Interrelationships between the Pseudorotation Parameters P and τ_m and the Geometry of the Furanose Ring†

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Abstract: The variations of the endocyclic bond angles in furanose rings as a function of the pseudorotation parameters P (phase angle of pseudorotation) and τ_m (amplitude of puckering) have been investigated. The effects of P and τ_m on the endocyclic angles have been separately analyzed. The geometries of the various puckers along the pseudorotational wheel for any chosen amplitude of puckering can now be derived from the equations given. The importance of the amplitude of puckering and its role in conformational preferences and dynamics are emphasized. Three modes of interconversion between the preferred puckered states C(2′)-endo and C(3′)-endo are discussed: (a) pseudorotation at constant amplitude of puckering; (b) pseudorotation with variable amplitudes of puckering; (c) inversion through the planar state. One or the other of these modes of interconversion may dominate depending on the nature of the intra- and intermolecular forces on the system (e.g., ring cyclization, bulky substitution, hydrogen bonding and stacking interactions in polynucleotides).

Introduction

It has long been recognized that the furanose moiety of nucleosides and nucleotides is not planar but puckered.[1-3] The many possible puckered states are best described in the framework of the pseudorotation concept,[4-6] whereby the phase of the amplitude of puckering rotates around the ring.

In contrast to cyclopentane, where pseudorotation is free, in nucleic acid constituents pseudorotation is hindered because of the substituents and it is found that there are two preferred puckered states which involve either the C(2′) or the C(3′) atom.[3] The geometries of these preferred puckered states are well defined.[3,7] Although some unusual puckerings have been observed in the solid state, not all the puckerings encountered in the pseudorotation circuit have been observed. Thus, the geometries of several puckerings in the pseudorotation path are unknown. Knowledge of the various relationships between furanose geometry and ring pucker is essential for a derivation of the energy potential underlying the dynamics of furanose rings.

Precise structural knowledge of furanose conformations has come from single-crystal X-ray diffraction studies of nucleosides and nucleotides. However, without an adequate mathematical formulation, the consequences of puckering on bond lengths and bond angles cannot be unequivocally extracted from the X-ray data. When they introduced pseudorotation for cyclopentane, Kilpatrick et al.[4] described the atomic out-of-plane displacements in terms of puckering amplitude and phase angle of pseudorotation. However, this description is not appropriate for analysis of crystallographic structural data, since the origin plane is unknown. Geise, Altona, and Romers[8] described a method for obtaining pseudorotation parameters in terms of the ring torsion angles instead of the atomic out-of-plane displacements and which was extended by Altona and Sundaralingam[6,9] to furanose rings of nucleic acids. According to this method, from the atomic coordinates, it is possible to determine unequivocally the various kinds of puckerings by two parameters, the phase angle of pseudorotation P and the degree of pucker τ_m which is the maximum torsion angle attainable in a pseudorotational pathway.

Dunitz[10] has related mathematically the out-of-plane displacements of Kilpatrick et al.[4] with the empirical relationships between torsion angles and the pseudorotational parameters of Geise et al.[8] Using these relationships we deduced systematically the effects of puckering on endocyclic angles in furanose rings in the framework of Altona and Sundaralingam[6] from an analysis of the numerous X-ray data. Recently, Cremer and Pople[11] devised an exact method for extracting the pseudorotation parameters from atomic coordinates and which would allow an analysis of the effects of puckering on geometry to be performed according to the description of Kilpatrick et al.[4] We will show that, for practical purposes, the approach of Altona and Sundaralingam[6] is fully adequate.[12] Further, their description in terms of torsion angles is preferable because of its usage in NMR analysis of sugar conformations.[9]

In this laboratory, the variations in the endocyclic bond lengths of the furanose ring with the pucker were also examined.[13] It was observed that, except for the bond lengths involving the O(4′) atom, the endocyclic bond lengths decrease with increasing value of the torsion angle about the bond (a decrease of about 0.02 Å between 0 and 40°). This effect was attributed to the nonbonded van der Waals interactions, which are greatest in the eclipsed conformation. The coordinates of the closed furanose rings at various P and τ_m values can be obtained upon request from M.S.

Method of Analysis

With increasing deviation from planarity, the average bond angle in a pentagon has to decrease from its planar value to achieve ring closure. Let each endocyclic angle be given by $\theta_i = 108° - \delta_i$, where δ_i is the decrease in the bond angle at atom i due to puckering. From Dunitz's equations,[10] it is possible to derive the equation relating the decrease in bond angle with the phase angle of pseudorotation (P) and the amplitude of puckering (q):

$$\delta_i = q^2[0.85 - 0.95 \cos (2P - 72i)] \qquad (1)$$

where i = 0, 1, 2, 3, 4 represents respectively O(4′), C(1′), C(2′), C(3′), C(4′). We follow the arbitrary convention adopted by Altona and Sundaraligam whereby $P = 0°$ corresponds to the symmetrical twist ${}^3_2$T conformation.[14] Dunitz[10] has provided a mathematical derivation of the empirical relationships between torsion angles and the pseudorotational parameters and showed that the maximum torsion angle attainable in a pseudorotation path is related to the amplitude of puckering by $\tau_m = 2.83q$, so that eq 1 can be written (after

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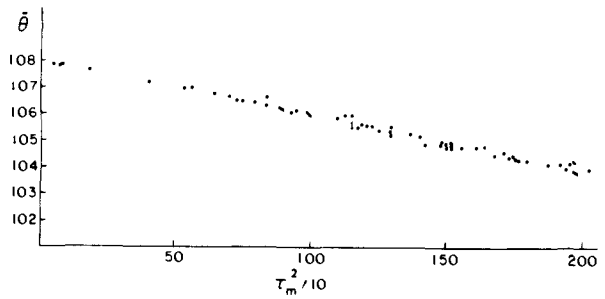
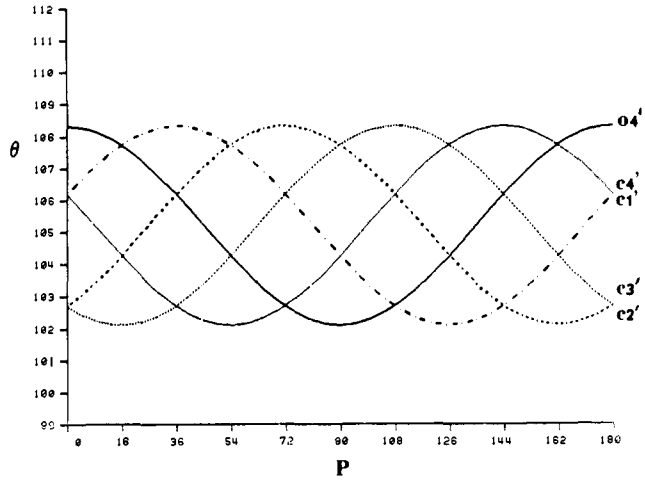


Figure 1. Plot of the average endocyclic bond angle against the square of the amplitude of puckering for a large variety of five-membered rings (furanose, oxolane, pyrrolidine rings, etc.) whose dimensions were accurately determined by X-ray crystallography. The carbocyclic rings (e.g., D ring of steroids), although appearing outside the range shown ($\tau_m^2 \sim 2500$), fall on the same line. Thus, substitution of a heteroatom for a methylene group in the ring generally flattens the ring.



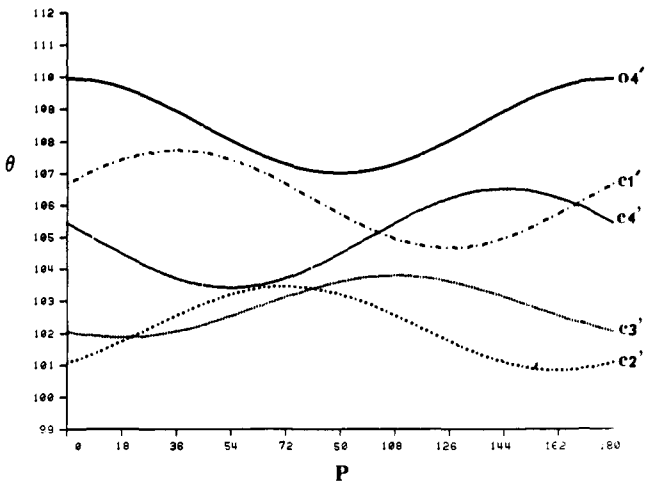


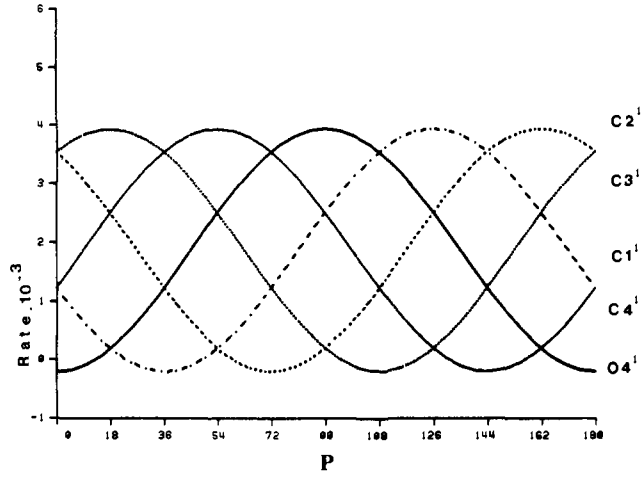
Figure 2. Theoretical (top) and experimental (bottom) variation of each endocyclic angle (θ) of a ribose ring at the mean value of amplitude of puckering, $\tau_m = 38.7°$, with the phase angle of pseudorotation, P.

conversion from radians to degrees)

$$\delta_i = \tau_m^2\,[0.00186 - 0.00207 \cos (2P - 72i)] \quad (2a)$$

$$\theta_i = 108° - \tau_m^2\,[0.00186 - 0.00207 \cos (2P - 72i)] \quad (2b)$$

Hence $\bar{\theta}$, the average bond angle, is equal to $\bar{\theta} = 108° - 0.00186\tau_m^2$ and is independent of the phase angle of pseudorotation, a result already deduced by Dunitz[10] and verified by a large number of five-membered rings. In Figure 1, we have plotted against τ_m^2 the average endocyclic bond angle of a



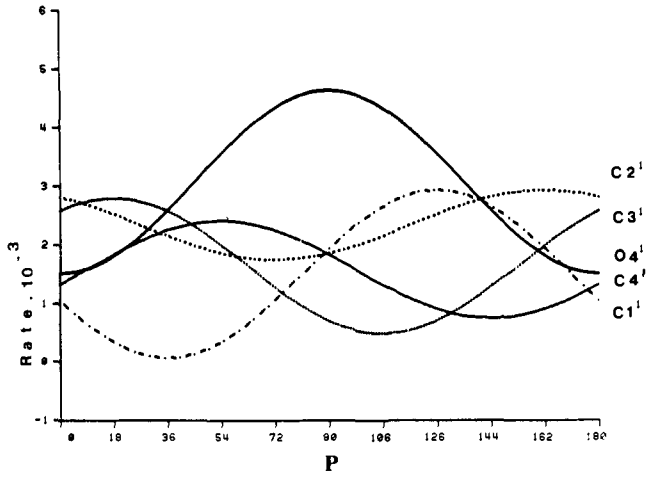


Figure 3. Theoretical (top) and experimental (bottom) variation with the phase angle of pseudorotation of the rate at which δ_i decreases with amplitude of puckering (see eq 2). Note that at each twist conformation the slope becomes negative (see ref 16).

large variety of saturated five-membered rings, as determined by X-ray crystallography. The points fall on a straight line with a slope of (-0.002 ± 0.0005), which is within one standard deviation of the value of $-0.001\,86$. It thus appears that the Altona and Sundaralingam[6] description of pseudorotation in terms of torsion angles is valid even for the finite displacements observed in crystal structures. We, therefore, extended the analysis to each endocyclic bond angle as a function of the pseudorotational parameters.

According to eq 2, in a regular pentagon, each endocyclic angle depends exclusively on τ_m^2, at a fixed value of P, with a slope depending on the chosen value of P. In the case of a pentagon with unequal bond lengths, one can write a similar equation:

$$\theta_i\,(P, \tau_m) = \theta_i^0 - \Delta_i(P)\tau_m^2 \quad (3a)$$

or

$$\theta_i\,(P, \tau_m) = \theta_i^0 - (\alpha_i + \beta_i \cos (2P - 72i))\tau_m^2 \quad (3b)$$

where θ_i^0, α_i, β_i are parameters to be determined experimentally. Since eq 3 are based on purely geometrical arguments, exo and endo puckerings are not distinguished. Consequently, in deriving eq 3 from eq 2, we assume that the effects on furanose geometry of the asymmetry (or the underlying chemical forces) introduced by the presence of the furanose oxygen and of the exocyclic substituents are incorporated into the parameters θ_i^0, α_i, β_i and that these effects are identical for exo and endo puckerings.

Table I. Values of the Coefficients A_i, B_i with Their Standard Deviations in the Equations $\theta_i = A_i + B_i \cos(2P - 72i)$, Where $i = 0, 1, 2, 3, 4$ for O(4′), C(1′), C(2′), C(3′), C(4′) for Various Fixed Values of τ_m[a]

	τ_m									
	20°		30°		38.7°		44°		ref 17	
endocyclic angle	A σ_A	B σ_B	A σ_A	B σ_B	A σ_A	B σ_B	A σ_A	B σ_B	A σ_A	B σ_B
O(4′)	111.72	−0.05	116.27	0.61	108.47	1.47	107.08	2.22	108.1	2.1
	0.3	0.5	0.15	0.2	0.1	0.1	0.2	0.3	0.2	0.2
C(1′)	107.82	−0.03	107.07	0.69	106.18	1.53	105.51	2.17	106.1	1.6
	0.07	0.09	0.05	0.06	0.01	0.01	0.05	0.07	0.1	0.2
C(2′)	104.81	0.70	103.54	0.93	102.13	1.32	101.11	1.60	102.5	1.5
	0.2	0.2	0.15	0.2	0.2	0.25	0.25	0.3	0.2	0.2
C(3′)	105.15	0.22	104.23	0.45	102.82	0.96	101.93	1.14	102.8	0.6
	0.3	0.5	0.1	0.15	0.1	0.1	0.1	0.1	0.15	0.2
C(4′)	106.62	0.71	105.86	1.09	104.95	1.55	104.28	1.91	104.9	1.5
	0.1	0.2	0.15	0.2	0.1	0.2	0.2	0.2	0.1	0.2
theoretical	107.26	0.83	106.33	1.86	105.21	3.10	104.40	4.01		

[a] The values presented in ref 17, where the effects of P and τ_m were not separated, are also given and compare well with the values for τ_m = 38.7°, which is the mean amplitude of puckering of ribofuranose rings.[6]

The method we have devised involves the following three steps:

(1) The published X-ray structures containing the ribose rings are classified according to their P values in ranges which are multiples of 18 ± 9°, i.e., in one of the five pairs of envelope conformations (^{3}E, $_3$E; ^{4}E, $_4$E; ^{0}E, $_0$E; ^{1}E, $_1$E; ^{2}E, $_2$E) or one of the five symmetrical twist conformations ($^3_2$T, $^2_3$T; $^3_4$T, $^4_3$T; $^0_4$T, $^4_0$T; $^0_1$T, $^1_0$T; $^2_1$T, $^1_2$T). Only the more accurate structures were considered (about 100 structures) and for most of these the average estimated errors in bond lengths and angles were about 0.006 Å and 0.4°, respectively.

(2) For each endocyclic angle θ_i, the dependence upon τ_m^2 for a fixed value of P is fitted to a straight line to yield θ_i and $\Delta_i(P)$:

$$\theta_i(P \text{ fixed}) = \theta_i^0 - \Delta_i(P)\tau_m^2 \qquad (4)$$

In the least-squares fitting process, each observation was given a weight inversely proportional to its standard deviation.

(3) From these equations, the dependence of the endocyclic bond angles on P at a fixed value of τ_m is obtained and fitted to an equation of the form

$$\theta_i(\tau_m \text{ fixed}) = A_i + B_i \cos(2P - 72i) \qquad (5)$$

In the least-squares fitting process at each value of τ_m, each observation was given a weight inversely proportional to the product $\tau_m\sigma_\Delta$, where σ_Δ is the standard deviation of $\Delta_i(P)$ obtained through the previous least-squares fit.

Because of the restriction of pseudorotation induced by the exocyclic substituents, the crystal structures are not equally distributed among the ten P ranges. In fact, in the P range between 54 and 126°, we could find only one ribose-containing structure. The endocyclic angles of rings in these unobserved P ranges can be derived from existing data by assuming a cosine function of period π in P. The extrapolation from known puckered states to unknown ones could be checked by considering some cyclic nucleo*side-tide* derivatives. The introduction of these usually improved the statistics and did not distort the results. Similar equations can be derived from deoxyriboses or arabinose rings. Owing to the scarcity of the data, however, this was not attempted in this paper.

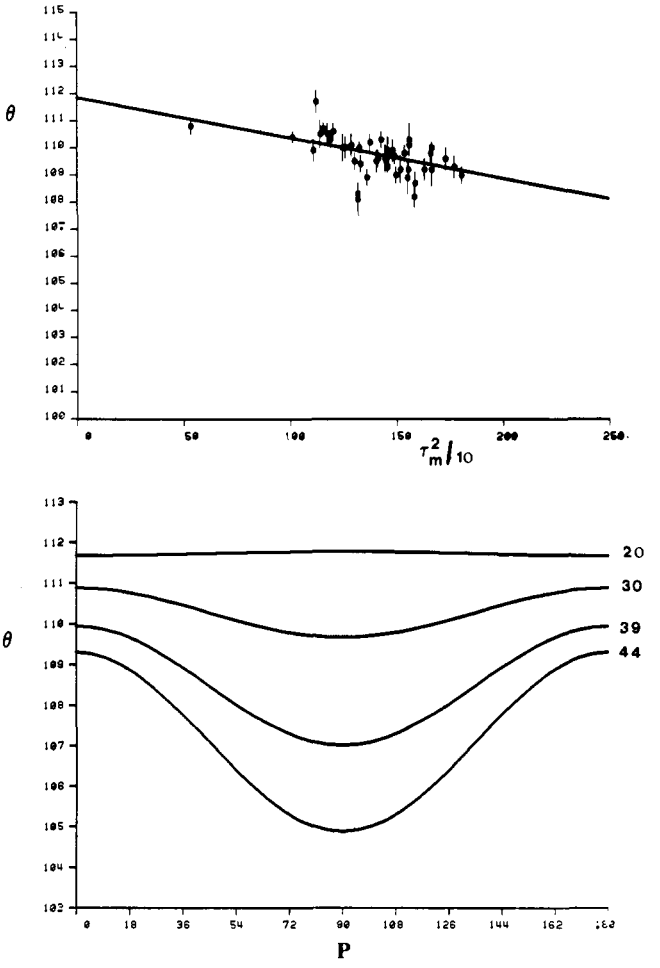


Figure 4. Top: variation of the endocyclic angle at O(4′) with the square of the amplitude of puckering for ribose rings for P values equal to 18 ± 9° (this value has been arbitrarily chosen as an illustrative example for step 2 of method). Bottom: variation of the endocyclic angle at O(4′) with the phase angle of pseudorotation for various values of τ_m (20, 30, 39, and 44°).

Results

Table I contains the values of A_i and B_i together with their standard deviations for several values of τ_m.[15] For comparison, the theoretical values deduced from eq 2b are also given. It appears that, in a ribofuranose ring, the effect of the phase angle of pseudorotation on each endocyclic angle is at all values of the amplitude of puckering much less than geometrically projected for a cyclopentane ring, since the experimentally determined B_i's are roughly half as large as theoretically projected. This is best illustrated in Figure 2, which shows the experimental and theoretical variation of the endocyclic angles at τ_m = 38.7°, the mean value of common nucleosides and nucleotides. Figure 2 shows also that, at a fixed τ_m, the amplitude of variation with P is such that it decreases in the order O(4′) ≥ C(1′) = C(4′) ≥ C(2′) ≥ C(3′) and is somewhat

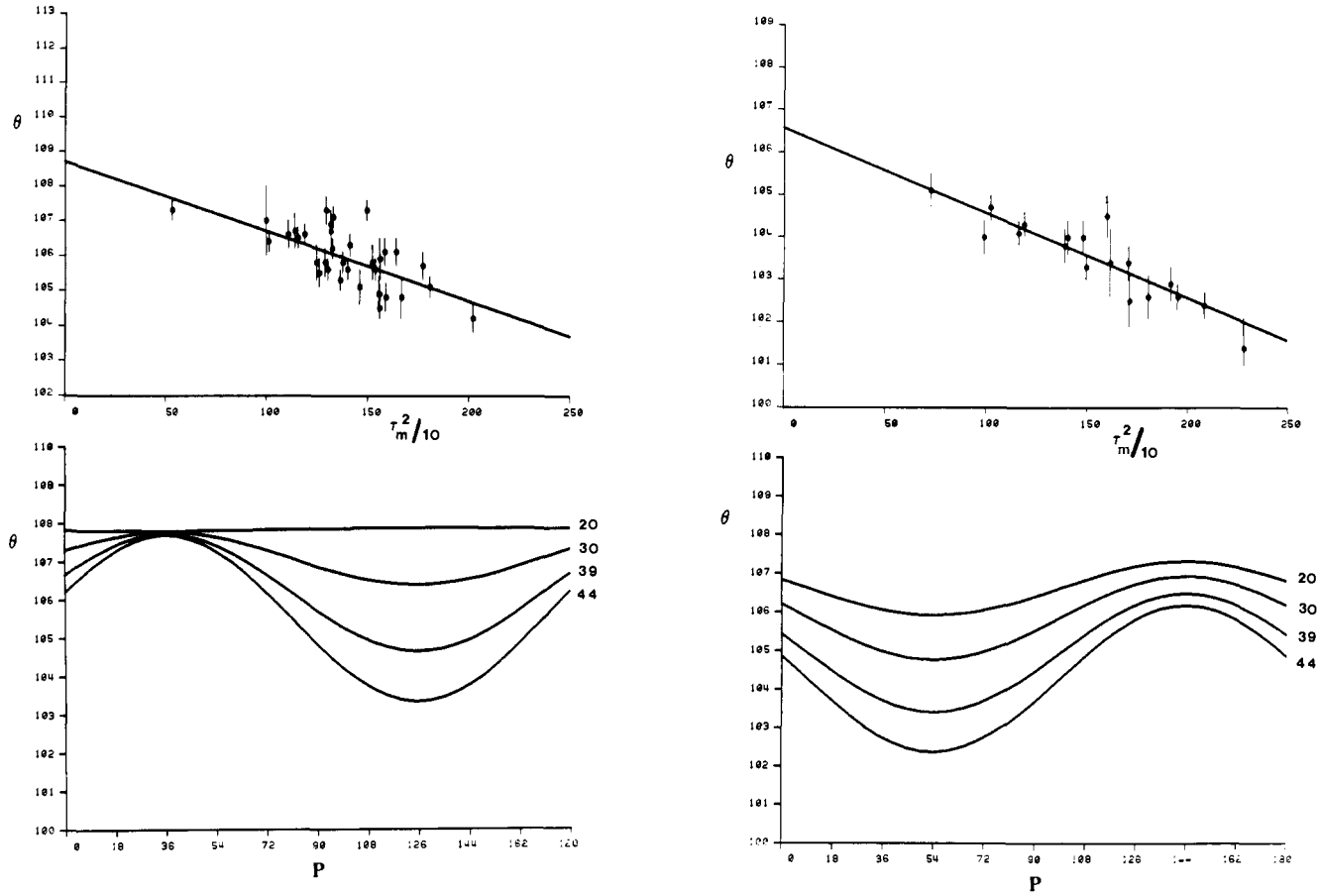


Figure 5. Left: top, variation of the endocyclic angle at C(1′) with the square of the amplitude of puckering for ribose rings for P values equal to 162 ± 9°; bottom, variation of the endocyclic angle at C(1′) with the phase angle of pseudorotation for various values of τ_m (20, 30, 39, and 44°). Right: top, variation of the endocyclic angle at C(4′) with the square of the amplitude of puckering for ribose rings for P values equal to 72 ± 9°; bottom, variation of the endocyclic angle at C(4′) with the phase angle of pseudorotation for various values of τ_m (20, 30, 39, and 44°).

Table II

compd	pucker type	av τ_m, deg	av dev, deg
3′,5′-cyclic nucleotides	^3_4T	44	1.0
2′,3′-cyclic nucleosides	^0E; $_0E$	23	1.2
6,2′-cycloarabinose nucleosides	$_4E$	43	0.4
2,2′-cycloarabinose nucleosides	^4E	25	0.5

paralleled by the absolute magnitude of the angle. This is also exemplified in Figure 3, where the theoretical and experimental variations of the rate at which Δ_i decreases with τ_m^2 are plotted against the phase angle of pseudorotation. The endocyclic angle at O(4′), which is shifted toward higher values than the tetrahedral value, has a stronger than expected variation, while the endocyclic angles at C(2′) and C(3′), which are shifted toward values much less than the tetrahedral value, have a weaker than expected variation. Geometrically, it is projected that the slope becomes negative.[16] This is not experimentally reproduced. Consequently, the endocyclic angle opposite to the bond with the highest torsion angle in a twist conformation (e.g., C(1′) in a ^3_4T, ^4_3T conformation or O(4′) in a ^2_3T, ^3_2T conformation) is not observed to increase with increasing amplitude of puckering. The decrease in the endocyclic angle of the puckered atom in an envelope conformation is, however, clearly noticeable.

The Internal C–O–C Angle at O(4′). It was noted a long time ago that the internal C–O–C angle is the only internal angle close to the tetrahedral bond angle in furanose rings.[3a] Figure 4 shows the dependence on τ_m^2 of the endocyclic angle at O(4′) at P = 18° and also the effect of P at various values of τ_m. It should be noted that, at τ_m = 38.7°, which is the average value for nucleosides and nucleotides, the variation of $\theta_{O4'}$ between P = 0° and P = 90° is about 3°. On the other hand, at τ_m = 30°, the variation is less than 2° and the value of $\theta_{O4'}$ at P = 90° is equal to that at P = 18°, τ_m = 38.7°, viz., 109.5°. Thus, other influences being equal, it could be expected that O(4′)-endo or O(4′)-exo puckerings would show a tendency for ring flattening. The mean value of the O(4′) angle in a flat ring is 112.9 ± 0.5°.

The Internal C–C–O Angles at C(1′) and C(4′). On the average, these angles have values significantly less than the tetrahedral value and which are intermediate between the internal C–O–C and C–C–C bond angles.[3a] Their behaviors upon variations in P or τ_m are, however, slightly different. Figure 5 shows the dependence on τ_m^2 and that on P for the endocyclic angles O(4′)–C(1′)–C(2′) and O(4′)–C(4′)–C(3′). At τ_m = 38.7°, the variation with the phase angle of pseudorotation of both angles is about 3°. However, at higher τ_m's, the variation of the angle at C(1′) is stronger than that of the angle at C(4′). In a flat ring, the angles at C(1′) and C(4′) would be 108.4 ± 0.5 and 107.2 ± 0.5°, respectively. It might be noted that, while the variation with P at τ_m = 38.7° is similar for the angles at O(4′), C(1′), and C(4′), in a flat ring the angle at O(4′) is about 5° larger than that at either C(1′) or C(4′).

The Internal C–C–C Angle at C(2′) and C(3′). These angles are the smallest ones in a furanose ring and are considerably less than the tetrahedral value for the most common puckerings. It was already observed that the values of the exocyclic angles around C(2′) and C(3′) depend on whether these atoms are puckered or not. Again, the variations in these bond angles

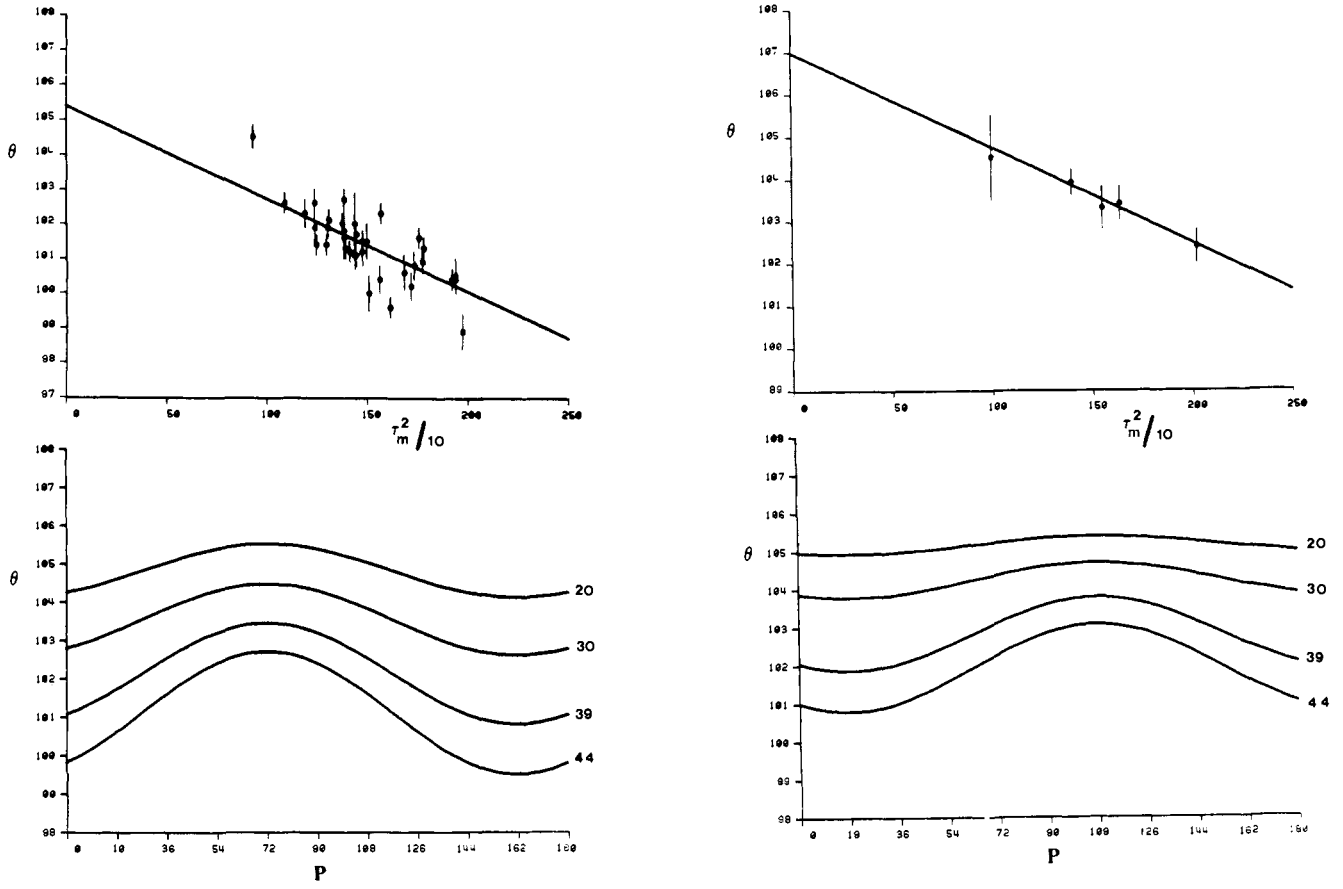


Figure 6. Left: top, variation of the endocyclic angle at C(2′) with the square of the amplitude of puckering for ribose rings for P values equal to $P = 144 \pm 9°$; bottom, variation of the endocyclic angle at C(2′) with the phase angle of pseudorotation for various values of τ_m (20, 30, 39, and 44°). Right: top, variation of the endocyclic angle at C(3′) with the square of the amplitude of puckering for ribose rings for P values equal to $P = 90 \pm 9°$; bottom, variation of the endocyclic angle at C(3′) with the phase angle of pseudorotation for various values of τ_m (20, 30, 39 and 44°).

with the pseudorotation parameters are different. At τ_m = 38.7°, the variation of C(1′)-C(2′)-C(3′) with P is less than 3° and that of C(2′)-C(3′)-C(4′) is even less than 2° (Figure 6). The dependence with P of the internal C-C-C angles is thus much less pronounced than in the case of the other endocyclic angles. In a flat ring, the angles at C(2′) and C(3′) would be 105.8 ± 0.5 and 106.0 ± 0.5°, respectively.

Recently, Murray-Rust and Motherwell[17] performed a computer analysis of numerous crystal structures of nucleosides. They analyzed the variations of the endocyclic angles of the five-membered ring with the phase angle of pseudorotation in terms of a cosine function of period π. However, these authors did not separate the effects of P and τ_m (Table I). Since they included highly substituted and cyclized structures, their conclusions were at variance with the "rigid" nucleotide concept[5,7] (see below).

Constrained Systems. Effects of Cyclization on Geometry. We have deduced the endocyclic angles of several cyclized nucleo*sides/-tides* from the aforementioned curves and compared them with the experimental values. The average value of the moduli of the differences between the observed and calculated values gives a good measure of the agreements ($\sum_{i=1}^{5}|\Delta\phi_i/5|$ where $\Delta\phi_i = \phi_i^{obsd} - \phi_i^{calcd}$). These are given in Table II. For 2,2′- and 6,2′-anhydroarabinonucleosides, the errors are about 0.5°, only slightly larger than for riboses, arabinoses, or deoxyriboses. However, for 2′,3′- and 3′,5′-cyclic nucleo*sides/-tides*, the errors are twice as large, independently of the average value of the amplitude of pucker. It would, therefore, appear that these cyclizations impose additional chemical constraints on the endocyclic angles of the five-membered ring, which are normally not present in furanose rings, as well as in 6,2′-cycloarabinose nucleosides and 2,2′-cycloarabinose nucleosides.

Importance of τ_m in Conformational Dynamics. In cyclopentane, any envelope or twist conformation has the same energy and pseudorotation is essentially free.[4] The introduction of an endocyclic substituent, like in tetrahydrofuran, leads to hindered pseudorotation, thereby stabilizing either the envelope (C_s) or the twist (C_2) conformation, with the substituent on the symmetry element, depending mainly on the difference between the torsional barriers.[18] The lowest energy path for interconversion is then usually the pseudorotation path at constant amplitude of puckering (constant τ_m) with an activation energy less than or equal to about 1.5 kcal mol^{-1}. In the presence of exocyclic substituents, as in nucleosides and nucleotides, more severe restriction of pseudorotation occurs because of nonbonded van der Waals interactions between the furanose exocyclic group (O2′ . . . O3′ at P = 90° or the base and the $-CH_2OH$ group at P = 270°). Cyclization between the exocyclic substituents of the furanose ring (e.g., 3′,5′-cyclic ribonucleotide monophosphate or 8,5′-cycloadenosine) can further restrict pseudorotation to one or two conformations, which are not necessarily observed in common nucleo*sides/-tides* (e.g., ${}^{1}_{0}T$ in the case of the 8,5′-cycloadenosine or ${}^{4}E$ and ${}_{4}E$ in the cases of the 2,2′- or 6,2′-cycloarabinonucleotides). These cases reveal that the furanose ring adapts itself to the intramolecular constraints by adopting not only preferential phase angles of pseudorotation, but also preferential amplitudes of puckering. For instance, the 2′,3′-cyclic ribonucleo*sides/-tides* are observed in various puckered states in the southern hemisphere with a much reduced amplitude of puckering compared to the common nucleo*sides/-tides* and

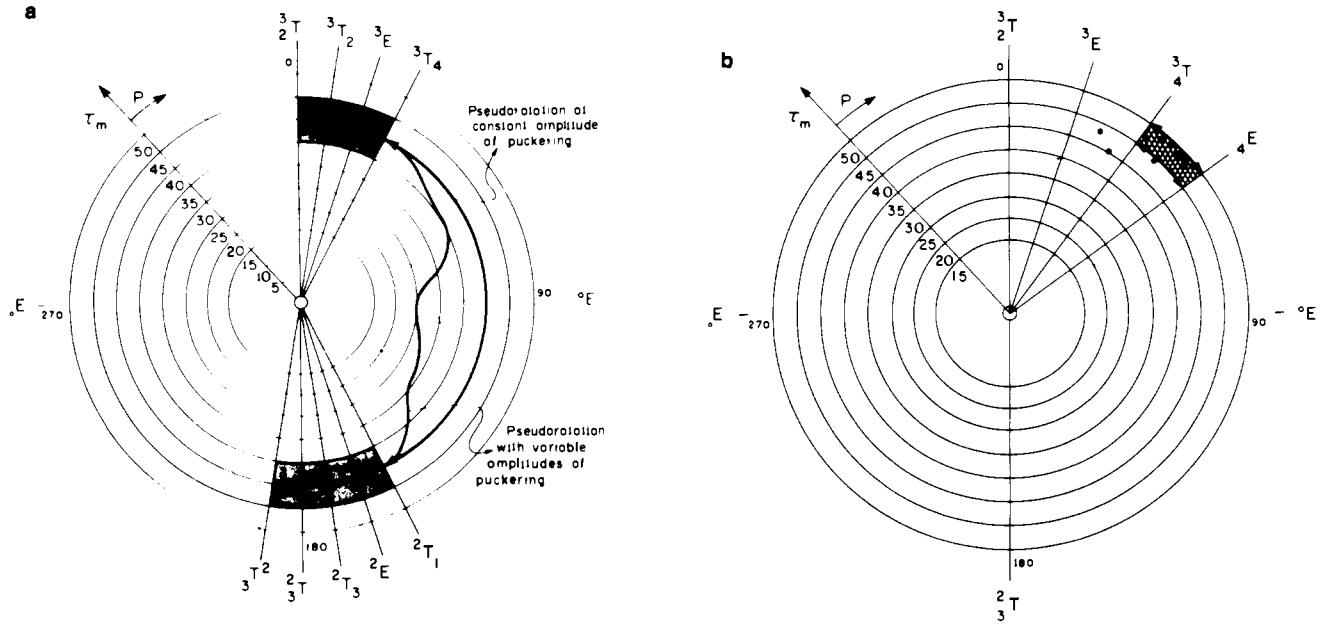


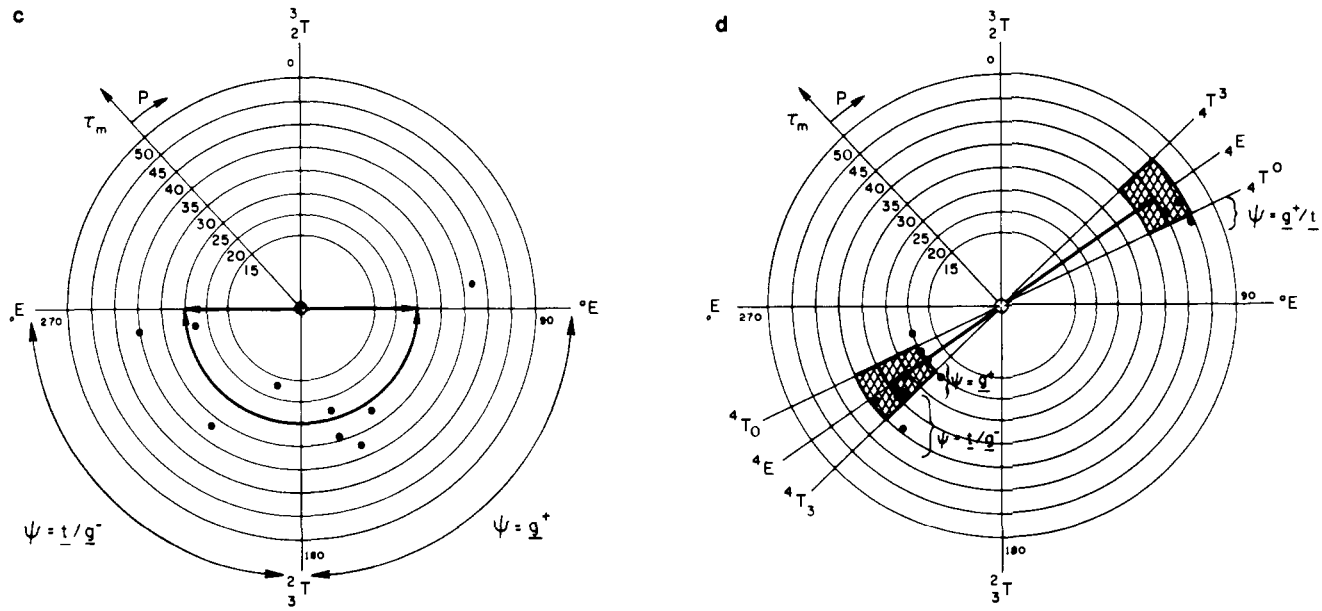
Figure 7. (**a**) The pseudorotational wheel (or (P, τ_m) plot) of furanose rings showing two pseudorotational pathways, one with constant amplitude of puckering and one with variable amplitudes of puckering. It is conceivable that the amplitude of puckering increases/decreases at some P values. The pseudorotation pathway through $P = 270°$ has not been shown, since it is thought to be energetically less favored (but not necessarily forbidden) because it requires the t (or g^-) conformation about C4′–C5′ and an expected flattening of the ring (see text). The interconversion through $P = 90°$ can occur with the preferred g^+ (or t) conformation about C4′–C5′, independently of the amplitude of puckering. The "higher energy" interconversion path through the planar state which goes through the center of the pseudorotation circle cannot be excluded. (**b**) (P, τ_m) plot of the furanose ring of 3′,5′-cyclic nucleotides which exhibit highly restricted pseudorotation. The dots represent the (P, τ_m) values of the following compounds: 3′,5′-cyclic uridine monophosphate (42, 48 and 48, 47°) (C. Coulter, *Acta Crystallogr., Sect. B*, **25**, 2055 (1969)); 5′-methylenadenosine 3′,5′-cyclic monophosphonate (37, 46°) (M. Sundaralingam and J. Abola, *J. Am. Chem. Soc.*, **94**, 5070 (1972)); 3′,5′-cyclic guanosine monophosphate (43, 44°) (A. K. Chwang and M. Sundaralingam, *Acta Crystallogr., Sect. B*, **30**, 1233 (1974)) and (44, 49°) (M. E. Druyan, M. Sparagana, and S. W. Peterson, *J. Cyclic Nucleotide Res.*, **2**, 373 (1976)); 3′,5′-cyclic inosine monophosphate (25, 42°) (unpublished results); 8-[*C*2-aminoethyl)amino]-3′,5′-cyclic adenosine monophosphate (44, 48°) (W. S. Sheldrick and E. Ricke, *Acta Crystallogr., Sect. B*, **34**, 2324 (1978)); 2′-acetyluridine-3′,5′-cyclophosphate benzyl triester (50, 47°) (W. Depmeier, J. Engels, and K. N. Klaska, *ibid.*, **33**, 2436 (1977); 5-acetyl-(3′,5′-*O*-isopropylidene-β-*O*-xylofuranosyl)uracil (31, 40°) (D. W. Jones, P. W. Rugg, G. Shaw, and J. M. Sowden, *J. Carbohydr., Nucleosides, Nucleotides*, **2**, 165 (1975)). (**c**) (P, τ_m) plot of the furanose ring of 2′,3′-cyclic nucleo*sides/-tides*, which are more flexible and can adopt a large number of conformational states ($90° \leq P \leq 270°$) and also appear to be able to interchange between them either through the pseudorotation path or by inversion through the planar state. The dots represent the (P, τ_m) values of the following compounds: 2′,3′-cyclic cytidine monophosphate (81°, 36°, and planar) (C. Coulter, *J. Am. Chem. Soc.*, **95**, 570 (1973)); 2′,3′-cyclic uridine monophosphate (174, 29°) (B. S. Reddy and W. Saenger, *Acta Crystallogr., Sect. B*, **34**, 1520 (1978)); 2′,3′-cyclophosphorothioateuridine (261, 23°)

give the only examples of planar ribofuranose rings (see Figure 7b). Also, the rigid chair conformation of the six-membered phosphate ring in 3′,5′-cyclic ribonucleotides forces the five-membered ring into a highly puckered twist conformation, C3′-endo–C4′-exo (Figure 7c). Further, it is observed that the amplitude of puckering is correlated with the conformation of an exocyclic substituent. Thus, in 2′,3′-cyclic ribonucleo*sides/-tides*, the conformation about C4′–C5′ (torsion angle ψ) is g^+ for P values between 90 and 185° and t or g^- for P values between 180 and 270° (Figure 7b). Also, the 6,2′-cycloarabinonucleosides (Figure 7c) are observed with the g^+ and t conformation about ψ and highly puckered states in the C4′-exo region (the g^- conformation is seemingly unfavored in the first quadrant, $0° \leq P \leq 90°$, because of unfavorable interactions between O5′ and O3′). However, the 2,2′-cycloarabinonucleosides, which favor C4′-endo puckers, have amplitudes of puckering higher than 30° only with the t or g^- conformation about C4′–C5′, while it is down to 20° with the g^+ conformation. (Some close contacts between O5′ and some atoms of the base exist when $\psi = g^+$ and these could be the limiting factor for the amplitude of puckering.) These observations strengthen the view that interconversion by pseudorotation occurs mainly through the O4′-endo conformation at $P = 90°$, where the C4′–C5′ bond can adopt its preferred g^+ conformation or the t conformation and show that pseudorotation through the O4′-exo conformation at $P = 270°$ necessitates not only the t or g^- conformation about ψ, but also a flattening of the ring by 10–20°.

These considerations lead to the possibility that the amplitude of puckering may vary depending on the intramolecular interactions encountered during pseudorotation. Such a variable-amplitude pathway (which in principle could also involve the planar state) may be important in the dynamics[19] of polynucleotides, since the adoption of the puckered states involving O4′, C1′, or C4′ could disrupt the base pair hydrogen bonding or/and the stacking interactions depending on the magnitude of the pucker. In Figure 7a two pseudorotation pathways are shown: one occurs at constant τ_m and the other with variable amplitudes of puckering.

Energy Barrier to Interconversion by Pseudorotation and Inversion. Several studies have attempted to compute the energy barrier to pseudorotation of the furanose moiety in β-nucleosides using either empirical partitioned potential energy functions[20] or quantum-mechanical methods.[21] The former method gave barriers of about 2.5–3.0 kcal mol^{-1} for the ribose and about 2.0 kcal mol^{-1} for the deoxyribose, while the latter method gave barriers of the order of 4 kcal mol^{-1} for the isolated ribose or deoxyribose ring and 5.0–6.0 kcal mol^{-1} for the corresponding nucleosides. In all these previous studies, the geometry of some preferred puckered state was used throughout the pseudorotation path without due consideration of the geometrical variations accompanying pseudorotation. This would be expected to give an overestimate of the energy barrier to pseudorotation. Two recent calculations are worth mentioning.

Cremer and Pople[16] have determined from ab initio calculations the potential energy surface in the (P, τ_m) space for oxolane (tetrahydrofuran) and shown that the lowest energy path for interconversion is the pseudorotation path at constant τ_m with an activation energy comprised between 0.3 and 1.3



(W. Saenger and F. Eckstein, *J. Am. Chem. Soc.*, **92**, 4712 (1970)); 2′,3′-*O*-isotropylidenadenosine (215°, 31°, and planar) (S. Sprang, O. C. Rohrer, and M. Sundaralingam, *Acta Crystallogr., Sect. B*, **34**, 2803 (1978)); 8-bromo-2′,3′-*O*-isopropylidenadenosine (145, 26°, and planar) (S. Fujii, T. Fujiwara, and K. Tomita, *Nucleic Acids Res.*, **3**, 1985 (1976)); 4,5-dimethoxycarbonyl-3-(2′,3′-*O*-isopropylidene-β-*O*-erythrofuranosyl)-1-*p*-nitrophenylpyrazole (261, 39°) (B. W. Liebich, *Acta Crystallogr., Sect. B*, **32**, 2549 (1976); 2′,3′-*O*-methoxymethyleneuridine (163, 23°) (A. J. deKok, C. Romers, H. P. M. de Leeuw, C. Altona, and J. H. van Boom, *J. Chem. Soc. Perkin Trans. 2*, 487 (1977)); 2′,3′-*O*-(2 carboxyethyl)ethylidenadenosine (198, 17°) (D. A. Jamiak, M. Noltemeyer, W. Saenger, and F. Seela, *Z. Naturforsch. C*, **33**, 169 (1978)). (**d**) (P, τ_m) plot of the arabinose ring of 2,2′- and 6,2′- or 8,2′-arabinosyl cyclic nucleosides. The interconversion between the two preferred domains occurs probably exclusively through the planar state (see also ref 31). The dots represent the (P, τ_m) values of the following compounds: 2,2′-anhydroarabinofuranosyluracil (227, 29 and 213, 34°) (D. Suck and W. Saenger, *Acta Crystallogr., Sect. B*, **29**, 1323 (1973)); 2,2′-anhydroarabinofuranosylcytosine (233, 27°) (T. Brennan and M. Sundaralingam, *Biochem. Biophys. Res. Commun.*, **52**, 1348 (1973)); 2,2′-anhydroarabinofuranosyl-3-deazauridine (69, 40 and 230, 27°) (W. L. B. Hutcheon and M. N. G. James, *Acta Crystallogr., Sect. B*, **33**, 2228 (1977)); 2,2′-anhydro-1,3′,5′-di-*O*-acetyl-β-*O*-arabinofuranosyl-5-chloro-6-oxocytosine (232, 18°) (Y. Yamagata, M. Koshibe, R. Tokuoka, S. Fujii, T. Fujiwara, T. Kanai, and K. Tomita, *ibid.*, **35**, 382 (1979)); 2,2′-anhydro-1-β-*O*-arabinofuranosyl-6-oxocytosine-5-dimethylsulfonium chloride (262, 21°) (see preceding reference); 2,2′-anhydro-1-β-*O*-arabinofuranosylcytosine 3′,5′-diphosphate (247, 22°) (Y. Yamagata, Y. Susuki, S. Fujii, T. Fujiwara, and K. Tomita, *Acta Crystallogr., Sect. B*, **35**, 1136 (1979)); 6,2′-anhydro-1-β-*O*-arabinofuranosyl-6-hydroxycytosine (63, 44°) (Y. Yamagata, S. Fujii, K. Kunai, K. Ogawa, and K. Tomita, *ibid.*, **35**, 378 (1979)); 7,2′-anhydro-β,*O*-arabinofuranosylorotidine (63, 44°) (J. L. Smith, Ph.D. Thesis, University of Wisconsin—Madison, 1978); 8,2′-anhydro-8-mercapto-9-β-*O*-arabinofuranosyladenine 5′-monophosphate (233, 33°) (K. Tanaka, S. Fujii, T. Fujiwara, and K. Tomita, *Acta Crystallogr., Sect. B*, **35**, 929 (1979)); 8,2′-anhydro-*O*-cycloarabinofuranosyladenine (217, 19°) (S. Neidle, G. L. Taylor, and P. C. Cowling, *ibid.*, **35**, 708 (1979)).

kcal mol^{-1}, depending on the type of basis set chosen. The energy barriers for interconversion through the planar state (inversion barrier) were calculated to be 1.2 and 2.6 kcal mol^{-1} for the C_s and C_2 conformation, respectively. Incidentally, it can be added that the bond angles deduced from their calculations are in good agreement with those inferred from our curves. The endocyclic angles at O(4′) are 113.11, 111.6, and 106.4° for a planar oxolane ring, the C_2 twist (P = 0°, τ_m = 38°), and the C_s envelope (P = 90°, τ_m = 37.5°), respectively. Our curves yield the following values: 113.1, 109.8, and 107.1°.

On the basis of consistent force field calculations, Levitt and Warshel[22] argued recently that the energy varies by only 0.5 kcal mol^{-1} along the pseudorotation path through O(4′)-endo in riboses and deoxyriboses and states that this leads to "extreme conformational flexibility" of the sugar ring. We have computed the geometries of the relaxed conformations published by these authors and noticed several values in marked disagreement with the values expected from our analysis. The largest and most serious discrepancy occurs in the values of the endocyclic angle at O(4′) for most conformations, which probably accounts for their low estimate of the activation energy of pseudorotation.

Thus, for the C(3′)-endo ribose with τ_m = 38.7° the endocyclic angle at O(4′) is 115.4°, for the C(2′)-endo ribose with τ_m = 40.4°, it is 116.3°, and for the O(4′)-endo ribose with τ_m = 41.6°, it is 112°. The values of the two preferred conformers and of the O(4′)-endo conformer are 5–6° larger than expected from the curves deduced above. The inversion barrier calculated by these authors is 3.5 kcal mol^{-1}.

NMR measurements of ^{13}C longitudinal relaxation rates have given an activation energy for internal motions associated with the ribose carbons in purine *ribo*nucleosides of 4.0–5.0 kcal mol^{-1}, independently of the relative population of the C3′-endo and C2′-endo puckered states.[23] It is not clear whether this activation energy is related to the pseudorotation barrier or to the inversion barrier. A similar activation energy, 5.21 kcal mol^{-1}, has been measured for the inversion process in cyclopentane.[32] To date, only one ribofuranose ring has been observed in the O4′-endo pucker, while four 2′-deoxyribofuranose rings have been observed with P values about 90°. Except for one case,[24] the compounds have unusual bases[25-27] or an unusual exocyclic substituent.[28] These results indicate that unusual puckerings (P values between 59 and 126°) may be adopted by both riboses and deoxyriboses, particularly under proper modifications, with the deoxyriboses showing higher propensity than the riboses.

In a work already mentioned,[17] the fact that a large portion of the pseudorotational wheel is accessible to the furanose ring was considered in opposition to the rigid" nucleotide concept.[5,7] This conclusion is based on two misunderstandings. The first misunderstanding results from the fact that the "rigid" nucleotide concept was not devised to be applied to chemically constrained systems. Secondly, the "rigid" nucleotide concept does not contend that the common nucleotides are rigidly held in one conformation. It contends, on the contrary, that nucleotides have preferred conformational domains for each torsion angle and that nucleotides have a restrained flexibility within each of these domains. The "rigid" nucleotide concept does not forbid less preferred conformations like the O4′-endo

pucker; it only considers them less likely to occur. The inherently preferred conformers lead to the secondary helical structures commonly observed and the restrained flexibility in each torsion angle leads to a variety of possible tertiary superhelical structures, while the less preferred conformers are found in loops and bends necessary for complex tertiary structures.[29,30]

Conclusions

The furanose ring plays a pivotal role in the conformations and topology adopted by nucleic acids. A precise knowledge of the furanose geometry is thus necessary for investigating the effects of the two pseudorotation parameters, P and τ_m, on the helical and superhelical parameters of nucleic acids as well as for understanding the dynamics of the conformational variations and transitions observed in nucleic acids. Equations have been developed in this paper correlating the geometries of the furanose ring with the pseudorotation parameters. Whenever the furanose geometry is altered, either in energy calculations or in structural studies, one should guarantee that the resulting geometry of the altered ring conforms to the restraints outlined here.

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(15) A comparison between eq 3b and 5 gives $A_i = \theta_i^0 - \alpha_i\tau_m^2$ and $B_i = -\beta_i\tau_m^2$. The first relationship is obeyed and yields the endocyclic angles of a hypothetical planar furanose ring. However, it is not possible to obtain values for β_i independent of τ_m.
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