Stereochemistry of Nucleic Acids and Their Constituents. XVIII.[1] Conformational Analysis of α Nucleosides by X-Ray Crystallography[2]

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Abstract: In general, the preferred conformations of the α nucleosides in the solid state are opposite (enantiomeric) to those of the β nucleosides. The favored stereochemical relationship between the base and the sugar is anti and the glycosidic torsion angle χ_{CN} is centered about −50° which is enantiomerically related to the anti range found in the β nucleosides. The furanoside ring puckerings that are preponderant in α nucleosides are C(2′)-exo, C(3′)-exo, and C(4′)-endo, which contrasts with the C(3′)-endo and C(2′)-endo in the β nucleosides. An abbreviated nomenclature system for conformations of furanoside rings is given.

Unlike the β nucleosides the α nucleosides are neither widespread in nature nor incorporated into the nucleic acids. Nevertheless, they are of considerable interest because they are constituents of the important coenzymes vitamin B_{12} and nicotinamide–adenine dinucleotide.[3] Detailed information on the conformations of nucleic acid constituents is now available mainly through single-crystal X-ray diffraction studies.[4] However, until very recently, the paucity of accurate crystal structural data on α nucleosides has prevented an understanding of their conformational properties. Recently, we reported the accurate crystal structures of α-D-pseudouridine monohydrate[5] and α-D-2′-amino-2′-deoxyadenosine monohydrate.[6] These results together with some previously available structural data have permitted us to make some important inferences regarding the preferred conformations of α nucleosides.

The conformations of the α nucleosides are described in terms of the torsion angles about the glycosidic bond, the exocyclic C(4′)–C(5′) bond, and the furanoside ring bonds. These angles were obtained by standard methods from the published atomic coordinates of the available crystal structures, and are listed in the appropriate tables below.

Conformation about the Glycosidic Bond. The orientation of the base ring relative to the furanoside ring is described by the glycosidic torsion angle χ_{CN}.[7] All of these angles (Table I) are in the anti range and lie within a narrow range of values, from −30° to −72° and centered about −50°, for the known compounds. Thus, the anti conformation is the preferred conformation for the α nucleosides. It is noteworthy that the anti range found for the α nucleosides bears an enantiomeric relation to the preferred anti range exhibited by the β nucleosides.[4]

In α-pseudouridine where there is a glycosidic C–C bond instead of the usual C–N bond, the χ angle is +3°. In the vitamin B_{12} derivatives the α nucleoside is in a loop and the base site N(7) (Figure 1) is bonded to the cobalt atom, while the 3′-phosphate end is connected to the corrin ring. This will explain the apparent rigidity to rotation of the loop[8] and the remarkable constancy in the glycosidic torsion angles (average −44°), Table I.

Conformation about the C(4′)–C(5′) Bond. As expected the three possible staggered arrangements for the rotation of the C(5′)–O(5′) bond about the exocyclic C(4′)–C(5′) bond are observed for the α nucleosides, Figure 2. The data, Table I, indicate that the conformational preference is in the following order: gauche-gauche > gauche-trans > trans-gauche, which is similar to the order found for the β nucleosides.[4]

It has been speculated previously that the preferred gauche-gauche conformation in the β nucleosides and the anti glycosidic angle were most probably due to the attractive potential (C–H---O) involving the interaction of the O(5′) atom and the C(6)-H group of the pyrimidine nucleosides or the C(8)-H group of the purine nucleosides.[9,10] This explanation appears to be

(1) Part XVII of this series is by M. A. Viswamitra, B. S. Reddy, G. Hung-Yin Lin, and M. Sundaralingam, *J. Amer. Chem. Soc.*, **93**, 4565 (1971).

(2) This paper was presented in part at the Joint Conference of the Chemical Institute of Canada and the American Chemical Society held in Toronto, Canada, May 24–29, 1970, Abstract of Papers No. 36.

(3) S. F. Velik in "Light and Life," W. D. McElroy and H. B. Glass, Ed., Johns Hopkins Press, Baltimore Md., 1961, p 106. Neither the α nucleoside moiety of vitamin B_{12} nor that of nicotinamide contain a common nucleic acid base. In the former, the base is a benzimidazole derivative, Figure 2, while in the latter the base is a pyrimidine derivative. As far as the author is aware, no case is yet known of a naturally occurring α nucleoside with a nucleic acid base.

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

(5) D. C. Rohrer and M. Sundaralingam, *J. Amer. Chem. Soc.*, **92**, 4950 (1970).

(6) D. C. Rohrer and M. Sundaralingam, *ibid.*, **92**, 4956 (1970).

(7) The original definition for this angle, referred to as ϕ_{CN}, was given by J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960). Subsequently other definitions have also been used, *viz.*, M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.*, **13**, 930 (1965); ref 4; and W. Saenger and K. H. Scheit, *J. Mol. Biol.*, **50**, 153 (1970). The abbreviation χ is used here only to conform to the accepted abbreviation (*J. Mol. Biol.*, **52**, 1 (1970)) used for the torsion angles of the amino acid side groups of a polypeptide chain. The definition of χ_{CN} used here is that given in ref 4, with positive angles in the range 0 to +180° and negative angles (0 to −180°) in the range 180–360°. The approximate relation between the definitions of Sundaralingam (S) and Donohue and Trueblood (DT) is $\chi_{CN}(S) \approx -\phi_{CN}(DT)$.

(8) It was pointed out earlier[4] that the conformations of the phosphodiester functions are also similar for all of the vitamin B_{12} compounds, and that these conformations were significantly different from the conformations commonly found in double stranded nucleic acids and polynucleotides. It is suggested here that the conformation of the nucleotide loop of vitamin B_{12} will serve as a model for the phosphodiester conformation in the loop regions of transfer RNA.[4] The available data also strongly suggest that the conformational "flexibility" in the polynucleotides dwells on the ester P–O(3′) and P–O(5′) bond.[4]

Table I. Torsion Angles about the Glycosidic Bond and the Exocyclic C(4′)–C(5′) Bond in α Nucleosides

Compound	Ring puckerings	χ_{CN}, deg	ϕ_{00}, deg	ϕ_{CO}, deg	O(2′)–C(2′)–C(3′)–O(3′), deg
α-Pseudouridine monohydrate (C–C glycosidic bond)[c]	C(2′)-exo	+3[a]	70 (gauche)	−170 (trans)	35
Vitamin B_{12} (wet)[d]	C(2′)-exo	−47	−44 (gauche)	74 (gauche)	34
Vitamin B_{12} (air dry)[e]	C(2′)-exo	−42	−60 (gauche)	66 (gauche)	41
Vitamin B_{12} 5′-phosphate[f]	C(2′)-exo	−45	−58 (gauche)	53 (gauche)	50
α-D-2′-Amino-2′-deoxyadenosine monohydrate[g]	C(3′)-exo	−60	53 (gauche)	171 (trans)	−37[b]
5-[1-(2′-Deoxy-α-D-ribofuranosyl)]uracilyl disulfide[h]					
Residue 1	C(3′)-exo	−30	174 (trans)	70 (gauche)	
Residue 2	C(3′)-exo	−72	−49 (gauche)	68 (gauche)	
5-[1-(2′-Deoxy-α-D-ribofuranosyl)]uracilyl methylsulfide[i]					
Molecule 1	C(4′)-endo	−66	−70 (gauche)	48 (gauche)	
Molecule 2	C(4′)-endo	−52	53 (gauche)	170 (trans)	
Vitamin B_{12} coenzyme[j]	C(3′)-endo	−40	−67 (gauche)	53 (gauche)	43

[a] The glycosidic bond here is a C–C bond. [b] The torsion angle here involves the 2′-amino group and the 3′-hydroxy group. The amino group forms an intramolecular hydrogen bond to the 3′-oxygen. [c] See ref 5. [d] D. C. Hodgkin, J. Lindsey, R. A. Sparks, K. N. Trueblood, and J. G. White, *Proc. Roy. Soc., Ser. A*, **266**, 494 (1962). [e] C. Brink-Shoemaker, D. W. J. Cruickshank, D. C. Hodgkin, M. J Kamper, and D. Pilling, *ibid., Ser. A*, **278**, 1 (1964). [f] S. W. Hawkinson, C. L. Coulter, and M. L. Greaves, *ibid.*, in press. [g] See ref 6. [h] E. Shefter, M. P. Kotick, and T. J. Bardos, *J. Pharm. Sci.*, **5**, 1293 (1967). [i] G. W. Frank, private communication. [j] P. G. Lenhert, *Proc. Roy. Soc., Ser. A*, **303**, 45 (1968).

untenable since even the α nucleosides tend to prefer the gauche-gauche conformation even though now the interaction between the O(5′) atom and the base is not possible because of the configurational inversion at

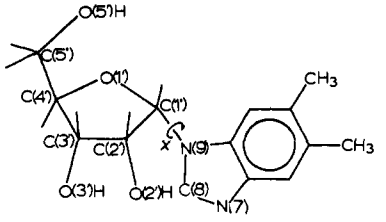

Figure 1. The numbering adopted for the α nucleoside part of the vitamin B_{12} compounds.

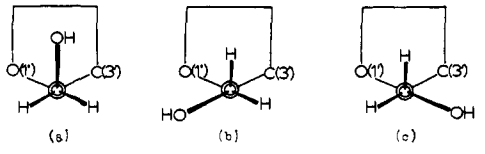

Figure 2. The three possible staggered conformations about the C(4′)–C(5′) bond: (a) gauche-gauche, (b) gauche-trans, and (c) trans-gauche.

C(1′). Therefore, there must be some other explanation for this preferred conformation. It is conceivable that dipole–dipole interactions involving the O(1′) and O(5′) atoms are more favored in the gauche-gauche conformation than in the gauche-trans.

Conformation of the Furanoside Ring. The furanoside ring plays a central role in the determination of the conformations of nucleosides.[11] The conformation of this ring is considered in terms of the puckering relative to the best four-atom and three-atom planes, and the torsion angles about the ring bonds.[11,12] The deviation of the puckered atom from the best four-atom least-squares plane and the mode of puckering are given in column 3, Table II. In general, the atoms

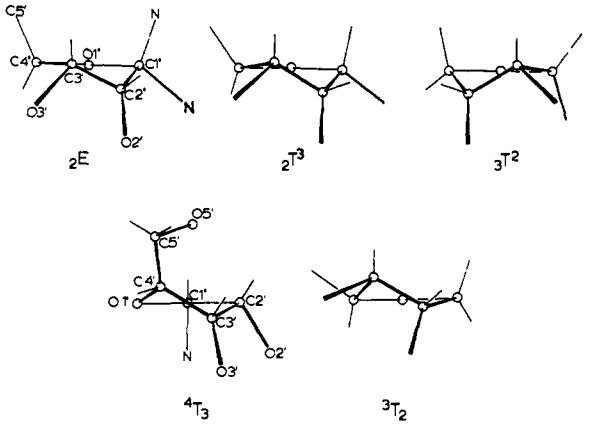

Figure 3. The different furanoside ring conformations found in α nucleosides.

defining the best four-atom least-squares plane do not lie in the plane.[11] Therefore, it is more accurate to present the conformation of the furanoside ring in terms of a three-atom plane, giving the displacements of the two remaining atoms from this plane. As a rule the three atoms that are common to both the best four-atom plane and the next best four-atom plane are chosen to define this plane: column 3, Table II. The deviations are considered to be significant if they are at least about 2.7 times the estimated standard deviation in the atomic coordinates.[13] If both the atoms are

(9) S. Furberg, C. S. Petersen, and Chr. Romming, *Acta Crystallogr.*, **18**, 313 (1965).
(10) E. Shefter and K. N. Trueblood, *ibid.*, **18**, 1067 (1965).
(11) M. Sundaralingam, *J. Amer. Chem. Soc.*, **87**, 599 (1965).
(12) G. M. Brown and H. A. Levy, *Science*, **141**, 921 (1963).
(13) This is a standard practice in crystallography; see D. W. J. Cruickshank and A. P. Robertson, *Acta Crystallogr.*, **6**, 698 (1953).

Table II. Furanoside Ring Conformations in α Nucleosides

Compound	Esd, A	Puckering and out-of-plane deviation from best four-atom plane	Puckering and out-of-plane deviation from three-atom plane
α-Pseudouridine monohydrate	0.004	C(2′)-exo $_2$E 0.57	C(2′)-exo-C(3′)-exo $_2$E 0.58 0.01
Vitamin B_{12} (wet)	0.04	C(2′)-exo $_2$E 0.74	C(2′)-exo-C(3′)-endo $_2$E 0.72 0.03
Vitamin B_{12} (air dry)	0.04	C(2′)-exo $_2$E 0.74	C(2′)-exo-C(3′)-endo $_2T^3$ 0.60 0.18
Vitamin B_{12} 5′-phosphate	0.04	C(2′)-exo $_2$E 0.78	C(2′)-exo-C(3′)-endo $_2T^3$ 0.57 0.26
α-D-2′-Amino-2′-deoxyadenosine monohydrate	0.006	C(3′)-exo $_3$E 0.46	C(3′)-exo-C(2′)-endo $_3T^2$ 0.34 0.15
5-[1-(2′-Deoxy-α-D-ribofuranosyl)]uracilyl disulfide			
Residue 1	0.006	C(3′)-exo $_3$E 0.53	C(3′)-exo-C(2′)-endo $^2_3$T 0.35 0.22
Residue 2	0.006	C(4′)-endo ^{4}E 0.39	C(4′)-endo-(3′)-exo $^4_3$T 0.23 0.21
5-[1-(2′-Deoxy-α-D-ribofuranosyl)]uracilyl methyl sulfide			
Molecule 1	0.01	C(4′)-endo ^{4}E 0.44	C(4′)-endo-C(3′)-exo ^{4}T$_3$ 0.32 0.15
Molecule 2	0.01	C(4′)-endo ^{4}E 0.47	C(4′)-endo-C(3′)-exo $^4_3$T 0.28 0.25
Vitamin B_{12} coenzyme	0.04	C(3′)-endo ^{3}E 0.74	C(3′)-endo-C(2′)-exo ^{3}T$_2$ 0.49 0.34

Table III. Torsion Angles Involving the Furanoside Ring Hydrogen Atoms

	H(1′)–H(2′), deg	H(1′)–H′(2′), deg	H(2′)–H(3′), deg	H′(2′)–H(3′), deg	H(3′)–H(4′), deg	H(4′)–H(5′), deg	H(4′)–H′(5′), deg
α-Pseudouridine monohydrate	−38		49		143	65	−175
α-D-2′-Amino-2′-deoxyadenosine monohydrate	−26		46		98	61	176
5-[1-(2′-Deoxy-D-ribofuranosyl)]-uracilyl disulfide							
Residue 1	−35	86	74	−32	−82	−76	174
Residue 2	−109	21	101	−30	−100	−56	63

Table IV. The Puckering and the Torsion Angles about the Ring Bonds in α Nucleosides

Compound	Puckering	τ_0, O(1′)–C(1′)	τ_1 C(1′)–C(2′)	τ_2 C(2′)–C(3′)	τ_3 C(3′)–C(4′)	τ_4 C(4′)–O(1′)
α-Pseudouridine monohydrate (C–C glycosidic bond)	C(2′)-exo	23	−35	34	−22	0
Vitamin B_{12} (wet)	C(2′)-exo	29	−46	46	−31	1
Vitamin B_{12} (air dry)	C(2′)-exo	24	−44	45	−35	8
Vitamin B_{12} 5′-phosphate	C(2′)-exo	24	−41	43	−37	10
α-D-2′-Amino-2′-deoxyadenosine monohydrate	C(3′)-exo	−6	22	−30	27	−13
5-[1-(2′-Deoxy-α-D-ribofuranosyl)]-uracilyl disulfide						
Residue 1	C(3′)-exo	−9	28	−34	30	−14
Residue 2		10	8	−21	28	−24
5-[1-(2′-Deoxy-α-D-ribofuranosyl)]-uracilyl methyl sulfide						
Molecule 1	C(4′)-endo	13	6	−22	30	−28
Molecule 2	C(4′)-endo	12	10	−26	34	−30
Vitamin B_{12} coenzyme	C(3′)-endo	13	−38	46	−44	20

significantly displaced, then the conformation is referred to as a twist (T) "hair-chair," whereas if only one of the atoms is significantly displaced the conformation is referred to as "envelope" (E). The abbreviated conformational nomenclature[14] is also given in Table II, where the subscript and superscript numbers denote

the numbering of the out-of-plane atoms. The superscript denotes that the deviation is on the same side (endo) of the plane as C(5′), while the subscript denotes the deviation is on the opposite side (exo) of C(5′). Priority is given to the atom that exhibits the primary[12] pucker and it appears before the letter, while the atom showing the secondary[11] pucker follows the letter.[14] While the results are based on X-ray data, the conformational designations would apply in general, *e.g.*, to results based on other data, such as nmr.

The twist (T) "half-chair" (Table II and Figure 3) is certainly the predominantly occurring conformation in α nucleosides. A similar conformational preference was found for the β nucleosides.[4] However, there is an important difference between the preferred twist conformations found in the α and β nucleosides. In the β nucleosides the C(3′)-endo–C(2′)-exo ($^3T_2$) [or C(3′)-endo] and C(2′)-endo–C(3′)-exo ($^2T_3$) [or C(2′)-endo] conformations were found to be preferred, while the opposite (or enantiomeric) conformations C(2′)-exo–C(3′)-endo ($_2T^3$) [or C(2′)-exo] and C(3′)-exo–C(2′)-endo ($_3T^2$) [or C(3′)-exo] and a new conformation C(4′)-endo–C(3′)-exo ($^4T_3$) [or C(4′)-endo] occur in the α nucleosides. A noteworthy feature is that the $_2T^3$ and $_2E$ conformations are exhibited by the ribosides only, while the $_3T^2$ and $^4T_3$ conformations are exhibited by the deoxyribosides. The only compound that exhibits a conformation ($^3T_2$) that is commonly found in the β nucleosides is vitamin B_{12} coenzyme, Table II. It should be pointed out here that, with the exception of the 3′,5′ cyclic nucleotides,[15] the C(2′)-exo and C(4′)-endo conformations have not so far been observed for the β nucleosides. The characteristic conformational properties of the α nucleosides on one hand and the β nucleosides on the other are mainly attributable to the differences in the steric interaction between the base and the sugar.

For the purposes of relating proton magnetic resonance coupling constants to the conformations of nucleosides in solution it is important to consider the torsion angles involving the furanoside ring hydrogen atoms. These angles are given in Table III for the structures where the hydrogen atoms have been determined by X-ray diffraction. In Table IV the torsion angles involving the furanoside ring bonds are given because they give the most precise description of the conformation of a ring.[11,12]

Acknowledgment. I am grateful to the National Institutes of Health of the United States Public Health Service for research support through Grant GM 17378.

(14) The nomenclature used here is a modification of the original proposal (L. C. Cross, private communication) by the British Carbohydrate Nomenclature Committee. If the deviations of the atoms from the three-atom plane are identical within the errors of the experiment then the furanoside ring possesses a symmetrical half-chair conformation. The displaced atoms are then shown on the same side of the letter T (see Table II).

(15) Cyclic 3′,5′-adenosine monophosphate (K. Watenpaugh, J. Dow, L. H. Jensen, and S. Furberg, *Science*, **159**, 206 (1968)); cyclic 3′,5′-uridine monophosphate (C. Coulter, *Acta Crystallogr., Sect. R*, **25**, 2055 (1969)); 5′-methylene analog of cyclic 3′,5′-adensosine monophosphate (M. Sundaralingam and J. Abola, *Nature (London)*, in press).

Proton Magnetic Resonance Study on Adenine Dideoxynucleoside Monophosphate with Emphasis on the Furanose Conformation[1a-c]

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Abstract: Spectral assignment of the base protons, H-1′,H-2′, and H-2″, of both the dAp and pdA portions of dApdA has been made. The results indicate that dApdA has an anti,anti right-handed conformation with extensive base–base interaction. Four coupling constants, $J_{1'-2''}$ (cis), $J_{1'-2'}$ (trans), $J_{2'-3'}$ (cis), and $J_{2'-3'}$ (trans), of the furanose of both the dAp and pdA portions of dApdA, 3′-dAMP, and 5′-dAMP were determined by first-order analysis of 220-MHz spectra and 100-MHz spectra. The data show that the coupling constants between the dAp and pdA portions of dApdA are similar to those of 3′-dAMP and 5′-dAMP, while the coupling constants of 3′-dAMP and 5′-dAMP are not the same. Through the application of the Karplus equation, four dihedral angles, $\phi_{1'-2''}$ (cis), $\phi_{1'-2'}$ (trans), $\phi_{2'-3'}$ (cis), and $\phi_{2'-3''}$ (trans), were determined. These analyses suggest that the furanose conformation for 3′-dAMP and dAp in dApdA is that of C(2′)-endo (envelope) or C(2′)-endo–C(3′)-exo (twisted form), while the furanose conformation for 5′-dAMP and pdA in dApdA is that of a rapid equilibrium between C(2′)-endo and C(3′)-endo. From the temperature and solvent studies of the $J_{1'-2'}$ values of 5′-dAMP and dApdA, it is suggested that the formation of intermolecular stacks of 5′-dAMP or the formation of intranucleotidyl stacking of dApdA does not cause a change in the furanose conformation.

Proton magnetic resonance has been used successfully to study the conformation and the interaction of nucleosides, nucleotides, and dinucleotides in aqueous solution.[3-6] However, most of these stud-

(1) (a) Part IV of a series entitled "Studies of the Conformation and Interaction of Dinucleoside Mono- and Diphosphates." (b) This work was supported in part by a grant from the National Science Foundation (GB-8500) and a grant from the National Institutes of Health (GM 16066-03). (c) Presented in part at 160th National Meeting of the American Chemical Society, Chicago, Ill., 1970.

(2) American Cancer Society, Postdoctoral Fellow.

(3) P. O. P. Ts'o, N. S. Kondo, R. K. Robins, and A. D. Broom, *J. Amer. Chem. Soc.*, **91**, 5625 (1969); P. O. P. Ts'o, M. P. Schweizer,