series have similar conformations of the cyclic phosphate and ribose rings. This conclusion is consistent with the ^1H nmr data,[2] which showed that the cyclic phosphate ring is locked in the chair conformation. The conformational information obtainable from ^{13}C chemical shifts and $^{13}C-^{31}P$ coupling constants is shown to be less reliable than that available from proton nmr data in the case of mononucleotides. In larger molecules such as dinucleoside monophosphates and polynucleotides[4,5] the ^{13}C nmr data are more useful.

A ^{13}C and ^1H Nuclear Magnetic Resonance Study of the Conformations of 2′,3′-Cyclic Nucleotides[1a]

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Abstract: The ^1H nmr spectra of the 2′,3′-cyclic nucleotides of uridine, cytidine, adenosine, and guanosine were analyzed to second order to yield $^1H-^1H$ and $^1H-^{31}P$ coupling constants. Fourier-transformed ^{13}C nmr spectra yielded $^{13}C-^{31}P$ coupling constants. The coupling constants indicate that the ribofuranose and cyclic phosphate rings are in rapid equilibrium between puckered forms and that the average conformations do not have planar ribofuranose rings. In the conformational equilibria the pyrimidine nucleotides tend toward the 3′-endo (2′-exo) conformers, that of adenosine tends (less) toward the 2′-endo (3′-exo) conformers, whereas that of guanosine manifests no significant preference for any of the principal conformers. Increasing the temperature from 30 to 75° decreases the observed conformational preferences. The exocyclic hydroxymethyl groups of the pyrimidine nucleotides demonstrate a preference for the unsymmetrical trans–gauche (or gauche–trans) rotamer, whereas those of the purine nucleotides prefer the gauche–gauche rotamer. The conformations of these compounds in aqueous solution are more flexible than in the solid state where rigid planar ribofuranose rings have been observed.

The decomposition of ribonucleic acids catalyzed by pancreatic ribonuclease involves a two-step mechanism.[2] The first step is cleavage of phosphorus–oxygen bonds in the backbone chain of the ribonucleic acid by transesterification to yield 2′,3′-cyclic nucleotides; the second is hydrolysis of the cyclic nucleotides to yield specifically 3′-ribonucleotides. The degradation of ribonucleic acids by alkali, although believed to occur by a similar two-step mechanism, is less specific and yields mixtures of 2′- and 3′-ribonucleotides.[3]

Recent studies have investigated the mechanism of this enzymatic decomposition[4,5] with a special interest in the stereochemistry of the second step of the reaction[4] and the enthalpy of hydrolysis of various 2′,3′-cyclic nucleotides.[6] The conformations of the 2′,3′-cyclic nucleotide intermediates and the influence of stereochemistry on the ribonuclease degradation of ribonucleic acids is not fully understood.

Only limited X-ray crystallographic data related to the 2′,3′-cyclic nucleotides are available.[7,8] Since such data are necessary for static conformations, they may not always reflect the situation in solution. We have examined the conformations of several 2′,3′-cyclic nucleotides (2′,3′-UMP, 2′,3′-CMP, 2′,3′-AMP, 2′,3′-GMP[9]) in aqueous solution with the aid of proton and carbon-13 nmr spectroscopy. Of particular interest are the conformations of the furanose and cyclic phosphate rings; the $^1H-^{31}P$ and $^{13}C-^{31}P$ coupling constants are particularly helpful in this case. A preliminary report of this work has appeared.[10]

Experimental Section

The 2′,3′-cyclic nucleotides were purchased from Schwarz Bioresearch and Sigma Chemical Co. and were used without further purification. Whenever possible the nucleotides were studied as their sodium salts, although the barium salt of 2′,3′-UMP and the pyridinium salt of 2′,3′-GMP were studied, the latter being converted to the pyridinium salt because of the insolubility of the barium salt. The internal reference, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), was a product of E. Merck, Germany. The nucleotides were 0.15 *M* in D_2O containing 0.08 *M* DSS. The pD of each solution was adjusted to 7.2 (pD = pH + 0.4[11]) by the addition of small amounts of dilute NaOD and DCl. The samples were lyophilized from D_2O to reduce the concentration of HDO. Proton and ^{13}C nmr spectra were obtained on a Varian XL-100-15 spectrometer with fast Fourier transform accessory. Proton noise decoupling was utilized in the case of the ^{13}C spectra. The spectra were run at 30° (^1H) and 37° (^{13}C).

Results and Discussion

Complete ^1H nmr spectra were taken at 100 MHz. Assignments to specific hydrogens were made by refer-

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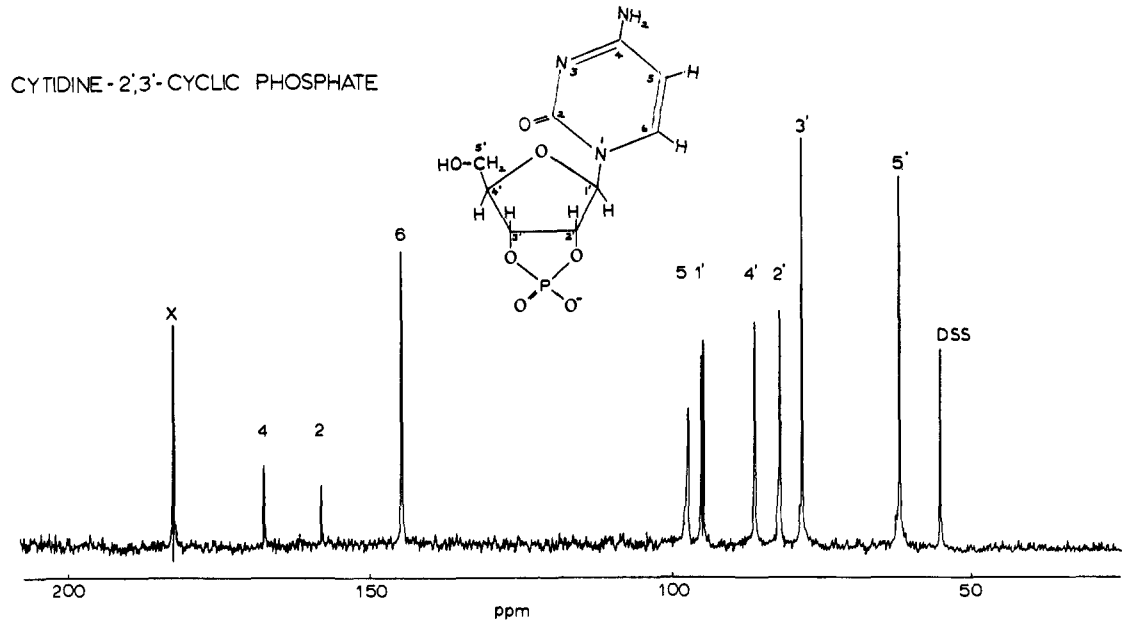

Figure 1. The 25.2-MHz ^{13}C nmr spectrum of a 0.15 *M* solution of 2',3'-cytidine cyclic monophosphate (sodium salt) in D_2O, pD 7.2, at 37°. The spectrum is proton noise decoupled and X indicates a spurious instrument "glitch." Chemical shifts are expressed in parts per million dowfield from the resonance of tetramethylsilane in a concentric capillary tube.

ence to earlier work,[12-15] by $^1H-^1H$ decoupling, and by $^1H-^{31}P$ decoupling. Spectral analyses (Table I) were performed using standard methods[16] and the computer program LAOCNPLT.[17]

Table I. Proton Data of 2',3'-Cyclic Nucleotides

Proton	Compound U (Ba)	C (Na)	A (Na)	G (Na)	G (Py)
	(a) Chemical Shifts (ppm from DSS)				
1'	5.898	5.851	6.132	6.106	6.171
2'	5.139	5.136	5.303	5.404	5.419
3'	4.917	4.947	5.084	5.164	5.169
4'	4.297	4.312	4.445	4.451	4.496
5'	3.895	3.920	3.909	3.940	3.944
5''	3.815	3.846	3.855	3.884	3.900
2			7.973		
5	5.857	6.014			
6	7.699	7.671			
8			8.147	7.937	8.207
	(b) Coupling Constants (Hz)				
Nuclei					
1'2'	3.0	2.7	4.4	3.7	3.4
2'3'	6.9	6.7	6.8	6.8	6.8
3'4'	5.5	5.4	3.9	4.4	4.2
4'5'	3.6	3.6	2.5	3.6	2.5
4'5''	5.7	5.7	4.8	4.8	5.8
5'5''	−12.5	−12.4	−12.7	−12.6	−12.8
5 6	8.1	7.5			
2'P	6.9	6.5	10.6	8.9	9.1
3'P	11.5	12.1	7.6	9.5	9.3
(2'P + 3'P)	18.4	18.6	18.2	18.4	18.4

The derived ^{13}C parameters are given in Table II and the ^{13}C spectrum of 2',3'-CMP in Figure 1. Assignment of the ^{13}C resonances was made with reference to previous work on nucleotides and nucleosides,[10,18,19] with attention to the revised assignment of the 2'- and 3'-resonances.[10,19]

Table II. Carbon-13 Data of 2',3'-Cyclic Nucleotides

Carbon	Molecule U (Ba)	C (Na)	A (Na)	G (Na)
	(a) Chemical Shifts (ppm from TMS)			
1'	93.74	94.97	90.60	90.24
2'	81.74	82.11	81.56	81.60
3'	78.26	78.54	78.78	78.73
4'	86.25	86.37	86.43	86.47
5'	62.12	62.32	62.40	62.32
2	152.42	158.23	153.70	154.85
4	167.41	167.77	149.24	152.15
5	103.46	97.30	119.83	117.49
6	144.79	144.91	156.40	159.70
8			141.69	139.35
	(b) Coupling Constants (Hz)			
Nuclei				
1'P	6.8	6.3	3.8	4.8
2'P	2.5	2.5	1.8	2.3
3'P	0.7	0.5	1.0	1.0
4'P	2.5	2.2	4.3	3.5
5'P	0.2	<0.2	<0.2	<0.2

A. The Furanose and Cyclic Phosphate Ring Conformations. The furanose and cyclic phosphate ring conformations are defined by dihedral angles between the planes containing the atoms: (a) $H_{1'}C_{1'}C_{2'}$ and

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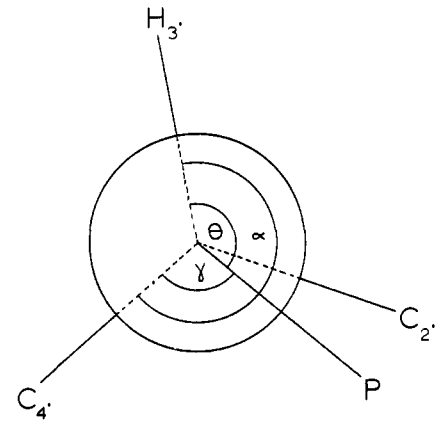

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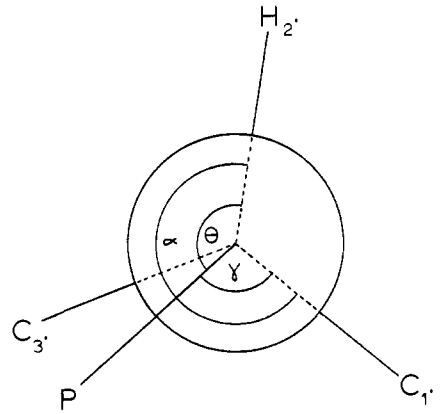

II

Figure 2. Dihedral angles in the cyclic phosphate ring: (I) about the $O_{3'}C_{3'}$ bond showing the angles $\theta(O_{3'}C_{3'})$, $\gamma(O_{3'}C_{3'})$, and $\alpha(O_{3'}C_{3'})$; (II) about the $O_{2'}C_{2'}$ bond showing the angles $\theta(O_{2'}C_{2'})$, $\gamma(O_{2'}C_{2'})$, and $\alpha(O_{2'}C_{2'})$.

$C_{1'}C_{2'}H_{2'}$, $\phi(C_{1'}C_{2'})$; $H_{2'}C_{2'}C_{3'}$ and $C_{2'}C_{3'}H_{3'}$, $\phi(C_{2'}C_{3'})$; $H_{3'}C_{3'}C_{4'}$ and $C_{3'}C_{4'}H_{4'}$, $\phi(C_{3'}C_{4'})$; (b) $PO_{2'}C_{2'}$ and $O_{2'}C_{2'}H_{2'}$, $\theta(O_{2'}C_{2'})$; $PO_{3'}C_{3'}$ and $O_{3'}C_{3'}H_{3'}$, $\theta(O_{3'}C_{3'})$; (c) $PO_{2'}C_{2'}$ and $O_{2'}C_{2'}C_{1'}$, $\gamma(O_{2'}C_{2'})$; $PO_{3'}C_{3'}$ and $O_{3'}C_{3'}C_{4'}$, $\gamma(O_{3'}C_{3'})$; see Figure 2. A discussion of the possible conformations of furanose rings and the dependence of the ^1H–^1H couplings on the dihedral angles of group a above has been given in detail previously.[12–15,20] The conformational relationships between group b and ^1H–^{31}P couplings and between group c and ^{13}C–^{31}P couplings will be discussed below.

It is clear from Table I that the ^1H–^1H coupling constants of the furanose ring in the 2′,3′-cyclic nucleotides do not correspond to those of any possible rigid conformation, either buckled or planar.[14,15,20] We conclude that the furanose ring is flexible, and, like most other nucleosides[10,12–15,21] and nucleotides,[10,15] is in a conformational equilibrium. In this case the conformations involved in the equilibrium are restricted by the cyclic phosphate ring to smaller amplitudes of pucker.

(20) M. Smith and C. D. Jardetzky, *J. Mol. Spectrosc.*, **28**, 70 (1968).
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Certain trends are noteworthy in the ^1H–^1H couplings. On comparing those in Table I with the predicted couplings for rigid planar or buckled conformations of the furanose ring,[14,15,20] it can be seen that the pyrimidine nucleotides 2′,3′-UMP and 2′,3′-CMP have similar conformational equilibria of the furanose rings with preferences in the direction of the 3′-endo (2′-exo) puckered form. The couplings for the purine nucleotides, 2′,3′-AMP and 2′,3′-GMP, are also similar but with more variation than in the case of the pyrimidine nucleotides. There is very little conformational preference manifest in the couplings, suggesting that all conformations have roughly equal populations. The ^1H–^{31}P couplings are more useful in ascertaining conformational preferences when they become this slight (*vide infra*). Secondly, throughout the series of 2′,3′-cyclic nucleotides the $J_{2'3'}$ coupling is approximately constant at 6.8 Hz. This no doubt reflects the constraint on the twisting of the furanose ring about the $C_{2'}C_{3'}$ bond due to the presence of the cyclic phosphate ring and implies that in all cases the amount of rotational flexibility about this bond is approximately constant. Molecular models suggest that the maximum variation in $\phi(C_{2'}C_{3'})$ is $\pm 20°$ from the eclipsed conformation ($\phi(C_{2'}C_{3'}) = 0°$).

The ^1H–^{31}P and ^{13}C–^{31}P vicinal couplings yield information about the conformations of the furanose and cyclic phosphate rings. The relationship between the vicinal ^1H–^{31}P coupling constants and dihedral angles in cyclic compounds has been demonstrated previously.[22] In the 3′,5′-cyclic nucleotides,[10,23] where the furanose and cyclic phosphate rings are rigid, the average trans vicinal ^1H–^{31}P coupling constant is 20.9 Hz and the average gauche coupling is 1.8 Hz. These values and the results of White and Verkade[22d] have led us to the following relationship between $^3J_{HP}$ and dihedral angle θ[23a]

$$^3J_{HP} = J_A \cos^2\theta + J_B \cos\theta = 16.3 \cos^2\theta - 4.6 \cos\theta$$

Using this equation we obtain the dihedral angles θ shown in Table IIIA. It is evident that for small changes in dihedral angle, large coupling constant differences are produced. Dihedral angles estimated from molecular models of rigid conformations are shown in Table IIIB. *It is again clear that these compounds do not have planar rigid conformations, since in this case the ^{31}P couplings to $H_{2'}$ and $H_{3'}$ should be identical.* The estimated dihedral angles are therefore the time-average values resulting from rapid interconversion between conformers.

It is evident from Table III that the purine and pyrimidine cyclic nucleotide data fall into two categories depending solely on the type of base present. The ^1H–^{31}P couplings and dihedral angles indicate that in the rapid equilibria the ribofuranose rings of the pyrimidine nucleotides tend toward a 3′-endo (2′-exo) pucker, while that of adenosine tends (less) toward a

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Table III. Dihedral Angles

Nucleotide	A. Calculated from Proton–Phosphorus and Carbon–Phosphorus Vicinal Coupling Constants: Dihedral angles from $^1H-^{31}P$ coupling constants $\theta(O_2{\cdot}C_2{\cdot})$	$\theta(O_3{\cdot}C_3{\cdot})$	Sum	Dihedral angles from $^{13}C-^{31}P$ coupling constants $\gamma(O_2{\cdot}C_2{\cdot})$	$\gamma(O_3{\cdot}C_3{\cdot})$	Sum	$\alpha(O_2{\cdot}C_2{\cdot})^a$	$\alpha(O_3{\cdot}C_3{\cdot})^a$
2′,3′-UMP[b]	122	136	258	157	124	281	279	260
2′,3′-CMP	121	137	258	153	122	275	274	259
2′,3′-AMP	133	124	257	134	137	271	267	261
2′,3′-GMP	128	130	258	141	132	273	269	262

Conformation of ribose ring	B. Predicted for Rigid Conformations of the Ribofuranose and Phosphorane Rings[c]: POCH dihedral angles $\theta(O_2{\cdot}C_2{\cdot})$	$\theta(O_3{\cdot}C_3{\cdot})$	Sum	POCC dihedral angles $\gamma(O_2{\cdot}C_2{\cdot})$	$\gamma(O_3{\cdot}C_3{\cdot})$	Sum	$\alpha(O_2{\cdot}C_2{\cdot})$	$\alpha(O_3{\cdot}C_3{\cdot})$
2′-Endo	135	105	240	105	135	240	240	240
2′-Exo	105	135	240	135	105	240	240	240
3′-Endo	105	135	240	135	105	240	240	240
3′-Exo	135	105	240	105	135	240	240	240
Planar	120	120	240	120	120	240	240	240

[a] $\alpha(O_2{\cdot}C_2{\cdot}) = \theta(O_2{\cdot}C_2{\cdot}) + \gamma(O_2{\cdot}C_2{\cdot})$ and $\alpha(O_3{\cdot}C_3{\cdot}) = \theta(O_3{\cdot}C_3{\cdot}) + \gamma(O_3{\cdot}C_3{\cdot})$. [b] Nucleotides as sodium salts except 2′,3′-UMP (barium salt). [c] In the models, four atoms of the ribose ring define a plane and the fifth atom is above (endo) or below (exo) this plane. The phosphorane ring is in its "time average" conformation with the OPO atoms defining a plane and with the carbon atoms equally spaced above and below this plane.

2′-endo (3′-exo) pucker, and that of guanosine has no conformational preference detectable within the limitations of this method. It is of interest to note that the sums of the $^1H-^{31}P$ couplings and dihedral angles thus obtained are constant within the limitations of experiment and theory, indicating the interlinked constraints of the furanose and cyclic phosphate ring systems.

It has also been demonstrated that vicinal $^{13}C-^{31}P$ coupling constants are capable of showing a conformational dependence.[10a,b,19,24] From data available on ^{13}C nmr studies of 3′,5′-cyclic nucleotides[10a,b,24] it has been possible to estimate the $^{31}POC\ ^{13}C$ trans coupling in a cyclic phosphate ring to be 8 Hz. The correct form of the relationship between $^3J_{PC}$ and γ must be determined by a study of a large number of conformationally pure compounds. Therefore, we take the simplest possible relationship

$$^3J_{CP} = J_0 \cos^2 \gamma = 8 \cos^2 \gamma$$

to estimate the time-average POCC dihedral angles in the 2′,3′-cyclic nucleotides (Table III). The values are consistent with the conclusions reached on the basis of the vicinal $^1H-^{31}P$ coupling constants. The pyrimidine nucleotides have a slight preference for the 3′-endo (2′-exo) conformation. For the purine nucleotides the $^3J_{C1',P}$ and $^3J_{C4',P}$ must be considered equal within the accuracy of the method. This demonstrates the superiority of the $^3J_{H,P}$ for this type of analysis, as has been emphasized recently.[23a,24]

Although the above dihedral angle considerations as regards $^1H-^{31}P$ and $^{13}C-^{31}P$ couplings are tentative, they are consistent with the conclusions drawn from the $^1H-^1H$ coupling constants. Variation of the J_A and J_B values by ± 1 Hz in the case of the $^1H-^{31}P$ couplings and of J_0 by ± 1 Hz in the case of the $^{13}C-^{31}P$ couplings does not invalidate the conclusions. It should, however, be noted that with the J_A and J_B values used for the $^1H-^{31}P$ and J_0 for the $^{13}C-^{31}P$ coupling dihedral angle dependence the calculated angles are somewhat larger than expected from the molecular models of the 3′-endo, 2′-endo, or planar forms of the furanose ring. In this context, the sum of the dihedral angles, $\alpha = \theta + \gamma$ (Figure 2), associated with the $O_{2'}C_{2'}$ and $O_{3'}C_{3'}$ bonds, although rather large (Table III), exhibits some interesting trends. It can be seen that the angle, α, associated with the $O_{3'}C_{3'}$ bond is constant throughout the series while that associated with the $O_{2'}C_{2'}$ bond is not—that for pyrimidine nucleotides being larger than for the purine nucleotides. This suggests a greater distortion at $C_{2'}$ in the pyrimidine nucleotides than in the purine nucleotides with the distortions about the $C_{3'}$ atom being constant throughout the series. Without further experiment it is only possible to speculate as to the reason for this, but it may involve interaction of $H_{2'}$ with the base. This has been suggested[25] to explain earlier ^1H nmr data for nucleosides, especially those thought to have the base in the syn conformation. X-Ray crystallography of 2′,3′-CMP[7] indicates that the base is syn with respect to rotation about the $C_{1'}$–N bond, with the carbonyl oxygen O_2 of the base over the ribose ring. If this is so in solution then distortion of the furanose ring in 2′,3′-UMP and 2′,3′-CMP might be expected to be larger at the $C_{2'}$ atom due to proximity of $H_{2'}$ and the base carbonyl oxygen. Such an interaction cannot occur in the purine nucleotides.

We have also studied 2′,3′-CMP and 2′,3′-AMP at 75° and found the variation in the $^1H-^1H$ coupling constants to be small (≤ 0.2 Hz) while the $^1H-^{31}P$ couplings (2′,3′-CMP: $J_{H2',P} = 6.8$ Hz, $J_{H3',P} = 11.5$ Hz; 2′,3′-AMP: $J_{H2',P} = 10.2$ Hz, $J_{H3',P} = 7.9$ Hz) vary appreciably. The variation in the $^1H-^{31}P$ coupling constants in going from 30 to 75° demonstrates the shift of both the purine and pyrimidine nucleotides away from their preferences at 30° for some conformers toward a time-averaged planar furanose ring system, *i.e.*, toward equal preference for the four principal buckled conformations. The changes in the $^{13}C-^{31}P$ coupling constants upon increasing temperature, although smaller, are consistent with the changes in conformation indicated by the $^1H-^{31}P$ couplings.

B. Conformation of the Exocyclic CH_2OH Group.

(24) R. D. Lapper, H. H. Mantsch, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **95**, 2878 (1973).

(25) (a) M. P. Schweizer, private communication; (b) F. E. Hruska, A. A. Smith, and J. G. Dalton, *J. Amer. Chem. Soc.*, **93**, 4334 (1971).

It has been demonstrated in the case of several nucleotides and nucleosides[12-15,21] that the exocyclic CH_2OH group is interconverting rapidly between the three principal staggered rotamers, and that usually a preference for one of them is manifest (that is, the CH_2OH group spends a longer time in one of the isomeric conformational states). A complete description of the method and its limitations has been given by Blackburn, *et al.*[12] Application of the method with the parameters used previously[12] yields the rotamer populations shown in Table IV.

Table IV. Calculated Populations of the Three Classical 60° Staggered Rotamers[a] of the 5′-CH_2OH Group

Nucleotide	P_I	P_{II}	P_{III}
2′,3′-UMP	0.35	0.46	0.19
2′,3′-CMP	0.35	0.46	0.19
2′,3′-AMP	0.60	0.34	0.06
2′,3′-GMP(Na)	0.47	0.34	0.19
2′,3′-GMP(Py)	0.48	0.47	0.05

[a] Rotamer I is gauche–gauche, II is gauche–trans, III is trans–gauche.[12-15,21]

It is evident that the exocyclic CH_2OH groups in the pyrimidine 2′,3′-cyclic nucleotides show a preference for a trans–gauche rotamer or the gauche–trans rotamer depending on the absolute assignment of the $H_{5'}$ and $H_{5''}$ resonances. This assignment apparently cannot be clarified by the argument suggested recently by Remin and Shugar,[26] because a specific deshielding effect is not manifest in the 5′ or 5″ proton resonances (at least not on comparing U, 3′-UMP, and 2′,3′-UMP, the only relevant compounds for which high resolution data are available at present). Similarly, the purine cyclic nucleotides show a preference for the gauche–gauche rotamer, a conclusion which is independent of the assignments for $H_{5'}$ and $H_{5''}$. An interesting decrease in the population of the trans–gauche rotamer of 2′,3′-GMP occurs on going from the sodium to the pyridinium salt.

C. Comparison with X-Ray Crystallographic and Calorimetric Data. The only available X-ray crystallographic data are for 2′,3′-CMP[7,27] and uridine 2′,3′-cyclophosphorothioate.[8] The former shows a predominantly syn base conformation (torsional angles of 253–242°) while the latter exhibits an anti conformation (torsional angle of 13°). In both compounds the four carbon atoms of the furanose ring are coplanar, as are $O_{3'}$, $C_{3'}$, $C_{2'}$, and $O_{2'}$. In 2′,3′-CMP both the gauche–gauche and gauche–trans conformers about $C_{4'}$–$C_{5'}$ were observed[7] whereas in the uridine compound only the trans–gauche was present.[8]

Thus, the conformations of these compounds in aqueous solution and single crystals are very different. The strain suggested in ref 7 is relieved by puckering of the furanose ring. Because the present nmr data cannot unequivocally indicate anything about the conformation of the base relative to the glycosidic bond, one can only speculate that the differences between the solution conformations of the purine and pyrimidine 2′,3′-cyclic nucleotides are due to interactions between the bases and the atoms ($H_{2'}$, $H_{3'}$, $OH_{5'}$) of the furanose ring. This is also suggested by differences in the enthalpies of hydrolysis[6] of purine 2′,3′-cyclic nucleotides (A, −9.4 kcal mol^{-1}; G, −9.5 kcal mol^{-1}) and pyrimidine 2′,3′-cyclic nucleotides (C, −8.1 kcal mol^{-1}; U, −7.8 kcal mol^{-1}). We are presently undertaking nuclear Overhauser[28,29] experiments to explore the conformations about the glycosidic bonds in these compounds.[30]

D. Comparison with Other Nmr Data. Since the completion of these studies we have received a preprint of a ^1H nmr study by Lavallee and Coulter on 2′,3′-UMP and 2′,3′-CMP.[31] Their data are in qualitative agreement with our present and earlier[10] reports, with the exception of the H–P coupling constants, which in their case they acknowledge are only approximate since ^1H–^{31}P decoupling was not done. Their conclusion that the base in 2′,3′-CMP is locked in the syn conformation, as it is in the solid state, suggests that the preference of the pyrimidine 2′,3′-cyclic nucleotides for the unsymmetrical rotamers about C4′–C5′ and their distortions at C2′ may be due to unfavorable interactions with the keto group at C2.

Conclusion

At normal temperatures the 2′,3′-cyclic nucleotides show slight preferences in their furanose ring conformational equilibria which depend on the base. The preference is toward the 3′-endo (2′-exo) conformation for the pyrimidine nucleotides, toward the 2′-endo (3′-exo) conformation for 2′,3′-AMP, and negligible for 2′,3′-GMP. The conformations involved in such equilibria are restricted by the cyclic phosphate ring to smaller amplitudes of pucker than those associated with nucleosides. It appears that the overall conformations of the furanose and cyclic phosphate rings are more distorted than molecular models would indicate. The conformations of the uridine and cytidine 2′,3′-cyclic phosphates are appreciably different from those found in the solid state.

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