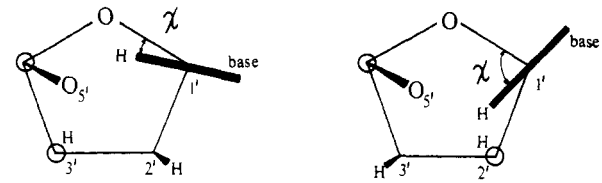
Proton	Chemical Shift Differences (ppm)[a] 5′-Ribonucleotides Δδ(AMP-5′–UMP-5′)	5′-Deoxyribonucleotides Δδ(dAMP-5′–dUMP-5′)
H(1′)	0.063	0.113
H(2′)	0.359	0.421
H(2″)		0.238
H(3′)	0.123	0.165
H(4′)	0.096	0.109
H(5′),H(5″)	−0.021	0.0

[a] A negative sign denotes upfield shifts.

explained by a preferred anti conformation for 5′-ribo- and deoxyribonucleotides, a result in accord with earlier conclusions (Danyluk and Hruska, 1968; Feldman and Agarwal, 1968; Schweizer *et al.*, 1968). In the anti conformation the charged phosphate group is located closer to H(8) than H(2) and therefore exerts a greater deshielding effect upon the former. A favorable projection of the negatively charged phosphate group over the furanose ring has already been confirmed by the present data (*cf.* preceding sections) showing preferred gg conformations about C(4′)–C(5′) and C(5′)–O(5′). Analogous shift differences are noted for H(8) of guanine nucleotides and again can be attributed to a preferred anti conformation about the glycosidic bond.

Consideration of shift data for pyrimidine nucleotides, Table VIII, shows that δ H(5) and δ H(6) of 5′-nucleotides are both downfield relative to the signals for 2′ and 3′ isomers. Moreover, the shift difference is greater for H(6), *e.g.*, $\Delta\delta$ = +0.22 ppm for H(6) and +0.086 ppm for H(5) of UMP-5′ relative to UMP-3′. The pyrimidine 5′-deoxyribonucleotides show much the same trend. Since the shifts for H(5) and H(6) are nearly constant for CMP-2′(3′) and UMP-2′(3′), where the phosphate group is remote from the pyrimidine ring, it is reasonable to ascribe the deshieldings in corresponding 5′-nucleotides to a preferred anti conformation. In this conformation the largest deshielding effect would be exerted at H(6) as observed.

It is tempting to make a quantitative estimate of deshielding magnitudes and syn–anti ratios using a conformational model derived from nmr data (previous sections). However, the actual magnitudes of such shift changes depend upon many factors such as electrostatic charge on the phosphate group, conformational equilibria in the furanose ring and exocyclic groups, possible intramolecular H bonds (Feldman and Agarwal, 1968) to name a few. Nevertheless, it is useful to point out a few interesting interrelationships. Within the accuracy of the present analysis significant differences are found in the N ⇄ S equilibrium composition of 5′-ribonucleotides (~40% N type) and 5′-deoxyribonucleotides (~30% N type). Also, the relative population of the gg conformer I is higher for 5′-ribonucleotides (~60%) than for 5′-deoxyribonucleotides (~45%). Either one or both of these may ultimately be a causative factor for greater deshielding magnitudes in 5′-ribonucleotides and by inspection the syn–anti equilibrium. In this connection recent NOE measurements (Tran-Dinh Son *et al.*, 1972a) showing that an increase in pD produces an increase in syn conformer in several guanine nucleotides is of considerable interest. A concomitant increase in N conformer population with pD increase is also found when furanose ring coupling data for GMP-2′ (Tran-Dinh Son *et al.*, 1972b) and GMP-5′ (Tran-Dinh Son *et al.*, 1972a) are analyzed in terms of pseudorotational parameters (preceding sections). There is, accordingly, a fair amount of indirect evidence pointing to an interdependence among key conformational features of mononucleotides. Further studies to develop such correlations quantitatively are now under way.



Polyribonucleotides — C(3′)-endo (N type) — $\chi \sim 15$

Polydeoxyribonucleotides — C(2′)-endo (S type) — $\chi \sim 80$

FIGURE 8: Schematic diagram for the nucleotidyl unit found in polynucleotides in the fibrous state.

The existence of a preferred anti conformation also explains in part the observed downfield chemical shift of ribose ring protons in purine derivatives relative to those in pyrimidine nucleotides, Table I. This downfield shift occurs in both ribo- and deoxyribonucleotides and increases in magnitude in the order H(5′)(5″) < H(4′) < H(1′) < H(3′) < H(2′)(2″), Table IX. As the comparison is made for sets of molecules having like furanose ring and exocyclic group conformations, the origin of these deshieldings must be at the base ring, a conclusion further indicated by the rough parallel between shift effect and distance of a proton from the base ring. A consideration of molecular models having conformational features established from nmr and crystal structure data,[12] Figure 8, shows that the downfield shift trends in purine nucleotides can be accounted for qualitatively by the anisotropy in diamagnetic susceptibility of the purine ring. All of the ribose ring protons lie within the deshielding region of the base ring, the largest effect being felt at H(2′).

5. *Summary*

A number of conclusions can be made regarding conformational properties of nucleotides based on the nmr results of this work.

(i) An analysis of coupling constant data in terms of pseudorotational parameters reveals that the D-ribose rings of purine and pyrimidine 5′-ribonucleotides favor an S-type conformation [C(2′)endo, C(3′)exo] over an N-type in the approximate ratio 60:40. The ring conformations do not vary significantly with the type of base ring and values for

[12] The glycosidic torsional angles depicted for anti-N and anti-S conformers in Figure 8 are taken from X-ray data of polynucleotides in the fibrous state (Sundaralingam, 1973). Similar trends are observed in the X-ray crystallographic data for both β-pyrimidine [C(3′)-endo, 28(±24); C(2′)-endo, 90(±24)] and β-purine nucleosides [C(3′)-endo, 18(±4); C(2′)-endo, 57(±4)] though to a less marked extent for 5′-nucleotides [C(3′)-endo, 38(±19), four structures only; C(2′)-endo, 41(±3)].

the pseudorotational parameters in solution fall in the ranges for those observed for mononucleotides in the crystalline state. No dramatic change of ring pucker or pseudorotational angle occurs in going from crystal to solution phases.

(ii) A pseudorotational analysis of ring proton couplings also shows that an S-type conformer is favored for D-deoxyribose rings of purine and pyrimidine 5′-deoxyribonucleotides. The N ⇄ S equilibrium lies somewhat more in favor of the S conformer, *i.e.*, 70:30, in deoxyribonucleotides than in ribonucleotides, and is a consequence of substitution of a hydroxyl group by a hydrogen atom. The Karplus expression used in the pseudorotational analysis for D-deoxyribose rings differs from that for ribonucleotides reflecting a change in substituent electronegativity at C(2′). No advantage is gained at the present state of analysis in using adjusted Karplus expressions for individual molecular fragments of the ring system.

As with the ribonucleotides, the final derived pseudorotational parameters for deoxyribonucleotides do not change much between nucleotides and fall within ranges found in the crystalline state.

In both sets of nucleotides a rapid determination of furanose ring equilibrium compositions can be made simply by evaluating the ratio $J_{1'2'}:J_{3'4'}$.

(iii) Proton couplings across the C(4′)–C(5′) bond can be analyzed in terms of a simple gg, tg, gt rotamer conformational model. In all molecules studied, there is a marked preference for the gg rotamer. This preference is somewhat greater in the ribonucleotides and is connected in some way with the furanose ring conformations and possibly base–ribose ring orientations in ribo- and deoxyribonucleotides.

(iv) An analysis of ^{31}P–H(5′)(5″) couplings on the basis of a similar rotamer equilibrium model, gg, tg, gt, shows that there is again a preference for the gg rotamer in all of the nucleotides.

In both cases (iii) and (iv) the conformational features found in solution are the same as those existing in the crystalline state. When taken together, the conformational results (iii) and (iv) are consistent with an all-trans bonding arrangement for atoms comprising the ribose–phosphate backbone. Such an arrangement is further confirmed by the observation of a four-bond long-range ^{31}P–O(5′)–C(5′)–C(4′)–H(4′) coupling in the nucleotides.

The apparent constancy of this backbone conformation across a wide variety of nucleotides in different phases is surprising, and tends to support a suggestion put forward recently of a "rigid" nucleotidyl unit (Rubin *et al.*, 1972; Sundaralingam, 1973; Yathindra and Sundaralingam, 1973). It should be kept in mind, however, that nucleotide structures are actually dynamically "averaged" in solution and that conformer populations and geometries show subtle but real changes with solvent conditions (temperature, pH, ionic strength). The nmr data further suggest an interdependence between the main conformational features of a nucleotide; *i.e.*, rotamer populations about C(4′)–C(5′) appear correlated with furanose ring geometry (% N).

(v) The base-ring proton chemical shift differences between corresponding 5′- and 3′-nucleotides are consistent with a favored anti orientation of base and ribose rings in all of the nucleotides. It was also found that ribose ring proton shift differences between purine and pyrimidine nucleotides can be qualitatively explained by an "in-plane" deshielding effect of purine rings in the former case. The existence of a rough correlation of base-ring proton shifts in 5′-ribo- and deoxyribonucleotides with exocyclic rotamer population [about C(4′)–C(5′)] and the N ⇄ S equilibrium further confirms the interdependence among key conformational features of the nucleotides.

Finally, it is of some interest to comment on the relevance of structural data determined from nmr measurements to nucleotidyl unit structures in polynucleotides, as derived from X-ray diffraction studies. As reviewed briefly earlier, the X-ray data show a number of general structural similarities in polynucleotides. Among these are the relative constancy in torsion angle along the ribose phosphate backbone and the preponderance of anti orientations for base–ribose rings. Where differences occur these are mainly (though not always) in furanose ring conformation, *e.g.*, the deoxyribose ring of DNA tends to exist in a S-type conformation (C(2′)-endo) while the ribose ring of RNA favors an N-type conformation (C(3′)-endo). A pictorial representation of these differences and the related base–ribose ring orientations is shown in Figure 8. Both of the generalized structures for the ribose–phosphate fragment are accommodated by the nmr coupling constant results, provided a rapidly equilibrating conformer mixture is assumed for the furanose ring and exocyclic phosphate group. A concordance between X-ray structures and shift data is also apparent. Thus an anti S-type conformation, Figure 8, would predict a deshielding of H(2′) and H(3′) of purine nucleotides relative to 5′-pyrimidine nucleotides, as is in fact observed. The structures in Figure 8 further suggest a significant anti N-type conformer contribution in order to account for deshielding of H(1′) and H(4′). From this general consideration it would appear that conformational features observed for nucleotidyl units in polynucleotides can serve as useful starting models for conformational analyses based on nmr solution data. Conversely structural features established from nmr measurements relate closely to those in the solid state.

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