(3) (a) G. Montaudo, V. Librando, S. Caccamese, and P. Maravigna, *J. Am. Chem. Soc.*, **95**, 6365 (1973); (b) G. Montaudo and P. Finocchiaro, *J. Org. Chem.*, **37**, 3434 (1972); (c) M. R. Willcott, III, R. E. Lenkinski, and R. E. Davies, *J. Am. Chem. Soc.*, **94**, 1742 (1972); (d) R. E. Davies and M. R. Willcott, III, *ibid.*, **94**, 1744 (1972); (e) H. L. Ammon, P. H. Mazzocchi, W. J. Kopecky, Jr., H. J. Tamburini, and P. H. Watts, Jr., *ibid.*, **95**, 1968 (1973); (f) I. M. Armitage, L. D. Hall, A. G. Marshall, and L. G. Werbelow, *ibid.*, **95**, 1437 (1973); (g) J. W. ApSimon and H. Beirbeck, *Tetrahedron Lett.*, 581 (1973); (h) D. J. Chadwick and D. H. Williams, *Chem. Commun.*, 128 (1974); (i) P. V. De Marco, B. J. Cerimele, R. W. Crane, and A. L. Thakkar, *Tetrahedron Lett.*, 3539 (1972); (j) A. J. Rafalski, J. Barciszewski, and M. Karonski, *J. Mol. Struct.*, **19**, 223 (1973); (k) G. E. Hawkes, D. Leibfritz, D. W. Roberts, and J. D. Roberts, *J. Am. Chem. Soc.*, **95**, 1659 (1973).
(4) (a) W. G. Bentrude, H.-W. Tan, and K. C. Yee, *J. Am. Chem. Soc.*, **94**, 3264 (1972); (b) W. G. Bentrude and H.-W. Tan, *ibid.*, **95**, 4666 (1973).
(5) B. C. Mayo, *Chem. Soc. Rev.*, **2**, 49 (1973).
(6) R. M. Wing, J. J. Uebel, and K. K. Andersen, *J. Am. Chem. Soc.*, **95**, 6046 (1973).
(7) P. A. Cunningham, D. E. Sands, W. F. Wagner, and M. F. Richardson, *Inorg. Chem.*, **8**, 22 (1969).
(8) The value of $\phi = 140°$ arose from some preliminary calculations in which ϕ was allowed to vary in the range 120–160°. The best fit between observed and calculated LIS was obtained for $\phi = 140°$ in all the compounds investigated.
(9) G. Montaudo, S. Caccamese, V. Librando, and P. Maravigna, *Tetrahedron*, **29**, 3915 (1973).
(10) K. L. Williamson, D. R. Center, R. Emch, M. Alexander, A. E. Burroughs, C. Chua, and M. E. Bogel, *J. Am. Chem. Soc.*, **96**, 1471 (1974).
(11) H. M. McConnell and R. E. Robertson, *J. Chem. Phys.*, **29**, 1361 (1958).
(12) This corresponds to the *R* factor commonly used in x-ray crystallography. (a) W. C. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965); (b) W. C. Hamilton, "Statistics in Physical Science", Ronald Press, New York, N.Y., 1964.
(13) The following considerations apply in the case of a fast equilibrium between the two species and are tied to the time scale of observation.
(14) E. F. Nifant'ev, A. A. Borisenko, I. S. Nasonovskii, and E. I. Matrosov, *Dokl. Akad. Nauk SSSR*, **196**, 28 (1971).
(15) W. G. Bentrude, H.-W. Tan, and K. C. Yee, *J. Am. Chem. Soc.*, **97**, 573 (1975).
(16) I. D. Blackburne, A. R. Katritzky, and Y. Takeuchi, *J. Am. Chem. Soc.*, **96**, 682 (1974).
(17) (a) R. S. Edmundson and E. W. Mitchell, *J. Chem. Soc. C*, 2091 (1968); (b) M. Kainosho and T. Shimozawa, *Tetrahedron Lett.*, 865 (1969); (c) J. P. Majoral, R. Kraemer, J. Devillers, and J. Navech, *Bull. Soc. Chim. Fr.*, 3917 (1970); (d) J. P. Marjoral and J. Navech, *ibid.*, 95, 1331 (1971); (e) J. P. Majoral, R. Pujol, J. Navech, and R. Mathis, *Tetrahedron Lett.*, 3755 (1971); (f) D. W. White, G. K. McEwen, R. D. Bertrand, and J. G. Verkade, *J. Chem. Soc. B*, 1454 (1971); (g) R. F. M. Whitej, *J. Mol. Struct.*, **6**, 75 (1970); (h) A. R. Katritzky, M. R. Nesbit, J. Michalski, Z. Tulimowski, and A. Zwierzak, *J. Chem. Soc. B*, 140 (1970); (i) R. S. Edmundson and E. W. Mitchell, *J. Chem. Soc. C*, 752 (1970); (j) W. G. Bentrude and J. H. Hargis, *Chem. Commun.*, 1113 (1969).
(18) See, e.g., J. P. Majoral, R. Pujol, and J. Navech, *C. R. Acad. Sci., Ser. C*, **274**, 213 (1972); J. P. Dutasta, A. Grand, J. B. Robert, and M. Taieb, *Tetrahedron Lett.*, 2659 (1974) (P–S compounds); and reference 17f.
(19) J. F. Caputo and A. R. Martin, *Tetrahedron Lett.*, 4547 (1971); R. R. Fraser and Y. Y. Wigfield, *Chem. Commun.*, 1471 (1970); G. E. Hawkes, D. Leibfrits, D. W. Roberts, and J. D. Roberts, *J. Am. Chem. Soc.*, **95**, 1659 (1973); K. L. Williamson, D. R. Clutter, R. Ench, M. Alexander, A. E. Burroughs, C. Chua, and M. E. Bogel, *ibid.*, **96**, 1471 (1974).
(20) W. G. Bentrude and J. H. Hargis, *J. Am. Chem. Soc.*, **92**, 7136 (1970).
(21) Mazhar-ul-Haque, C. N. Caughlan, J. H. Hargis, and W. G. Bentrude, *J. Chem. Soc. A*, 1786 (1970).
(22) "Tables of Interatomic Distances and Configuration in Molecules and Ions", *Chem. Soc. Spec. Publ.*, **No. 18**, (1965).

Aqueous Solution Conformation of Rigid Nucleosides and Nucleotides

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Abstract: The aqueous solution conformations of 18 different nucleoside derivatives in which various segments of the molecules were locked were investigated by NMR spectroscopy. In one set of the compounds the exocyclic linkage and the ribose were frozen; in another set the base and the ribose moieties were fused. Within these two broad categories conformations of the derivatives related to each other as enantiomers, α,β anomers and oxy and deoxy analogues were examined. Even though significant differences exist in the intimate details of their conformations, in general it was found that when the exocyclic linkage and the ribose were frozen the sugar ring exhibited ^3E conformation and the cyclic phosphate a chair form, the base showing accessibility of syn conformation. In those cases when the base and the ribose were fused, the sugar ring displayed ^2E pucker for the β derivatives and ^3E pucker for α derivatives with the magnitude of the sugar–base torsion angle being the same in both cases ($\chi \simeq 290°$). In addition, strong intramolecular perturbation between the phosphate backbone and the base was detected in the β cyclic series, while it was absent in the α cyclic series. As expected, the enantiomeric pairs displayed identical conformational features. In the case of 2′-deoxy-3′,5′-cyclic AMP, the possibility of the ribose ring existing as an unusual $^3E \rightleftarrows {}^0E$ equilibrium is discussed.

Introduction

Nuclear magnetic resonance spectroscopy has been used as a powerful tool (comparable to the use of x-ray crystallography in solid state) to obtain direct molecular parameters in solution. When the flexible molecules are studied in solution, their conformations are interpreted as states of dynamic equilibrium between the energy minimal conformers. For instance, in the conformational studies of nucleosides and nucleotides in recent years,[1-17] the glycosidic conformation has been described as syn $\rightleftarrows$ anti, the ribofuranose ring as $^2E \rightleftarrows {}^3E$, the C(4′)–C(5′) bond as gg $\rightleftarrows$ g/t, and the C(5′)–O(5′) bond as g′g′ $\rightleftarrows$ g′/t′ equilibrium with preference for one or the other conformer. In this paper, NMR studies are presented of several rigid nucleosides and nucleotides in which either the exocyclic linkage and the ribofuranose ring are fixed (e.g., 3′,5′-cyclic nucleotides I and II) or the base moiety and the sugar ring are fused (e.g., β-D- (or L-) 2′,O^2-cyclic arabinonucleosides (III) and α-D-2′,O^2-cyclic nucleosides (IV)). Several novel compounds, α-nucleosides and nucleotides, were also studied. As usual, the conformations about the flexible regions of the molecules were expressed in terms of dynamic equilibrium between the minimum energy conformers. The conformations of the inflexible portions (except for the base) of the molecules were described in terms of dihedral angles computed from the appropriate Karplus equations. The pure ^2E and ^3E conformations observed in the present case were compared with the models postulated in pseudorotational pathway.[18,19] Comparative studies of the interactions between the base and the exocyclic backbone were also undertaken. A comparison of the data of oxy and deoxy 3′,5′-cyclic nucleotides was made to obtain information about the anisotropic effect of hydroxyl groups. Among the 18 different nucleoside deriv-

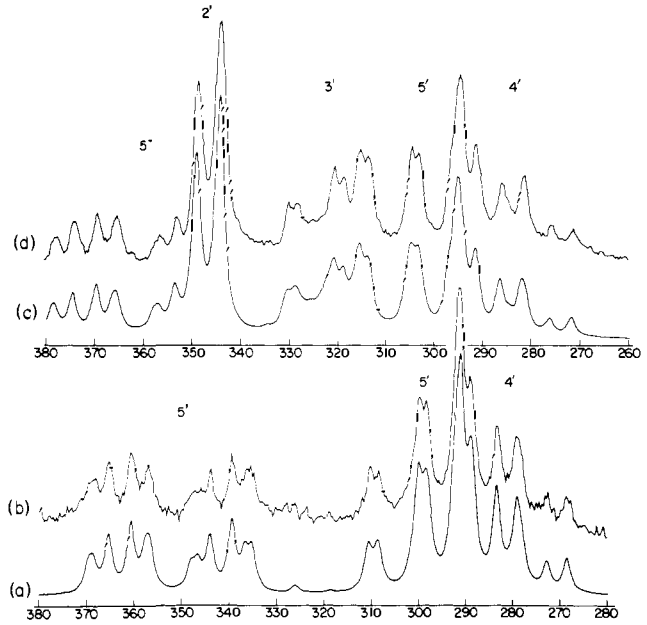
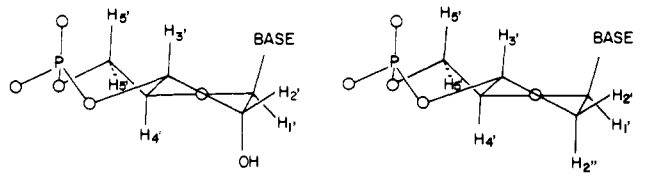
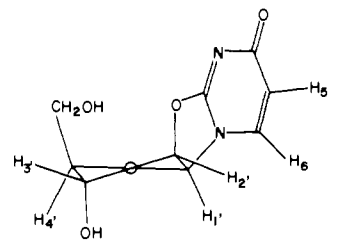
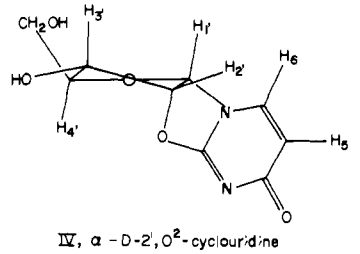


Figure 1. The 270-MHz ^{1}H NMR spectra of 3′,5′-cyclic GMP (cGMP) (b), and 3′,5′-cyclic CMP (cCMP) (d) with the corresponding simulated spectra (a) and (c) below. The chemical shift (Hz) is measured from internal tetramethylammonium chloride. For cGMP (b) only the region of H(4′), H(5′), and H(5″) is shown; for cCMP H(2′), H(3′), H(4′), H(5′), and H(5″) are shown.







atives whose solution conformations are reported here, two of them were reported earlier by Smith and co-workers.[20]

Experimental Section

The 3′,5′-cyclic nucleotides were purchased from Sigma Chemical Co., β-D- (or L-) 2′,O^2-cyclic arabinonucleosides, the corresponding 5′-nucleotides, α-nucleosides and the corresponding 5′-nucleotides, as well as α-D-2′,O^2-cyclic nucleosides were bought from Terra-Marine Bioresearch. All the samples were lyophilized three times from

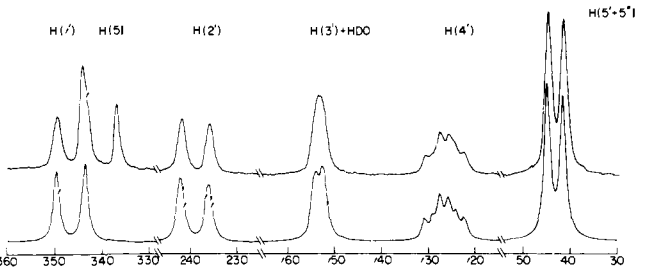


Figure 2. The 100-MHz ^{1}H NMR spectrum of β-D-2′,O^2-cyclic cytosine arabinonucleoside (β-D-c-C) hydrochloride (top) and the simulated spectrum (bottom). The peaks of H(6) are not shown. The chemical shifts are expressed in hertz from internal tetramethylammonium chloride.

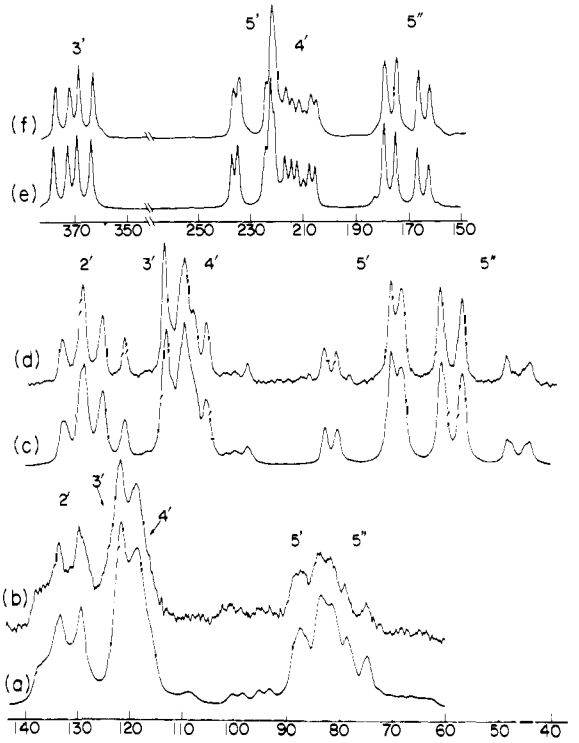


Figure 3. ^{1}H NMR spectra of α-5′-CMP (b), α-cytidine (d), and α-2′,O^2-cyclocytidine hydrochloride (f) with their corresponding simulated spectra (a), (c), and (e) below. Spectra of α-5′-CMP and α-cytidine are recorded at 100 MHz and that of the cyclic compound (f) at 300 MHz. The chemical shifts are expressed in hertz from internal tetramethylammonium chloride.

99.8% D_2O and the final solution was made up in "100%" D_2O from Bio-Rad Laboratories at a concentration of 0.1 M, pD 7.4.

The ^{1}H NMR spectra were recorded at 100, 270, or 300 MHz at 26 °C using tetramethylammonium chloride (TMA) as an internal standard. Details of the 100-MHz instrumentation are given elsewhere.[21] The 270-MHz spectra were obtained using Bruker HX-270 system at Southern New England High Field Facility at New Haven, Conn. The HX-270 system is equipped with a BNC data system and is capable of performing 16K transforms. The 300-MHz spectra were recorded at the Institute of Polymer Science, University of Akron, Akron, Ohio. This Varian 300-MHz system is interfaced to a 620 Varian Data System capable of performing 16K transforms.

Results and Discussion

A. Analysis of Spectra. The various ^{1}H NMR spectra were analyzed by simulation iteration methods using LAOCOON III. Figures 1, 2, and 3 show some samples of experimental and simulated spectra. A complete set of chemical shift and coupling constant data for the compounds investigated in this report is presented in Tables I, II, and III.

B. 3′,5′-Cyclic Nucleotides. 1. Conformation of the Phosphate Ring. In 3′,5′-cyclic nucleotides the phosphate group is

Table I. Chemical Shifts,[a] Coupling Constants,[b] and Dihedral Angles[c] of 3′,5′-Cyclic Nucleotides[d]

	cAMP		cGMP		cUMP		cCMP		dcAMP		dcTMP	
$\delta_{1'}$	2.911		2.743		2.638		2.637		3.305		3.135	
$\delta_{2'}$	1.482		1.550		1.354		1.284		−0.397		−0.680	
$\delta_{2''}$									−0.397		−0.597	
$\delta_{3'}$	1.509		1.778		1.220		1.193		1.726		1.503	
$\delta_{4'}$	1.131		1.047		1.038		1.060		0.854		0.721	
$\delta_{5'}$	1.143		1.107		1.113		1.126		1.103		1.096	
$\delta_{5''}$	1.349		1.304		1.329		1.339		1.272		1.260	
$\delta_{2 \text{ or } 5}$	4.999		4.658		2.680		2.890		5.067			
$\delta_{8 \text{ or } 6}$	4.988				4.456		4.493		4.978		4.261	
	J_{ij}	ϕ_{ij}	J_{ij}	ϕ_{ij}	J_{ij}	ϕ_{ij}	J_{ij}	ϕ_{ij}	J_{ij}	ϕ_{ij}	J_{ij}	ϕ_{ij}
$J_{1'2'}$	0.7	102	0.7	102	0.7	102	0.7	102	5.2		2.4	115
$J_{1'2''}$									5.2		8.7	14
$J_{2'2''}$									−14.0		−13.4	
$J_{2'3'}$	5.7	37	5.3	40	5.3	40	5.1	41	9.1		7.8	23
$J_{2''3'}$									9.1		10.9	164
$J_{3'4'}$	9.8	156	9.8	156	10.2	159	9.8	156	9.4	153	9.6	154
$J_{4'5'}$	10.7	163	10.7	163	10.7	163	10.7	163	10.7	163	10.7	163
$J_{4'5''}$	4.5	44	4.7	43	4.5	44	4.9	42	4.6	44	4.7	43
$J_{5'5''}$	−9.3		−9.5		−9.5		−9.7		−9.7		−9.3	
$J_{5'P}$	1.7	62	1.9	61	1.9	61	1.9	61	2.2	60	2.2	60
$J_{5''P}$	21.4	164	21.6	165	21.4	164	21.2	161	20.6	160	20.6	160
$J_{3'P}$	2.2	60	2.1	60	2.2	60	2.2	60	2.0	61	2.2	60
$J_{2'P}$	0.7		0.7		0.7		0.7		0.7		0.7	
$J_{1'P}$	0.7		0.7		0.7		0.7		0.7		0.7	
$^{N}\tau_m$[e]	38		40		42		41					

[a] The chemical shifts (ppm) are measured from the internal reference tetramethylammonium chloride (TMA). [b] The coupling constants (Hz) are checked by computer simulation. [c] The dihedral angles (deg) are computed from the Karplus equation: $^3J_{HH} = 10.5 \cos^2 \phi_{HH} - 1.2 \cos \phi_{HH}$ and $^3J_{HP} = 18.1 \cos^2 \phi_{HP} - 4.8 \cos \phi_{HP}$. [d] The abbreviations of the 3′,5′-cyclic nucleotides are: cAMP = 3′,5′-cyclic AMP; cGMP = 3′,5′-cyclic GMP; cUMP = 3′,5′-cyclic UMP; dcAMP = 2′-deoxy-3′,5′-cyclic AMP; dcTMP = 2′-deoxy-3′,5′-cyclic TMP [e] The values of $^{N}\tau_m$ (deg) are obtained by the method described in ref 8 and 19.

Table II. NMR Data of β-Cyclic Compounds[a]

	β-D-c-C	β-L-c-C	β-D-c-U	β-L-c-U	β-D-c-O	β-L-c-UMP
$\delta_{1'}$[b]	3.468	3.464	3.360	3.362	3.934	3.289
$\delta_{2'}$	2.394	2.390	2.290	2.291	2.271	2.307
$\delta_{3'}$	1.534	1.531	1.486	1.487	1.518	1.506
$\delta_{4'}$	1.267	1.262	1.219	1.222	1.186	1.211
$\delta_{5'}$	0.433	0.430	0.394	0.394	0.397	0.506
$\delta_{5''}$	0.433	0.430	0.394	0.394	0.397	0.506
δ_5	3.413	3.409	3.108	3.019	3.683	3.030
δ_6	4.944	4.941	4.743	4.744		4.748
$J_{1'2'}$[c]	6.0	5.9	5.9	5.9	5.9	5.9
$J_{2'3'}$	0.7	0.7	0.7	0.7	0.7	1.9
$J_{3'4'}$	1.8	1.8	1.7	1.8	1.6	3.9
$J_{4'5'}$	3.4	3.3	4.0	4.0	3.8	6.1
$J_{4'5''}$	3.4	3.3	4.0	4.0	3.8	6.1
Σ[d]	6.8	6.6	8.0	8.0	7.6	12.2
$J_{5'5''}$[e]	−12.0	−12.0	12.0	12.0	−12.0	−12.0
$J_{5'P}$						6.5
$J_{5''P}$						6.5
Σ'[f]						13.0

[a] The abbreviations of the compounds are: β-D- (or L-) c-C = β-D- (or L-) 2′,O^2-cyclic arabinocytidine; β-D- (or L-) c-U = β-D- (or L-) 2′,O^2-cyclic arabinouridine; β-D-c-O = β-D-2′,O^2-cyclic arabinoorotidine; β-L-c-UMP = β-L-2′,O^2-cyclic UMP. [b] The chemical shifts (ppm) are measured from the internal reference tetramethylammonium chloride (TMA). [c] The coupling constants (Hz) are checked by computer simulation. [d] $\Sigma = J_{4'5'} + J_{4'5''}$. [e] The coupling constant $J_{5'5''}$ is set arbitrarily. [f] $\Sigma' = J_{5'P} + J_{5''P}$.

present as part of a six-membered ring comprising O(3′)-P-O(5′)-C(5′)-C(4′)-C(3′). Information about the geometry of this ring can be obtained from ring coupling constants. The derived data for 3′,5′-cyclic AMP (cAMP), 3′,5′-cyclic GMP (cGMP), 3′,5′-cyclic UMP (cUMP), 3′,5′-cyclic CMP (cCMP), 2′-deoxy-3′,5′-cyclic AMP (dcAMP), and 2′-deoxy-3′,5′-cyclic TMP (dcTMP) are listed in Table I. The perspective structures of the ribose and phosphate rings are shown in I and II. The magnitude of the coupling constants (Table I) reveals that the conformations of the cyclic phosphate rings, in general, are constant throughout the array of compounds examined. The data indicate that the phosphate ring exists in a *chair form* in which H(3′) and H(4′), H(4′) and H(5′), and H(5″) and P are almost trans to each other; H(3′) and P, and H(5′) and P are almost gauche to each other (I, II).

One can compute the P-O-C-H and H-C-C-H dihedral angles using appropriate Karplus equations. Blackburn et al.[20] and Davies and Danyluk[22] have proposed the following Karplus dependence for P-O-C-H linkage in nucleic acid components.

$$^3J_{HP} = 16.3 \cos^2 \phi_{HP} - 4.6 \cos \phi_{HP}$$

However, in order to correlate data observed by Donaldson and Hall[23] (J_t = 22.8 Hz and J_g = 1.4 Hz) with those observed by Giese[24] in crystal (ϕ_{HP_t} = 174° and ϕ_{HP_g} = 66°), the above equation should be revised to

$$^3J_{HP} = 18.1 \cos^2 \phi_{HP} - 4.8 \cos \phi_{HP}$$

Several Karplus relationships[19,25-27] are available to calculate the vicinal HH dihedral angles. The one selected for the present study is that proposed by Altona and Sundaralingam,[19] i.e.,

$$^3J_{HH} = 10.5 \cos^2 \phi_{HH} - 1.2 \cos \phi_{HH}$$

The computed dihedral angles for the phosphate ring in the various compounds are given in Table I. In cAMP, cGMP, cUMP, cCMP, dcAMP, and dcTMP, the computed values (Table I) of $\phi_{3'4'}$ (153-159°), $\phi_{4'5'}$ (163°), $\phi_{4'5''}$ (42-44°), $\phi_{5'P}$ (60-62°), $\phi_{5''P}$ (160-165°), and $\phi_{3'P}$ (60-61°) indicate that

Table III. NMR Data of α-Nucleosides, α-Nucleotides, and α-2′,O²-Cyclonucleosides

	α-Cytidine	α-5′-CMP	δ-2′,O²-Cyclocytidine		α-Uridine	α-5′-UMP	α-2′,O²-Cyclouridine	
	δ_i	δ_i	δ_i	$\Delta\delta_i$[b]	δ_i	δ_i	δ_i	$\Delta\delta_i$[b]
$\delta_{1'}$[a]	2.955	2.991	3.353	0.398	2.972	3.023	3.241	0.269
$\delta_{2'}$	1.288	1.334	2.414	1.126	1.302	1.376	2.311	1.009
$\delta_{3'}$	1.139	1.222	1.237	0.098	1.137	1.224	1.184	0.047
$\delta_{4'}$	1.061	1.174	0.713	−0.348	1.110	1.209	0.687	−0.423
$\delta_{5'}$	0.736	0.887	0.763	0.028	0.717	0.811	0.761	0.044
$\delta_{5''}$	0.544	0.762	0.574	0.030	0.534	0.720	0.572	0.038
δ_5	2.855	2.864	3.443	0.588	2.686	2.684	3.051	0.365
δ_6	4.562	4.605	4.941	0.379	4.613	4.660	4.739	0.061
	J_{ij}	J_{ij}	J_{ij}	ϕ_{ij}[d]	J_{ij}	J_{ij}	J_{ij}	ϕ_{ij}[d]
$J_{1'2'}$[c]	3.8	4.1	5.5	38	3.8	4.4	5.4	39
$J_{2'3'}$	4.3	4.3	5.4	39	4.3	4.4	5.4	39
$J_{3'4'}$	8.2	8.0	9.2	152	8.0	8.0	9.2	152
$J_{4'5'}$	2.5	2.4	2.3		2.6	2.4	2.3	
$J_{4'5''}$	4.6	4.5	4.7		4.6	4.5	4.6	
Σ[e]	7.1	6.9	7.0		7.2	6.9	6.9	
$J_{5'5''}$	−12.5	−12.0	−13.0		−12.7	−11.8	−13.0	
$J_{5'P}$		5.0				5.4		
$J_{5''P}$		5.8				5.6		
Σ'[f]		10.8				11.0		

[a] The chemical shifts (ppm) are measured from the internal reference tetramethylammonium chloride. [b] $\Delta\delta_i$ is the chemical shift difference between the cyclic and noncyclic α-nucleoside. [c] The coupling constants (Hz) are checked by computer simulation. [d] The dihedral angles are calculated from the Karplus equation: $^3J_{HH} = 10.5\cos^2\phi_{HH} - 1.2\cos\phi_{HH}$. [e] $\Sigma = J_{4'5'} + J_{4'5''}$. [f] $\Sigma' = J_{5'P} + J_{5''P}$.

the phosphate ring has a flattened chair conformation which *shows little conformational sensitivity to variation in base and pentose constitutions, attesting to the rigidity of the ring system.* The observed deviation of the dihedral angles from the ideal trans (180°) and gauche (60°) conformations is due to: (i) a real difference in bond angles and bond lengths between the phosphate ring of cyclic nucleotides and the ideal chair conformation; (ii) molecular strain between the fused ribose and phosphate rings; and (iii) the uncertainty in the value of constants used in the Karplus equations. As mentioned before there are several Karplus relations available to compute ϕ_{HH}, and we have discussed in extenso elsewhere[14] that there could be errors as much as ±10% in the computed torsion angles. However, we have shown[12,15,28] that the data are extremely reliable for *comparing* conformational preferences among a series of analogous compounds.

2. Conformation of the Ribose Ring. In aqueous solution the ribofuranose ring of 3′ and 5′ mononucleotides exist as a flexible ring system[6,8,9,14] which is best described by a C(2′)-endo ⇄ C(3′)-endo ($^2E \rightleftarrows {}^3E$) equilibrium.[14,21,29] In the 3′,5′-cyclic nucleotides, because of the fusion between the ribose ring and the exocyclic linkage, it is likely that the flexibility of the ring is considerably reduced. In cAMP, cGMP, cUMP, and cCMP the magnitude of $J_{1'2'}$ is 0.7 Hz and that of $J_{3'4'}$ lies in the narrow range of 9.8–10.2 Hz. The data indicate that the ribose ring in these compounds exists almost entirely in ^3E conformation and that the conformation of the ribose is so rigid that it shows no significant sensitivity to the nature of the base. The observation that the sum (Table I) $J_{1'2'} + J_{3'4'}$ (10.5–10.9 Hz) is about 1.0–1.5 Hz greater than that reported for 3′ and 5′ mononucleotides[8,9] does not necessarily mean that the furanose ring in the cyclic nucleotides is abnormally puckered compared with those in the 3′ and 5′ mononucleotides. In fact, the observed values for $J_{2'3'}$ (Table I) are those expected of an ideal ^3E pucker,[8,9] indicating that the magnitudes of $J_{1'2'}$ and $J_{3'4'}$ are to some extent influenced by ring strain that is possibly present in the fused system. The amplitude of pucker τ_m can be computed from the values of $J_{1'2'} + J_{3'4'}$ and $J_{2'3'}$ using procedures outlined elsewhere,[8,9,14,19] and the results for cAMP, cGMP, cUMP, and cCMP are summarized in Table I. The observed range of 38–42° lies within the domain exhibited by 3′ and 5′ ribonucleotides.[8,9,14,19]

The ribose moiety of the deoxy analogues dcTMP and dcAMP shows some interesting features. In dcTMP, the value for $J_{3'4'}$ is 9.6 Hz, indicating that the ring pucker is almost exclusively ^3E. However, the values of $J_{2'3'}$ and $J_{1'2'} + J_{3'4'}$ in dcTMP are 7.8 and 12 Hz, respectively. The corresponding values in 3′dTMP and 5′dTMP are 6.6–6.8 and 10.6–11.2 Hz, respectively;[8,30] i.e., in dcTMP both $J_{2'3'}$ and $J_{1'2'} + J_{3'4'}$ increase by ≃ 1.1 Hz. We believe that the significant increase in $J_{2'3'}$ is due to flattening of the pentose moiety as a result of 3′,5′ cyclization. Such flattening of the ring on cyclization would also cause a decrease in $J_{1'2'} + J_{3'4'}$. This is contrary to what is observed. This can be rationalized on the ground that the anticipated decrease in $J_{1'2'} + J_{3'4'}$ due to flattening is more than compensated by the increase in their value due to molecular strain discussed above. Comparison of the values of $J_{2'3'}$ in cUMP, cCMP, and dcTMP (Table I) reveals that this coupling constant in the deoxy compound has increased by a substantial 2.6 Hz.[31] Part of this increase (≃0.5 Hz) is due to differences in the electronegativity and inductive effects between OH(2′) and H(2″); most of it could be explained if one could postulate that the pentose in dcTMP is flatter than in the oxy analogues. This flattening obviously results from the removal of the interaction between OH(2′) and O(3′) groups as the molecule undergoes oxy → deoxy transition. It should be emphasized that in the above discussion we have attempted to describe *relative conformational differences* among a series of analogous systems using accurately measured coupling constants (0 ± 0.1 Hz). Even though there could be errors as much as ±10% between the actual torsion angles and those computed,[14] this error is the same throughout the series. Hence, as elaborated elsewhere,[12,15,28] the conclusions reached with respect to relative conformational differences among the present series of compounds should be correct.

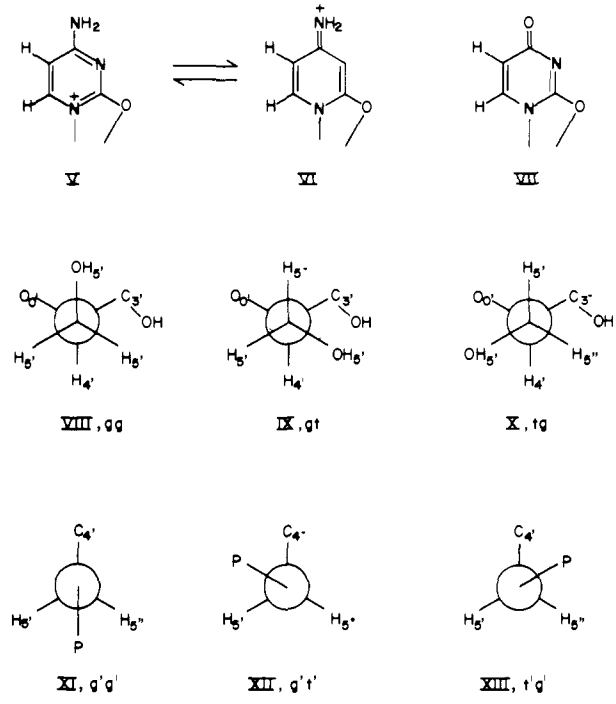
In the case of dcAMP, determination of the pentose conformation is complicated by the equivalence of H(2′) and

H(2″) chemical shifts. One cannot determine the individual values for $J_{1'2'}$, $J_{1'2''}$, $J_{2'3'}$, and $J_{2'3''}$ and they could have any values which can satisfy the observed sum $J_{1'2'} + J_{1'2''}$ or $J_{2'3'} + J_{2''3'}$. Based purely on the observed magnitude of 9.4 Hz for $J_{3'4'}$ in dcAMP (Table I), one may conclude that the deoxy ribose ring in dcAMP exists in an almost pure ^3E conformation. However, the observed sums for $J_{1'2'} + J_{1'2''}$ and $J_{2'3'} + J_{2''3'}$ are consistent with an unusual situation in which $J_{1'2'} = J_{1'2''} = 5.2$ Hz and $J_{2'3'} = J_{2''3'} = 9.1$ Hz. If this were true, conformationally this means that in dcAMP the linkage C(4′)-O(O′)-C(1′)-C(2′)-C(3′) of the pentose is so flexible that the ring exists as a $^3E \rightleftarrows {}^0E$ equilibrium; i.e., the deoxyribose ring in dcAMP undergoes pseudorotation from ^3E to ^0E conformation without significant change of the C(3′)-C(4′) bond conformation (or $J_{3'4'}$ value), in which the four carbon atoms of the ring are nearly in plane and the ring oxygen is puckered in the direction of the exocyclic linkage. This is the first time NMR data which are consistent with a $^3E \rightleftarrows {}^0E$ equilibrium have been obtained for a nucleotide derivative. It should be noted that pseudorotation from ^3E to ^2E is impossible because of the locked C(3′)-C(4′) bond conformation and as such the pseudorotation should stop at the ^0E stage.

In addition to vicinal coupling, the geminal coupling also illuminates some aspects of the molecular conformation. It is generally accepted that the geminal coupling constant of a saturated compound is dependent on the bond angle.[32,33] The dependences are: $^2J_{HH\ 105°} \simeq -20$ Hz, $^2J_{HH\ 109°} \simeq -12$ Hz, $^2J_{HH\ 125°} \simeq 0$ Hz, and $^2J_{HH>125°} > 0$ Hz. In the cyclic phosphate ring, the magnitude of the geminal coupling $J_{5'5''}$ is −9.5 Hz, suggesting that the H(5)-C(5′)-H(5″) angle is about 114° (larger than the tetrahedral angle 109.5°). In the deoxy compounds, the magnitude of $J_{2'2''}$ (−13.5 Hz) indicates that angle H(2′)-C(2′)-H(2′) is about 108°, suggesting that its complementary angle C(1′)-C(2′)-C(3′) is larger than the tetrahedral angle (109.5°)—again an indication of the flattening of the deoxy ribose ring. However, the thrust of the argument is weakened by the fact that the correlation between J_{gem} and geminal angle also depends on electronegativities of substituent groups and no simple way exists to sort out the influence of this factor.

The solution conformations of the ribose and cyclic phosphate moiety are essentially in agreement with the data reported in crystal,[34-39] attesting the ability of 3′,5′-cyclic nucleotides to exist in solution with a rigid ribophosphate conformation. It further shows that the Karplus dependency for $^3J_{CP}$ deduced by Smith and co-workers from 3′,5′-cyclic nucleotides is correct.[20,40] Blackburn et al.[20] concluded that dcTMP exists in C(4′)-exo conformation without taking into consideration the effect of ring flattening on coupling constants.

3. Conformation about the Glycosidic Linkage. It is generally accepted that the glycosidic bond of 5′ and 3′ mononucleotides in aqueous solution exists as an equilibrium system of anti and syn conformers with preference for the anti arrangement. Several theoretical calculations[41-43] project that the anti arrangement for the base is stabilized by interaction with the phosphate and that such stabilizing interactions occur only when the C(4′)-C(5′) bond is in the gg (VIII) conformation. These projections were experimentally confirmed by extensive NMR studies on 8-bromoadenosine, 8-methioadenosine, 8-azaadenosine, 8-azaguanosine, 6-azauridine, and their corresponding 5′-phosphate derivatives.[11,12,17,44] In the case of 3′,5′-cyclic nucleotides, the C(4′)-C(5′) bond is fixed in trans-gauche (X) and the C(5′)-O(5′) bond in gauche′-trans′ (XII) conformations, and the stabilizing interactions for the anti conformation between the backbone and the base do not exist. Hence, syn conformational domains should be accessible for these molecules. Quantitative estimation of the syn,anti populations is not at present possible because limiting chemical



shift data in the pure syn,anti conformers are not available. One could make very reasonable qualitative conclusions from the following arguments.

(i) In Figures 4 and 5 are illustrated the theoretically computed[45] relative chemical shift profiles for the ribose protons as a function of χ_{CN} changes in adenosine. The data show that the chemical shift of ribose protons (especially 2′ and 3′) are highly sensitive to torsional variation about the glycosidic bond and that the direction and magnitude of the shifts are also dependent on the mode of sugar pucker. Comparison of δH(2′) and δH(3′) in cAMP with that in the acyclic analogues 3′-AMP and 5′-AMP indicate that δH(2′) is shifted *upfield* (0.187 ppm) and δH(3′) *downfield* (0.055 ppm) in cAMP compared to the acyclic analogues. These reported shifts are the average of values obtained from both of the acyclic components. Using the chemical shift trends in Figures 4 and 5, one may rationalize these observations by either or both of the following events: (a) χ_{CN} remains in the anti range for 3′-AMP, 5′-AMP, and cAMP and that changes in δH(2′) and δH(3′) merely reflect an increase in the population of ^3E conformer for the ribose (30–40% ^3E for 3′-AMP and 5′-AMP[11,14,46,47] and ≃100% for cAMP); (b) in cAMP, the population of syn conformers (χ_{CN} = 180–200°) increases compared to 3′-AMP and 5′-AMP. Arguments presented below enable to make a distinction between the above two possibilities.

(ii) In Figures 4 and 5, one is dealing with relative changes in chemical shifts as a function of χ_{CN}, assuming that all chemical shifts are zero when χ_{CN} is zero. As is actually observed in the molecules with considerable presence of syn conformation,[11] in the 3′,5′-cyclic nucleotides, the H(3′) of the purine and H(2′) of the pyrimidine cyclic nucleotides appear at considerably lower fields compared to the corresponding protons in the regular 5′-mononucleotides. The inference is that in the cyclic nucleotides, the syn domain has become accessible.

(iii) Comparison of the H(3′) chemical shift data for 5′-AMP and cAMP as well as 5′-GMP and cGMP (Table I and ref 11 and 12) show that cyclization causes H(3′) of cGMP to shift (0.48 ppm) to lower field more than the H(3′) of cAMP (0.19 ppm). This is most likely due to the influence of the C(2)NH_2 group in the syn populations of cGMP.

Thus the data appear to indicate that, for the 3′,5′-cyclic nucleotides investigated in this report, the base exists as an

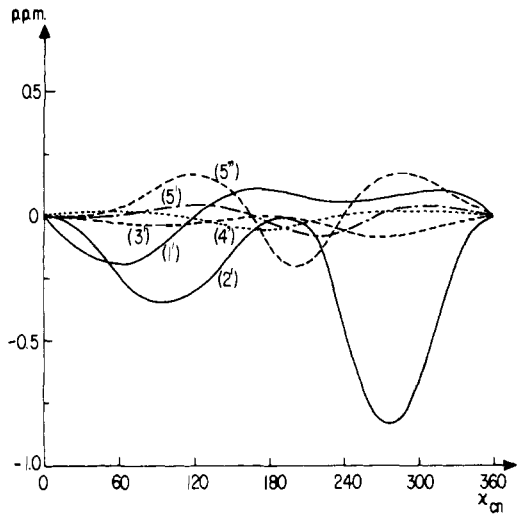


Figure 4. Theoretically computed changes in ribose proton chemical shifts as a function of χ_{CN} in ^2E adenosine (details in ref 45).

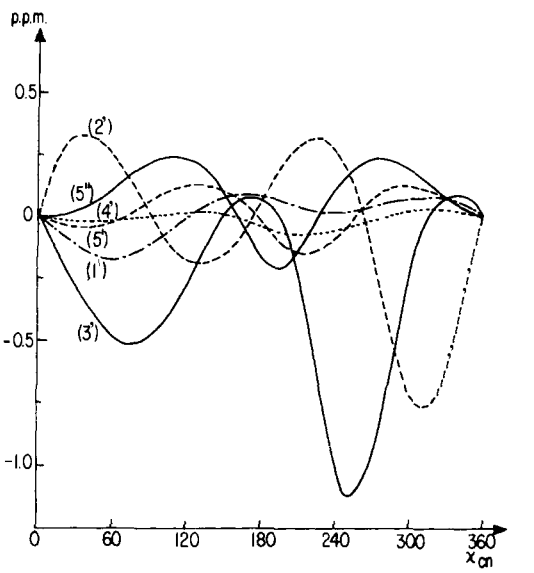


Figure 5. Theoretically computed changes in ribose proton chemical shifts as a function of χ_{CN} in ^3E adenosine (details in ref 45).

equilibrium mixture of conformers in the syn and anti domains. The ratio of the two types of conformers remains ambiguous. The crystal data for the glycosidic conformation are: syn and anti for cAMP,[39] syn for cGMP,[38] and anti for cUMP.[34,36,37]

4. Steric Dependence of Shielding Associated with Ring Hydroxyl Groups. The 3′,5′-cyclic nucleotides have a rigid ribophosphate structure and hence provide an opportunity to examine whether the shielding effects of substituents exert steric control. When the chemical shifts of H(1′) and H(3′) in 3′,5′-cyclic pyrimidine nucleotides were compared to those of the respective protons in the deoxy analogues (Table I), it is found that the 2′-OH group shifts H(1′) and H(3′) to higher fields, the H(1′) undergoing a larger shift (0.50 vs. 0.28 ppm). In other words, a vicinal hydroxyl group shields the gauche hydrogen more than the trans one (I, II). Contrarily, the H(4′) which is four-bond separated from 2′-OH is shifted to lower field (0.32 ppm) in the oxy series compared to the deoxy ones (II). A similar phenomenon is observed (Table I) in the cAMP series; however, the observed effects are small because of the $^3E \rightleftarrows {}^0E$ equilibrium and the lack of rigidity in dcAMP. These observations of the steric dependence of shielding associated with ring hydroxyl groups are very useful in analyzing NMR spectra of carbohydrates.[48]

5. Environmental Differences Between the H(5′) and H(5″) in 3′,5′-Cyclic Nucleotides. Conformationally the environmental differences between the H(5′) and H(5″) in 3′,5′-cyclic nucleotides are: (i) H(5′) is axial and H(5″) is equatorial in the six-membered cyclic phosphate structure; (ii) H(5′) is gauche to phosphorus, while H(5″) is trans to it; (iii) H(5′) is adjacent to an oxygen atom of the phosphate, while H(5″) is not. The first two occurrences tend to shift H(5′) upfield and H(5″) downfield while the third produces opposite effects.[48-50] The overall effect of the above three factors is that the H(5′) appears at a higher field compared to H(5″) (Table I).

C. β-D- (or L-) 2′,O²-Cyclic Arabinonucleosides and Nucleotides. 1. Conformation of the Arabinofuranose Ring. The fusion of the sugar ring and the base (III) through the 2′,O²-ether linkage restricts the conformational domains these molecules can occupy. A complete set of chemical shift and coupling constant data for β-D-2′,O²-cyclic arabinocytidine (β-D-c-C), β-L-2′,O²-cyclic arabinocytidine (β-L-c-C), β-D-2′,O²-cyclic arabinouridine (β-D-c-U, III), β-L-2′,O²-cyclic arabinouridine (β-L-c-U), β-D-2′,O²-cyclic arabinoorotidine (β-D-c-O), and β-L-2′,O²-cyclic 5′UMP (β-L-c-UMP) are listed in Table II. The data indicate that the enantiomers β-D-c-C and β-L-c-C as well as β-D-c-U and β-L-c-U have identical conformations. The pentose coupling constant data (Table II) for the cyclic arabinonucleosides show that the sugar pucker is insensitive to base variation. Such insensitivity is due to the restriction of conformational freedom by the fusion between the sugar ring and the base. This creates a rigid geometry for the pentose ring. The dihedral angles, computed from the Karplus equation (see section B1, B2 for equation and errors) are: $\phi_{H1'H2'} = 36°$, $\phi_{H2'H3'} = 102°$, and $\phi_{H3'H4'} = 110°$. These values for arabinosides (III) fit a model in which the sugar ring is fixed in the ^2E conformation and the base is oriented at a torsion angle $\chi_{CN} \simeq 290°$. The effect of 5′-phosphorylation on the conformation of the fused ring system can be obtained by comparing the ring coupling constant data for β-L-c-U and β-L-c-UMP. The data (Table II) show that 5′-phosphorylation increases $J_{2'3'}$ by 1.2 Hz and $J_{3'4'}$ by 2.1 Hz and it has *no effect* on $J_{1'2'}$. This is an indication that the pentose moiety in both the nucleoside and the nucleotide display ^2E conformation, but the sugar moiety in the nucleotide is flattened due to repulsion between the phosphate and the 2′,O² ether group.

2. Conformation about the Glycosidic Bond. The observed sugar–base torsion angle of 290° is midway between that traditionally accepted for syn and anti conformations. Such a conformation would be expected to generate chemical shift variations for all protons in the molecules compared to those of the unfrozen analogues.[51] The 2′,O² cyclization would cause a change of electron distribution in the base and this would contribute toward differences in chemical shifts between the cyclized and noncyclized arabinosides. In cyclocytidine, the base is in the form of a hydrochloride, which in turn, would be in equilibrium between states V and VI, in which state V is aromatic, while in cyclouridine, the base is solely in the non-aromatic VII form. Consequently, the base protons in cyclocytidine experience additional downfield shift (0.2 ppm) during cyclization compared to those in cyclouridine.

3. Conformation about the C(4′)–C(5′) Bond. The population distribution of conformers about the C(4′)–C(5′) bond (VIII, IX, and X) can be computed using the expressions:[10,11,17]

$$\%gg\ (VIII) = (13 - \Sigma)/10 \times 100$$

$$\%g/t\ (IX + X) = 100 - \%gg$$

where $\Sigma = J_{4'5'} + J_{4'5''}$. These empirical equations should be modified to reflect the more reasonable values of $J_g = 2.0$ Hz and $J_t = 11.7$ Hz.[8] The modified expression is

$$\%gg = (13.7 - \Sigma)/9.7 \times 100$$

The gg population computed from Σ values in Table II is 72% for cyclocytidine and 60% for cyclouridine and cycloorotidine. The extra gg population (12%) in cyclocytidine is most likely due to a stabilizing interaction between the hydrochloride base moiety and the 5′-OH group in the gg conformation. Data in Table II show that in these cyclic compounds, the 5′ geminal hydrogens display an unusual magnetic equivalence in contrast to the noncyclic analogues[51] and the regular pyrimidine nucleosides.[3,6,52] This is merely a reflection of the intramolecular interaction between the 2′,O² ether group and the C(4′)-C(5′)H_2OH system. Phosphorylation at OH(5′) would be expected to introduce extraordinary repulsion between the backbone and the frozen base, causing a depopulation of the gg conformers in an effort to relieve the resulting tension. Therefore it is not surprising to discover that the gg population in β-L-2′,O²-cyclic UMP is 16%, only a quarter of that exhibited by the corresponding nucleoside. This, as well as the exocyclic bond in galactose derivatives,[48] is among the few cases in which the gg population is so rare.

4. Conformation about the C(5′)–O(5′) Bond. In the case of nucleosides, the population distribution of conformers about the C(5′)–O(5′) bond in aqueous solution cannot be determined by NMR methods because the proton of the OH(5′) exchanges with D_2O and no coupling constants can be derived. In the case of β-L-2′,O²-cyclic UMP, this can be accomplished from the magnitude of Σ′ ($J_{5'P} + J_{5''P}$, Table II). The available equations are[10,11,17]

$$\%g'g' \text{ (XI)} = (24 - \Sigma')/18 \times 100$$

$$\%g'/t' \text{ (XII + XIII)} = 100 - \%g'g'$$

In view of the crystal[24] and NMR data[23] on phosphate esters (see Section B1) the expression for g′g′ population should be modified. The modified expression is

$$\%g'g' = (25 - \Sigma')/20.8 \times 100$$

Using this expression and the data in Table II, %g′g′ population was computed for β-L-2′O²-cyclic UMP. The value obtained (58%) is considerably lower than that reported for regular 5′-mononucleotides[8,10-12,17] and indicates the impact of repulsion between the phosphate and the 2′,O² ether groups on C(5′)–O(5′) torsion. The observed percentage population of g′g′ conformer in β-L-2′,O²-cyclic UMP is comparable to those in 8-Br-5′-AMP, 8-methio-5′-AMP, 8-aza-5′-AMP, 8-aza-5′-GMP, and 6-aza-5′-UMP,[11,12,17] i.e., nucleotide derivatives in which considerable repulsive interaction exists between the phosphate and the substituted base. These observations are consistent with the firmly established[10-12,41-43,53] correlations between gg and g′g′ conformations in mononucleotides. It appears *that rotation of the C(4′)–C(5′) bond is of primary importance in reducing the stress while that of C(5′)–O(5′) bond is secondary during the reorientation of the backbone conformation.*

D. α-Nucleosides, α-Nucleotides, and α-2′,O² Cyclonucleosides. 1. Conformation of the Ribofuranose Ring. A complete set of chemical shift and coupling constant data for α-cytidine, α-5′-CMP, α-2′,O²-cyclocytidine, α-uridine, α-5′-UMP, and α-2′,O²-cyclouridine (IV) is given in Table III. The conformation of the ribofuranose ring in the noncyclic α derivatives can be described as a $^2E \rightleftharpoons {}^3E$ equilibrium.[14] The observed values for $J_{3'4'}$ in the acyclic compounds fall in the range of 8.0–8.2 Hz, suggesting an overwhelming preference (over 85%) for the ^3E pucker. In the corresponding cyclic systems α-2′,O²-cyclocytidine and α-2′,O²-cyclouridine, the values of $J_{3'4'}$ increase by 1.0–1.2 Hz, indicating the presence of an almost pure ^3E conformation. Furthermore, the magnitude of $J_{2'3'}$ (4.3 Hz) in the noncyclic α-derivatives is smaller than that proposed for the pseudorotation model,[19] (4.7–6.2 Hz) suggesting that the sugar ring of the molecules puckers to a greater extent (or higher amplitude) in order to attain the maximal dihedral angles of N(1)-C(1′)-C(2′)-O(2′) and O(2′)-C(2′)-C(3′)-O(3′). The high amplitude of pucker of the ribose ring shown in α-nucleosides does not appear in the case of the corresponding cyclic derivatives ($J_{2'3'}$ = 5.4 Hz). This is obviously due to the formation of the 2′,O² ether linkage. Both the dihedral angles of H(1′)-C(1′)-C(2′)-H(2′) and H(2′)-C(2′)-C(3′)-H(3′) are computed to be 39°.

2. Conformation about the Glycosidic Bond. The glycosidic conformation in the acyclic α molecules is probably not exactly the same as that shown in the corresponding β anomers, since no interactions between the α base and the exocyclic linkage exist. Even though no direct experiments were conducted to determine sugar–base torsional preferences, it is reasonable to assume that, for the acyclic α anomers, both the syn and anti conformational domains are accessible. One anticipates a preference for the anti domain because of the possible repulsion between the oxo(2) and the OH(2′) groups in the syn conformer. Comparison between α-cytidine (or α-uridine) and α-5′-CMP (or α-5′-UMP) indicates that phosphorylation does not cause any significant shift of the base protons—obviously due to the distance between these groups in the α system.[54]

The glycosidic conformation in the α-cyclic compounds is fixed at a torsion angle of about 290° (IV). Similar to that in β-2′,O²-cyclic arabinonucleosides and nucleotides discussed previously, this conformation is midway between the traditional syn and anti states. As a result of conformational and electronic changes, H(1′), H(2′), H(4′), H(5), and H(6) of the molecules exhibit considerable chemical shift changes (Table III, compare the data for α-cytidine and α-cyclocytidine; α-uridine and α-cyclouridine). But H(3′), H(5′), and H(5″) display insignificant shifts since they are away from the base moiety. Again, the aromaticity of the base in α-cyclocytidine shifts the base protons 0.25 ppm more downfield as compared to the nonaromatic uridine analogue (Table III).

3. Conformation about the C(4′)–C(5′) and C(5′)–O(5′) Bonds. The sum of coupling constants $J_{4'5'}$ and $J_{4'5''}$ (Σ) and $J_{5'P}$ and $J_{5''P}$ (Σ′) provide information about the distribution of conformers about C(4′)–C(5′) and C(5′)–O(5′) bonds, respectively (VIII–XIII). The coupling constant sums (Σ) in Table III for α compounds, cyclic or noncyclic, nucleoside or nucleotide, show (indiscriminatively) the same conformational distribution about the C(4′)–C(5′) bond. This is simply because in the α anomers the constitution is such that an interaction between the backbone and base is not possible. In assigning the geminal protons, it is found that the lower field hydrogen couples (2.5 Hz) with H(4′) less than does the higher field proton (4.6 Hz). Furthermore, considering the repulsion between OH(5′) and OH(3′) in the gt conformation, it is likely that the gg and tg conformations are preferred along the C(4′)–C(5′) bond. The computed populations are found to be gg 68%, gt 5%, and tg 27%. The assignment is in agreement with that proposed for regular nucleosides and 3′-mononucleotides by Remin and Shugar.[52]

The computed g′g′ population about the C(5′)–O(5′) bond in α-5′-CMP and α-5′-UMP was found to be over 70% and this reflects the tendencies of the conformational distribution along the C(5′)–O(5′) bond in the absence of interaction between the exocyclic backbone and the base. The small difference between this population and that shown by β-nucleotides[8,10,16] indicates the insensitivity of the C(5′)–O(5′) conformation to the interaction between the exocyclic linkage and the base, once again suggesting that the C(5′)–O(5′) bond plays a less impressive role than does the C(4′)–C(5′) bond in alleviating the intramolecular tension in β-nucleotides.

Conclusion

This report not only reveals the conformations of the 18

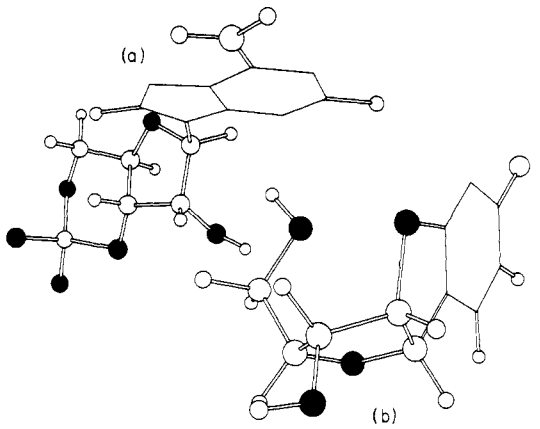


Figure 6. Three dimensional perspectives of the conformations of cAMP (a) and β-D-2′-O²-cyclic arabinouridine.

molecules investigated, but also examines the repercussions of restricting the conformational freedom of various segments of a nucleotide. The 3′ 5′-cyclic nucleotides are observed as existing in the $|{}^3E$, syn $\rightleftharpoons$ anti| conformation with the cyclic phosphate group in the chair form (Figure 6a). The sugar pucker in dcTMP displays greater flattening than does the oxy analogue. The deoxy ribose ring in dcAMP may exist in an unusual ${}^3E \rightleftharpoons {}^0E$ equilibrium. The conformation of β-D- (or L-) 2′,O²-cyclic arabinonucleosides is found to be |gg(60) $\rightleftharpoons$ g/t(40), ${}^2E$, $\chi \simeq 290°$| for uracil and orotidine derivatives (Figure 6b) and |gg(72) $\rightleftharpoons$ g/t(28), ${}^2E$, $\chi \simeq 290°$| for the cytidine analogue, the number in parentheses being the percent population. Phosphorylation of 2′,O²-cyclic β-arabinosides at 5′ causes extraordinary repulsion between the backbone and frozen base, so much so the backbone in the nucleotides prefers the g/t conformation. The conformation for α-nucleosides is |gg(68) $\rightleftharpoons$ g/t(32), ${}^2E(20) \rightleftharpoons {}^3E(80)$, syn $\rightleftharpoons$ anti|, that for α-nucleotides is |g′g′(73) $\rightleftharpoons$ g′/t′(27), gg(70) $\rightleftharpoons$ g/t(30), ${}^2E(21) \rightleftharpoons {}^3E(79)$, syn $\rightleftharpoons$ anti|. α-2′,O²-Cyclic nucleosides display a |gg(70) $\rightleftharpoons$ g/t(30), ${}^3E$, $\chi \simeq 290°$| conformation. In addition the following observations are made: (i) The shielding effect of ring hydroxyl groups is strongly geometry dependent. (ii) In β-nucleosides and nucleotides intramolecular interactions exist between the base and the backbone, and the C(4′)–C(5′) bond responds to this interaction by torsional variation and the rotational variation about the C(4′)–C(5′) bond is of primary importance in eliciting the compensatory conformations.

We have been informed by Dr. S. S. Danyluk that he has already completed the complete analyses of the ¹H NMR spectra of cAMP, cGMP, cUMP, and cCMP.

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References and Notes

(1) M. P. Schweizer, A. D. Brown, P. O. P. Ts'o, and D. P. Hollis, *J. Am. Chem. Soc.*, **90**, 1042 (1968).
(2) M. P. Schweizer and R. K. Robins, *Jerusalem Symp. Quantum Chem. Biochem.*, **5**, 324 (1973).
(3) T. Schleich, B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *Biochemistry*, **11**, 137 (1972).
(4) I. C. P. Smith, H. H. Mantsch, R. D. Lapper, R. Deslauriers, and T. Schleich, *Jerusalem Symp. Quantum Chem. Biochem.* **5**, 381 (1973).
(5) F. E. Hruska, *Jerusalem Symp. Quantum Chem. Biochem.*, **5**, 345 (1973).
(6) F. E. Hruska, A. A. Grey, and I. C. P. Smith, *J. Am. Chem. Soc.*, **92**, 4088 (1970).
(7) S. S. Danyluk and F. E. Hruska, *Biochemistry*, **7**, 1038 (1968).
(8) D. B. Davies and S. S. Danyluk, *Biochemistry*, **13**, 4417 (1974).
(9) D. B. Davies and S. S. Danyluk, *Biochemistry*, **14**, 543 (1975).
(10) R. H. Sarma, R. J. Mynott, D. J. Wood, and F. E. Hruska, *J. Am. Chem. Soc.*, **95**, 6457 (1973).
(11) R. H. Sarma, C. H. Lee, F. E. Evans, N. Yathindra, and M. Sundaralingam, *J. Am. Chem. Soc.*, **96**, 7337 (1974).
(12) C. H. Lee, F. E. Evans, and R. H. Sarma, *J. Biol. Chem.*, **250**, 1290 (1975).
(13) F. E. Evans and R. H. Sarma, *FEBS Lett.*, **41**, 253 (1974).
(14) F. E. Evans and R. H. Sarma, *J. Biol. Chem.*, **249**, 4754 (1974).
(15) F. E. Evans and R. H. Sarma, *J. Am. Chem. Soc.*, **97**, 3215 (1975).
(16) D. J. Wood, R. J. Mynott, F. E. Hruska, and R. H. Sarma, *FEBS Lett.*, **34**, 323 (1973).
(17) D. J. Wood, F. E. Hruska, R. J. Mynott, and R. H. Sarma, *Can. J. Chem.*, **51**, 2571 (1973).
(18) L. D. Hall, P. R. Steiner, and C. Pedersen, *Can. J. Chem.*, **48**, 1155 (1970).
(19) C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **95**, 2333 (1973).
(20) B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *J. Am. Chem. Soc.*, **95**, 2873 (1973).
(21) R. H. Sarma and R. J. Mynott, *J. Am. Chem. Soc.*, **95**, 1641 (1973).
(22) D. B. Davies and S. S. Danyluk, "International Symposium on NMR Spectroscopy", University of Surrey, 1972.
(23) B. Donaldson and L. D. Hall, *Can. J. Chem.*, **50**, 2111 (1972).
(24) H. J. Giese, *Recl. Trav. Chim. Pays-Bas*, **86**, 362 (1967).
(25) R. J. Abrahams, L. D. Hall, L. Hough, and K. A. McLauchlan, *Chem. Ind. (London)*, 213 (1962).
(26) R. J. Abrahams, L. D. Hall, L. Hough, and K. A. McLauchlan, *J. Chem. Soc.*, 3699 (1962).
(27) R. U. Lemieux, J. D. Stevens, and R. R. Fraser, *Can. J. Chem.*, **40**, 1958 (1962).
(28) C. H. Lee and R. H. Sarma, *J. Am. Chem. Soc.*, **97**, 1225 (1975).
(29) R. H. Sarma and R. J. Mynott, *J. Chem. Soc., Chem. Commun.*, 975 (1972).
(30) D. J. Wood, F. E. Hruska, and K. K. Ogilve, *Can. J. Chem.*, **52**, 3353 (1974).
(31) In dcTMP, the observed magnitude of $J_{2'3'}$ is such that the Altona–Sundaralingam[19] expressions are no longer applicable to calculate the amplitude of pucker.
(32) J. R. Dyer, *"Applications of Absorption Spectroscopy of Organic Compounds"*, Prentice-Hall, Englewood Cliffs, N.J., 1965, p 116.
(33) A. J. Pople and A. A. Bothner-By, *J. Chem. Phys.*, **42**, 1339 (1965).
(34) C. L. Coulter, *Acta Crystallogr., Sect. B*, **25**, 2055 (1969).
(35) M. Sundaralingam and J. Abola, *J. Am. Chem. Soc.*, **94**, 5070 (1972).
(36) C. L. Coulter, *Science*, **159**, 888 (1968).
(37) C. L. Coulter, *Acta Crystallogr., Sect. B*, **26**, 441 (1970).
(38) A. K. Chwang and M. Sundaralingam, *Nature (London), New Biol.*, **244**, 136 (1973).
(39) K. Watenpaugh, J. Dow, L. H. Gensen, and S. Furberg, *Science*, **159**, 206 (1968).
(40) R. D. Lapper, H. H. Mantsch, and I. C. P. Smith, *J. Am. Chem. Soc.*, **95**, 2878 (1973).
(41) H. Berthod and B. Pullman, *FEBS Lett.*, **33**, 147 (1973).
(42) W. Olson, *Biopolymers*, **12**, 1787 (1973).
(43) N. Yathindra and M. Sundaralingam, *Biopolymers*, **12**, 297 (1973).
(44) M. P. Schweizer, J. T. Witkowski, and R. K. Robins, *J. Am. Chem. Soc.*, **93**, 277 (1971).
(45) C. Giessner-Bettre and B. Pullman, manuscript in preparation.
(46) C. H. Lee, F. E. Evans, and R. H. Sarma, *FEBS Lett.*, **51**, 73 (1975).
(47) F. E. Evans, C. H. Lee, and R. H. Sarma, *Biochem. Biophys. Res. Commun.*, **63**, 106 (1975).
(48) C. H. Lee and R. H. Sarma, *Biochemistry*, **15**, 697 (1976).
(49) F. A. L. Anet, *J. Am. Chem. Soc.*, **84**, 1053 (1962).
(50) R. U. Lemieux, R. K. Kulling, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).
(51) D. Doris, C. H. Lee, and R. H. Sarma, unpublished data on arabino nucleosides and nucleotides.
(52) M. Remin and D. Shugar, *Biochem. Biophys. Res. Commun.*, **48**, 636 (1972).
(53) W. K. Olson and P. J. Flory, *Biopolymers*, **11**, 25 (1972).
(54) R. H. Sarma and N. O. Kaplan, *Biochem. Biophys. Res. Commun.*, **36**, 780 (1969).