Conformational Analysis of the Sugar Ring in Nucleosides and Nucleotides. Improved Method for the Interpretation of Proton Magnetic Resonance Coupling Constants

C. Altona and M. Sundaralingam* [1]

Contribution from the Crystallography Laboratory, Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706.
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Abstract: Torsion angles ϕ_{HH} between the sugar C–H bonds were deduced from X-ray data on 60 nucleosides and nucleoside phosphates with the aid of the pseudorotation model for five-membered rings. Two pseudorotation ranges were considered classified as type N [C(2′)-exo, C(3′)-endo] and type S [C(2′)-endo, C(3′)-exo] conformers, each characterized by a narrow range of the phase angle of pseudorotation P. The various values of ϕ_{HH} along the ring bonds, and hence the corresponding coupling constants, must obey certain interrelationships. It was shown that the coupling $J_{2'3'}$ as well as the sum $J_{1'2'} + J_{3'4'}$ should be practically independent of the position of the N $\rightleftarrows$ S conformational equilibrium; values taken from the literature were then used to extract a set of Karplus parameters valid for the system in question. The effect of pseudorotation (within each of the two ranges of P) on the coupling constants is explored. For all practical purposes, the percentage of S-type conformer in aqueous solution of the common nucleosides and nucleoside phosphates can be calculated by taking $10J_{1'2'}$. The purine ribosides show a small conformational preference for the S type conformer ($\Delta G°_{av} = -0.12$ kcal mol^{-1}), whereas the pyrimidine derivatives slightly favor the N-type ribose conformer ($\Delta G°_{av} = +0.16$ kcal mol^{-1}). Published coupling constants of two compounds in which a keto group must lie over the sugar ring, orotidine and β-cyanuric acid ribonucleoside, were reinterpreted as follows: (i) the ribose ring in these compounds is "flatter" than in the normal series, (ii) the N type conformer is favored over the S type by 0.4–0.5 kcal mol^{-1}, and (iii) the N type rings are characterized by a higher P value than normally occurs. On the basis of known coupling cosstants of dinucleoside phosphates at various temperatures, we proposed a three-state dynamic conformational equilibrium model for the common dimers: (i) unstacked S type, (ii) unstacked N type, and (iii) fully stacked N type. The 2′,5′ dimers, A2′p5′A and A2′p5′C, behave in another manner; two stacked forms seem to be present, differing in the conformation of the 2′-ribose residue. The deoxyribose ring in all common deoxyribonucleosides and their 5′-phosphates show equilibrium compositions that are substantially biased toward the S type conformer over a wide temperature range. This preference may be due to an entropy, rather than to an enthalpy, difference.

In a previous paper,[2] we introduced a new description of furanose ring conformations which is based on the concept of pseudorotation and allows unequivocal determination of the exact conformation of each furanoid ring in terms of two parameters, the "phase angle of pseudorotation," P, and the degree of pucker, τ_m. The values of P and τ_m for 63 purine and pyrimidine substituted sugar rings were calculated from the known endocyclic torsion angles as determined by X-ray methods. Recognizing that the classical conformational notation (C(2′)-exo, C(3′)-endo, C(2′)-endo, C(3′)-exo) may easily lead to misunderstandings[3] and is also rather superficial, we introduced a new notation: type N conformers comprise all conformations that occupy the northern half of the pseudorotational circle ($P = 0 \pm 90°$), which half-includes the classical C(2′)-exo and C(3′)-endo conformations; type S conformers occupy the southern half of the circle ($P = 180 \pm 90°$), and includes the classical C(2′)-endo and C(3′)-exo forms (Figure 1). It was shown[2] that two relatively narrow pseudorotational ranges are preferred by the β sugars, each range occupying less than 10% of the total pseudorotational pathway ($0 \rightarrow 360°$ in terms of P), which ranges are separated by two wide "forbidden regions." Figure 2 illustrates diagrammatically our concept[2] of two potential energy wells, type N and type S, respectively, separated by two barriers opposing pseudorotation, appoximately at $P = 90°$ and at $P = 270°$. Writing the equilibrium equation as N $\rightleftarrows$ S, the value of $\Delta G°$ is taken positive if N has the lowest energy. Both positive and negative $\Delta G°$ values occur, section E. The great majority of N type β-pyrimidine sugars show P values ranging from 2 to about 25°; in few cases the upper end of this range is extended to maximally $P = 34°$. In the type S conformations a greater spread is seen; for the ribonucleosides P lies between 139 and 175° with the purines generally preferring a smaller range in the lower end of the scale ($P_{av} = 158°$), whereas the pyrimidines are usually found at the higher end ($P_{av} = 170°$). The deoxypyrimidines show a wider range of P values, up to $P = 214°$.

It was also argued[2] that the combined wealth of information from the total solid-state data, combined with the concept of pseudorotation, constitutes a reliable and accurate basis for the interpretation of physical measurements in solution. It is this solid-state information, combined with well-established rules in conformational analysis, that we propose to utilize in the present paper in order to extract new information on the conformational behavior in solution from published proton magnetic resonance coupling constants

(1) Author to whom correspondence should be addressed.
(2) C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.*, **94**, 8205 (1972).
(3) In the literature one occasionally finds notations like, *e.g.*, C(2′)-endo $\rightleftarrows$ C(3′)-exo, suggesting the existence of a dynamic equilibrium situation between these two conformations. This is incorrect; the two forms are not separated by a potential energy barrier but rather form part of an allowed region of conformations; *i.e.*, of a single continuous potential energy pocket.[2] This remark also holds for a notation C(2′)-exo $\rightleftarrows$ C(3′)-endo; see also Figure 2.

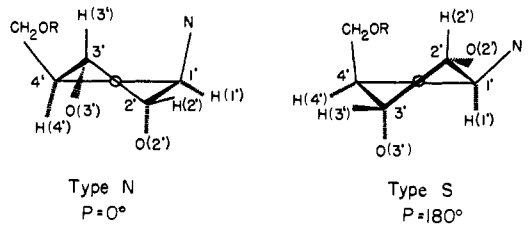

Figure 1. Perspective views of the N and S sugars.

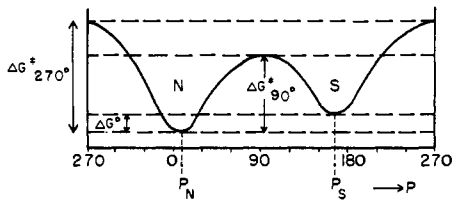

Figure 2. Potential energy wells for the N and S conformers.

as well as to establish guidelines for future work in this field, especially with most accurate pmr Fourier transform techniques now on the horizon. We will limit our discussion to β-substituted ribosides and deoxyribosides; α compounds and 3′,5′-cyclic phosphates will be reported on at a later date.

Results and Discussion

A. Method. We first briefly outline the steps necessary to deduce the torsion angles between carbon–hydrogen bonds (ϕ_{HH}), the Karplus parameters, the vicinal proton–proton coupling constants as function of the N ⇄ S equilibrium composition, and the possible effects of pseudorotational shift of the minimum energy geometries.

(i) The torsion angles, ϕ_{HH}, are deduced indirectly from X-ray data, using the pseudorotational model (section B).

(ii) On the basis of these ϕ_{HH} values, it is found that two important pieces of information, $J_{2'3'}$ and the sum $J_{1'2'} + J_{3'4'}$, should be practically independent of the N/S equilibrium constant K and hence can be used to extract the necessary Karplus parameters (section C).

(iii) Once these parameters are determined, the effect of pseudorotation within each energy minimum is explored (*i.e.*, the relation between J's and P), section D.

(iv) The observed J's and their mutual relationships can now be used to deduce the equilibrium constant and thus the free energy difference between S and N type conformers in the purine and pyrimidine mononucleosides and nucleotides. The temperature dependence of the couplings in several ribodinucleoside phosphates yields interesting information regarding the conformation of the ribose rings in the stacked form, section E.

B. Torsion Angles ϕ_{HH}. It is generally recognized that knowledge of the vicinal proton coupling constants in principle should permit us to determine the stereochemistry of the ribose ring,[4-8] but the classical approach taken is to estimate ϕ_{HH} values from Dreiding or other models, either for a limited number of classical endo and exo "envelope" (E) geometries[8] or for all 20 symmetical (E and T) geometries met on the pseudorotational circle.[7] Next, literature values for the parameters to be used in the Karplus equation[9] are chosen and "model" coupling constants calculated and compared with the measured J's. Although this approach has been reasonably successful in the past, mainly because it has shown conclusively that "the molecule is not frozen in a given conformation but exists in an equilibrium between two or more buckled forms"[8] [β-pseudouridine in this example], several objections may be raised against it.[10]

Of course, individual structure analyses may vary in accuracy, and individual molecules may show to some extent a typical behavior due to special crystal packing effects; therefore, we have adopted a statistical procedure to find the "best" ϕ_{HH} values. Two approaches are now possible. (a) Hydrogen coordinates have been determined by X-ray methods in about 50% of all structures studied. It is, therefore, possible to collect the available information and compute the mean value of ϕ_{HH} for the two conformational ranges, type N and type S, which mean value should be centered near the midpoints of the preferred ranges. However, the error in these hydrogen atomic coordinates is fairly large, yielding errors in the torsion angles of individual molecules of over 10°; the root-mean-square standard deviation in the mean values (Table I) is estimated to be ±4°. (b) All information on the torsion angles between carbon, oxygen, and nitrogen atoms is collected and the additional assumption made that in the Newman projection the carbon–hydrogen vectors are located midway (in a few cases a slight bias of ±1° toward the result of method a was introduced) between the relevant carbon–non-hydrogen atom vectors. This assumption is equivalent to that of approximately equal C–C–H bond angles. Again, the mean values are taken.[14] The results of the two

(4) C. D. Jardetzky, *J. Amer. Chem. Soc.*, **82**, 299 (1970); **84**, 62 (1962).
(5) S. I. Chan and J. H. Prestegard, *ibid.*, **91**, 2843 (1969).
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(7) M. Smith and C. D. Jardetzky, *J. Mol. Spectrosc.*, **28**, 70 (1968).
(8) F. E. Hruska, A. A. Grey, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **92**, 4088 (1970).
(9) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); *J. Amer. Chem. Soc.*, **85**, 2870 (1963).

(10) First, mechanical models cannot be expected to faithfully reproduce torsion angles in molecules that show large deviations from tetrahedral bond angles, five-membered rings in particular. In ring D of steroids deviations from trigonal symmetry (sum of any two adjacent torsion angles = 120°) of over 10° have been noted;[11a] in fact, a mathematical relation between deviations in torsion angles and non-tetrahedral bond angles has been worked out.[11b] Secondly, the Karplus "parameters" are known to vary from molecular system to molecular system[12] and should be extracted, if possible, for the system of interest by combining experimentally determined geometries and nmr data. Fortunately, the ribose system now stands practically second to none among the five-membered rings studied by X-ray crystallographic methods[13] and its behavior is well understood.[2]
(11) (a) C. Altona in "Conformational Analysis," G. Chiurdoglu, Ed., Academic Press, New York, N. Y., 1971, p 1; (b) H. J. Geise C. Altona, and C. Romers, *Tetrahedron*, **23**, 439 (1967).
(12) L. J. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Vol. 5, Pergamon Press, Elmsford, N. Y., 1969, p 292, and references therein.
(13) The number of ribose structures studied with reasonable accuracy now has risen to over 60; the number of steroids, terpenoids, and their analogs studied may be close to this number.
(14) The nonhydrogen atom (C, O, N) torsion angles were computed from published atomic coordinates using program WIL: C. Altona and S. T. Rao, unpublished results. See ref 2 for the list of structures available. Of the type N structures, three were omitted from the calculation of the mean values. Of the type S structures, four were omitted. The omitted structures all show abnormally high values of P (ref 2)

Table I. Hydrogen–Hydrogen Torsion Angles (ϕ) of D-Ribose and D-Deoxyribose in Nucleosides and Nucleotides Obtained by Three Different Methods (See Text)[a]

Conformer type		$\phi_{1'2'}$ trans, deg	$\phi_{1'2''}$ cis, deg	$\phi_{2'3'}$ cis, deg	$\phi_{2''3'}$ trans, deg	$\phi_{3'4'}$ trans, deg
N	(b) Indirect	89	−32	43	166	−158
N	(a) Direct[b]	92	*a*	41	*a*	−162
N	Mechanical model[c]	105	−15	45	165	−165
S	(b) Indirect	158	39	−39	80	−96
S	(a) Direct[b]	155	*a*	−39	*a*	−100
S	Mechanical model[c]	165	45	−45	75	−105

[a] Not calculated due to insufficient number of deoxyriboses studied by X-ray methods. [b] Due to large inaccuracies in the hydrogen atom coordinates these values may be in error by about 4°. [c] Reference 7.

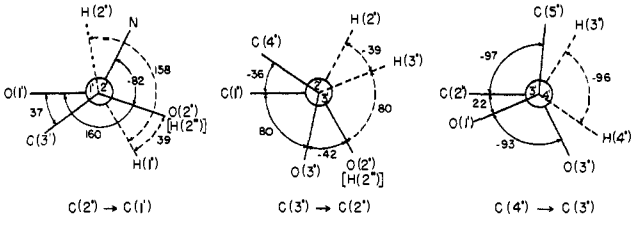


Figure 3. Newman projections along the three bonds C(1′)–C(2′), C(2′)–C(3′), and C(3′)–C(4′), N type.

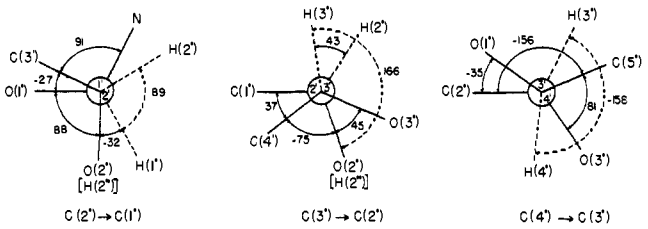


Figure 4. Newman projections along the three bonds C(1′)–C(2′), C(2′)–C(3′), and C(3′)–C(4′), S type.

methods as well as those obtained from mechanical models[7] are presented in Table I. In the remainder of this paper we will use the more accurate angles obtained by method b. Figures 3 and 4 show the mean values of the torsion angle about the C(1′)–C(2′), the C(2′)–C(3′), and the C(3′)–3(4′) bonds for type N (C(3′)-endo) and type S (C(2′)-endo) conformers, respectively; the endocyclic torsion angles about these bonds are denoted τ_1, τ_2, and τ_3. Deviations up to 8° from trigonal projection symmetry (120° symmetry) occur. It is important to note that the angles obtained by method b may be expected to correspond closely to geometries of "ideal" type N and type S conformations occupying the center of the naturally occurring N and S pseudorotational ranges and possessing a mean amplitude of pucker τ_m.[2] In other words, the endocyclic torsion angles τ_1, τ_2, and τ_3 shown in Figures 3 and 4 should be related by the pseudorotation eq 1–3.[15]

$$\tau_1 = \tau_m \cos (P - 144) \quad (1)$$

$$\tau_2 = \tau_m \cos P \quad (2)$$

$$\tau_3 = \tau_m \cos (P + 144) \quad (3)$$

It will be shown that this is indeed the case; furthermore, eq 1–3 constitute an invaluable aid in the calculation of τ's and the corresponding ϕ_{HH} values for other parts of the pseudorotational ranges, see below.

C. Calculation of Karplus Parameters. The Karplus equation[9] relates vicinal coupling constants, J_{HH}, and the torsion angle, ϕ_{HH}, between vicinal C–H vectors.[16a] The originally proposed relationship[9] $J_{HH} = J_0 \cos^2 \phi_{HH} - 0.28$, which utilizes different values of J_0 for $0 \leq \phi \leq 90°$ and $90° \leq \phi \leq 180°$, has been modified[16b] to a convenient and equivalent three-parameter equation

$$J_{HH} = A \cos^2 \phi_{HH} - B \cos \phi + C \quad (4)$$

which is valid for the entire range $0 \leq \phi \leq 180°$. The parameters A, B, and C are regarded[16b] as adjustable parameters, to be extracted from geometrical data on the system to be investigated. Recent MO calculations of the H–C–C–H fragment couplings in ethane[17] differ from the results of Karplus[9] in that the value of J_{HH} at $\phi_{HH} = 90°$ is predicted to be +0.2 Hz instead of −0.28 Hz[9] [equal to the constant C in eq 4]. In view of this theoretical uncertainty regarding the value of J_{HH} at a torsion angle of 90°, we prefer to set $C = 0$ Hz and concentrate our attention on the parameters A and B. It is a well-documented fact that the nmr spectra of mononucleosides and nucleotides reflect a time average of equilibrating (N ⇄ S) molecular species,[2,8,18–26] and in any attempt to delineate

and are thought to be less representative of the normal behavior of nucleosides and nucleotides. No attempt was made to specify separate values for purines and pyrimidines; the only significant difference found (±2°) is in the torsion angles about the C(3′)–C(4′) bond (τ_3) of the type S conformers. This is in line with our observation[2] that type S purines center about a slightly lower P value than type S pyrimidines (compare Figure 1 in our previous paper[2] which shows that τ_3 exhibits greater dependence on P than τ_1 or τ_2 in the S region). However, $\phi_{3'4'}$ being close to 90°, no observable difference in $J_{3'4'}$ of type S purines and pyrimidines is expected.

(15) These equations follow from eq 1 in ref 2.

(16) (a) It has been stressed[9] that this equation is approximate only insofar that the calculated vicinal coupling constants for ethane follow only roughly a cosine square law. Furthermore, electronegativity of substituents, their orientation (gauche or anti) with respect to the coupling proton(s),[12] as well as ring size[16b] (five-membered ring systems appear to show different parameters from the similarly substituted six-membered ones) all influence the shape of the curve relating J_{HH} and ϕ_{HH}. (b) C. Altona, H. R. Buys, H. J. Hageman, and E. Havinga, *Tetrahedron*, **23**, 2265 (1967); C. Altona and A. P. M. van der Veek, *ibid.*, **24**, 4377 (1968), and references therein.

(17) R. C. Fahey, G. C. Graham, and R. L. Piccioni, *J. Amer. Chem. Soc.*, **88**, 193 (1966).
(18) F. E. Hruska and S. S. Danyluk, *ibid.*, **90**, 3266 (1968).
(19) S. I. Chan and J. H. Nelson, *ibid.*, **91**, 168 (1969).
(20) B. W. Bangerter and S. I. Chan, *ibid.*, **91**, 3910 (1969).
(21) B. J. Blackburn, A. A. Grey, I. C. P. Smith, and F. E. Hruska, *Can. J. Chem.*, **48**, 2866 (1970).
(22) F. E. Hruska, K. K. Ogilvie, A. A. Smith, and H. Wayborn, *ibid.*, **49**, 2449 (1971).
(23) F. E. Hruska, A. A. Grey, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **92**, 214 (1970).
(24) H. Dugas, B. J. Blackburn, R. K. Robins, R. Deslauriers, and I. C. P. Smith, *ibid.*, **93**, 3468 (1971).
(25) F. E. Hruska, A. A. Smith, and J. G. Dalton, *ibid.*, **93**, 4334 (1971).
(26) T. Schleich, B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *Biochemistry* **11**, 137 (1972).

Karplus parameters for the ribose ring, one must seek out coupling constants or sums or differences of coupling constants that are to a good approximation independent of the equilibrium constant K. Fortunately, the geometries of N and S type ribose rings in the centers of their pseudorotational ranges[27a] are indeed such that it is possible to extract two experimental values which lead to two independent linear equations that can be solved for the Karplus parameters A and B.

A scrutiny of Table I shows that the torsion angle $\phi_{2'3'}$-*cis*- has practically the same value in N and S type conformations (43 and 39°, respectively); hence $J_{2'3'}$ should show little or no dependence on K. Indeed, observations show (section E) that $J_{2'3'}$ varies but little from compound to compound and we chose an average value of 5.1 Hz (see Table VII) to represent a 1:1 equilibrium mixture and average values of the ribose pseudorotation parameters P_N, P_S, τ_{mN}, τ_{mS}. Hence, the mean values $\cos \phi_{2,3} = 0.75$ and $\cos^2 \phi_{2,3} = 0.57$ are substituted in eq 4, with C = 0 as was explained above.

$$5.1 = 0.57A - 0.75B \qquad (5)$$

The second linear equation is derived as follows. Both couplings $J_{1'2'}$ and $J_{3'4'}$ depend not only on the geometries but as well on the mole fractions of conformer N (X_N) and conformer S ($X_S = 1 - X_N$) present in dynamic equilibrium and can be written

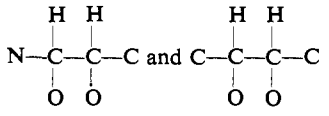
$$J_{1'2'} = X_N J_{89°} + (1 - X_N) J_{158°}$$

$$J_{3'4'} = X_N J_{159°} + (1 - X_N) J_{96°}$$

Assuming $J_{89°}$ and $J_{95°}$ to be very small or zero,[27b] the sum of these couplings is seen to be a constant.

$$J_{1'2'} + J_{3'4'} = X_N J_{158°} + (1 - X_N) J_{158°} = J_{158°}$$

(27) Our assumption that the N and S type ribose rings in solution occupy the centers of the pseudorotational ranges that occur in the crystal may not be valid for individual molecules,[2] but again we choose the statistical approach and (for the sake of having a starting point) we select coupling constant values that represent a good average value of a series of ribosides thought to show an equilibrium constant K close to unity. It turns out that the internal consistency of the nmr data not only supports our assumptions but allows for more detailed interpretations than was possible heretofore. As more accurate coupling constant data become available in the future, the values presently adopted may well need slight revision, perhaps of the order of a few tenth of a hertz, but this will not affect the general validity of our conclusions. The nmr data presently used will be presented in section E. (b) Dropping this simplificating assumption would revise the calculated parameters by 0.1–0.2 Hz. In view of the uncertainty regarding the "best" value of $J_{90°}$ we prefer to adopt this simplification for the time being. (c) It should be stressed that our new values are valid only for the ring system from which they were derived, *viz.*, a five-membered ring containing the fragments



The second fragment occurs twice (about the C(2')–C(3') and C(3')–C(4') bond). We are aware of a hypothesis[8] that the presence of a nitrogen atom in the first fragment might cause a decrease in $J_{1'2'}$ not related to a change in ϕ_{HH}. This hypothesis was based on a comparison of coupling constants in uridine (U) with those in β-pseudouridine (βΨ) in which latter molecule the nitrogen is replaced by a carbon. If true, the above derivation of Karplus parameters should be modified to account for the effect of the nitrogen. Fortunately, a more recent and accurate analysis[26] of the coupling constants in U now shows conclusively that the magnitude of such an effect, if present, remains well within the limit of experimental error. The 0.2 Hz difference between $J_{1'2'}$ of U and βΨ is explained as due to a small difference in the respective K's (see section E).

Selecting a "best" value $J_{1'2'} + J_{3'4'} = 10.1$ Hz (see Table VII) ($\phi_{HH} = 158°$) we write

$$10.1 = 0.68A + 0.93B \qquad (6)$$

Solving eq 5 and 6 for A and B we find: $A = 10.5$ Hz and $B = 1.2$ Hz. These values yield a J *vs.* ϕ curve[27c] that is close to some curves utilized previously for five-membered rings (Table II).

Table II. Karplus Curves for Five-Membered Ring Systems[a]

Ref	J_0	J_{30}	J_{60}	J_{90}	J_{120}	J_{150}	J_{160}
b	9.3	6.8	2.0	0	3.2	8.9	11.7
c	9.0	6.7	2.0	−0.3	2.7	8.7	11.7
d	9.0	6.7	2.0	−0.3	2.3	7.5	10.1
e	9.4	7.1	2.5	0.4	3.0	8.0	10.4

[a] Coupling constants are given in Hz. [b] This work. [c] Reference 21. [d] R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLauchlan, *J. Chem. Soc.*, 3699 (1962). [e] Reference 16.

It should be mentioned here that the new parameters should not be applied to deoxyriboses without appropriate correction for the electronegativity effect on coupling constants; we will return to this subject in a following section.

D. Effect of Pseudorotation on Coupling Constants. Equations 1–3 (section B) will now be used in two ways, first as a check on the internal consistency of the τ values, and second as a means of calculating ribose τ values and hence the corresponding ϕ_{HH} values at any desired point P along the pseudorotational pathway. Table III shows the result of this calculation as well as the corresponding coupling constants calculated from eq 4 with $A = 10.5$ and $B = 1.2$ Hz; for each conformer type (N and S) the P values chosen represent the lower limit, the center and the approximate upper limit of the pseudorotational ranges occupied by ribose moieties in the crystalline state. Throughout the calculation, a value of $\tau_m = 38°$ was taken.[28] Note that the calculation of ϕ_{HH} for the lower and upper limits of the P ranges involves the reasonable assumption that small pseudorotations have no effect on the bond angles so that a given change in τ causes a change of equal magnitude in ϕ_{HH}.

The solid-state data[2] have shown that the amplitudes of pucker τ_m exhibit a fairly large spread in magnitude, 35–45°. This spread has been attributed,[2] at least in part, to crystal-packing effects; another plausible cause might be found in experimental errors, τ_m being much more sensitive to errors in the atomic coordinates than P. Whatever the case may be, the effect of "flattening" (small τ_m) or increased "pucker" (large τ_m) relative to the average value used in the present work should be discussed here. Although, quantitatively speaking, changes in τ_m are hard to disentangle from changes in P, certain rough conclusions can be drawn. We have to keep in mind, however, that a complete description of a riboside in dynamic conformational equilibrium (N $\rightleftarrows$ S) in solution requires knowledge of five parameters: P_N, P_S, τ_{mN}, τ_{mS}, and K(S/N), whereas at most only three coupling constants[29] are available, $J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$

(28) We ignore the small difference (1–1.5°) in τ_m of purines and pyrimidines, purines being slightly more puckered on the average.[2]

(29) Temperature- or solvent-induced shifts of the coupling constants, when they occur, are of great interest since the information content of the couplings then increases sharply (section E).

Table III. Endocyclic Torsion Angles τ, Derived Torsion Angles between Carbon–Hydrogen Vectors ϕ and Calculated[a] J_{HH} Coupling Constants for N and S Type Conformers as Function of P[a]

P,[a] deg	τ_{calcd}[b]	τ_m,[c] deg	ϕ_{trans}, deg	J_{trans},[d] Hz	ϕ_{cis}, deg	J_{cis},[d] Hz
			Type N			
	τ_1	τ_1	$\phi_{1'2'}$	$J_{1'2'}$	$\phi_{1'2''}$	$J_{1'2''}$
3	−29.5	(−30)	86	0.0	−35	(6.1)
10	−26.4	−27	89	0.0	−32	(6.5)
25	−18.4	(−19)	97	0.3	−24	(7.7)
	τ_2	τ_2	$\phi_{2''3'}$	$J_{2''3'}$	$\alpha_{2'3'}$	$J_{2'3'}$
3	38.0	(38)	167	(11.1)	44	4.6
10	37.4	37	166	(11.0)	43	4.7
25	34.4	(34)	163	(10.7)	40	5.2
	τ_3	τ_3	$\alpha_{3'4'}$	$J_{3'4'}$		
3	−31.9	(−33)	156	9.9		
10	−34.1	−35	158	10.1		
25	−37.3	(−38)	161	10.5		
			Type S			
	τ_1	τ_1	$\phi_{1'2'}$	$J_{1'2'}$	$\alpha_{1'2''}$	$J_{1'2''}$
145	38.0	(39)	160	10.4	41	(5.1)
161	36.3	37	158	10.1	39	(5.4)
175	32.6	(33)	154	9.5	35	(6.1)
	τ_2	τ_2	$\alpha_{2''3'}$	$J_{2''3'}$	$\alpha_{2'3'}$	$J_{2'3'}$
145	−31.1	(−31)	35	(0.0)	−34	6.2
161	−35.9	−36	80	(0.1)	−39	5.4
175	−37.9	(−38)	78	(0.2)	−41	5.1
	τ_3	τ_3	$\phi_{3'4'}$	$J_{3'4'}$		
145	12.4	(12)	−106	1.1		
161	21.8	22	−96	0.2		
175	28.7	(29)	−89	0.0		

[a] Reference 2 and text. [b] Calculated from eq 1–3, $\tau_m = 38°$. [c] Calculated from published atomic coordinates, see text. Values shown in parentheses were derived from curve matching with τ_{calcd} values. [d] Calculated from the ϕ_{HH} values utilizing eq 4 and $A = 10.5$, $B = 1.2$, and $C = 0$ Hz. J values for deoxyriboses, shown in brackets, are uncorrected for electronegativity effects.

(in addition, the P's and τ_m's are thought of as being time averages, since different (syn–anti) base conformations and (trans–gauche) C(5′)–O(5′) side-chain rotamers may exert an effect on these parameters). According to eq 1–3, a change in τ_m causes a proportional change in all τ's. An increase in τ_m will cause: (a) an increase of all cis ϕ_{HH} values, hence a decrease in $J_{2'3'}$; (b) an increase of all trans ϕ_{HH} values between protons that form a pseudoaxial pair (ϕ range 150–170°), correlated to an increase in J trans (as well as in $J_{1'2'} + J_{3'4'}$); (c) a small and varying effect on couplings between protons that form a pseudoequatorial pair (ϕ range 76–106°). Summarizing, abnormally puckered ribose rings are expected to show abnormally small values of $J_{2'3'}$ combined with abnormally large values of $J_{1'2'} + J_{3'4'}$; flattened rings will show the opposite trend.

The effect of pseudorotation on the various couplings may oppose the effect of τ_m. In view of the "reasonable" behavior of the great majority of couplings in β-ribosides studied thus far, we prefer to concentrate our attention on the information regarding the equilibrium constant and, in some cases, a rough estimate of P_N and/or P_S that can be extracted from these couplings. For this reason, Table IV has been prepared, which shows the predicted coupling constants for various N ⇄ S equilibrium compositions. This was done with the ribose ring adopting average P_N and P_S values (center of the range, case A) as well as for the possible end-of-range combinations of P_N and P_S. If desired, other combinations can be calculated from the data given in Table III and a three-dimensional surface constructed for each of the couplings for K values of interest. Due to the fact that the *couplings cannot assume random values, independent of each other, but must obey interrelationships governed by the law of pseudorotation*, several interesting predictions are now possible. Confining our discussion to the 20–80% range of compositions, one sees that knowledge of $J_{1'2'}$ alone[30] suffices to pinpoint the equilibrium composition to within ±5%, assuming the absence of error in the J value. However, accurate knowledge of all three coupling constants, especially in cases where the equilibrium composition is strongly biased one way or the other, should allow us to draw conclusions concerning the pseudorotational state of the predominant conformer. For example, if the bias is toward the type N conformer ($J_{1'2'} < 4$ Hz), a relatively large value of $J_{2'3'}$, combined with a large value of $J_{1'2'} + J_{3'4'}$, leads to the conclusion that the type N ribose ring probably prefers the higher end of the P_N range (keeping in mind the possible complication of "flattening" *vs.* "puckering" discussed above). Preluding on the material presented in section E we note that shifts of the equilibrium constant may cause $J_{2'3'}$ as well as the sum $J_{1'2'} + J_{3'4'}$ to increase or decrease, depending on P_N and P_S.

E. Equilibrium Compositions. A mononucleoside molecule is built up from the furanose ring by the addition of a number of side chains (base, two OH groups, one CH_2OH group). Each of these side chains is capable, at least in principle, of orienting itself in either two (base) or three different ways (conformers or rotameric

(30) A warning[8] has been issued to the effect that first-order measurement of the width of the H(1′) "doublet" may cause errors of up to 1 Hz in $J_{1'2'}$. Indeed, various published values of $J_{1'2'}$ of the same compound (uridine, 3′-uridine monophosphate and 5′-adenosine monophosphate, Table VI, are good examples) differ by as much as 0.4–0.5 Hz.

Table IV. Predicted $^3J_{HH}$ Coupling Constants (Hz) for Various Compositions of the Conformational Equilibrium Mixture[a]

P_N, deg	% N / % S	100 / 0	80 / 20	60 / 40	40 / 60	20 / 80	0 / 100	P_S, deg	Case
10	$J_{1'2'}$	0.0	2.0	4.0	6.1	8.1	10.1	162	A
	$J_{2'3'}$	4.7	4.8	5.0	5.1	5.3	5.4		
	$J_{3'4'}$	10.1	8.1	6.1	4.2	2.2	0.2		
	$J_{1'2'} + J_{3'4'}$	10.1	10.1	10.1	10.3	10.3	10.3		
3	$J_{1'2'}$	0.0	2.1	4.2	6.2	8.3	10.4	145	B
	$J_{2'3'}$	4.6	4.9	5.2	5.6	5.9	6.2		
	$J_{3'4'}$	9.9	8.1	6.4	4.7	2.8	1.1		
	$J_{1'2'} + J_{3'4'}$	9.9	10.2	10.6	10.9	11.1	11.5		
25	$J_{1'2'}$	0.3	2.1	4.0	5.8	7.7	9.5	175	C
	$J_{2'3'}$	5.2	5.2	5.2	5.1	5.1	5.1		
	$J_{3'4'}$	10.5	8.4	6.3	4.2	2.1	0.0		
	$J_{1'2'} + J_{3'4'}$	10.8	10.5	10.3	10.0	9.8	9.5		
3	$J_{1'2'}$	0.0	1.9	3.8	5.7	7.6	9.5	175	D
	$J_{2'3'}$	4.6	4.7	4.8	4.9	5.0	5.1		
	$J_{3'4'}$	9.9	7.9	5.9	4.0	2.0	0.0		
	$J_{1'2'} + J_{3'4'}$	9.9	9.8	9.7	9.7	9.6	9.5		
25	$J_{1'2'}$	0.3	2.3	4.3	6.4	8.4	10.4	145	E
	$J_{2'3'}$	5.2	5.4	5.6	5.8	6.0	6.2		
	$J_{3'4'}$	10.5	8.6	6.7	4.9	3.0	1.1		
	$J_{1'2} + J_{3'4'}$	10.8	10.9	11.0	11.3	11.4	11.5		

[a] The calculation was carried out for five combinations of P_N and P_S, see text.

sites) of presumably different enthalpy content. The rotameric sites of each side chain are separated from one another by enthalpy barriers opposing completely "free" rotation, otherwise no distinct geometrical preferences would occur, contrary to experiment.[31] On the other hand, in all cases encountered so far, the barriers are sufficiently low to allow interconversions between conformers to take place at a rate which is rapid on the nmr time scale. This phenomenon is often described by the term "time average blend of conformers." We prefer to omit reference to any particular time scale and use the more general term: dynamic conformational equilibrium, or equilibrium.

Conformational analysis of mononucleosides would be a straightforward matter if the equilibrium populations of the various rotameric sites (partial equilibria) were independent of the ribose conformation and of each other. However, strong correlations are known to exist in which the ribose ring and the nature of the base play dominant roles. Some combinations of conformational features seem distinctly preferred over others.[32]

Let us analyze the present status of knowledge before turning our attention to the conformational analysis of the ribose ring proper. It should be recognized that the total number of allowed conformers in solution must be fairly large[2,31] (Table V). For example, type S purines show little preference for either anti or syn orientation of the purine moiety in the crystal; moreover, both anti and syn orientations allow the existence of gauche–gauche (gg) and gauche–trans (gt) rotamers about the C(4′)–C(5′) bond.[31] Hence, type S purines may exist in at least four different conformers and in many more if the number of possible rotamers about the bonds C(2′)–O(2′), C(3′)–O(3′), and C(5′)–O(5′) is taken into account. On the other hand, type N purines show exclusive preference for the anti orientation in the solid, but both gg and gt conformers occur. It follows that one should expect the existence of a dynamic equilibrium between at least six distinct purine mononucleoside conformers in solution.[33] Similarly, the number of allowed combinations of pyrimidine nucleosides is four (Table V).

Some selected experimental evidence for the existence of partial conformational equilibria of nucleosides in solution will be briefly discussed, and the original conclusions reinterpreted where necessary.

(a) Measurement of hydroxyl proton coupling constants of the common nucleosides (in dry Me_2SO-d_6 solution) has shown[34] that all possible OH rotamers are present in significant, though not necessarily equal, amounts.

(b) Measurement of $J_{4'5'}$ and $J_{4'5''}$ has indicated (for pyrimidine nucleosides at least[21,23,25,28]) that the exocyclic CH_2OH group interconverts between the three principal conformers, gg, gt, and tg. Following a suggestion from the solid state,[31,35] Hruska, Smith, and Dalton[25] found a correlation between the puckering of the ribose and the CH_2OH conformational equilibria. This means that a shift of the equilibrium from N to S type conformer (= increasing $J_{1'2'}$) allows an increase in the relative populations of the gt and tg forms (= increasing sum $J_{4'5'} + J_{4'5''}$). This finding is in complete accord with the information from the solid state (Table V). Similar nmr data on purines seem to be lacking, but from Table V we predict that

(31) M. Sundaralingam, *Biopolymers*, 7, 821 (1969).

(32) Conformers that have been found to occur in two or more crystal structures are considered to be "allowed." The term conformer denotes distinct geometrical aspects such as syn *vs.* anti orientation of a side chain or N *vs.* S geometry of the ribose ring. The term conformation denotes the exact species-specific geometry.

(33) In a formal sense 324 distinct conformers exist if all possible rotamer combinations of ribose mononucleosides are considered. A large number of these will be present in trace amounts due to their unfavorable enthalpy content compared to the allowed forms. It seems largely a matter of taste (and of the limit of detectability of the experimental method used) whether or not a certain conformer is classified "allowed" or "forbidden." The "overall" equilibrium contains so many conformations that study of a single one is not feasible except in the solid state. Nevertheless, one may hope that studies of partial equilibria in the long run will yield a reliable enthalpy and entropy scheme for the overall equilibrium.

(34) D. B. Davies and S. S. Danyluk, *Can. J. Chem.*, **48**, 3112 (1970).

(35) H. R. Wilson and A. Rahman, *J. Mol. Biol.*, **56**, 129 (1971).

Table V. Number of Allowed Conformers of Mononucleosides[a] According to Information from the Solid State[b]

	Base		C(5′)–O(5′)			Number[c] of allowed combinations
	Anti	Syn	gg	gt	tg	
Type N purines	+	−	+	+	−	2
Type S purines	+	+	+	+	−	4
Total states for purines						6
Type N pyrimidines	+	(−)[d]	+	−	−	1
Type S pyrimidines	+	−	+	+	+	3
Total states for pyrimidines						4

[a] The number of possible OH rotamers has not been taken into account. If these are included, the number of allowed combinations should be multiplied by 27 in the case of ribosides, by 9 in the case of deoxyribosides. [b] A plus sign signifies that the conformer is found in more than one crystal structure, a minus sign signifies that no examples have occurred. [c] Obtained by multiplying the number of allowed orientations of the base by the number of allowed C(5′)–O(5′) rotamers. In no case do we imply equality of the energy levels of the allowed combinations. [d] Only one case has been found. For the time being, we assume that this conformation plays only a minor role in solution. Only one example of the syn conformation is known.[2]

similarly constructed correlation graphs will turn out to be rather flatter for purines compared to pyrimidines, if there is any correlation at all.

(c) Measurement of ultrasonic relaxation processes[36] at various temperatures revealed that the purine nucleosides show a relaxation process due to the syn → anti transition of the glycosidic bond and associated with an activation energy of 6.2 kcal mol^{-1}, whereas pyrimidines show at most a trace of a relaxation effect. These findings can be rationalized on the basis of the information from the solid-state data and from the coupling constants presented below. In purine nucleosides, the N-syn combination is strongly disfavored with respect to the N-anti conformer, but syn and anti forms of S type purines seem roughly isoenergetic.[37-40] The position of the N ⇄ S equilibrium slightly favors the S conformer (50–60%, see below); hence in the total equilibrium of purine nucleosides the syn form is expected to occur in substantial amounts, perhaps up to 25–30%. The syn form of both N and S type pyrimidine nucleosides of the common series seem to occur in relatively low concentrations and their presence may be quite difficult to detect.

(d) A wealth of chemical shift data has accumulated in the literature. Upfield shifts in the H(6) pyrimidine resonances have been associated[26] with increasing glycosidic torsion angles (χ_{CN}[31]). Recent studies[21,24,41] of the 2-keto anisotropic effects upon the ribose chemical shifts of pyrimidine nucleosides in aqueous solution allowed the qualitative conclusion that the anti form normally is the only one detected. On the other hand, certain hindered compounds like the 6-methylpyrimidines exist primarily in the syn form;[41] the anti–syn shift is correlated with a small shift of the equilibrium constant K(S/N), see below.

(e) Long-range ($5J$) spin–spin coupling interaction between H(5) and H(1′)[42] supports these conclusions, but, due to the relative weakness of the long-range coupling phenomenon, this technique is inherently less sensitive than the chemical shift method.[43] A similar remark can perhaps be made concerning the use of the nuclear Overhauser effect.[44]

(f) Optical rotatory dispersion (ORD) and circular dichroism (CD) have recently developed into powerful tools for syn–anti conformational assignments in nucleosides.[44-47] Calculations of the rotational strength R_A as a function of the glycosidic torsion angle have been carried out by several authors.[44-46] Thus far reasonable qualitative agreement with experiment has been obtained. However, in the absence of unequivocal conformational models, CD observations are inherently unable to distinguish single conformers from appropriately weighted mixtures of conformers.[44] We will return to this point later on.

(g) The ribose spin–spin coupling constants have in the past not been interpreted to yield *quantitative* information with regard to the position of the dynamic equilibrium between the two principal conformers of the ribose ring. Hruska, *et al.*,[8,18,21-25] carried out a detailed study of these couplings in a variety of pyrimidine nucleosides. From the lack of a significant temperature dependence for either chemical shifts or coupling constants, these authors concluded that the energy difference between the "conventional ring puckered" conformations[48] is small.[49] In the remainder of this paper, we propose to go a step further and deduce quantitatively the relative populations of type N and type S ribose and deoxyribose conformations.

(36) L. M. Rhodes and P. R. Schimmel, *Biochemistry*, **10**, 4426 (1971).
(37) S. T. Rao and M. Sundaralingam, *J. Amer. Chem. Soc.*, **92**, 4963 (1970).
(38) S. S. Tavale and H. M. Sobell, *J. Mol. Biol.*, **48**, 109 (1970).
(39) D. W. Miles, L. B. Townsend, M. J. Robins, R. K. Robins, W. H. Inskeep, and H. Eyring, *J. Amer. Chem. Soc.*, **93**, 1600 (1971).
(40) A. V. E. Haschemeyer and A. Rich, *J. Mol. Biol.*, **27**, 69 (1967).
(41) M. P. Schweizer, J. T. Witkowski, and R. K. Robins, *J. Amer. Chem. Soc.*, **93**, 277 (1971).
(42) F. E. Hruska, *Can. J. Chem.*, **49**, 2111 (1971).
(43) This also seems true for the long-range proton–fluorine spin–spin coupling in 5-fluoropyrimidine nucleosides; see R. J. Cushley, I. Wempen, and J. J. Fox, *J. Amer. Chem. Soc.*, **90**, 709 (1968).
(44) P. A. Hart and J. P. Davis, *ibid.*, **93**, 753 (1971).
(45) D. W. Miles, W. H. Inskeep, M. J. Robins, M. W. Winkley, R. K. Robins, and H. Eyring, *ibid.*, **92**, 3872 (1970).
(46) N. H. Teng, M. S. Itzkowitz, and I. Tinoco, *ibid.*, **93**, 6257 (1971).
(47) M. Ikehara, S. Vesugi, and K. Yoshida, *Biochemistry*, 11, 830 (1972).
(48) Four conventional puckered forms were considered by these authors: 3′-endo and 2′-exo, now grouped under type N form, 3′-exo and 2′-endo, now type S form, and a single eclipsed form, whereas in fact two eclipsed conformations are envisaged:[2] the doubly equatorial one at $P = 90°$ and the doubly axial conformation at $P = 270°$.
(49) The argument that the absence of a temperature dependence of the ribose couplings[6,18,21-25] is necessarily correlated to approximately equal populations of the conformers in equilibrium implies the statement that $\Delta S° = 0$ and $\Delta H° = 0$ and should be augmented as follows. Let us consider two components in conformational equilibrium. In general, change of temperature will not affect the equilibrium constant when the *enthalpy* difference $\Delta H°$ is zero. This point follows from the equation $-RT \ln K = \Delta H° - T\Delta S°$, which reduces to $R \ln K = \Delta S° \approx$ constant for $\Delta H° = 0$. One calculates population ratios of 50/50, 62/38, 73/27, and 82/18 for reasonable $\Delta S°$ values of 0, 1, 2, and 3 eu, respectively, and $\Delta H° = 0$; see ref 54.

In earlier studies, deductions regarding ribose conformations often have been based on an essentially static approach, that is, by calculating interproton "torsion angles" ϕ_{ij} straightaway from one or more measured values of J_{HH} ($J_{1'2'}$, $J_{2'3'}$, $J_{3'4'}$) although it was recognized that these ϕ_{ij} values are apparent angles only.[8] However, the apparent ϕ_{ij}'s are *not* the weighted arithmetic mean of the ϕ_{HH}'s of each conformer participating in the equilibrium and have little physical significance. We prefer to employ the usual linear relationship between the time-average J and the J of each participating conformer

$$J_{obsd} = X_I J_I + X_{II} J_{II} \quad (7)$$

where the sum of the mole fractions $\Sigma X_i = 1$.

The J values J_N and J_S for the pure N and S type ribose conformers are taken from Table IV.[50a]

Mononucleosides and Nucleotides. Table VI shows some examples of $J_{1'2'}$ values of β-purines and β-pyrimidines taken from the literature, the calculated equilibrium compositions, and the free energy difference ΔG°_{25} in D_2O. The purine ribosides show a small but clear-cut preference for the S type ribose conformer ($\Delta G^\circ_{av} = 0.12$ kcal mol^{-1}), whereas in the pyrimidine series the N type conformer is favored ($\Delta G^\circ_{av} = +0.16$ kcal mol^{-1}). Note that this trend is opposite to that expected on the basis of the entropy of mixing terms alone (Table V). Therefore, the different behavior of β-purines on the one hand and β-pyrimidines on the other may reflect a genuine enthalpy difference, but solvent structure and hydrogen bonding may also play a certain part.

Table VI. Some Examples of $J_{1'2'}$ Values (Hz) of β-Purines and β-Pyrimidines Taken from the Literature. The Calculated Equilibrium Composition near 25° (% N + % S = 100) Is Shown As Well As the Corresponding Free Energy Difference ΔG_{25}°

Compd	$J_{1'2'}$[a]	% N[a]	ΔG°_{25}[b]
	Purines		
Adenosine	6.1	40	−0.25
Adenosine 5′-phosphate	5.6,[c] 5.1,[d] 5.3[e]	48	−0.07
Adenosine 3′-phosphate	5.8,[c] 5.6[d]	43	−0.17
Inosine	5.6[c]	45	−0.12
Mean of 11 purines[c]	5.6[c]	45	−0.12
	Pyrimidines		
5-Iodocytidine	2.8[f]	72	0.56
Cytidine 5′-phosphate	3.2[c]	68	0.45
Cytidine	3.7,[c] **4.0**[f]	60	0.25
Uridine	4.4,[i] 4.5,[g] **4.8**[h]	52	0.05
Uridine 5′-phosphate	4.6,[c] 4.8[g]	53	0.07
Uridine 3′-phosphate	4.3,[c] 4.3,[g] **4.8**[h]	52	0.05
β-Pseudouridine	5.0,[c] 5.0[i]	50	0.0
Mean of 12 pyrimidines[c]	4.3[c]	57	0.16

[a] $J_{1'2'}$ values in bold type were measured at 100 or 220 MHz and extracted from computer analysis; therefore, these values are judged most accurate and utilized to calculate the equilibrium composition (assuming case A, Table IV). [b] In kcal mol^{-1}; the reported temperatures range from 23 to 30° but no correction for differences from 25° were applied here. The second decimal place may not be significant. The solvent was D_2O in all cases. [c] P. O. P Ts'o, private communication to M. S., to be published. [d] Reference 18. [e] I. Feldman and R. P. Agarwal, *J. Amer. Chem. Soc.*, **90**, 7329 (1968). [f] Reference 25. [g] J. H. Prestegard and S. I. Chan, *ibid.*, **91**, 2843 (1969). [h] Reference 26. [i] Reference 8. [j] Reference 21.

In the pyrimidine series we find some interesting phenomena. Uridine and its 3′- and 5′-phosphate are quite close to an equilibrium constant of unity; in contrast, the three cytidines are definitely biased toward the N type conformer (ΔG° = 0.25–0.56 kcal mol^{-1}).[50b] No straightforward explanation for the difference in behavior of uridines and cytidines in solution presents itself, the structural differences between the respective base moieties being located at positions 4 and 5, remote from the ribose ring. Also, without careful separation of enthalpy and entropy factors[54] one has yet little ground for speculation.

Table VII shows examples of purine and pyrimidine compounds for which all three vicinal coupling constants of the ribose ring have been measured. Unfortunately, data on purines are scarce and general conclusions concerning the geometry of the purine ribose ring in solution cannot yet be drawn. One sees that 5′-AMP fits case D slightly better than case A in Table IV; perhaps the ribose ring of this particular mononucleotide in solution prefers N and S geometries that are shifted slightly from the "average" ones. If this interpretation is correct, the calculated percentage N type conformer given in Table VI should be revised downward by about 3%. More data on various β-purine mononucleosides are needed, however, before judgment can be passed.

The β-pyrimidine series in general shows constant behavior with respect to $J_{2'3'}$ and to the sum $J_{1'2'} + J_{3'4'}$. In all cases where the pyrimidine base is known to occur predominantly anti, $J_{2'3'}$ is found in the small range 5.0–5.5 Hz; the sum $J_{1'2'} + J_{3'4'}$ ranges from 9.5 to 10.8 Hz at room temperature. These are the observations that, combined with theoretical considerations and solid-state data, led us to adopt the procedure discussed in section C. Equilibrium compositions and ΔG° values may be calculated, if desired, from the data presented in Tables IV and VI, except in the cases of orotidine[56] (O) and β-cyanuric acid ribonucleoside[24] (β-CAR). In β-CAR both the 2 and 6 positions of the pyrimidine ring carry a keto group, one of which must lie over the ribose ring; this situation is analogous to the syn conformer of the common pyrimidine nucleosides. Comparison of the nmr data for O and β-CAR led to the conclusion[56] that the same situation occurs in O as well. Dugas, *et al.*,[24] concluded that the conformational

(50) (a) Of course one has to keep in mind, as has been argued in section D, that J_N and J_S themselves represent time-average couplings because the various orientations of the side chains may have slightly different effects on the ground state geometry of the ribose ring. The couplings in case A, Table IV, seem of sufficient reliability when only $J_{1'2'}$ is known and even better in cases where our proposed "probes for geometry" $J_{2'3'}$ and $J_{1'2'} + J_{3'4'}$ do not differ significantly from the "best" standard values of 5.1 and 10.1 Hz, respectively. For all *practical* purposes, the percentage S conformer calculated by taking $10 \times J_{1'2'}$ or $10 \times (10.1 - J_{3'4'})$ seems good enough. (b) In the crystalline state, cytidine occurs with an N type ribose conformation,[51] but in cytidine 3′-phosphate, S type ribose conformations are found in both its crystalline modifications.[52,53] Evidently, crystal packing effects may overcome a free energy bias of 0.25–0.50 kcal mol^{-1}.

(51) S. Furberg, C. S. Peterson, and C. Rømming, *Acta Crystallogr.*, **18**, 313 (1965).

(52) M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.*, **13**, 914 (1965).

(53) C. E. Bugg and R. E. Marsh, *ibid.*, **13**, 914 (1965).

(54) We stress the importance of measuring accurate coupling constants of selected mononucleosides and -nucleotides over a wide range of temperatures and especially in the range 4–25°. Our analysis[55] of the variable-temperature data given in ref 18 for adenosine 5′-phosphate (5′-AMP) and adenosine 3′-phosphate (3′-AMP) indicates that in 5′-AMP both ΔH° and ΔS° are rather close to zero, but for 3′-AMP we find $\Delta H^\circ \approx -0.9$ kcal mol^{-1}, $\Delta S^\circ \approx -2.6$ eu.

(55) C. Altona and M. Sundaralingam, to be published..

(56) F. E. Hruska, *J. Amer. Chem. Soc.*, **93**, 1795 (1971).

Table VII. Some Examples of β-Purines and β-Pyrimidines for Which All Three Vicinal Ring Coupling Constants (Hz) Have Been Measured at 100 or at 220 MHz

Compd	$J_{1'2'}$	$J_{2'3'}$[a]	$J_{3'4'}$	$J_{1'2'} + J_{3'4'}$	Temp, °C
		Purines			
Adenosine 5′-phosphate (5′-AMP[d])	5.3	4.8	3.8	9.1	27
e	5.0	5.0	4.5	9.5	27
f	5.6	4.8	3.8	9.4	*c*
		Pyrimidines			
Uridine[g]	4.4	5.3	5.5	9.9	28
h	4.8	5.2	5.4	10.2	23
h	5.1	5.2	5.1	10.2	80
β-Pseudoruidine[i] (βψU)	5.0	5.0	5.2	10.2	30
i	5.2	5.0	5.2	10.4	70
Uridine 3′-phosphate[h] (3′-UMP)	4.8	5.2	5.5	10.3	23
h	5.0	5.3	5.5	10.5	88
4-Thiouridine[j] (4-SU)	3.9	5.4	5.6	9.5	25
Orotidine[k] (O)	3.6	6.3[b]	7.0	10.6[b]	50
β-Cyanuric acid riboside[l] (β-CAR)	3.9	6.4[b]	6.6	10.5[b]	30
	4.0	6.1[b]	6.2	10.2[b]	75
β-Pseudouridine 5′-phosphate[h]	5.3	5.3	5.5	10.8	23
	5.5	5.5	5.6	11.1	75

[a] The mean value of these couplings were used in eq 5 and 6. [b] These numbers were omitted from the mean. [c] Temperature not stated. [d] Footnote *e*, Table VI. [e] S. Fujiwara and M. Uetsuki in "Recent Developments of Magnetic Resonance in Biological Systems," S. Fujiwara and L. H. Piette, Ed., Hiroka Publishing Co., Tokyo, 1968, p 1. [f] Footnote *c*. Table VI. [g] Reference 21. [h] Reference 26. [i] Reference 8. [j] Reference 22. [k] F. E. Hruska, *J. Amer. Chem. Soc.*, **93**, 1795 (1971). [l] Reference 24.

Table VIII. Sample Calculation[a] of $J_{1'2'}$ and $J_{3'4'}$[b] as Function of the Amplitude of Pucker (τ_m) of the Ribose Ring

		Step I	Step II	Step III	Step IV		Step V		Step VI		
τ, deg	$J_{2'3'}$	$\phi_{2'3'}$, deg	τ_2, deg	P, deg	τ_1, deg	τ_3, deg	$\phi_{1'2'}$, deg	$\phi_{3'4'}$, deg	$J_{1'2'}$	$J_{2'3'}$	sum
					Type N						
32	6.3	33.5	27.5	30.7	−12.7	−31.9	102.7	156.0	0.8	9.9	10.7
36	6.3	33.5	27.5	40.2	−8.6	−35.9	106.9	159.9	1.2	10.4	11.6
40	6.3	33.5	27.5	46.6	−5.2	−39.3	110.3	163.3	1.7	10.7	12.4
44	6.3	33.5	27.5	51.3	−2.1	−42.4	113.4	166.4	2.1	11.1	13.3
					Type S						
32	6.3	−33.5	−30.5	162.4	30.4	19.0	152.1	−98.8	9.3	0.4	9.7
36	6.3	−33.5	−30.5	147.9	35.9	13.4	147.6	−104.4	10.0	1.0	11.0
40	6.3	−33.5	−30.5	139.7	39.9	9.5	161.6	−108.3	10.6	1.4	12.0
44	6.3	−33.5	−30.5	133.9	43.3	6.0	165.0	−111.8	11.0	2.0	13.0

[a] The six steps necessary to carry out the calculation are outlined in the text. [b] Coupling constants are in Hz. The value for $J_{2'3'}$ chosen (6.3 Hz) seems representative for pyrimidine ribosides in which a keto group lies over the ribose ring (β-CAR and O, Table VII).

equilibrium (N ⇄ S in the present notation) in β-CAR compared to uridine has shifted toward the N type form. A more quantitative analysis of the couplings can now be made along the lines discussed in section D.

The geometry-dependent coupling constant $J_{2'3'}$ in O and β-CAR (6.3–6.4 Hz at 25°) clearly falls outside the usual range. This fact suggests that unfavorable interactions between the keto group and atoms of the ribose ring not only affect the relative energies of the N and S conformers and hence the equilibrium constant, but also compel the five-membered ring to adapt its geometry in some way. Before we proceed to investigate this adaptation in more detail, some assumptions have to be made. First, it seems likely that *both* N and S type ribose geometries are changed, though not necessarily in the same manner. Lacking information on $J_{2'3'}$ of the "pure" conformers, we adopt a value of 6.3 Hz for both. Secondly, we assume that the steric interactions do not affect the relationship between the τ angles and the corresponding ϕ_{HH} angles (Table III). Our task is then to deduce pseudorotation parameters P and τ_m in such a way that we obtain a correct value for the second geometry dependent piece of information, $J_{1'2'} + J_{3'4'}$ (10.5–10.6 Hz). The six steps involved in this procedure are straightforward and will be illustrated[57] here for the type N conformer. Step I: Given $J_{2'3'} = 6.3$ Hz, it follows from eq 4 with $A = 10.5$ Hz and $B = 1.2$ Hz, that the torsion angle between the carbon–hydrogen bonds $\phi_{2'3'}$ is 33.5°. Step II: In order to utilize the pseudorotation equations, one must find the corresponding endocyclic torsion angle τ_2. Table III serves our purpose. We note that $\tau_2 \cong \phi_{2'3'} - 6°$; therefore we take $\tau_2 = 27.5°$. Step III: From eq 2, one now calculates the phase angle of pseudorotation P. In the present example, we choose $\tau_m = 32°$ (*vide infra*) and find $P = 30.7°$. Step IV: From eq 1 and 3 we calculate the corresponding angles $\tau_1 = -12.7°$ and $\tau_3 = -31.9°$. Step V: Table III is again used, this time in reverse sense to yield $\phi_{1'2'} = 102.7°$ and $\phi_{3'4'} = 156.0°$. Step VI: Finally, eq 4 gives us the corresponding coupling constants $J_{1'2'} = 0.8$ Hz and $J_{3'4'} = 9.9$ Hz; their sum is 10.7 Hz.

The results of a series of such calculations for various values of τ_m are presented in Table VIII. Note that the sum $J_{1'2'} + J_{3'4'}$ is remarkably dependent on the exact value of τ_m so that, once τ_2 is determined from $J_{2'3'}$,

(57) For the sake of clarity we retain throughout the procedure one more digit than is warranted.

the parameters τ_m and P can be pinpointed uniquely. One has here a powerful tool which allows fairly accurate determination of geometry in solution, of the flattening or puckering, and of pseudorotational shifts of the ribose ring relative to the ring in "normal" nucleosides and nucleotides.

"Best" values (not shown in Table VIII) for the type S conformer of O and β-CAR are: $\tau_m = 34°$, $P = 153.8°$, $J_{1'2'} = 9.7$ Hz, $J_{3'4'} = 0.7$ Hz. For the equilibrium compositions of O (at 50°) and β-CAR (at 30°) we find 69 and 65% N type conformer, respectively; the corresponding $\Delta G°_T$ values are 0.51 and 0.37 kcal mol^{-1}, respectively. We conclude that the interactions between the keto group and the ribose ring are accommodated more easily by the N type ribose ring than by the S form. However, the N and (presumably) S type geometries are different from those in uridine; the ribose rings of O and β-CAR are about 6° flatter, the N type rings have pseudorotated toward a higher P value (31° compared to the standard value of 11°). No X-ray information on O or β-CAR seems available to date, but we may compare our results with the data on 4-thiouridine[58] (4-SU) which compound occurs in the solid (but not in solution[22]) in the syn form with N type ribose conformation. From our previous paper,[2] we have for 4-SU in the crystalline state: $P = 34.2°$ and $\tau_m = 41.0°$. This P value appears close to that calculated for O and β-CAR in solution; the respective τ_m values disagree. The disagreement is not deemed serious because it is known[2] that the same molecular species in different crystal field surroundings may show a relatively far greater variation in τ_m than in P.

Dinucleotides. The coupling constants of the ribose protons in dinucleotides often show a surprisingly large temperature dependence[6,18,20,59,60] compared to the monomers. It seems generally agreed that this temperature effect results from changes in the intramolecular base-stacking interaction but a quantitative treatment of the stacking ⇄ destacking equilibrium based on the couplings between the ribose protons is still lacking. We have recently completed such an analysis, the results of which will be published elsewhere.[55]

Suffice to say for the moment that the percentages of N type ribose rings present in the mixtures were calculated from the $J_{1'2'}$ values[61] and are shown in Table IX. Combining these results with evidence from optical methods[62] now leads to some very interesting conclusions. It is seen that A3'p5'A contains about 75–80% N type ribose rings at the lowest temperature used (4°), while ultraviolet, optical rotatory dispersion, and circular dichroism data[62] strongly suggest that at this temperature stacking is still only about 70% complete. As the temperature *increases*, both the amount of stacked form[62] and the percentage N type conformer *decrease*. This observation strongly suggests that if one were able to drop the temperature sufficiently to approach a 100% stacking mode, one would also end up with a 100% pure N type ribose ring. In other words, we propose the hypothesis that A3'p5'A *in its stacked conformer contains*, within the limits of experimental error, *only one kind of ribose conformer*, which we will call the ordered N-stacked form, Ns.[63] It automatically follows that the amount of S type ribose rings present in the mixture, which amount increases with increasing temperature, exists in a completely or nearly completely unstacked form: S-unstack. Now in the mononucleoside phosphates, an N ⇄ S conformational equilibrium exists (Table VI) with an equilibrium constant approaching unity at elevated temperatures. Exactly the same behavior is seen in the dinucleoside phosphates. At temperatures where destacking is virtually complete[62] (Table IX), a roughly 50/50 equilibrium exists between S-unstack and N-unstack conformers, Su and Nu, respectively. We are tempted at this point to postulate that the interactions between the 3'- and the 5'-substituted ribose moieties of the *unstacked dimer* are relatively unimportant,[64] so that the thermodynamical properties of the equilibrium Nu ⇄ Su of 3'A and 5'A can be approximated by the properties of the N ⇄ S

Table IX. Some Examples of $J_{1'2'}$ Values (Hz) of Dinucleotides Taken from the Literature[a,b]

Temp, °C	Solvent	$J_{1'2'}$	% N	$J_{1'2'}$	% N
	A3'p5'A	A3' residue		A5' residue	
4	D_2O	2.5	75	2.0	80
30	D_2O	3.2	68	3.5	65
60	D_2O	4.5	55	4.1	59
30	DMSO	~6.6	~35	~5.8	43
	A3'p5'C	A3' residue		C5' residue	
4	D_2O	2.4	76		
39	D_2O	3.7	63	2.0	80
60	D_2O	4.5	55	3	70
	A2'p5'A	A2' residue		A5' residue	
4	D_2O	4.5	55	3.0	70
30	D_2O	4.5	55	2.7	73
60	D_2O	5.2	49	4.0	60
30	DMSO	5.9	42	5.8	43
	A2'p5'C	A2' residue		C5' residue	
4	D_2O	6.7	34	<1	>90
30	D_2O	6.6	35	<1	>90
60	D_2O	6.6	35	<1	>90
30	DMSO	5.4	47		
	A5'p5'A			A5' residue	
4	D_2O			4.3	57
30	D_2O			4.6	54
60	D_2O			4.7	54
30	DMSO			5.7	44

[a] Reference 60. [b] The calculated equilibrium composition (% N + % S = 100) at various temperatures is shown.

(58) W. Saenger and K. H. Scheit, *J. Mol. Biol.*, 50, 153 (1970).
(59) K. N. Fang, N. S. Kondo, P. S. Miller, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, 93, 6647 (1971).
(60) (a) N. S. Kondo, H. M. Holmes, L. M. Stempel, and P. O. P. Ts'o, *Biochemistry*, 9, 3749 (1970); (b) P. O. P. Ts'o, N. S. Kondo, M. P. Schweizer, and D. P. Hollis, *ibid.*, 8, 997 (1969).
(61) No special conformational effects need be invoked to explain the observed coupling constants in dinucleoside phosphate; the conformations of the ribose ring (defined by P and τ_m) in the dimers studied so far by X-ray analysis fall in the same ranges as those of the monomers (ref 2).
(62) J. T. Powell, E. G. Richards, and W. B. Gratzer, *Biopolymers*, 11, 235 (1972), and references therein.
(63) This conclusion is not entirely new. Several authors[18-20] have interpreted their nmr data to mean that the ribose ring puckering in stacked 3',5' dimers is "more" 3-endo (N type) and that this changes "toward" 2'-endo (S type) with unstacking. The present approach, however, seems capable of yielding more quantitative information than was previously possible, and at the same time reconciles some apparent contradictions.
(64) The S type ribose ring of purines in principle allows the existence of a syn–anti equilibrium and for certain purposes it may be necessary to split the S-unstacked state of purine dimers into syn-Su and anti-Su states. Furthermore, the *unstacked* dimer conformers are supposed to be more or less random in the sense that the ribose equilibrium parameters $\Delta H°$ (S/N) and $\Delta S°$ (S/N) for any particular residue depend more on the nature and orientation of the base and on the position of the phosphate link rather than on the instantaneous conformer (N or S) adopted by the neighboring ribose unit. It follows that one may speak of (unstacked) substrates which exist in conformational equilibrium: 3'N-5'N, 3'N-5'S, 3'S-5'N, and 3'S-5'S.

equilibrium in 3′-AMP and 5′-AMP, respectively. Summarizing, we propose the existence of a *three-state dynamic equilibrium model* for 3′,5′-dinucleoside phosphates:[64] Su in equilibrium with Nu, which in turn exists in thermodynamic equilibrium with the ordered Ns conformer, the latter being the one occurring in RNA and in the A form of DNA.

Our proposal is at first sight in conflict with a recent statement[62] to the effect that in dimers the stacking equilibrium can be operationally regarded as a two-state system. Indeed, the ultraviolet absorption, circular dichroism, and optical rotatory dispersion data of Powell, *et al.*,[62] are incompatible with a multistate scheme in which more than two *optically* distinguishable substates exist. Our point is that the differences between the optical and thermodynamical properties of Nu and Su type conformers are probably second order compared to the difference between these properties of Nu and the ordered Ns conformers. Calculations on circular dichroism of pyrimidine nucleosides[45] indeed suggest that changing the conformation of the ribose ring from N to S type should not have a dramatic effect on the rotational strength if the base remains in the anti conformer. We wish to mention that our thermodynamic analysis[55] of the stacking–unstacking equilibrium in ApC and CpA, based on our proposed three-state model and using published nmr coupling constants, yielded $\Delta H°$ unstack and $\Delta S°$ unstack values in good agreement with those derived by various authors from optical measurements.[62,65] A two-state nmr model is not compatible with the optically derived thermodynamic parameters. The fact that crystalline U3′p5′A exists in an *unstacked* conformation which nevertheless has the ribose ring of each unit assuming N type geometry[2] lends support to our line of reasoning. No nmr data on his compound are available to us, unfortunately, but it seems rather likely that various conformational equilibria do occur in solution.

Let us briefly survey several interesting features displayed by the data in Table IX. Similar arguments, as given above, hold for both residues of A3′p5′C. We conclude that in the stacked form of this dimer the ribose ring assumes the N type conformer exclusively in both its residues. At 60° the amount of stacked Ns form is only about 15%;[55] subtracting this percentage from the total of N type ribose present in the 5′C residue, we see that the Nu/Su equilibrium ratio (65/35) matches well with the equilibrium conditions of the monomer 5′-CMP[66] (Table VI).

Different situations appear to exist in the 2′,5′ dimers. It has been suggested by several authors[60,67] that the 2′,5′ dimers at 25° have an extensive degree of base stacking, as monitored by optical properties and chemical shifts. The temperature dependence of these properties[60] in A2′p5′A, interpreted in terms of the shift of the stack–unstack equilibrium toward the unstacked forms at higher temperature, was found to be generally smaller than in the corresponding 3′,5′ dimer, though still substantial. The temperature shift in A2′p5′C is anomalously small.[60,67] How well do these findings correlate with the temperature dependence of the nmr couplings? Unfortunately, the $J_{1'2'}$ values of the 5′A residue seem internally inconsistent, but for the time being and awaiting further nmr results, we are tempted to explain the data by assuming that the 5′A ribose ring in the stacked form exists in the N type conformer. The 5′C residue in A2′p5′C clearly shows even greater preference for the N type ribose than is seen in the 3′,5′ dimer, especially at higher temperatures. Even at 60° only 10% or less of the Su form is present in the mixture, corresponding to maximally about 20% of the unstacked Nu form. This means that at 60° this dimer still contains 70% or more of the stacked Ns forms. Now comparing these results with the percentage N type ribose in the corresponding A2′ residues, we come to the interesting conclusion that the A2′ residues in A2′p5′A and A2′p5′C contain more, respectively much more, S type ribose than expected *if the stacked form contained exclusively 2′-N type conformers*. This evidence points to the presence of *two different stacked forms* in A2′p5′A and A2′p5′C, written as A(N)2′p5′A(N) and A(S)2′p5′A(N), respectively, and similarly for A2′p5′C. Ts'o, *et al.*,[60] suggested that both dimers may contain a considerable percentage of left-handed stack. An exciting possibility now comes to mind: if it is true that right-handed stacking of ribonucleoside dimers requires both ribose rings to assume N type geometry exclusively, could then the 2′S–5′N combination somehow cause left-handed stacking? Much experimental work at various temperatures, especially some combination of optical and nmr data, seems called for in order to find an adequate answer. On the other hand, A2′p5′U in the solid state exists as[2] A(S)2′p5′U(N), but in a right-handed pattern.[31] Finally, A5′p5′A is known (from chemical shifts and optical evidence) to contain an appreciable amount of stacked conformer.[60] Judging from the temperature effect on the measured quantities, a "normal" shift toward the unstacked forms occurs at higher temperatures. Surprisingly enough, this shift is only weakly reflected by the temperature shift of the $J_{1'2'}$ coupling. We are tempted to conclude that the ribose rings in the stacked form of the 5′,5′ dimer exist somehow in both N and S type conformers. The question whether or not right-handed and left-handed stacked forms occur side by side remains open for the time being. However, any proposed model should account for the magnetic equivalence of the same protons in both residues,[60] which equivalence requires fast conformational exchange between the various conformers, fast enough on the nmr time scale to give rise to symmetry on a time-average basis.

Deoxyribose Monomers. The coupling constants (Table X) can be interpreted in the same manner as before, except that the absence of the 2′-hydroxyl group necessitates the application of a small electronegativity correction to the calculated couplings in which the protons H(2′) and H(2″) participate. A study of Table III shows that the couplings $J_{1'2'}$ (cis), $J_{2'3'}$ (cis), and the sum $J_{1'2'}$ (trans) + $J_{2'3'}$ (trans) should be fairly invariant with respect to shifts in the conformational equilibrium constant as well as to shifts in the pseudorotation parameter P. Therefore, the magnitude of the electronegativity correction (+0.3 Hz) was chosen so as to bring the calculated values of these couplings in line with the observed ones. Unexplained inconsistencies

(65) R. C. Davis and I. Tinoco, *Biopolymers*, 6, 223 (1968), and references therein.

(66) $J_{1'2'}$ of 5′-CMP remains constant (3.3 Hz) in the temperature range 30–60°.[64b]

(67) M. M. Warshaw and C. R. Cantor, *Biopolymers*, 9, 1079 (1970).

Table X. Calculated[a] and Some Experimental Coupling Constants (Hz) of the Deoxyribosyl Unit

	N type calcd	dU[b] expt	5′-dAMP[c] expt	3′-dAMP[c] expt	S type calcd
$J_{1'2'}$ (trans)	0.3	6.5	6.9	8.1	10.4
$J_{1'2'}$ (cis)	6.8	6.7	6.6	6.0	5.7
Sum	7.1	13.2	13.5	14.1	16.1
$J_{2'3'}$ (cis)	5.0	6.8	5.8	6.3	5.7
$J_{2''3'}$ (trans)	11.3	3.5	3.8	2.9	0.4
$J_{1'2'}$ (tr) + $J_{2''3'}$ (tr)	11.6	10.0	10.7	11.0	10.8
$J_{3'4'}$ (trans)	10.1	4.0			0.2
% N type[d]	100	~30	~30	~22	0
$\Delta G°_{20}$		~−0.5	~−0.5	~−0.8	

[a] An electronegativity correction of +0.3 Hz was applied to all calculated couplings (Table III) in which H(2′) and H(3′) participate, see text. [b] Reference 26. [c] Reference 59. [d] Based on the calculated and observed values of $J_{1'2'} + J_{1'2''}$.

(up to 1 Hz) remain, but one has to keep in mind that the conformational properties of the deoxyribosyl unit, as revealed by X-ray studies, are presently less well established compared with those of the ribose ring. Data on deoxyribopurines are particularly scarce.[2] We feel that a detailed analysis of the geometry of the deoxyribose ring in solution must await the availability of more complete experimental results (X-ray and nmr). Nevertheless, some interesting conclusions[68] seem already warranted.

(i) The deoxyribose rings in dU, 5′-dAMP, and 3′-dAMP show N ⇄ S equilibrium compositions that are substantially biased toward the S type conformer, but a state of "conformational purity" is not attained in any of the three compounds discussed.

(ii) A substantial change in temperature (from 6 to >80° in the case of 3′-dAMP and 5′-dAMP[59] and from 23 to 80° in the case of dU[26]) causes at the most a slight shift in the coupling constants. The shift in equilibrium constant K with temperature is therefore also small, although K is far removed from unity. This suggests that the enthalpy difference between N and S conformers is close to zero, with the S form having the greater entropy.[49]

The splitting pattern of the H(1′) signal in deoxyribosides yields the *sum* of the coupling $J_{1'2'}$ (cis) + $J_{1'2''}$ (trans). Table X shows that this information already allows one to draw conclusions concerning the equilibrium composition, especially where a series of analogous compounds are compared. It is of interest to find that the values of $J_{1'2'} + J_{1'2''}$ in the common deoxyribonucleosides and their 5′-phosphates[69] (dA, dU, T, 5′-dAMP, 5′-dCMP, 5′-dUMP, and 5′-TMP) all occur in the narrow range 13.0–14.1 Hz, corresponding roughly to 65–80% of S conformer.[70] In other words, the conformational equilibrium of the deoxyribose ring in all monomers investigated thus far by nmr spectroscopy appears biased toward the same conformer that occurs in B-DNA. Paucity of nmr data of the corresponding dimers prevents a fruitful discussion of their conformational properties at this stage.

(68) Qualitative interpretations of the deoxyribose couplings in terms of representing rapid time averages of couplings due to various conformers were given by the original authors.[26,59]

(69) P. O. P. Ts'o, private communication to M. S., to be published.

Conclusion

The important result of this work lies in the recognition that application of the well-established rules of conformational dynamics and energetics,[71] combined with abundant information from X-ray studies[2] and utilizing the pseudorotation model of five-membered rings,[2,10,11] yields a self-consistent and quantitative picture of some of the conformational properties of the building blocks of RNA and, to a lesser extent, of those of DNA. For the time being we prefer to avoid interpretation of the results in terms of specific intramolecular interactions.

After completing this manuscript, we received an informative preprint from Hruska[72] concerning correlations between some ribose and side-chain nmr coupling constants in aqueous solution. His starting model is in one respect similar to ours, *i.e.*, he considers the conformational equilibrium conditions between two (not four) ribose conformers, noted + and − (equivalent to our N and S type forms, respectively).

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(70) These percentages correspond to $\Delta G°$ values between −0.4 and −0.8 kcal mol^{-1}. Of course, one realizes that even a small revision of the lower and upper limits calculated for $J_{1'2'} + J_{1'2''}$ (7.1 and 16.1 Hz, respectively) has an appreciable effect on $\Delta G°$ in these biased systems.

(71) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Wiley, New York, N. Y., 1965.

(72) F. E. Hruska, communication to M. S.