Conformational Properties of the Furanose Phosphate Backbone in Nucleic Acids. A Carbon-13 Nuclear Magnetic Resonance Study†

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ABSTRACT: Carbon-13 nuclear magnetic resonance spectra of the adenylic acid and uridylic acid series (monomer, dimer, and polymer) are obtained either as a function of temperature or pH value. The previous assignments of the C2′ and C3′ resonances in the spectra of poly(uridylic acid), poly(adenylic acid), and in the Np portion of UpU and ApA spectra are interchanged. In the mononucleotides studied, the rotamer distribution of ϕ (C5′-O5′) is not affected by the secondary ionization of the phosphate, but ϕ' (C3′-O3′) is affected by this ionization. The conformation of the backbone in dimers and polymers as revealed by ϕ and ϕ' depends both on the base unit and the temperature. Conformation analysis of ϕ in terms of rotamer distribution was accomplished using the conventional three-rotamer model, while ϕ' was analyzed using a nonconventional two-rotamer model (gt', $\phi' \approx 210° \rightleftharpoons tg'$, $\phi' \approx 270°$) in rapid equilibrium. A correlation among base-stacking interaction, furanose conformation (C2′-endo ⇌ C3′-endo), and furanose phosphate conformation (tg' rotamer ⇌ gt' rotamer for ϕ'; gg rotamer ⇌ (tg + gt) rotamer for ϕ) is demonstrated. The data indicate that a strong base-stacking interaction in ribosyl dinucleoside monophosphates and polynucleotides elicits the C3′-endo (furanose), gt' (ϕ'), and gg (ϕ) conformations. The observed differences in the comparative study of the uridylic acid and adenylic acid series with respect to the furanose and furanose phosphate backbone conformations clearly delineate the role and the importance of base-base stacking as a major force in determining the backbone conformation of RNA in aqueous solution.

The conformational analysis of nucleic acids in solution has been greatly facilitated during the past 15 years by nuclear magnetic resonance spectroscopy (Ts'o, 1974; Smith et al., 1973; Davies and Danyluk, 1974, 1975; Kondo and Danyluk, 1976). The majority of NMR[1] studies have been on the hydrogen-1 nucleus, with lesser amounts on the carbon-13 and phosphorus-31 nuclei. ^{13}C NMR spectroscopy is particularly useful in studying the sugar–phosphate backbone conformation via the carbon–phosphorus coupling constants, since the backbone has not been very amenable to study by proton magnetic resonance. The early work of Smith and co-workers (Mantsch and Smith, 1972; Lapper et al., 1972, 1973; Smith et al., 1973) demonstrated that it was possible to obtain conformational information concerning the backbone of nucleic acids from the vicinal coupling constants, $^3J_{C2',P3'}$, $^3J_{C4',P3'}$, and $^3J_{C4',P5'}$. From these coupling constant values, it is possible to obtain angular preferences of the bonds ϕ (C5′-O5′) and ϕ' (C3′-O3′). Although rotational preferences of these bonds were apparent, no significant changes in rotamer preferences were demonstrated by varying the base moiety (e.g., UpU and ApA) or by varying the temperature (Schleich et al., 1975, 1976). The conclusion from these investigations was that very little rotation, if any, of the ϕ and ϕ' bonds occurs in these nucleic acid backbones.

In the present study, the dimers, UpU and ApA, have been reinvestigated, along with the polymers, poly(A), poly(U), poly(2′-Me)A, and poly(2′-Me)U. Our results lead us to exchange the previous assignments of resonances for the C2′ and C3′ of poly(U) (Mantsch and Smith, 1972), poly(A) (Smith et al., 1975), and the C2′ and C3′ (Np portion) of UpU and ApA (Smith et al. 1973). The data also indicate significant differences in the vicinal carbon–phosphorus coupling constant values among these nucleic acids. These coupling constants have been analyzed to determine rotamer distribution for the C5′-O5′ (ϕ) and C3′-O3′ (ϕ') bonds. Analysis of bond ϕ using the conventional three-rotamer model indicated a preference in distribution for the gg rotamer. Bond ϕ' was analyzed using both the conventional three-rotamer model and the nonconventional two-rotamer model. The preference for the two-rotamer model is discussed along with the interrelationship of the furanose and the furanose phosphate conformations. The data suggest that C3′-endo (furanose), gt' (ϕ), and gg (ϕ) are preferred backbone conformations in ribosyl dimers and polyribonucleotides when these molecules have extensive base-stacking interaction.

Experimental Section

Materials. The poly(2′-Me)A and poly(2′-Me)U were synthesized according to published procedures (Tazawa et al., 1972). All other nucleic acids were obtained from commercial sources (P-L Biochemicals, Sigma, and Boehringer Mannheim).

Instrumentation. Most data were obtained from a JEOL FX-100 NMR spectrometer operating in the Fourier-transform mode at 25.05 MHz, using quadrature phase detection with 10-mm diameter sample tubes. Some data were obtained from a JEOL PFT-100P spectrometer. Data tables were usually 16K words with spectral bandwidths from 2500 to 3000 Hz, providing a computer resolution of 0.30–0.37 Hz.

Methods. The JEOL spin-simulation software was sometimes used with the FX-100 to obtain more accurate determinations of chemical shifts and coupling constants. This was usually the case in determining the two $^3J_{C4',P}$ values in polymers, since these resonances were quartets or pseudotriplets which were broadened at lower temperatures. This procedure

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[1] Abbreviations used are: NMR, nuclear magnetic resonance; UpU, uridylyl(3′-5′)uridine; ApA, adenylyl(3′-5′)adenosine; poly(A), poly(adenylic acid); poly(U), poly(uridylic acid); poly(2′-Me)A, poly(C2′-*O*-methyladenylic acid); poly(2′-Me)U, poly(C2′-*O*-methyluridylic acid); EDTA, (ethylenedinitrilo)tetraacetic acid.

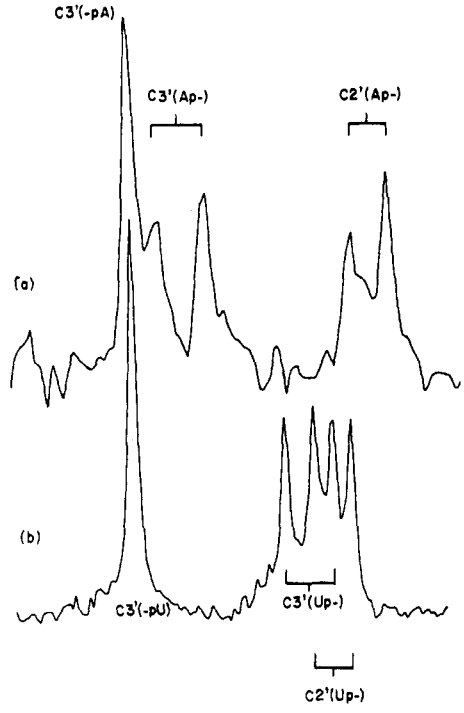

FIGURE 1: Partial ^{13}C NMR spectrum of (a)ApA, 50 °C, and (b)UpU, 27 °C.

may account, in part, for our ability to determine these coupling constants which were only reported at high temperature in other studies (Smith et al., 1975). The partial spectra of UpU and ApA in Figure 1 illustrates the spectrum resolution and the assignment of the C2′ and C3′ (Np portion).

The coupling constant data reported here and previously by others (Smith et al., 1973, 1975 Kotowycz and Hayamizu, 1973; Schleich et al., 1975) are in varying degrees of agreement. There is a range of about 1 Hz in reported coupling constant values for some of the mononucleotides, a range of 2–3 Hz for the dinucleotides, and for polynucleotides a 1–2-Hz range. There may be several reasons for these variations: (1) the computer resolution of the NMR spectrum from which the data were obtained; (2) the method of data extraction, by computer software or manually; (3) the stability of the NMR spectrometer and the resultant resolution of the spectrum; (4) trace paramagnetic metal ions in commercial sources of nucleic acids; (5) the pH of the NMR solution (for mononucleotides). All of these factors were given particular attention in obtaining our data. The manual procedure for determination of J values (by measurement of peak separations of an expanded plot) coupled with spectral spin simulations appeared to yield the most consistent results. The addition of small amounts of EDTA (~1 mM) eliminated any paramagnetic-ion broadening effects.

The concentrations of solutions in NMR studies usually were 0.05 M as expressed in phosphate unit, although 3′- and 5′-UMP solutions were at 0.5 M. Several compounds were studied at various concentrations: poly(A) and poly(U) (0.05–0.15 M), 3′-AMP (0.05–0.27 M), 5′-AMP (0.05–0.5 M). Although in some cases the chemical shift was observed to be concentration dependent, no significant changes in the carbon–phosphorus coupling constants were observed.

Results and Discussion

Assignment of the ^{13}C Resonances from Furanose Carbons in the Mononucleotides. The ^{13}C resonances from the furanose carbons of 5′-UMP and 5′-AMP are assigned according to the work of Dorman and Roberts (1970), as modified by Mantsch and Smith (1972). The assignment of the resonances to the C2′ and C3′ carbons of 3′-UMP and 3′-AMP is not as straightforward due to the similarity of chemical shifts and coupling constant values of these two ^{13}C resonances, as illustrated in Figure 2. The indicated assignments in Figure 2 are made by comparison with 5′-AMP, and by the spectral information of 3′-AMP at different pH's. In Figure 3, a similarity is seen in the pH dependence of the chemical shift due to the ionization of the secondary phosphate group of C5′ in 5′-AMP and the assigned C3′ of 3′-AMP, which is different from the assigned C2′ of 3′-AMP. The $^2J_{C5',P}$ of 5′-AMP is compared also with the assigned $J_{C,P}$ of the C2′ and C3′ of 3′-AMP in Table I. The $^2J_{C,P}$ couplings show only a slight pH dependence (~0.2 Hz), while $^3J_{C,P}$ couplings of the 3′-mononucleotides vary nearly 2 Hz. This observation, which will be discussed in the succeeding section, supports the assignment based on chemical shifts.

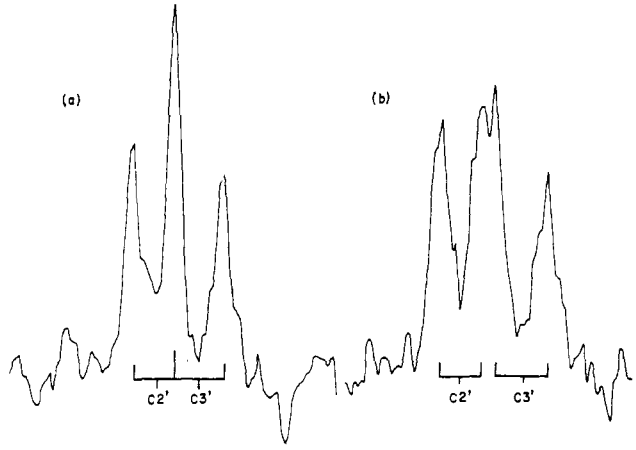

FIGURE 2: The ^{13}C NMR spectrum of C2′ and C3′ of (a) 3′-UMP, pH 4.2, 30 °C, and (b) 3′-AMP, pH 6.3, 33 °C.

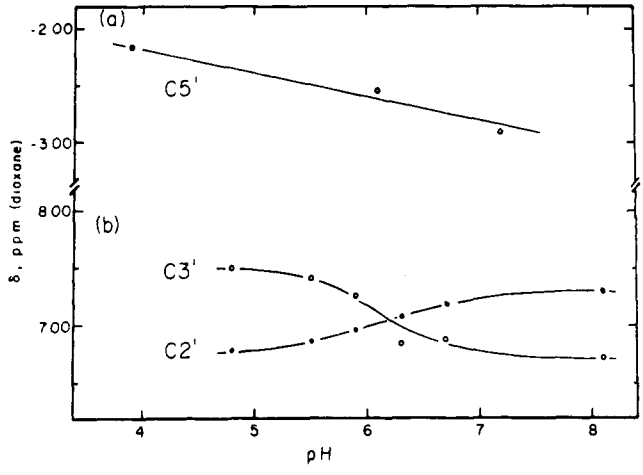

FIGURE 3: Dependence of the chemical shift on pH of (a) 5′-AMP (C5′) and (b) 3′-AMP (C2′ and C3′).

Effect of pH and the Ionization of the Secondary Phosphate Group on the ^{13}C–^{31}P Coupling Constants in Mononucleotides. The $J_{C,P}$ values of 3′-UMP, 5′-UMP, 3′-AMP, and 5′-AMP (Table I) show varying degrees of dependence on pH. The $^2J_{C,P}$ values from both the 3′- and 5′-mononucleotides show only small variations (~0.3 Hz) upon ionization of the secondary phosphate in the pH range from 4 to 8. In the case

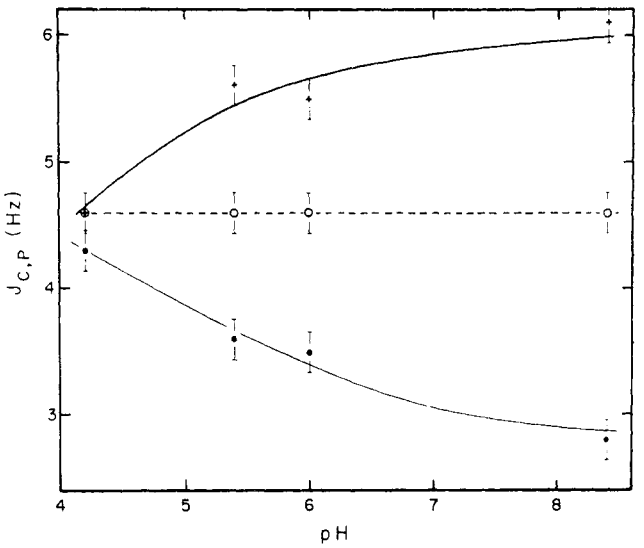

FIGURE 4: The dependence of $^3J_{C2',P}$ (·), $^2J_{C3',P}$ (O), and $^3J_{C4',P}$ (+) of 3'-UMP on pH.

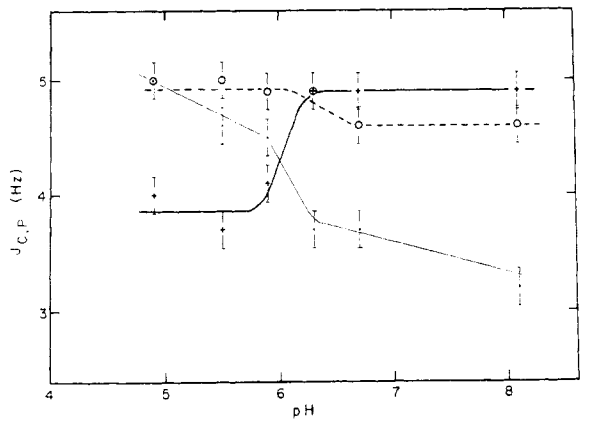

FIGURE 5: The dependence of $^3J_{C2',P}$ (·), $^2J_{3',P}$ (O), and $^3J_{C4',P}$ (+) of 3'-AMP on pH.

of $^3J_{C,P}$, the values from 5'-mononucleotides show no significant change, while the $^3J_{C2',P}$ and $^3J_{C4',P}$ values from the 3'-isomer vary greatly (Figures 4 and 5). These $^3J_{C,P}$ values from cyclic nucleotides have been used in the pioneering work of Smith and co-workers (Mantsch and Smith, 1972; Lapper et al., 1972, 1973; Smith et al., 1973) in demonstrating the existence of a Karplus relationship between the rotation and the $^3J_{C,P}$ values of the ϕ (C5'-O5') and ϕ' (C3'-O3') bonds. The distribution of the rotamer population of these acyclic phosphates can be calculated based on the assumptions that these vicinal coupling constants have the same dihedral angular dependence as the cyclic mononucleotides, and rotation of the ϕ and ϕ' bonds are free and sufficiently fast in the NMR time scale.

The conventional analyses based on the distribution of population among three staggered rotamers of ϕ (Figure 6) were adopted without apparent problems. From the equation of Govil and Smith (1973):

$$^3J_{C,P} \simeq 9.5 \cos^2\theta - 0.6 \cos\theta \qquad (1)$$

where θ is the dihedral angle between the planes ^{13}CCO and $CO^{31}P$, the values of $^3J_{trans} = 10.1$ Hz and $^3J_{gauche} = 2.1$ Hz are calculated. It must be noted that subsequent calculations of rotamer distributions are dependent upon the applicability

TABLE I: pH Dependence of the ^{13}C-^{31}P Coupling Constants of Uridylic and Adenylic Acids due to the Ionization of the Secondary Phosphate.

Compd	pH	$^2J_{C3',P}$	$^3J_{C2',P}$	$^2J_{C5',P}$	$^3J_{C4',P}$
5'-UMP	4.1			4.3	8.8
	6.3			4.7	8.5
	8.4			4.6	8.8
3'-UMP	4.2	4.6	4.2		4.6
	6.3	4.6	3.5		5.5
	8.4	4.6	2.8		6.1
5'-AMP	4.1			4.9	8.5
	6.3			4.7	8.7
	7.2			4.9	8.5
3'-AMP	4.8	5.0	5.0		4.0
	5.5	4.9	4.6		3.7
	5.9	4.9	4.3		4.1
	6.3	4.9	3.7		4.9
	6.7	4.6	3.7		4.9
	8.1	4.6	3.4		4.9

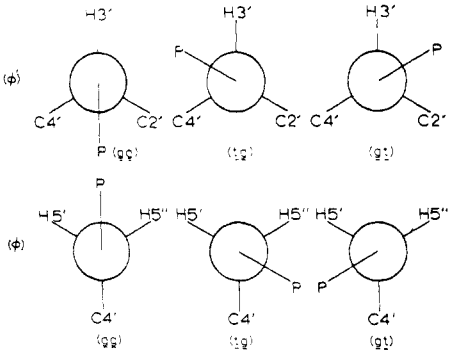

FIGURE 6: Classical staggered rotamers in nucleic acid backbone for bond ϕ' (C3'-O3') and for bond ϕ (C5'-O5').

of eq 1, which has been used for both ϕ and ϕ'. For instance, since the electronegativity of the substituents of C2' and C4' may not be the same, the application of the same equation to the analysis of both $^3J_{C2'P}$ and $^3J_{C4'P}$ may lead to certain inaccuracies. Nevertheless, using these parameters, the distribution of rotamer populations for the 5'-mononucleotides is approximately 85% *gg* and 15% *gt* + *tg*. These rotamer populations are in reasonable agreement with those calculated by Davies and Danyluk (1975) (72–77% for *gg*, 28–32% for *gt* + *tg*) using proton-phosphorus-31 coupling constants. The problem of employing this approach for the analyses of the coupling constants related to ϕ in the 3'-mononucleotides will be discussed in a later section.

Assignment of the Furanose Carbons in the Dimer and Polymer. The published assignments of C2' and C3' of UpU (Smith et al., 1973; Schleich et al., 1974), ApA (Smith et al., 1973; Schleich et al., 1974, 1975) (3' portions), poly(U) (Mantsch and Smith, 1972; Smith et al., 1973; Govil and Smith, 1973; Schleich et al., 1974), and poly(A) (Smith et al., 1975) have placed the C2' resonance to lower-field positions relative to the C3' in all cases. Our data indicate that in the published data of these four nucleic acids actually the assignment (for the C2' resonances vs. C3' resonances) should be with the C3' resonance located at lower-field position than the C2'. This conclusion is reached by comparison of (1) the coupling constants of the ribose-homopolynucleotides, poly(U) and poly(A), and with the C2'-*O*-methylated homopolynucleotides, poly((2'-Me)U) and poly((2'-Me)A); (2) the dimer

TABLE II: ^{13}C-^{31}P Coupling Constants (Hz) of Monomers, Dimers, and Polymers.

Carbon	Residue	3′-AMP	5′-AMP	ApA		poly(A)		poly(2′-Me)A	3′-UMP	5′-UMP	UpU		poly(U)		poly(2′-Me)U
		35°	35°	20°	70°	35°	75°	60°	35°	35°	27°	60°	35°	80°	60°
C2′	Np-	4.9		3.7	4.6	2–3	4.0	3.7	4.2		4.2	4.7	4.8	4.6	4.1
C3′	Np-	4.8		6.1	5.5	4–5	5.5	4.5	4.6		4.6	5.5	5.5	5.5	4.8
C4′	Np-	3.9		5.5	4.3	4–5	5.3		4.6		4.9	4.8	4.0	4.3	
	-pN		8.5	9.8	8.5	8–9	8.1			8.5	8.9	8.5	8.5	8.5	
C5′	Np-														
	-pN		4.9	4.6	4.5	*a*	4.6			4.5	5.3	5.5	5.3	6.1	4.8

[a] Not resolved.

TABLE III: Temperature Dependence of the Chemical Shifts ($\Delta\delta_t$)[a] of C2′, C3′, and C5′ in Dimers and Polymers.

	$\Delta\delta_t$ (ppm)		
	C2′	C3′	C5′
UpU	0.0	0.24	0.26
poly(U)	−0.01	0.07	0.10
poly(2′-Me)U	0.02	0.39	0.35
ApA	0.17	0.48	0.40
poly(A)	0.30	1.77	1.06
poly(2′-Me)A	0.32	1.01	0.75

[a] $\Delta\delta_t = \delta_{60°} - \delta_{27°}$.

TABLE IV: ^{13}C NMR Chemical Shifts of Furanose Carbons in Dimers[a] and Polymers[a]

	δ (ppm)[b]				
	C1′	C2′	C3′	C4′	C5′
UpU (Up portion) (35°)	22.68	6.38	6.51	16.87	−6.13
poly(U) (35°)	21.77	6.22	7.47	15.82	−1.58
poly(2′-Me)U (36°)	20.08	14.64	5.48	15.63	−2.07
ApA (Ap portion) (34°)	22.33	6.47	7.27	17.35	−5.64
poly(A) (26°)	22.37	6.86	5.26	14.25	−2.64
poly(2′-Me)A (27°)	18.76	14.9	4.73	14.9	−2.58

[a] Concentration: 0.03–0.05 M, pD 7–8. [b] Relative to dioxane (internal reference).

and polymer coupling constants with the respective mononucleotides; and (3) the temperature dependence of the chemical shift of C2′, C3′, and C5′ in the dimers and polymers.

In the first comparison, the ^{13}C NMR spectra of C2′-*O*-methylribose derivatives make possible the unequivocal assignment of the C2′ and C3′, since methyl substitution at the furanose C2′-OH of the monomer causes C2′ to shift downfield approximately 9 ppm. For poly(2′Me)U at 60 °C the $^2J_{C3',P}$ and $^2J_{C5',P}$ are similar (both are 4.8 Hz) and different from $^3J_{C2',P}$ (4.1 Hz). This same similarity between $^2J_{C3',P}$ and $^2J_{C5',P}$ and the same difference between $^3J_{C2',P}$ are evident in the coupling constants assigned to these respective carbons in poly(U) and UpU in Table II. The $^2J_{C3',P}$ and $^3J_{C2',P}$ for poly(2′Me)A at 60 °C are 4.5 and 3.7 Hz, respectively; these also agree with the relative magnitude of these couplings for poly(A) and ApA.

A second method was the comparison of $J_{C,P}$ values from the monomer with those from dimer and polymer. This was generally more useful for the adenylic acids, where the assigned $^3J_{C2',P}$ of the dimer and polymer changed significantly from the monomer. Such a variation of a $^3J_{C,P}$ is not unexpected from a conformational change in the backbone of the dimer and polymer, while a change in conformation would not be expected to alter a two-bond coupling like $^2J_{C3',P}$.

In all studies of the dimers and polymers, the resonances of C3′ and C5′ have shown a characteristically large temperature dependence of the chemical shift ($\Delta\delta_t$) in comparison to resonances of C2′. The data in Table III indicate that C3′ and C5′, which are two bonds away from the phosphorus, have large and comparable $\Delta\delta_t$ values, while C2′, which is three bonds away from the phosphorus, has a much smaller $\Delta\delta_t$ value. Since the resonances of the furanose carbons can be easily assigned for the C2′-*O*-methylated polymers and since the assigned C2′, C3′, and C5′ resonances of the ribosyl dimers and polymers show the same $\Delta\delta_t$ dependencies as the C2′-*O*-methyl and polymers, our assignments of the resonances of the five furanose carbons listed in Table IV are strongly supported by these data.

Conformational Analyses of C3′-O3′ (ϕ') Rotation in 3′-Mononucleotides and Dinucleoside Monophosphates. In analyzing the rotamer distribution of bond angle ϕ', the calculations using the conventional approach of the three rotamers, i.e., $\phi' = 60°$ (*gg* or trans), 180° (*gt*), and 300° (*tg*) (Figure 6), the $^3J_{C,P}$ data (Table I), and eq 1 indicate the *gg* (or trans, $\phi' = 60°$) rotamer to be significantly populated to the extent of 30–50%. This result is not in agreement with the theoretical calculations (Pullman et al., 1972; Olson and Flory, 1972a,b; Olson, 1973, 1975a,b,c; Perahia et al., 1974a,b; Yathindra and Sundaralingam, 1974; Tewari et al., 1974; Broyde et al., 1975) and the crystal structures of 3′-nucleotides (Sundaralingam, 1969) and dinucleoside monophosphates (Rubin et al., 1972; Sussman et al., 1972; Rosenberg et al., 1973; Hingerty et al., 1975) determined by x-ray diffraction. In these studies, the trans rotamers ($\phi' = 60°$) were found to be only slightly populated or nonexistent. This discrepancy has been noted by Lee et al. (1976) and by Cheng and Sarma (1977, submitted). The conclusion based on theoretical investigations can be readily demonstrated with the manipulations of the space-filling CPK models. The model of the ApA dimer shows the difficulty in achieving base-base stacking interaction with the ϕ' in the trans conformation and the extensive steric interference upon transfering from the gauche rotamers (either *gt* or *tg*) to the trans rotamer. Therefore, it appears unreasonable to adopt this three-rotamer distribution model for the analysis of ϕ' in the 3′-nucleotidyl unit, which affords the same high percentage of trans population (36–42%) for UpU, poly(U), ApA, and poly(A) at 27–60 °C range (Table V), while all these compounds have vastly different extents in base-base stacking under these conditions.

TABLE V: Calculated Rotamer Distributions of ϕ' in Uridylic and Adenylic Acids.

Compd	Temp (°C)	Three-Rotamer Model[a]			Two-Rotamer Model[b]	
		f_{gt}	f_{tg}	f_{gg}	$f_{gt'}$	$f_{tg'}$
UpU	27	0.38	0.26	0.36	0.54	0.46
	60	0.33	0.28	0.39	0.52	0.48
poly(U)	27	0.24	0.35	0.41	0.46	0.54
	60	0.25	0.33	0.42	0.46	0.54
ApA	20	0.43	0.20	0.37	0.60	0.40
	60	0.31	0.31	0.38	0.50	0.50
poly(A)	75	0.40	0.24	0.36	0.58	0.42

[a] Calculated from eq 1 and $J_{C2',P3'} = f_{gt}J_{gauche} + f_{tg}J_{trans} + f_{gg}J_{gauche}$; $J_{C4',P3'} = f_{gt}J_{trans} + f_{tg}J_{gauche} + f_{gg}J_{gauche}$; $f_{gt} + f_{tg} + f_{gg} = 1$. [b] Calculated from eq 1 and 5-7.

A similar discrepancy was also encountered in the analyses of the $^3J_{H,P}$ data for the ϕ' in the 3'-ribonucleotides (Davies and Danyluk, 1975; Cheng and Sarma, 1977, submitted). From the Karplus relationship, $J_{H,P} = 18.1 \cos^2\theta - 4.8 \cos\theta$, the classical trans (*gg*) and gauche (*gt* and *tg*) rotamers yield a calculated $^3J_{H,P}$ value of 22.9 and 2.1 Hz, respectively. On this basis, the observed values of $^3J_{H3',P3'}$ of 7-8 Hz would predict a significant population of trans rotamer ($\phi' = 60°$), a prediction in conflict with the theoretical calculations and the crystal structure described above. In recognization of this problem, Ezra et al. (1977, submitted) and Cheng and Sarma (1977, submitted) proposed the following modification of the conventional three-rotamer model: (1) arbitrarily depopulate the trans rotamer ($\phi = 60°$); (2) modify the classical gauche positions to $\phi' = 210°$ and 270°, at which the rotamers are either fixed or in rapid equilibrium.

We have attempted to analyze the $^3J_{C,P}$ data of ϕ' with the same approach in a stepwise manner. In the first step, the trans rotamer ($\phi' = 60°$) is assumed to be absent. Then, if the entire population consists of only the classical gauche rotamers, i.e., rotamers with $\phi' = 180°$ (*gt*) and 300° (*tg*), the calculated $^3J_{C2',P3'}$ and $^3J_{C4',P4'}$ will have the value of either 2.1 or 10.1 Hz based on the Karplus relationship (eq 1). In the case that there is a rapid equilibrium between the pure *gt* and *tg* forms, then a constant sum $^3J_{C2',P3'} + {}^3J_{C4',P3'} \approx 12.2$ Hz, should be observed. The experimental data in Table I did not indicate the existence of either one of these two situations. The $^3J_{C2',P3'}$ and $^3J_{4',P3'}$ are 3.4-5.0 Hz and 3.7-4.9 Hz, respectively, and the sum is about 8.5 Hz for 3'-AMP and 8.9 Hz for 3'-UMP. This result indicates that the $^3J_{C,P}$ data for ϕ' should be also analyzed with nonconventional gauche rotamers.

Employing the ϕ' values of 210° and 270° recommended by the $^3J_{H,P}$ data, dihedral angles (θ) of 90° (for C2'-C3'-O3'-P3' when $\phi' = 210°$ and for C4'-C3'-O3'-P3' when $\phi' = 270°$) and 150° (for C2'-C3'-O3'-P3' when $\phi' = 270°$ and for C4'-C3'-O3'-P3' when $\phi' = 210°$) are obtained for the bond between carbon and phosphorus atoms. From the Karplus relationship (eq 1), angles of $\phi = 90°$ and 150° yield $^3J_{C,P}$ values of 0 and 7.6 Hz, respectively. These values are not in satisfactory agreement with the observed data for either the fixed-rotamer or equilibrium-rotamer models. A slight modification of the ϕ' values (210-205° and 270-275°) produce θ values of 85° and 155°, and give $^3J_{C,P}$ values of 0.1 and 8.3 Hz, respectively. The data in Table I do not have values of 0.1 or 8.3 Hz for either $^3J_{C2',P3'}$ or $^3J_{C4',P3'}$, a condition required in the fixed rotamer model. However, the calculated sum ($^3J_{C2',P3'} + {}^3J_{C4',P3'} = 0.1 + 8.3 = 8.4$) is in good agreement with the observed sum for 3'-AMP (8.5 ± 0.3 Hz) and 3'-UMP (8.9 ± 0.1 Hz), consistent with the equilibrium-rotamer model. Therefore, the $^3J_{C,P}$ data can be calculated on the basis of the ϕ' values recommended by the $^3J_{H,P}$ data with a slight shifting of 205° to 275° (instead of 210° to 270°); for the sake of simplicity in discussion, the range of ϕ' is maintained as 210° to 270°, provided the rotamers are in rapid equilibrium.

To illustrate this question further, i.e., whether the rotamers are fixed at one of the two positions of the nonconventional angles (210° or 270°) or in rapid equilibrium between these two types, 3'-AMP is used as an example. At pH 5.9, the $^3J_{C,P}$ values in Table I indicate that the P3'-C2' or P3'-C4' dihedral angle should be ~45° or ~130°. In case the rotamers are fixed in one position, and when the angle C2'-C3'-O-P is 45°, then the angle C4'-C3'-O-P would be 165°, which leads to a corresponding value of ~9.4 Hz for $^3J_{C4',P3'}$. Similarily, when the angle C2'-C3'-O-P is 130°, then the angle C4'-C3'-O-P would be 110° which leads to a corresponding value of ~1.2 Hz. Neither one of these two values, 8.1 or 1.2 Hz, agrees with the observed value of 4.1 Hz. This deduction indicates that the putative nonconventional rotamers are not in a fixed conformation in the NMR time scale. Most likely, these rotamers are in rapid equilibrium between $\phi' \simeq 210°$ and 270°.

The effect of the secondary phosphate ionization on the rotation of ϕ' in 3'-mononucleotides was investigated. The data in Table I and Figures 4 and 5 indicate a small but significant change in both $^3J_{C2',P3'}$ and $^3J_{C4',P3'}$ values of 3'-AMP and of 3'-UMP from pH 5.5 to 8.1. In this range of the pH change, the secondary phosphate ionization is affected. Similarily, a small change of $^3J_{H3',P3'}$ for 3'-AMP and for 3'-UMP was detected in the pH range of 5.5 to 8.5 or 11 (Cozzone and Jardetzky, 1976). In analyzing the $^3J_{C,P}$ data, it was found that the sum of the $^3J_{C2',P3'} + {}^3J_{C4',P4'}$ remains *constant* at pH 4.2 to 8.4 for 3'-UMP (8.8-8.9 Hz) and at pH 5.5 to 8.1 for 3'-AMP (8.5 Hz). These data suggest that there is no significant alteration of the rotamer positions (210° or 270°) in a symmetric manner with respect to the C3'-H3' bond. While there can still be a shifting of rotamer angle in a nonsymmetrical manner in order to maintain a constant sum, such a change would be less likely and has to be fortuitous in nature. The most likely explanation for the change of individual $^3J_{C2',P3'}$ and $^3J_{C4',P3'}$ values, while maintaining a constant sum, is that the population distribution of these two nonconventional rotamers ($\phi' = 210°$ and 270°) in equilibrium has been changed. On the other hand, if a change in pH produces no changes in symmetry of the dihedral angle (i.e., $\theta = |-\theta|$) between H3' and P3', as is the case for rotamers $\phi' = 210°$ and 270°, the change of the population distribution of these two rotamers (210° and 270°) would not cause a change of the $^3J_{H3',P3'}$. This observed change of $^3J_{H3',P3'}$ (for 7.8-8.8 Hz for 3'-AMP and from 7.4-7.9 Hz) does suggest a small change in the symmetry of the two rotamer positions in the oscillation of the H3'-C3'-O-P dihedral angle. At this region of the Karplus relationship, a small shift of a few degrees can account for the change of $^3J_{H3',P}$ observed in the range of pH 5.5-8.5 or 11.

A conformational analysis of the furanose phosphate backbone concerning the rotamer distribution of ϕ' can now be performed using an approach similar to that applied to the C2'-endo ⇌ C3'-endo equilibrium states of the ribosylfuranose ring system (Davies and Danyluk, 1974). This furanose conformational analysis calculated the distribution of C2'-endo and C3'-endo forms using $^3J_{H1',H2'}$, $^3J_{H3',H4'}$, and eq 2-4:

$$f_{C3'\text{-endo}} = {}^3J_{H3',H4'}/({}^3J_{H1',H2'} + {}^3J_{H3',H4'}) \qquad (2)$$

or

TABLE VI: Comparison of Ribosylfuranose and Sugar Backbone Conformation.

Nucleic Acid	pH	Temp (°C)	$f_{C3'\text{-endo}}$	$f_{gt'}$
ApA	7.0			
Ap-		20	0.60	0.60
		70	0.48	0.48
UpU	7.0			
Up-		20	0.56	0.55
		60		0.53
		89	0.52	
3'-AMP	6.0	27	0.37	
	5.9	27		0.49
	7.0	27	0.38	
	6.7	27		0.57
3'-UMP	4.3	27	0.49	
	4.2	27		0.52
	8.3	27	0.57	
	8.4	27		0.69

$$f_{C2'\text{-endo}} = {}^3J_{H1',H2'}/({}^3J_{H1',H2'} + {}^3J_{H3',H4'}) \quad (3)$$

and

$$f_{C3'\text{-endo}} + f_{C2'\text{-endo}} = 1.0 \quad (4)$$

The furanose phosphate backbone conformation can also be subjected to a similar analysis to determine the distribution of gt' and tg' rotamers, using ${}^3J_{C2',P3'}$, ${}^3J_{C4',P3'}$ and eq 5-7:

$$f_{gt'} = {}^3J_{C4',P3'}/({}^3J_{C2',P3'} + {}^3J_{C4',P3'}) \quad (5)$$

or

$$f_{tg'} = {}^3J_{C2',P3'}/({}^3J_{C2',P3'} + {}^3J_{C4',P3'}) \quad (6)$$

and

$$f_{gt'} + f_{tg'} = 1.0 \quad (7)$$

The proton data of ApA from Lee et al. (1976) were analyzed using eq 2-4 and are listed in Table VI along with the calculated $f_{gt'}$ populations using eq 5-7. These calculations suggest that a relationship between the C3'-endo and gt' conformations (and between the C2'-endo and tg' conformations) probably exist for the angle ϕ' in the ribosylfuranose phosphate system. For the dimer data presented in Table VI, a close correlation (actually a 1:1 relationship) is observed, while for the mononucleotides only a loose coupling between the sugar and sugar phosphate conformations is found. It should be noted that these comparisons are only made with systems which have been shifted slightly from the 50/50 population distributions of the two conformations. Thus, correlations between C3'-endo and gt' still remain to be determined under the conditions where large deviations from midpoint occurs.

Dependence of the Furanose Phosphate Backbone Conformation on Temperature in Dimers and Polynucleotides. The coupling constant values (Table II), which semiquantitatively reflect the furanose phosphate backbone conformation of the mononucleotides, dinucleoside monophosphates, and polynucleotides, and the calculated rotamer distributions (Tables V and VI), can be compared (1) for a given base with respect to temperature variation or (2) at a given temperature with respect to uracil and adenine. Such a comparative analysis, taken in combination with the ^{1}H NMR data concerning the dimers and polymers (Alderfer et al., 1974; Kondo et al., 1970), provides a greater understanding about the governing

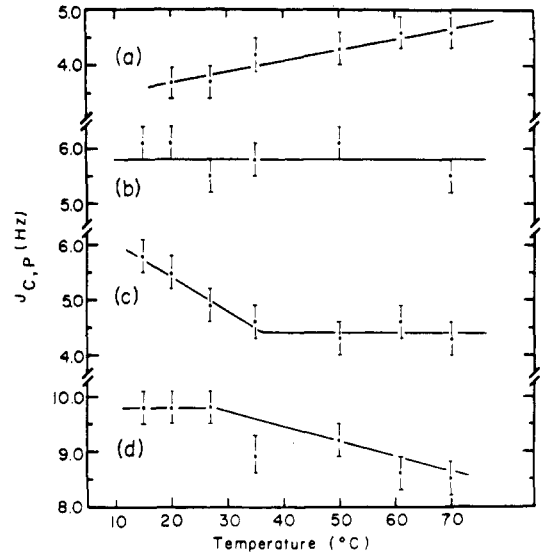

FIGURE 7: The temperature dependence of (a) ${}^3J_{C2',P}$, (b) ${}^2J_{C3',P}$, (c) ${}^3J_{C4,P3'}$, and (d) ${}^3J_{C4',P5'}$ in ApA.

forces of nucleic acid conformation. The coupling constants of the mononucleotides as the monoanion at pH 4 to 5 (Table II) are used as a point of reference in this comparison. Considering the uridylic acid series, comparison of ${}^3J_{C2',P}$ and ${}^3J_{C4',P}$ of monomer, dimer, and polymer indicates little change at low or high temperature. Thus, in terms of the dihedral angles of ϕ and ϕ', no significant alterations are detected. ^{1}H NMR data (Ts'o et al., 1969; Lee et al., 1976) have shown that bases in UpU do not have significant base-stacking interaction, as judged either from dimerization shift or furanose conformation changes with temperature. The situation for the adenylic acid series is considerably different where marked variations in both ${}^3J_{C2'P}$ and ${}^3J_{C4,'P}$ are observed when poly(A) is compared with 3'-AMP (Table II) and for ApA (Figure 7). It is well known that the adenyl compounds have extensive base-stacking properties. In the case of ApA, as the temperature increases then ${}^3J_{C2',P3'}$ increases, ${}^3J_{C4',P3'}$ decreases, and ${}^3J_{C4',P5'}$ decreases. Such temperature-dependent variations of $J_{C,P}$ values of ApA were not seen by others (Schleich et al., 1975, 1976). The reasons for these differences are not understood. These variations in backbone conformation with temperature can be easily understood from variations of the base-stacking interactions with temperature (Kondo et al., 1970). For ApA at low temperature, the larger fraction of gt' relative to tg' for bond ϕ' is not unexpected for a dimer which possesses an extensive stacking of bases, since the gt' rotamer facilitates a maximum amount of base-base overlap. Increasing the temperature reduces the amount of base-stacking in ApA, and the gt' rotamer is depopulated with the concomitant increase of the tg' population. At high temperature, the conformation of ϕ' in ApA is approaching that of the monomer 3'-AMP (Figure 6). A similar trend was observed for the furanose conformation of ApA (Kondo et al., 1970, 1972) and poly(A) (Alderfer et al., 1974) (monitored by $J_{H1',H2'}$). At low temperatures, the furanoses of the dimer and polymer were mainly in the C3'-endo conformation but reverted more to the C2'-endo form at higher temperature, assuming the main conformational form of the mononucleotide. These observations support the discussion in the preceding section and suggest that the furanose conformation and ϕ' conformation are probably interrelated (Table VI). It should also be noted for ApA (Table II and Figure 7) that ${}^3J_{C4',P5'}$ increases with

lowering in temperature, to a value larger than that from 5′-AMP. This suggests an increased population of the *gg* rotamer of the ϕ bond (C5′-O5′) as the result of increased base-stacking interactions. Included in Figure 7 for comparative purposes is the effect of temperature on $^2J_{C3',P}$ of ApA, which is negligible. It should be pointed out that in the case of poly(A) at low temperatures, the line widths of both the proton and carbon data preclude an accurate determination of the coupling constants; however, the data are highly suggestive of conformations which are predominantly, if not exclusively, C3′-endo (furanose), *gt′* (C3′-O3′), and *gg* (C5′-O5′). These observed differences in the uridylic acid and adenylic acid series, with respect to the furanose and furanose phosphate backbone conformations, clearly delineate the role and importance of base-base stacking as a major force in determining backbone conformations of RNA in aqueous solution.

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