Interdependence of Ring Conformations of 2',3'-Cyclic Mononucleotides by Nuclear Magnetic Resonance Spectroscopy

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The inter-relation between the conformational properties of the sugar and phosphate rings of 2',3'-cyclic mononucleotides has been investigated using ^{1}H and ^{13}C n.m.r. results. ^{13}C N.m.r. measurements of $^{3}J(^{31}P, ^{13}C)$ magnitudes have been made on a number of 2',3'-cyclic mononucleotides to supplement results available in the literature as well as those for $^{3}J(H,H)$ and $^{3}J(^{1}H, ^{31}P)$ in the same systems. It is found that certain spin coupling constants are constant, *i.e.* $J_{2',3'}$ 6.8 ± 0.1 ; $(J_{2',P} + J_{3',P})$ 18.4 ± 0.1 ; $(J_{1',2'} + J_{3',4'})$ 8.2 ± 0.3; and $(J_{P,C\text{-}4'} + J_{P,C\text{-}1'})$ 8.3 ± 0.4 Hz. The coupling constant behaviour does not support conformational models suggested by *X*-ray crystallographic results but it is consistent with a two-state symmetrical conformational model for both five-membered rings. Analysis of spin-coupling constants reveals that the puckering of the sugar and phosphate rings is about the same and that the conformational equilibria of both rings are related. It is shown that there is a direct correlation between sugar and phosphate ring conformations in which the cyclic phosphate adopts the O-3'-*endo*,O-2'-*exo* ($^{3}O_{2}$) conformation for the sugar ring in the N-type (C-3'-*endo*, C-2'-*exo*; $^{3}T_{2}$) conformation and the O-2'-*endo*, O-3'-*exo* ($^{2}O_{3}$) conformation for the sugar ring in the S-type (C-2'-*endo*, C-3'-*exo*; $^{2}T_{3}$) conformation. One consequence of the quantitative rationalisation of the conformational behaviour of 2',3'-cyclic mononucleotides is that it leads to the empirical determination of the electronegative substituent effect of the glycosyl nitrogen atom at C-1' for both ^{1}H–^{1}H ($J_{1',2'}$, δJ 0.7 Hz) and ^{31}P–^{13}C ($J_{P,C\text{-}1'}$, δJ −1.1 Hz) vicinal coupling paths.

Cyclic 2',3'-mononucleotides are intermediates in the hydrolysis of ribonucleic acids catalysed by pancreatic ribonuclease and a knowledge of their conformations is relevant to an understanding of the mechanism for RNase catalysis.[1] The structures and conformations of 2',3'-cyclic mononucleotides have been observed in the solid state by *X*-ray crystallography,[2–4] in solution by various spectroscopic methods [5–12] and have been the subject of theoretical calculations.[13,14] The results of *X*-ray crystallographic studies show that the sugar ring may exhibit a range of conformations varying from a normal N-type conformation (^{2}E; 2',3'-cCMP, free acid),[4] to the more unusual ^{0}E (O-4'-*endo*; 2',3'-cCMP) [3] and $_{0}E$ (O-4'-*exo*; 2',3'-UMP, S analogue) [2] conformations and even to an example of a planar sugar ring (2',3'-CMP); [3] these results indicate that crystal packing forces may be dominant in determining the conformation of the 2',3'-cyclic mononucleotides. In the solid state the cyclic phosphate ring is also puckered though the phosphate ring exhibits essentially one puckered conformation.

By analysis of ^{1}H, ^{13}C, and ^{31}P n.m.r. shift and coupling constant data a general picture has emerged in which the sugar ring and phosphate ring are deemed to be flexible and in rapid equilibrium between different puckered forms which vary little on changing the pH from 7.2 to 2.3 or on addition of lanthanide ions at the latter pH.[11,12] It was found that the degree of puckering of the sugar ring of 2',3'-cyclic mononucleotides is smaller than that observed for normal nucleosides and nucleotides.[11] On the other hand the N and S conformations of the sugar rings are similar with the N $\rightleftharpoons$ S equilibrium for pyrimidine 2',3'-cyclic nucleotides (60 : 40) being slightly different from the purine derivative (50 : 50).[11] Analysis of the phosphate ring conformation from lanthanide probe measurements indicated that an equilibrium might exist between different puckered forms but that closest fit to results was given by the ring being planar on average.[11]

There is a marked discrepancy between the results of *X*-ray crystallographic studies of 2',3'-cyclic mononucleotides and n.m.r. studies of the molecules in solution. From an analysis of these crystal structures and those for other 2',3'-bridged nucleosides, it was suggested by Murray-Rust and Motherwell [15] that the extra ring connecting C-2' and C-3' prevents pseudorotation of the sugar ring and an alternative path-way for ring deformation may have the sugar ring undergoing an equilibrium between envelope and planar structure, *i.e.* equilibria (1).[15]

$$^{0}E\ (\text{O-4'-}endo) \rightleftharpoons \text{planar} \rightleftharpoons {}_{0}E\ (\text{O-4'-}exo) \quad (1)$$

A more recent analysis of crystal structure results by Altona and his co-workers [16] indicated that, though this pathway may be important in furanosides with *cis*-fused five-membered rings, it would not apply to free nucleosides as suggested by the work of Murray-Rust and Motherwell.[15] The discrepancy between the results from n.m.r. and *X*-ray crystallographic studies is explored in this work using the wealth of n.m.r. spin-coupling constant data in the literature (proton–proton,[5,11] proton–phosphorus,[5,11] and carbon–phosphorus [5,7]) supplemented by present measurements of carbon–phosphorus spin coupling constants. In order to make meaningful comparisons of conformations derived from spin-coupling data and conclusions derived from lanthanide probe measurements, ^{13}C n.m.r. measurements have been made of 2',3'-cyclic mononucleotides under the same conditions used for the ^{1}H n.m.r. lanthanide probe measurements. The results do not agree with the conformations observed in the solid state [2–4] for either the sugar or phosphate rings but are closer to those determined in solution by lanthanide ion probe methods, except for the phosphate ring. By adopting a two-state symmetrical conformational model for both five-membered rings it is shown quantitatively that the conformations of both rings are directly interdependent and that derivation of the inter-relationship leads to an estimate of the purine and pyrimidine substituent electronegativity effect on vicinal coupling constants involving the glycosyl bond, *i.e.* $J_{1',2'}$ and $J_{P,C\text{-}1'}$.

Experimental

The 2',3'-cyclic mononucleotides of the common purine (adenine, guanine) and pyrimidine (cytosine, uracil) base

Table 1. Coupling constants of 2'3'-cyclic mononucleotides [a]

	Cation	T/K	pH	Sugar ring ^3J(HH)/Hz			
				1',2'	2',3'	3',4'	(1',2' + 3',4')
2',3'-UMP	Ba	303	7.2	3.0	6.9	5.5	8.5
		305	8.2	3.1 [b]	7.0	5.8	8.9
2'3'-CMP	Na	303	7.2	2.7	6.7	5.4	8.1
		305	7.3	3.1 [b]	6.9	5.4	8.5
			2.3	2.6	6.8	5.3	7.9
(+La^{III}) [d]			2.3	2.2	6.6	5.1	7.3 [d]
2',3'-m^{6}UMP		298		1.5	6.5	6.5	8.0
2',3'-AMP	Na	303	7.2	4.4	6.8	3.9	8.3
			2.3	3.5	6.8	4.6	8.1
(+La^{III}) [d]			2.3	3.5	6.6	4.4	7.9 [d]
	Na	303	7.2	3.7	6.8	4.4	8.1
	Pyr [c]	303	7.2	3.4	6.8	4.2	7.6
Average					6.8 (±0.1)		8.2 (±0.3)

	Cyclic phosphate							
	^3J(HP)/Hz				^3J(PC)/Hz			
	2',P	3',P	(2',P + 3',P)	Ref.	P,C-1'	P,C-4'	(P,C-1' + P,C-4')	Ref.
2',3'-UMP	6.9	11.5	18.4	5	6.8	2.5	9.3	5
				6	6.1	2.4	8.5	*e*
2'3'-CMP	6.5	12.1	18.6	5	6.3	2.2	8.5	5
				6	6.1	2.5	8.6	*e*
	7.5	10.7	18.2	11	6.1	2.4	8.5	*e*
(+La^{III}) [d]	8.8	11.0	19.8 [d]	11	5.5	2.5	8.0	*e*
2',3'-m^{6}UMP	4.2	14.6	18.8	*f*				
2',3'-AMP	10.6	7.6	18.2	5	3.8	4.3	8.1	5
	8.8	9.7	18.5	11	4.3	3.1	7.4	*e*
(+La^{III}) [d]	9.2	9.9	19.1 [d]	11	4.3	3.7	8.0	*e*
	8.9	9.5	18.4	5	4.8	3.5	8.3	5
	9.1	9.3	18.4	5				
Average			18.4 (±0.1)				8.3 (±0.4)	

[a] 100, 220, or 270 MHz ^{1}H n.m.r. spectra of cyclic nucleotides in D_2O solution with δ and J checked by computer simulation. 25 (ref. 5) or 15 MHz (this work) noise decoupled ^{13}C n.m.r. spectra. [b] $J(^{31}P$, H-1') 0.75 Hz. [c] Pyridinium salt. [d] La^{III} results omitted from average. [e] This work. [f] A. Rabczenko, personal communication.

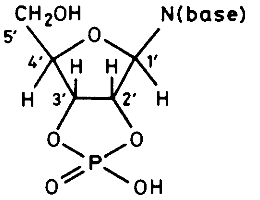

Figure 1. Molecular structure and atom numbering for 2',3'-cyclic mononucleotides

rings were purchased from Sigma Chemical Company. 100 mM solutions were prepared in ^2H_2O (used for internal lock) and the solution adjusted to the appropriate p^{2}H- (p^{2}H = pH + 0.4)[17] with NaO^{2}H and ^{2}HCl. Europium(III) chloride was used to make the solutions *ca.* 0.2M in lanthanide. It was not deemed necessary to use other lanthanides as previous work with ^{1}H n.m.r. showed that binding of La^{III} (80% of complex formed) caused only minor changes in ^{1}H–^{1}H and ^{1}H–^{31}P spin-coupling constants.[11]

Proton noise-decoupled 15 MHz ^{13}C n.m.r. spectra of the 2',3'-cyclic mononucleotides (at *ca.* 35 °C) were measured on a JEOLF X60 n.m.r. spectrometer. About 1 000 f.i.d.s were collected and the Fourier transform spectrum was calculated with minimum exponential weighting function so that small couplings (*ca.* 1 Hz) could be measured. Signal assignments were made by comparison with previous work[5] and the $^3J(^{13}C, ^{31}P)$ magnitudes of the nucleotides measured under the same solution conditions as ^{1}H n.m.r. measurements are listed in Table 1.

Discussion

The bicyclic structure of 2',3'-cyclic mononucleotides is shown in Figure 1 together with the atomic numbering scheme. The vicinal spin-coupling constants relevant to the conformational analysis of the sugar and phosphate rings of 2',3'-cyclic nucleotides are summarised in Table 1. The ^{1}H–^{1}H coupling constants monitor the conformations of the C-1'–C-2', C-2'–C-3' and C-3'–C-4' bonds of the sugar ring whereas the ^{1}H–^{31}P ($J_{2',P}$ and $J_{3',P}$) and ^{13}C–^{31}P ($J_{P,C-1'}$ and

$J_{P,C\text{-}4'}$) monitor the conformations of the C-2′–O-2′ and C-3′–O-3′ bonds and, together with $J_{2',3'}$, monitor the conformation of the cyclic phosphate ring. The results in Table 1 show that a number of key coupling constants remain constant for the series of molecules, *i.e.* $J_{2',3'}$ 6.8 ± 0.1, ($J_{2',P} + J_{3',P}$) 18.4 ± 0.1, ($J_{1',2'} + J_{3',4'}$) 8.2 ± 0.3, and ($J_{P,C\text{-}1'} + J_{P,C\text{-}4'}$) 8.3 ± 0.4 Hz. One might expect the constant magnitudes of these coupling constants to be an important feature of the conformational analysis of these molecules.

(1) *Conformational Models.*—(a) *Crystal structures.* The conformational model [15] suggested by consideration of crystal structure results for 2′,3′-cyclic mononucleotides and compounds similarly constrained by isopropylidene groups summarised in equation (1) is not consistent with the n.m.r. results for molecules in solution. The model predicts that (i) $J_{2',3'}$ is constant and independent of the conformational equilibrium, (ii) the magnitudes of $J_{1',2'}$ would always equal $J_{3',4'}$, excluding electronegativity effects on $J_{1',2'}$, and (iii) ($J_{1',2'} + J_{3',4'}$) is expected to vary with the populations of individual conformers.

Inspection of the results summarised in Table 1 shows that this situation is not observed for prediction (ii) and (iii) though it is for (i).

An analogous set of predictions would apply to the coupling constants that monitor the conformations of the phosphate ring if ring deformation between analogous puckered forms also occurred *via* a planar conformation: (iv) magnitudes of $J_{2',P} = J_{3',P}$ and $J_{P,C\text{-}1'} = J_{P,C\text{-}4'}$, excluding electronegativity effects on $J_{P,C\text{-}1'}$ and (v) ($J_{2',P} + J_{3',P}$) and ($J_{P,C\text{-}1'} + J_{P,C\text{-}4'}$) are expected to vary with conformer populations for a series of molecules. Again the results in Table 1 do not conform to such predictions and so the model in which conformational interconversion proceeds *via* a planar conformation is not valid for either five-membered ring of the 2′,3′-cyclic mononucleotides in solution.

(b) *Pseudorotational analysis.* It was shown by Altona and Sundaralingam [18,19] that one condition of the pseudorotational analysis applied to ‘ normal ’ ribose and deoxyribose rings is that $J_{2',3'}$ and ($J_{1',2'} + J_{3',4'}$) are constant. This same behaviour is found for the ribose ring in the bicyclic ring structure of 2′,3′-cyclic mononucleotides (Table 1) so one might expect that the sugar ring could be analysed in terms of its pseudorotational conformational properties bearing in mind the limitation that the solid state criteria upon which the pseudorotational analysis is based are not met by the 2′,3′-cyclic mononucleotides. The magnitudes of the degree of pucker (denoted by ψ_m) and phase angle of pseudorotation (denoted by *P*) of the sugar ring calculated from the ^{1}H n.m.r. results of 2′,3′-cyclic mononucleotides show that the degree of puckering for the sugar ring of 2′,3′-cyclic mononucleotides (ψ_m *ca.* 31°) is smaller than that found for ‘ normal ’ ribonucleotides (ψ_m *ca.* 39°),[19] and the pseudorotational angles (^{N}P *ca.* 6 and ^{S}P *ca.* 174°) that differ from those found for ‘ normal ’ ribonucleotides (^{N}P *ca.* 18 and ^{S}P *ca.* 162°).[19] The differences are outside experimental error as the variations of *P* and ψ_m from the averaged values are small, *i.e.* 5 in *P* and 1° in ψ_m. The smaller degree of puckering of the sugar ring when constrained by the 2′,3′-cyclic phosphate group for the mononucleotides in solution (31° *versus* 39°) is similar to that observed in the solid state for 2′,3′-cyclic mononucleotides and 2′,3′-isopropylidene nucleosides (29—32°, Table 5 of ref. 16) compared to ribonucleosides (36—41°, Table 4 of ref. 16). The changes in pseudorotational angles for the molecules in solution represent a displacement of the N conformer from a ‘ normal ’ ^{3}E conformation towards $^{3}_{2}T$ and a displacement of the S conformer from a ‘ normal ’ ^{2}E conformation towards $^{2}_{3}T$ for the 2′,3′-cyclic phosphate derivative. In principle, a pseudorotational description of the phosphate ring, analogous to that for the sugar ring, can be determined from $J_{2',3'}$ and both ($J_{2',P} + J_{3',P}$) and ($J_{P,C\text{-}4'} + J_{P,C\text{-}1'}$) magnitudes, if the Karplus relations for coupling in the appropriate coupling paths are sufficiently well characterised. Such a description is not necessary for the purpose of the present work where a simpler two-state symmetrical conformational model is used for both rings as it is adequate to interpret the observed vicinal spin-coupling constants and to develop the relationship between the conformations of both rings.

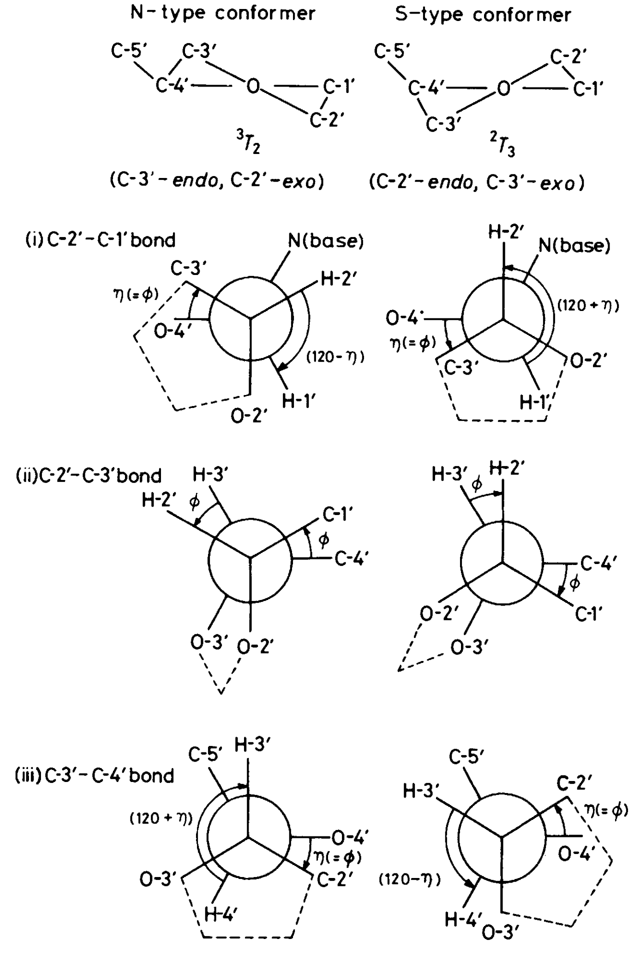

Figure 2. Bond rotations for a two-state conformational model of the β-D-ribofuranose ring of 2′,3′-cyclic mononucleotides

(c) *Two-state model.* The conformation of the sugar ring is described by rotation about five bonds but only the three bonds sensitive to vicinal coupling constants (C-1′–C-2′, C-2′–C-3′, C-3′–C-4′) are shown in Figure 2 for the two states labelled N-type (C-3′-*endo*, C-2′-*exo*; $^3T_2$) and S-type (C-2′-*endo*, C-3′-*exo*; $^2T_3$) conformations. The two states are represented by angles η (C-2′–C-1′ and C-3′–C-4′ bonds) and φ (C-2′–C-3′ bond) which are zero for the planar conformation. It is shown that φ can be determined from $J_{2',3'}$ and η from ($J_{1',2'} + J_{3',4'}$) observations and that magnitudes of φ and η have about the same values. The angles may be positive or negative corresponding to the two main puckered conformations and both are determined from observed ^3J because of the cosine dependence of the Karplus relation [20] shown in its generalised form [21] in equation (2) where θ represents a dihedral angle.

$$^{3}J = A\cos^{2}\theta - B\cos\theta + C \qquad (2)$$

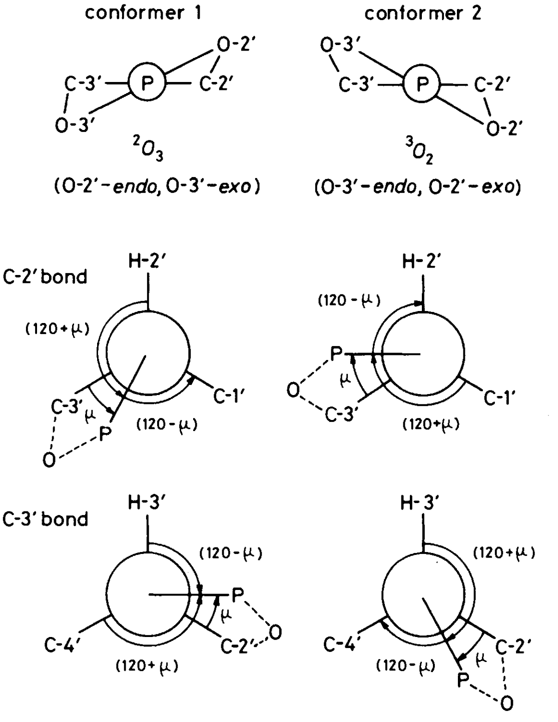

Figure 3. Bond rotations for a two-state conformational model of the phosphate ring of 2',3'-cyclic mononucleotides

The conformation of the phosphate ring is described by rotation about five bonds but only three are sensitive to vicinal coupling constants. The C-2'–C-3' bond is common to both rings and the two other bonds (C-2'–O-2' and C-3'–O-3' shown in Figure 3) are sensitive to ^1H–^{31}P and ^{13}C–^{31}P vicinal coupling constants. The angle μ represents the twist of the C-2'–O-2' and C-3'–O-3' bonds and is zero for a planar ring conformation. It is shown that μ may be determined from both $(J_{2',P} + J_{3',P})$ and $(J_{P,C\text{-}1'} + J_{P,C\text{-}4'})$ magnitudes and that the calculated values are also similar to φ. Again, the positive and negative angles correspond to the two main puckered conformations of the phosphate ring which are described, for convenience, as O-2'-*endo*, O-3'-*exo* (denoted $^2O_3$) and O-3'-*endo*, O-2'-*exo* (denoted $^3O_2$) conformations.

(2) *Sugar Ring*.—Let φ_N and φ_S represent the angles of twist for the N and S conformers, respectively, of the C-2'–C-3' bond. For a symmetrical two state model ($\varphi_N = \varphi_S = \varphi$) it is predicted that $J_{2',3'}$ magnitudes should be constant and independent of the sugar ring conformational equilibrium according to equation (3) where p_N and p_S represent the

$$J_{2',3'} = p_N J(\varphi_N) + p_S J(\varphi_S) = J(\varphi) \qquad (3)$$

relative populations of N and S conformers, respectively ($p_N + p_S = 1$). Using Karplus parameters for proton coupling constants (*A* 10.2, *B* 0.8, *C* 0 Hz)[10] derived for nucleosides and nucleotides by the method of Altona and Sundaralingam[19] the observed magnitude of $J_{2',3'}$ 6.8 Hz for the 2',3'-cyclic mononucleotides (Table 2) corresponds to φ ±31° (or ±141° which is inadmissible in the bicyclic structure). The magnitude of φ is less than that observed for ' normal ' ribose rings ($J_{2',3'}$ 5.2 Hz, φ ±41°) and consistent with results derived by pseudorotational analysis of the sugar ring.[10,19]

The sugar ring conformational behaviour is sensitive to observed $J_{1',2'}$ and $J_{3',4'}$ according to equation (4) where θ_N and θ_S represent the dihedral angles between vicinal protons in the N and S conformers.

$$J_{1',2'} = p_N J[\theta_N(1',2')] + p_S J[\theta_S(1',2')]$$

$$J_{3',4'} = p_N J[\theta_N(3',4')] + p_S J[\theta_S(3',4')] \qquad (4)$$

For a symmetrical conformational model equation (4) is simplified because of relations (5). Using equations (4) and (5) it is predicted that the sum of

$$\theta_N(1',2') = \theta_S(3',4') = (120 - \eta) \qquad (5a)$$

$$\theta_S(1',2') = \theta_N(3',4') = (120 + \eta) \qquad (5b)$$

$(J_{1',2'} + J_{3',4'})$ is constant and independent of conformer populations according to equation (6).

$$\Sigma J = (J_{1',2'} + J_{3',4'}) = J(120 - \eta) + J(120 + \eta) = -A\cos^2\eta + B\cos\eta + (3A/2 + 2C) \qquad (6)$$

The coupling constants observed for 2',3'-cyclic mononucleotides (Table 1) exhibit a constant $(J_{1',2'} + J_{3',4'})$ which is consistent with the use of a symmetrical two-state conformational model for the sugar ring of 2',3'-cyclic mononucleotides. Using the average $(J_{1',2'} + J_{3',4'}) = 8.2$ (±0.3) Hz observed for the 2',3'-cyclic mononucleotides, the angle η is calculated to be 29(±2)° which is similar in magnitude and within experimental error of φ (*ca.* 31°) calculated from $J_{2',3'}$ magnitudes.

(3) *Phosphate Ring*.—The conformation of the phosphorinan ring is described by rotation about five bonds but only the three bonds sensitive to vicinal coupling constants (C-2'–C-3', C-2'–O-2', and C-3'–O-3') are considered. The conformational equilibrium may be monitored by observed magnitudes of $J_{2',P}$ and $J_{3',P}$ according to equation (7) where p_1 and p_2 represent the relative populations and θ_1 and θ_2 represent the conformational angles for a two-state (1 and 2) conformational model. For a symmetrical pucker of the phosphate ring we have the simplification (8). The sums of $J_{2',P}$ and $J_{3',P}$

$$J_{2',P} = p_1 J[\theta_1(\text{H-2}',\text{P})] + p_2 J[\theta_2(\text{H-2}',\text{P})] \qquad (7a)$$

$$J_{3',P} = p_1 J[\theta_1(\text{H},3',\text{P})] + p_2 J[\theta_2(\text{H},3',\text{P})] \qquad (7b)$$

$$\theta_1(\text{H},2',\text{P}) = \theta_2(\text{H},3',\text{P}) = (120 - \mu) \qquad (8a)$$

$$\theta_1(\text{H},3',\text{P}) = \theta_2(\text{H},2',\text{P}) = (120 + \mu) \qquad (8b)$$

calculated from equations (7) and (8) are predicted to be constant and independent of the conformer populations p_1 and p_2.

$$\Sigma J' = (J_{2',P} + J_{3',P}) = J(120 - \mu) + J(120 + \mu) = -A\cos^2\mu + B\cos\mu + (3A/2 + 2C) \qquad (9)$$

For the 2',3'-cyclic mononucleotides the observed sum of coupling constants $(J_{2',P} + J_{3',P})$ was found to be constant (18.4 ± 0.1 Hz, Table 1) which is consistent with using the symmetrical two-state conformational model for the phosphate ring. Similar behaviour is expected and found for $(J_{P,C\text{-}4'} + J_{P,C\text{-}1'})$ magnitudes but these results are considered later.

The angle of puckering, μ, calculated from $\Sigma J'$ 18.4 ± 0.1 Hz by equation (9) using the appropriate Karplus parameters (*A* 18.1, *B* 4.8, *C* 0 Hz for HCOP)[22] may be ±33 or ±125°, though, because of the constraints of the bicyclic structure, only the smaller angles (±33°) are allowed. The calculated displacement angle μ (*ca.* 33°) is similar in magnitude and within experimental error of φ (*ca.* 31°) calculated from $J_{2',3'}$

Table 2. Sugar ring conformational properties of 2′,3′-cyclic mononucleotides

		Pseudorotational analysis [a]						Two-state model			
	pH	τ_m	^SP	^NP	^SX [b]	$K_{eq.}$ [c]	$K_{eq.}$ [d]	$\varphi_{2',3'}$ [f]	$\eta(1',2' + 3',4')$ [g]	^SX [h]	$K_{eq.}$ [i]
2′,3′-UMP	7.2	31	167	13	0.34	0.51	0.55	30	31 → 36	0.40	0.67
	8.2	31	162	18	0.32	0.47	0.53	30	34 → 38	0.40	0.66
2′,3′-CMP	7.2	32	176	4	0.32	0.47	0.50	32	28 → 33	0.39	0.63
	7.3	31	167	13	0.35	0.54	0.57	30	31 → 36	0.41	0.70
	2.3	31	177	3	0.32	0.47	0.49	31	27 → 32	0.38	0.62
(+La^{III})	2.3	33	188	352	0.30	0.43	0.43	32	22 → 28	0.36	0.57
2′,3′-m^{6}UMP		33	178	2	0.18	0.22	0.23	33	28 → 32	0.25	0.34
2′,3′-AMP	7.2	32	171	9	0.53	1.13	1.13	31	30 → 34	0.57	1.31
	2.3	31	175	5	0.43	0.75	0.76	31	28 → 33	0.48	0.91
(+La^{III})	2.3	33	178	2	0.45	0.82	0.80	32	27 → 32	0.49	0.95
2′,3′-GMP	7.2	31	175	5	0.45	0.82	0.84	31	28 → 33	0.50	1.00
	7.2 [e]	31	180	0	0.45	0.82	0.81	31	25 → 40	0.49	0.98

[a] Calculated from curves in ref. 10. [b] Calculated from $^SX = (^NJ_{3',4'} - J_{3',4'})/(^NJ_{3',4'} - {}^SJ_{3',4'})$ from ref. 10. [c] $K_{eq.} = {}^SX/{}^NX$. [d] $K_{eq.} \sim J_{1',2'}/J_{3',4'}$. [e] Pyridinium salt. [f] Calculated from equations (2) and (3). [g] Calculated from equation (6) using average Karplus parameters (*A* 10.2, *B* 0.8, *C* 0 Hz) for both coupling paths. The first angle corresponds to calculations using observed $J_{1',2'}$ (with $J_{3',4'}$) and the second angle corresponds to calculations using $J_{1',2'}$ increased by δJ(0.7 Hz) to account for substituent electronegativity. [h] Calculated from equation (17) with δJ 0.7 Hz. [i] $K = (J_{1',2'} + \delta J)/J_{3',4'}$.

Table 3. Phosphate ring conformational properties of 2′,3′-cyclic nucleotides

		Using $^3J(^1H,^{31}P)$			Using $^3J(^{31}P,^{13}C)$			
	pH	μ [a]	p_1 [b]	$K_{eq.}$ [c]	μ [d]	μ [e]	p_1 [f]	K [g]
2′,3′-UMP	7.2	33	0.38	0.60	41	41	0.27 → 0.30	0.37 → 0.43
	8.2				36	32	0.28 → 0.32	0.39 → 0.48
2′,3′-CMP	7.2	34	0.35	0.54	36	32	0.26 → 0.30	0.35 → 0.42
	7.3				37	33	0.29 → 0.33	0.41 → 0.50
	2.3	32	0.41	0.70	36	32	0.28 → 0.32	0.39 → 0.48
(+La^{III})	2.3	38	0.44	0.80	33	26	0.31 → 0.36	0.45 → 0.57
2′,3′-m^{6}UMP		34	0.22	0.29				
2′,3′-AMP	7.2	32	0.58	1.39	34	27	0.53 → 0.61	1.13 → 1.59
	2.3	33	0.48	0.91	29	17	0.42 → 0.49	0.72 → 0.96
(+La^{III})	2.3	36	0.48	0.93	33	26	0.46 → 0.53	0.86 → 1.15
2′,3′-GMP	7.2	33	0.48	0.94	35	30	0.42 → 0.49	0.73 → 0.95
	7.2	33	0.49	0.98				

[a] Calculated from equation (9) with *A* 18.1, *B* 4.8, and *C* 0 Hz for ^3J(POCH) magnitudes. Error ±2°. [b] Calculated from $p_1 = J_{2',P}/(J_{2',P} + J_{3',P})$. [c] Calculated from $K_{eq.} = p_1/p_2 = J_{2',P}/J_{3',P}$ [d] Calculated from equation (17) with *A* 10, *B* C 0 Hz (T. Schleich, B. P. Cross, and I. C. P. Smith, *Nucleic Acid Res.*, 1976, 3, 355) and no electronegativity correction to $J_{P,C\text{-}1'}$. [e] Calculated from equation (17) with *A* 6.4, *B* 1.3, *C* 1.2 Hz and a 1.1 Hz electronegativity correction to $J_{P,C\text{-}1'}$ (ref. 25). [f] Calculated from $p_1 = J_{P,C\text{-}4'}/(J_{P,C\text{-}1'} + J_{P,C\text{-}4'} + \delta J)$ with δJ 0 (first number) and δJ −1.1 Hz (second number). [g] Calculated from $K_{eq.} = J_{P,C\text{-}4'}/(J_{P,C\text{-}1'} + \delta J)$ with δJ 0 (first number) and δJ −1.1 Hz (second number).

magnitudes, indicating that the puckering of the sugar and phosphate rings in 2′,3′-cyclic mononucleotides are about equal.

The magnitudes of μ calculated from equation (9) for the series of 2′,3′-cyclic mononucleotides (Table 3) show that μ is approximately constant for measurements made at pH values 7.2 or 2.3 but that there appears to be a greater puckering of the phosphate ring for 2′,3′-CMP (μ 38°) and 2′,3′-AMP (μ 35°) on addition of lanthanide ion at pH 2.3. This result is not unexpected since the lanthanide ion binds to the phosphate oxygen atoms and their interaction might be sufficient to stabilise the phosphate ring in a more puckered form than without the lanthanide ion.

(4) *Relationship between Sugar and Phosphate Ring Conformations*.—(a) ^3J(HH) *and* ^{3}J(HP) *magnitudes and the electronegativity correction for* $J_{1',2'}$. The sugar ring conformational equilibrium is sensitive to $J_{1',2'}$ and $J_{3',4'}$ magnitudes whereas the phosphate ring conformational equilibrium is sensitive to $J_{2',P}$ and $J_{3',P}$ magnitudes.

The parameter of interest for exploring the conformer populations of the sugar ring is the difference in coupling constants $\Delta J (= J_{3',4'} - J_{1',2'})$ monitoring the conformations about the C-3′–C-4′ and C-1′–C-2′ bonds whereas the parameter of interest for exploring the conformer populations of the phosphate ring is the difference in coupling constants $\Delta J'$ ($= J_{3',P} - J_{2',P}$) which monitors the conformations about the C-3′–O-3′ and C-2′–O-2′ bonds. The relation between ΔJ and $\Delta J'$ for the 2′,3′-cyclic mononucleotides is shown in Figure 4 for the results summarised in Table 1. An approximately linear relationship is observed with a positive slope of *ca.* 0.42 and an intercept of 0.7 (±0.1) Hz. This linear correlation is predicted using equations resulting from the two-state conformational models for both five-membered rings.

In order that one Karplus relation may be used for both $J_{1',2'}$ and $J_{3',4'}$ coupling paths, observed $J_{1',2'}$ values are modified by a correction factor δJ according to equation (10), where

$$^RJ_{1',2'} = J_{1',2'} + \delta J \qquad (10)$$

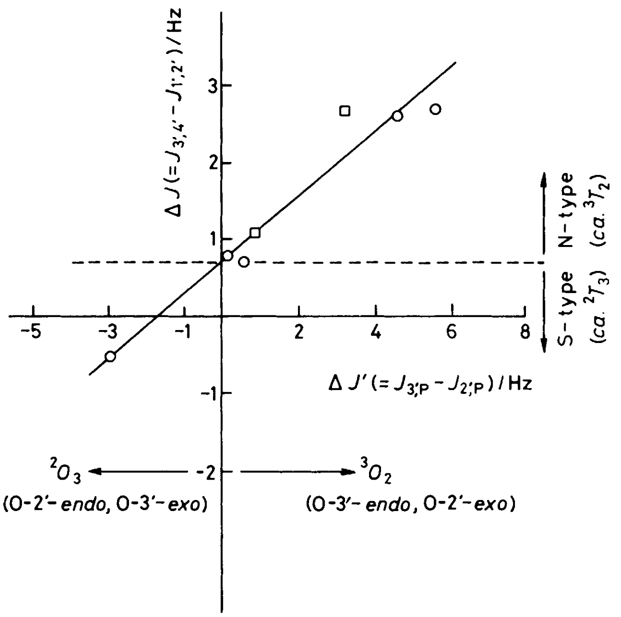

Figure 4. Relationship between sugar and phosphate ring conformational equilibria of 2′,3′-cyclic mononucleotides in terms of $\Delta J(=J_{3',4}-J_{1',2'})$ *versus* $\Delta J'(=J_{3',P}-J_{2',P})$ plot

the ' real ' or ' correct ' coupling constant is denoted by $^{R}J_{1',2'}$. An expression for the variation of ΔJ in terms of the conformer populations and conformer types of the sugar ring is shown in equation (11) which was derived from equations (4), (5), and (9) with $(p_N + p_S) = 1$. Taking account

$$(J_{3',4'} - {}^{R}J_{1',2'}) = (J_{3',4'} - J_{1',2'}) - \delta J = \Delta J - \delta J = (p_N - p_S)[J(120 + \eta) - J(120 - \eta)] \quad (11)$$

of the form of the Karplus relation in equation (2) and the range of angles of puckering in the bicyclic system ($-40° < \eta < 40°$), equation (11) shows that positive values of $(J_{3',4'} - J_{1',2'})$ correspond to a greater proportion of the N compared to the S conformer, making due allowance for δJ, because $J(120 + \eta)$ is greater than $J(120 - \eta)$. For the phosphate ring a similar equation may be derived for $\delta J'$ though, in this case, no electronegativity correction is expected for the ^{31}P–H-2′ and ^{31}P–H-3′ spin-coupling constants, *i.e.* equation (12).

$$\Delta J' = (J_{3',P} - J_{2',P}) = (p_2 - p_1)\,[J(120 + \mu) - J(120 - \mu)] \quad (12)$$

By analogy with equation (11) for the sugar ring, positive values of $(J_{3',P} - J_{2',P})$ correspond to a greater proportion of conformer 2 ($^3O_2$) than conformer 1 ($^2O_3$).

The nature of the relationship between ΔJ and $\Delta J'$ is given in equation (13) derived by combination of equations (11) and (12). Equation (13) predicts the linear relationship

$$\Delta J = \frac{(p_N - p_S)}{(p_2 - p_1)} \times \frac{[J(120 + \eta) - J(120 - \eta)]}{[J(120 + \mu) - J(120 - \mu)]} \times \Delta J' + \delta J \quad (13)$$

between $(J_{3',4'} - J_{1',2'})$ and $(J_{3',P} - J_{2',P})$ observed for the 2′,3′-cyclic mononucleotides in Figure 4 in which the intercept is δJ (= 0.7 Hz) and the slope (0.42) is given by the term which is the coefficient of $\Delta J'$. The electronegativity correction δJ has the expected sign (positive) for previous work [23,24] has shown that a substituent nitrogen atom would decrease coupling in the H-1′–H-2′ coupling path compared to the H-3′–H-4′ path for the sugar ring (Figure 1).

The relationship between the sugar ring and phosphate ring conformations is explored in the following manner. Knowledge of the angles of displacement of the sugar ($\varphi \sim \eta \sim 31°$) and phosphate ($\mu \sim 33°$) rings means that contributions to the slope of $J(120 - \eta)$ and $J(120 - \mu)$ are negligible:

$$i.e. \frac{[J(120 + \eta) - J(120 - \eta)]}{[J(120 + \mu) - J(120 - \mu)]} \sim \frac{[J(120 + \eta) + J(120 - \eta)]}{[J(120 + \mu) + J(120 - \mu)]} \sim \frac{\Sigma J(=8.2)}{\Sigma J'(=18.4)} \sim 0.44$$

Hence from the observed slope (0.42) and calculated J factor (0.44) it can be seen that the ratio of the sugar ring and phosphate ring conformational equilibria is constant and close to unity:

$$i.e.\ (p_N - p_S)/(p_2 - p_1) \sim 0.42/0.44 = 0.95.$$

Interpretation of the interdependence of the ring conformational equilibria using the two-state conformational models in this work is that the phosphate ring adopts the approximate $^3O_2$ (conformer 2) conformation for the sugar ring in the $^3T_2$ (N) conformation and the phosphate ring adopts an approximate $^2O_3$ (conformer 1) conformation for the sugar ring in the $^2T_3$ (S) conformation.

(b) *Relation between* ^3J(HH) *and* $^3J(^{31}P, {}^{13}C)$ *magnitudes.* The conformation of the O-2′–C-2′ and O-3′–C-3′ bonds of the phosphate ring of 2′,3′-cyclic mononucleotides may be monitored by ^3J (^{31}P, ^{13}C) magnitudes ($J_{P,C\text{-}1'}$ and $J_{P,C\text{-}3'}$ for O-2′–C-2′ bonds; $J_{P,C\text{-}2'}$ and $J_{P,C\text{-}4'}$ for O-3′–C-3′ bonds) as well as $J(^{31}P, {}^{1}H)$ magnitudes considered in the previous section. Only one set of ^{13}C, ^{31}P vicinal couplings for each bond is needed for the present analysis and the couplings considered in this work are $J_{P,C\text{-}1'}$ and $J_{P,C\text{-}4'}$ because vicinal $J_{P,C\text{-}2'}$ and $J_{P,C\text{-}3'}$ magnitudes are complicated by geminal coupling constants. By analogy with the results in the previous section the relationship between ^3J(HH) and $^3J(^{31}P, {}^{13}C)$ of cyclic nucleotides may be explored by a plot of ΔJ $(= J_{1',2'} - J_{3',4'})$ against $\Delta J''$ $(= J_{P,C\text{-}4'} - J_{P,C\text{-}1'})$ using the results in Table 1. Such a plot is linear but as both $J_{1',2'}$ and $J_{P,C-1'}$ are affected by electronegativity corrections for the nitrogen atom of the base, the relationship between ΔJ and $\Delta J''$ is explored by making empirical electronegativity corrections in the following manner.

The dependence of observed $J(^{31}P, {}^{13}C)$ magnitudes on the relative proportions of the two symmetrical conformers for the phosphate ring is given by equations (14), where p_1 and p_2 represent the conformer populations and $\delta J'$ represents the correction factor to $J_{P,C\text{-}1'}$ in order to account for the electronegativity effect of purine or pyrimidine base ($\delta J'$ -1.1 Hz).[25] The difference in observed ^{31}P, ^{13}C coupling

$$J_{P,C\text{-}1'} + \delta J' = p_1 J(120 - \mu) + p_2 J(120 + \mu) \quad (14a)$$

$$J_{P,C\text{-}4'} = P_1 J(120 + \mu) + p_2 J(120 - \mu) \quad (14b)$$

constants reflects the conformational behaviour of the phosphate ring according to equation (15).

$$\Delta J'' - \delta J' = (J_{P,C\text{-}4'} - J_{P,C\text{-}1'}) - \delta J' = -(p_2 - p_1)[J(120 + \mu) - J(120 - \mu)] \quad (15)$$

The relationship between sugar ring and phosphate ring conformations is shown in equation (16) which is derived by combination of equations (11) and (15). Equation (16)

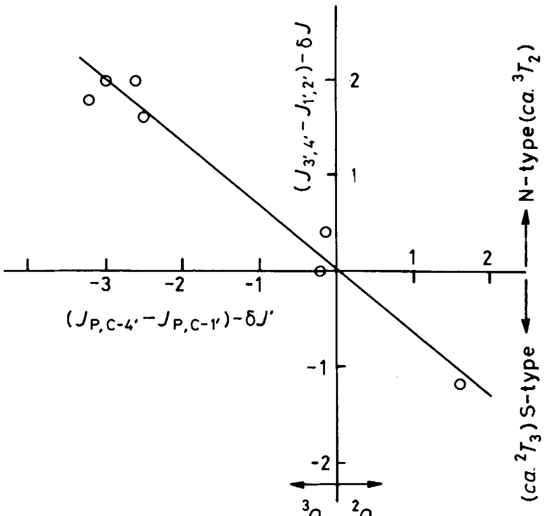

Figure 5. Relationship between sugar and phosphate ring conformational equilibria of 2′,3′-cyclic mononucleotides in terms of ΔJ *versus* $\Delta J''(= J_{\mathrm{P,C\text{-}4'}} - J_{\mathrm{P,C\text{-}1'}})$ plot making allowance for empirical substituent electronegativity correction for $J_{1',2'}(\delta J$ 0.7 Hz) and $J_{\mathrm{P,C\text{-}1'}}(\delta J'$ -1.1 Hz)

$$\frac{(J_{3',4'} - J_{1',2'}) - \delta J}{(J_{\mathrm{P,C\text{-}4'}} - J_{\mathrm{P,C\text{-}1'}}) - \delta J'} = -\frac{(p_{\mathrm{N}} - p_{\mathrm{S}})[J(120 + \eta) - J(120 - \eta)]}{(p_2 - p_1)[J(120 + \mu) - J(120 - \mu)]} \qquad (16)$$

predicts a linear relationship between $(J_{3',4'} - J_{1',2'} - \delta J)$ and $(J_{\mathrm{P,C\text{-}4'}} - J_{\mathrm{P,C\text{-}1'}} - \delta J')$ magnitudes with a negative slope that goes through the origin. The results for 2′,3′-cyclic mononucleotides in Table 1 plotted according to equation (16) exhibit a linear relationship with negative slope (*ca.* -0.65) that passes close to the origin as shown in Figure 5; this behaviour is consistent with the two-state symmetrical conformational model used for both five-membered rings and confirms the interrelation between them and confirms the magnitudes and signs of the electronegativity corrections for $J_{1',2'}(\delta J$ 0.7 Hz) and $J_{\mathrm{P,C\text{-}1'}}(\delta J'$ -1.1 Hz) magnitudes.

It should be noted that the sum of observed $^3J(^{31}\mathrm{P}, {}^{13}\mathrm{C})$ magnitudes is independent of the conformational equilibrium according to equation (17) and can be used to determine angle μ from observed $\Delta J''$ magnitudes. Magnitudes of angle μ calculated from $^3J(^{31}\mathrm{P}, {}^{13}\mathrm{C})$ are similar to those calculated from $^3J(^1\mathrm{H}, {}^{13}\mathrm{C})$ (Table 3), which in turn are similar to those (φ, η) calculated for the sugar ring.

$$\begin{aligned} \Sigma J'' + \delta J' &= (J_{\mathrm{P,C\text{-}1'}} + J_{\mathrm{P,C\text{-}4'}}) + \delta J' \\ &= J(120 + \mu) + J(120 - \mu) \\ &= -A\cos^2\mu + B\cos\mu + (3A/2 + 2C) \qquad (17) \end{aligned}$$

(5) *Equilibrium Compositions*.—(a) *Sugar ring*. The equilibrium composition of the sugar ring may be calculated for the two-state conformational model using equations (18) which were derived from equations (4), (5), and (10). These equations are similar to those derived by the pseudorotational analysis of the sugar ring [*i.e.* $p_{\mathrm{N}} = J_{3',4'}/(J_{1',2'} + J_{3',4'})$ and $p_{\mathrm{S}} = J_{1',2'}/(J_{1'2'} + J_{3',4'})$] [26] except that, in order to use one Karplus relation for both molecular fragments, observed $J_{1',2'}$ magnitudes are increased by the contribution for the electronegativity of the glycosyl nitrogen fragment observed in this work, *i.e.* δJ 0.7 Hz. It can be seen from equation (18) that the population of the N conformer calculated with δJ *ca.* 0.7 Hz is smaller than when it is not taken into account (*i.e.* $\delta J = 0$). The electronegativity correction of 0.7 Hz corresponds to a 4—8% decrease in the proportion of the N conformer and a corresponding increase in the proportion of the S conformer compared to the value in Table 2 derived by the full pseudorotational analysis of the sugar ring.

$$\begin{aligned} p_{\mathrm{N}} &= [J_{3',4'} - J(120 - \eta)]/[J(120 + \eta) - \\ &\quad J(120 - \eta)] \sim J_{3',4'}/(J_{1',2'} + J_{3',4'} + \delta J) \qquad (18a) \end{aligned}$$

$$p_{\mathrm{S}} \sim (J_{1',2'} + \delta J)/(J_{1',2'} + J_{3',4'} + \delta J) \qquad (18b)$$

The equilibrium constant may be calculated directly from observed $J_{1',2'}$ and $J_{3',4'}$ magnitudes according to equation (19). The expression for K reduces to that previously concluded from the pseudorotational analysis of the sugar ring ($K = J_{1',2'}/J_{3',4'}$) when no electronegativity correction for $J_{1',2'}$ was taken into account.[26] Magnitudes of K calculated for the 2′,3′-cyclic mononucleotides using equation (19) with a positive electronegativity correction (Table 2) are greater than when no correction is applied and the magnitude of the difference depends on the position of the equilibrium.

$$K = p_{\mathrm{S}}/p_{\mathrm{N}} = (J_{1',2'} + \delta J)/J_{3',4} \qquad (19)$$

(b) *Phosphate rings*. The composition of the phosphate ring conformational equilibrium for the symmetrical two-state conformational model may be calculated from equation (20) which is derived from equations (7) and (8). Magnitudes of p have been calculated for the J(HP) values of 2′,3′-cyclic mononucleotides and the results which are listed in Table 3 indicate that p_1 varies from 0.65 (preference for the $^3O_2$ conformation) to 0.42 (preference for the $^2O_3$ conformation) though many nucleotides have about equal proportions of both conformers. This result is in broad agreement with that determined by the lanthanide probe method, except that a 50 : 50 equilibrium of the two puckered conformations for the phosphate ring is *not* equivalent to a planar ring on average as suggested previously.[11]

$$p_1 = J_{2',\mathrm{P}}/(J_{2',\mathrm{P}} + J_{3',\mathrm{P}}) \qquad (20a)$$

$$p_2 = J_{3',\mathrm{P}}/(J_{2',\mathrm{P}} + J_{3',\mathrm{P}}) \qquad (20b)$$

The equilibrium constant for the puckering equilibrium ($K = p_1/p_2$) may be calculated directly from observed $J_{2',\mathrm{P}}$ and $J_{3',\mathrm{P}}$ magnitudes according to equation (21) and the values for the 2′,3′-cyclic nucleotides listed in Table 3 vary from 1.39 ($p_1 > p_2$) to 0.54 ($p_2 > p_1$) in line with conformer populations calculated individually from equation (20).

$$K = p_1/p_2 = J_{2,\mathrm{P}},/J_{3,\mathrm{P}} \qquad (21)$$

Conclusions

The relationship between the sugar and cyclic phosphate rings of 2′,3′-cyclic mononucleotides in solution has been investigated by results from ^{1}H and ^{13}C n.m.r. spectroscopy. The conformation of the sugar ring was monitored by ^3J(HH) magnitudes ($J_{1',2'}$, $J_{2',3'}$, $J_{3',4'}$) and the conformation of the phosphate ring was monitored by $^3J(^1\mathrm{H}, {}^{31}\mathrm{P})$, ($J_{2',\mathrm{P}}$ and $J_{3',\mathrm{P}}$), and $^3J(^{31}\mathrm{P}, {}^{13}\mathrm{C})$ magnitudes ($J_{\mathrm{P,C\text{-}1'}}$ and $J_{\mathrm{P,C\text{-}4'}}$) for molecules under the same solution conditions of pH and temperature and added lanthanide ion.

(1) The ^3J(HH) n.m.r. results on 2′,3′-cyclic nucleotides

are not consistent with a conformational model derived from *X*-ray crystallographic results in which the planar conformation of the pseudorotational pathway between O-1′-*endo* and O-1′-*exo* conformations.[15] Similarly the variations in $^3J(^1H, ^{31}P)$ and $^3J(^{31}P, ^{13}C)$ magnitudes of cyclic nucleotides are not consistent with only one type of puckered conformation of the phosphate ring suggested by the *X*-ray results.[2–4]

(2) A number of spin coupling constants are observed to be constant for the series of purine and pyrimidine 2′,3′-cyclic mononucleotides which is a prerequisite for a pseudorotational analysis of both five-membered rings. Such an analysis is not used in the present work because the necessary *X*-ray crystallographic results are not available and because the interdependence of the ring conformations can be explored using a simple two-state symmetrical conformational model for both five-membered rings.

(3) It is found that the puckering of the phosphate ring is approximately the same as that for the sugar ring if puckering of the rings is judged by the magnitudes of the angles of twist about appropriate bonds determined from analysis of vicinal spin coupling constants [$^3J(HH)$, $^3J(^1H, ^{31}P)$, and $J(^{31}P, ^{13}C)$] using appropriate Karplus relations.

(4) Using the two-state symmetrical conformational model the puckering of the sugar ring corresponds to $^3T_2$ (C-3′-*endo*, C-2′-*exo*) in the N conformation and to $^2T_3$ (C-2′-*endo*, C-3′-*exo*) in the S conformation whereas the puckering of the phosphate ring corresponds to $^3O_2$ (O-3′-*endo*, O-2′-*exo*) and $^2O_3$ (O-2′-*endo*, O-3′-*exo*) conformation.

(5) The interdependence of the conformations of the sugar and phosphate rings was demonstrated by the observed linear dependence of the plot of $\Delta J(= J_{3',4'} - J_{1',2'})$ against either $\Delta J'(= J_{3',P} - J_{2',P})$ or $\Delta J''(= J_{P,C\text{-}4'} - J_{P,C\text{-}1'})$ magnitudes. The slopes of the linear correlations were rationalised in terms of the two-state models for both rings such that the equilibrium of the sugar ring ($^2T_3 \rightleftharpoons {}^3T_2$) may be directly correlated with the equilibrium of the phosphate ring ($^2O_3 \rightleftharpoons {}^3O_2$) which might be expected considering that the rings have the C-2′–C-3′ bond in common.

(6) The intercepts of the linear correlations were rationalised in terms of electronegativity corrections caused by the glycosyl nitrogen atom of purine and pyrimidine bases *i.e.* $J_{1',2'}$ and $J_{P,C\text{-}1'}$. It was found that a substituent electronegativity correction of 0.7 Hz should be *added* to observed $J_{1',2'}$ magnitudes so that use of one Karplus relation for $J_{1',2'}$ and $J_{3',4'}$ is justified whereas the substituent electronegativity correction of 1.1 Hz should be *subtracted* from observed $J_{P,C\text{-}1'}$ so that one Karplus relation for $J_{P,C\text{-}1'}$ and $J_{P,C\text{-}4'}$ is justified.

The analysis of proton–proton, proton–phosphorus, and phosphorus–carbon spin-coupling constants of 2′,3′-cyclic mononucleotides confirms the conformational flexibility of both the sugar and phosphate five-membered rings, shows that the conformations of both rings are interdependent and should be taken into account for any explanation of the influence of stereochemistry on the degradation of ribonucleic acids by ribonuclease.

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