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Conformation of the Common Purine (β) Ribosides in Solution: Further Evidence for a Correlation between N-S State of the Ribose Moiety and Syn-Anti Equilibrium

H.-D. Lüdemann, O. Röder, E. Westhof, E. v. Goldammer and A. Müller

Lehrstuhl für Physik, Fachbereich Biologie,
Universität Regensburg

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Abstract. The solution conformations of adenosine, guanosine and inosine in liquid ND_3 have been determined by NMR. Comparison of the Karplus analysis of the proton HR spectra of the ribose moiety obtained in this solvent with the data from aqueous solutions of *A* and *I* proves that the conformations of the nucleosides are very similar in both liquids. From the analysis of the vicinal coupling constants of the ring protons it has been deduced that the *S* state C(2′)-endo is slightly preferred. The mole fraction in *S* approximates 0.6 for all three nucleosides. C-13 relaxation measurements have been applied in the determination of the correlation times for rotational diffusion. Only at temperatures below − 40° C is the pseudorotation of the furanoside ring slowed down sufficiently for it not to contribute to the measured relaxation rates. From NOE studies and T^1 measurements on the individual protons it is derived that the *N*, C(3′)-endo, form of the ribose is correlated with an anti conformation of the base ($Y \approx 210°$ to 220°) and the *S*, C(2′)-endo, form of the ribose with a syn conformation of the base ($Y \approx 30°$ to 50°). The glycosyl torsion angles derived for the two conformations of *A*, *G*, and *I* are equal within the limits of accuracy.

Key words: Nucleosides — Conformation — HRNMR — Relaxation — NOE.

1. Introduction

Static structures obtained by X-ray crystallography provide a firm basis for studying dynamic conformations adopted by molecules in solution. Crystal data on various purine ribosides have been reviewed by Sundaralingam (1973) who classified these compounds according to their three major modes of internal motion (see Fig. 1):

(i) pseudorotation (puckering) of the furanose ring of the pentose with two conformers: *N* or C(3′)-endo and *S* or C(2′)-endo (Altona and Sundaralingam, 1972)

(ii) rotation around the glycosydic bond with two states: syn and anti

(iii) rotation of the hydroxymethyl group around the C(4′)-C(5′) bond of the pentose with three rotamers: *gg*, *gt*, *tg* (Hruska, 1973).

For purine ribosides Sundaralingam (1973) found that the C(3′)-endo-state is linked to a glycosyl torsion angle in the anti range in all cases, while the C(2′)-endo-ribose is accompanied by the syn or the anti orientation with nearly equal frequencies. The reported ratio C(3′)-endo:C(2′)-endo is 1:4.

In crystals the conformers encountered may be determined by packing effects since energy differences between alternative states are small. Thus the question

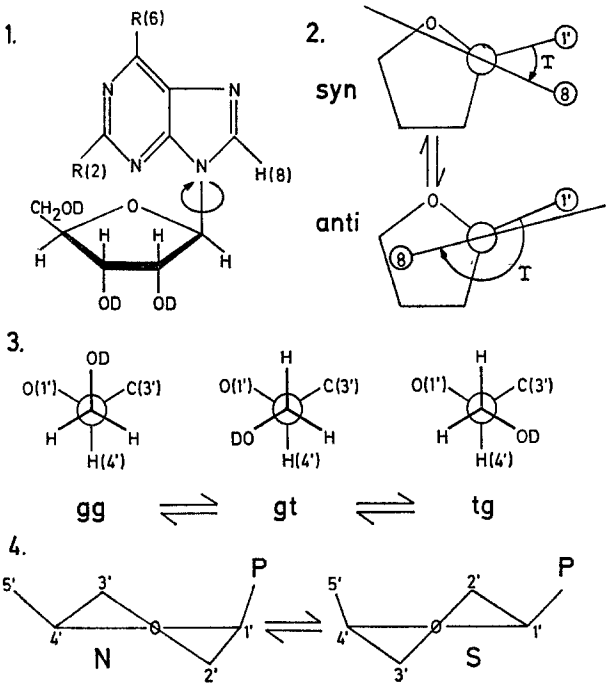

Fig. 1. Schematic representation of the possible internal motions in the purine(β)ribosides.
1.1

	A	*G*	*I*
R(2)	H	NH_2	H
R(6)	NH_2	OH	OH

1.2 Rotation around the glycosydic bond with definition of *Y* (Davis and Hart, 1972).
1.3 Newman projection along the C(4′)-C(5′) bond showing the three possible rotamers *gg*, *gt*, *tg*.
1.4 Pseudorotation of the ribose moiety linking the *N* and *S* conformations

arises as to whether a correlation exists between the ribose puckering and the syn-anti equilibrium in solutions of purine nucleosides.

From an analysis of high resolution proton magnetic resonance (PMR) spectra of purine ribosides in aqueous solutions a dynamic equilibrium was deduced between the *N* and *S* corformers of the ribose with a slight preponderance of the latter (Altona and Sundaralingam, 1973). However, the experimental basis for these conclusions is limited resting on only two substances: adenosine and inosine. Measurements on aqueous solutions of purine nucleosides are generally hampered by low solubilities. Furthermore, considerable association of solute molecules occurs which may interfere in uncontrolled ways with the conformational states, thus jeopardizing a straightforward interpretation.

When considering the utilization of other solvents the modifying influence of solute-solvent interactions such as hydrogen bonding and charge transfer must be taken into account. For a comparison with aqueous systems liquid ammonia was considered the most suitable since its donor and acceptor capacities in forming hydrogen bridges are similar to those of water. Ammonia also possesses good

solubilities for purine nucleosides but lacks the complicating quality of inducing association of the solute. Besides, PMR spectra of nucleosides are much better resolved in ammonia than in water due to the higher viscosity of water. Finally, spectra can be followed to temperatures sufficiently low to slow down internal mobility between different conformations. It turned out that all nucleosides tested had solubilities in ammonia of at least 0.6 molal between $-66°$ C and $+50°$ C.

High resolution PMR spectra registered in CW operation were complemented by dynamic methods in order to acquire sufficient data for a thorough analysis. Nuclear Overhauser Enhancements (NOE) of protons and relaxation rates of H-1 and C-13 nuclei were measured. The results permit one to derive the dynamic configurations. The conformations of four purine ribosides have now been studied: adenosine, guanosine, inosine and xanthosine which was investigated previously (Lüdemann *et al.*, 1974). It is concluded that in solution the $C(2')$-endo or S-state is linked to a glycosyl torsion angle of $Y \approx 50°$ to $90°$ (syn)[1] and that the $C(3')$-endo or N-state is correlated with $Y \approx 210°$ to $230°$ (anti). The temperature dependence of the $N \rightleftarrows S$ equilibrium in ammonia is small and is determined by the base moiety. As the temperature is reduced, the mole fraction in N decreases for A and G, increases for X, and shows indifferent behaviour for I.

We have also measured high resolution PMR spectra of aqueous solutions of adenosine and inosine which have been investigated previously (Altona and Sundaralingam, 1972; Hruska, 1973) and compare them with older NOE values (Lüdemann and v. Goldammer, 1973). The results did not permit an analysis with the same scope for the reasons indicated above. However, they are sufficient for a comparison with ammonia solutions. As expected, major deviations from the conformational relations have not been found.

2. Experimental

Substances and Sample Preparation

Adenosine, guanosine and inosine were purchased from Papierwerke Waldhof-Aschaffenburg AG, Mannheim, BRD. Paramagnetic ions were removed by passing aqueous solutions of the nucleosides through a Dowex 50 WX 8 column. Preliminary proton relaxation results obtained on G and I showed that this procedure was not sufficient to remove all paramagnetic impurities. After at most three recrystallisations from water and repeated ion exchange treatments the relaxation times of I and G remained constant. Some of the samples were in addition treated with a chelating ion exchanger (Chelating resin A 1, Serva, Heidelberg, BRD), without further improvement. Exchangeable protons were removed by recrystallisation from deuterium oxide. The trideuteroammonia (99% deuterated, Merck, Sharp and Dohme Ltd., Pointe Claire, Canada) was kept over solid potassium deuterooxide for at least 24 hrs before use to remove residual moisture.

The detailed procedure of sample preparation has been given previously (Lüdemann and v. Goldammer, 1973; Lüdemann *et al.*, 1974). All proton spectra and

[1] In this paper the glycosyl torsion angle will be defined according to the convention suggested by Davies, J. B. and Hart, P. A. (1972). In this reference the correlation between this convention and the definitions adopted by M. Sundaralingam (1969) and J. Donohue and K. N. Trueblood (1960) is given for comparison.

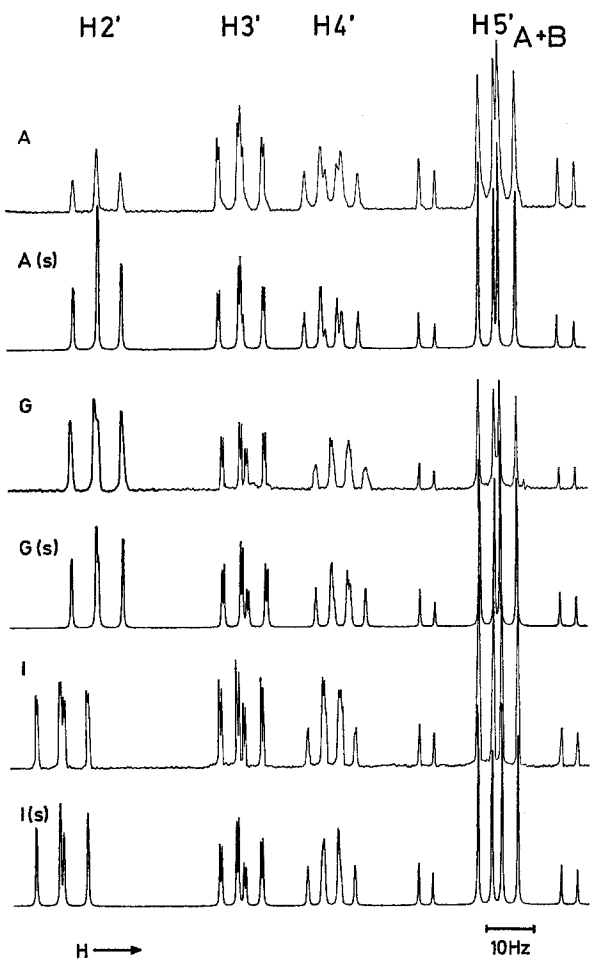

Fig. 2. Experimental proton high resolution spectra of *A*, *G*, and *I* of 0.12 molal solutions in ND_3 at + 40° C covering the region of the protons H(2') to H($5'_A$) and H($5'_B$) compared with the spectra obtained by computer simulation *A*(s), *G*(s), and *I*(s) under insertion of the coupling constants given in Table 2

relaxation studies were done on 0.12 molal solutions of the nucleosides. The C-13 relaxation rates were obtained in large volume tubes (i.d. 9 mm, o.d. 12 mm, Wilmad Glass Corp., Buena, N.J., USA).

Spectra

The experiments were performed on a Varian XL-100-15 FT-NMR-spectrometer connected to a Varian 620i-computer. The variable temperature unit was modified to allow the spectrometer to run unattended over periods up to 10 hrs. Details of the experimental procedures have been given previously (Lüdemann and v. Goldammer, 1973; Lüdemann *et al.*, 1974). The longitudinal relaxation rates determined by a *t*-90°-*t*-180°-*t*-90° pulse sequence (Freeman and Hill, 1971) are

reproducable to ± 10%. Values used in the calculations are taken from smoothed curves drawn through the experimental points. The limit of error is estimated as ± 5%. The nuclear Overhauser enhancements (NOE) are averages of at least 20 determinations and judged to be reliable to ± 0.01. Coupling constants obtained from the analysis of the high resolution proton spectra are reproducible to ± 0.05 Hz. This low value is largely due to the fact that small variations in temperature do not influence the coupling constants.

3. Theoretical Section

General

The possibility of quantitative conformational analysis of flexible molecules and specifically nucleosides and nucleotides by application of nuclear magnetic resonance relaxation techniques is a controversial issue of the current literature (Schirmer *et al.*, 1972; Son *et al.*, 1972). Hence it seems indicated to point out the statistical mechanics involved. McConnell's equation (McConnell, 1958) which underlies Eq. (1) to (4) given below is valid only if τ_c, the rotational correlation time, is significantly shorter than any inverse rate constant k^{-1} characterizing the conformational motions that modify the intramolecular proton-proton-vectors. This will be the case if the energy of activation of all modes of motion involved is at least one order of magnitude higher than the thermal energy. The Boltzmann equation under these conditions yields the result, that in the ensemble average at most one molecule in 1000 is activated, while the vast majority of molecules is locked in a few fixed conformations. The intermediate region II $R_d \ll k \ll \tau_c^{-1}$ Schirmer *et al.* (1972) applies to the nucleosides at the lowest temperatures.

Relaxation Studies

In organic molecules, consisting only of H, C, N, and O, the nuclear Overhauser enhancements $f_d(s)$ of the protons in a multispin system are given by Noggle and Schirmer (1971), Schirmer *et al.* (1972)

$$f_d(s) = \frac{1}{2} \sum_s \frac{\langle \varrho_{ds} \rangle}{\langle R^1_d \rangle} - \frac{1}{2} \sum_{n \neq d,\, s} \frac{\langle \varrho_{dn} \rangle}{\langle R^1_d \rangle} f_n(s) \,. \tag{1}$$

The subscript d refers to the nucleus detected, s to the nucleus saturated, and n includes all other protons interacting with d or s. It is assumed as usual, that it is sufficient to include only the protons in the sum over n. $\langle R^1_d \rangle$ is the total direct relaxation rate of spin d given by

$$\langle R^1_d \rangle = \sum_{n \neq d} \langle \varrho_{dn} \rangle + \varrho^*_d \,. \tag{2}$$

Here, $\langle \varrho_{ij} \rangle$ is the direct dipole-dipole relaxation rate between the two protons i and j. In the extreme narrowing case this rate is given by

$$\langle \varrho_{ij} \rangle = \gamma_H^4 \cdot \hbar^2 \cdot \tau_c \frac{1}{\langle r^6_{ij} \rangle} \,, \tag{3}$$

with γ_H the magnetogyric ratio of the proton, r_{ij} the distance between the two nuclei and τ_c the correlation time of the interaction between the two spins. The term ϱ^*_d includes all other intra- and intermolecular relaxation paths of spin d. In order to make NOE studies a sensitive tool in the determination of conformations,

ϱ_d^* has to be kept as small as possible. In a previous paper (Lüdemann *et al.*, 1974) we determined ϱ^* from the relaxation rates of the H(6) proton in purine riboside to be $\varrho^* \leq 0.008\ s^{-1}$ at $-60°$ C. This is negligible compared to the R^1 values given below.

In rigid approximately spherical molecules the rotational correlation time τ_c is the same for all protons. The longitudinal relaxation rates R^1 of C-13 atoms bound directly to a proton are completely described by the interaction between this proton and the C-13 spin and is given by

$$R^1 = N \cdot \gamma_H^2 \cdot \gamma_C^2 \cdot \hbar^2 \frac{\tau_c}{r^6_{CH}}, \tag{4}$$

where N is the number of protons directly bound, r_{CH} the carbon hydrogen bond length. The latter has been determined in the case of the nucleosides by X-ray crystallography. Measuring the C-13 relaxation rates and applying (4) yields τ_c.

Molecular Geometry

The bond lengths and bond angles that are independent of the conformation of the purine nucleosides were taken from average values of the crystal data published (Voet and Rich, 1970; Sundaralingam, 1973). For the C-H bond lengths a uniform value of 0.102 nm was taken. The proton high resolution spectra were analysed and simulated using the computer program LAOCN-3 (QCPE)[2]. The vicinal coupling constants obtained in this way were subjected to the analysis proposed by Altona and Sundaralingam (1973). In this procedure it is assumed that the observed coupling constants J_{obs} are linear time averages of the couplings of the two conformers N and S:

$$J_{obs} = [N]\, J_N + [S]\, J_S. \tag{5}$$

From this analysis the mole fractions of the ribose in the two states $[N]$ and $[S]$, and the phase angles of pseudorotation are obtained. The Karplus equation (Karplus, 1963)

$$J_{HH} = A \cdot \cos^2 \varphi_{HH} - B \cdot \cos \varphi_{HH} \tag{6}$$

with $A = 10.5$ Hz and $B = 1.2$ Hz yields the torsion angles φ_{HH} between the vicinal proton vectors. The geometry of the ribose moiety is now uniquely determined.

Using the computer program n°136 of QCPE the distances between the ribose protons in the N and S states were calculated from the torsion angles derived above with the bond lengths and bond angles taken from solid state data (Voet and Rich, 1970; Sundaralingam, 1973). The distances between the ribose protons and the base proton H(8) for various values of the glycosyl-torsion-angle Y were determined in the same way. These are shown in Fig. 3.3 and 3.4.

4. Results and Discussion

Determination of the Rotational Correlation Times

In a previous paper on xanthosine in liquid deuteroammonia it was found that the internal motions do not contribute to the relaxation of the ribose carbons 1′ to

[2] Quantum Chemistry Program Exchange (Indiana University Chemistry Dept.) Program No. 111.

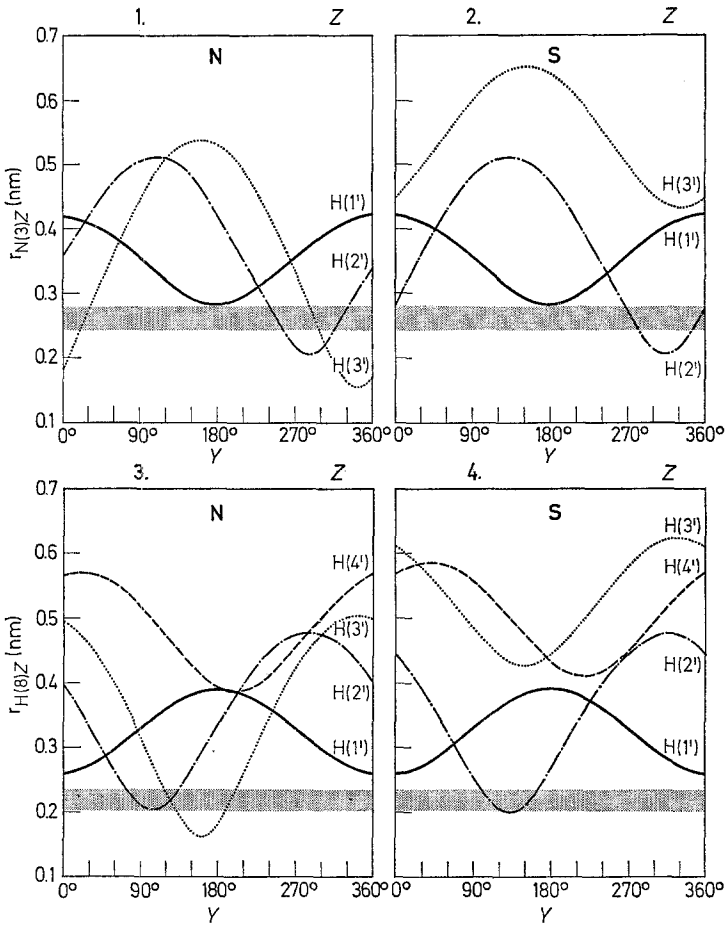

Fig. 3. Distances between base- and ribose-atoms in the *N* and *S* state as a function of the glycosyl-torsion angle *Y*. The upper limit of the shaded zone between 0.2 and 0.3 nm represents the sum of the van der Waals radii of the atoms involved, while the lower limit gives the shortest distance possible.

3.1 ribose in *N*, distances between N(3) and H(1′) to H(3′)
3.2 ribose in *S*, distances between N(3) and H(1′) to H(3′)
3.3 ribose in *N*, distances between H(8) and H(1′) to H(4′)
3.4 ribose in *S*, distances between H(8) and H(1′) to H(4′)

4′ only at temperatures below — 40° C. The same result was obtained with the three nucleosides investigated here. At temperatures below — 40° C the longitudinal relaxation rates of all carbons bound to one proton are identical within experimental error, while at higher temperatures the rates for the sugar carbons are lower than the values for the tertiary base carbons. Eq. (1) to (3) can consequently only be applied at low temperatures. The best description of the nucleosides at higher temperatures is achieved by a qualitative extrapolation of the low temperature results. Table 1 gives the average longitudinal relaxation rates of the individual carbons and the τ_c values derived by application of (4) assuming $r_{CH} = 0.102$ nm.

Proton High Resolution Spectra

The vicinal coupling constants J_{ij} of the ribose protons of *A*, *G*, and *I* at various temperatures obtained from the computer analysis are given in Table 2. The experimental spectra of *A*, *G*, and *I* obtained at + 40° C are compared with the computersimulated spectra in Fig. 2. In addition the analysis of the spectra of *A* and *I* in deuterium oxide is presented. The sum $(J_{1'2'} + J_{3'4'})$ is very similar for all three nucleosides and agrees closely with the results derived for *X* (Lüdemann *et al.*, 1974). It is however slightly lower than any of the values calculated by Altona and Sundaralingam. As a consequence the sum of $[N]$ and $[S]$ deviates from 1.00. Agreement could be obtained by introducing somewhat different amplitudes of pseudorotation for the two states or by modification of the constants A and B in Eq. (6). The decrease of $(J_{1'2'} + J_{3'4'})$ with temperature found in all cases investigated is regarded as due to small changes of the average angle φ_{HH} with temperature. In approximate calculations of the mole fractions $[N]$ and $[S]$ the following coupling constants have been applied:

$$J_{2'3'}(N):4.6 \quad J_{1'2'}(N):0.0 \quad J_{3'4'}(N):9.9$$

$$J_{2'3'}(S):5.1 \quad J_{1'2'}(S):9.5 \quad J_{3'4'}(S):0.0.$$

These values correspond to a phase angle of pseudorotation $P \approx 3°$ in the *N* state and $P \approx 175°$ in the *S* state. Some qualitative comments on the temperature dependence of the $N \rightleftarrows S$ equilibrium can be given from the data presented in Table 2. The decrease of $J_{3'4'}$ by ~ 0.5 Hz in *A* and *G*, dissolved in deuteroammonia, with falling temperature shows that in these nucleosides the *N* form has a slightly higher energy. On the other hand much smaller changes are found in *I* and even the opposite effect is observed in xanthosine (Lüdemann *et al.*, 1974). This reveals that in the purine nucleosides the nature of the base determines which of the two conformers of the ribose moiety possesses the lower free energy. In any case the effects found are small and it is obvious that this equilibrium is influenced by slight differences in the solvation properties caused either by the nature of the base substituents or by a change of solvent. In the aqueous solutions of *A* and *I* the increase in *S* with decreasing temperature is a little more pronounced. In water, however, the temperature dependent stacking equilibria between the nucleoside molecules introduces an additional parameter likely to influence the $N \rightleftarrows S$ equilibrium (Hruska, 1973). In the calculations of the glycosyl torsion angles given below, the mole fractions used were $[N] = 0.40$, $[S] = 0.60$ for *G* and *I* while *A* is described by $[N] = 0.45$, $[S] = 0.55$. These values are judged to be reliable within $\pm$ 0.05. An error in the mole fractions of this magnitude influences the results derived for the glycosyl torsion angle *Y* only by a few degrees. Having established the position of the protons bound to the ring carbons 1′ to 4′, the location of the two 5′ protons at the exocyclic-CH_2OD group remains to be determined. Hruska *et al.* (1970, 1973) proposed an approximative formula for the determination of the relative population of the three rotamers. Application of this procedure at — 60° C leads to a mole fraction of 0.65 to 0.70 for the *gg*-rotamer. Considering the approximations involved in the determination of the coupling constants, we assume that this value gives the lower limit for the population of the *gg*-rotamer.

Table 1. Averaged longitudinal relaxation rates of the tertiary base carbons $\overline{R^1}$ C(2), C(8) and the ring carbons of the ribose moiety $\overline{R^1}$ C(1′)-C(4′) and rotational correlation times calculated from (4). Concentration: 0.6 molal. Solvent: ND_3

Nucleoside	Adenosine				Guanosine[a]				Inosine			
Temperature (° C)	−60	−44	−30	−13	−60	−45	−30	−15	−60	−45	−30	−15
$\overline{R^1}$ C(2), C(8) (s^{-1})	3.8	2.15	1.50	1.05	3.7[b]	2.9[b]	2.3[b]	1.82[b]	4.7	3.6	2.4	1.72
$\overline{R^1}$ C(1′)-C(4′) (s^{-1})	3.7	2.13	1.31	0.98	3.6	2.8	2.0	1.39	4.6	3.6	2.2	1.52
τ_c (ps)	115	67			114	89			145	121		

[a] Concentration: 0.12 molal. The R^1 of *G* showed a slight concentration dependence of unknown origin. Consequently all measurements of this compound were done at the same concentration.

[b] R^1 C(8) only.

Table 2. Vicinal coupling constants of the ribose protons in *A*, *G*, and *I*. For comparison the results obtained in D_2O solutions of *A* and *I* are included. $J_{1'2'} + J_{3'4'}$ is a measure for the phase angles of pseudorotation in the two states

Nucleoside	Adenosine						Guanosine				Inosine					
Solvent	ND_3				D_2O		ND_3				ND_3				D_2O	
Concentration (molal)	0.12				0.025		0.12				0.12				0.10	
Temperature (° C)	+40	−2	−30	−60	+80	+10	+40	−2	−30	−60	+40	−2	−30	−60	+80	+10
J_{ij} (Hz)																
1′2′	5.0	5.1	5.1	5.1	5.8	5.8	5.5	5.7	5.7	5.6	5.7	5.7	5.7	5.6	5.5	5.6
2′3′	5.0	5.0	4.85	4.8	5.1	5.0	5.1	5.0	5.1	5.2	5.0	5.0	5.0	5.0	5.3	5.3
3′4′	4.4	4.3	4.0	3.9	3.9	3.4	3.85	3.6	3.6	3.3	3.5	3.4	3.4	3.3	4.4	3.8
$4'5'_A$	3.4	3.6	3.8	3.8	3.1	3.0	3.2	3.3	3.3	3.3	3.0	3.1	3.1	3.3	3.4	3.0
$4'5'_B$	3.5	3.2	3.0	3.0	4.0	3.4	3.3	3.2	3.0	3.0	3.3	3.1	2.9	2.8	4.5	3.9
1′2′ + 3′4′	9.4	9.4	9.1	9.0	9.7	9.2	9.35	9.3	9.3	8.9	9.2	9.1	9.1	8.9	9.9	9.4
[*N*]	0.44	0.43	0.40	0.39	0.39	0.34	0.39	0.36	0.36	0.33	0.35	0.34	0.34	0.33	0.44	0.38
[*S*]	0.53	0.54	0.54	0.54	0.59	0.52	0.58	0.60	0.60	0.59	0.60	0.60	0.60	0.59	0.58	0.59

[*S*] mole fraction of nucleoside in the *S* state.

[*N*] mole fraction of nucleoside in the *N* state calculated by application of the coupling constants given in the text.

Table 3. Longitudinal relaxation rates of the single protons of *A*, *G*, and *I* 0.12 molal in ND_3

Nucleoside	Adenosine						Guanosine						Inosine					
Temperature (° C)	+ 40	+ 25	0	− 30	− 45	− 60	+ 40	+ 25	0	− 30	− 45	− 60	+ 40	+ 25	0	− 30	− 45	− 60
Proton																		
1′	0.051	0.058	0.083	0.144	0.193	0.26	0.132	0.171	0.23	0.34	0.47	0.71	0.063	0.086	0.118	0.20	0.26	0.33
2′	0.148	0.164	0.22	0.36	0.50	0.84	0.20	0.24	0.37	0.74	1.11	1.54	0.131	0.158	0.24	0.41	0.58	1.00
3′	0.132	0.156	0.22	0.39	0.56	0.91	0.21	0.25	0.37	0.69	1.05	1.55	0.134	0.160	0.25	0.42	0.56	0.91
4′	0.114	0.133	0.190	0.32	0.43	0.63	0.20	0.23	0.32	0.53	0.83	1.11	0.122	0.148	0.22	0.38	0.48	0.71
$5'_{A+B}$	0.43	0.49	0.72	1.15	1.67	2.86	0.52	0.76	1.28	2.04	2.50	4.00	0.42	0.50	0.74	1.25	1.67	2.86
2	0.007	0.009	0.015	0.027	0.038	0.059	—	—	—	—	—	—	—[a]	—[a]	0.026	0.042	0.054	0.074
8	0.063	0.083	0.116	0.22	0.31	0.44	0.18	0.21	0.29	0.48	0.67	1.11	—[a]	—[a]	0.16	0.27	0.38	0.53

[a] The resonance signals H(2) and H(8) of *I* coalesce at these temperatures and are not measurable.

Longitudinal Relaxation Rates of the Protons

The results obtained for the protons of the three nucleosides which are not exchangeable are collected in Table 3. From the data obtained for H(2) in A and I it is obvious that this proton must be fairly remote from the other protons of the nucleoside in all conformations existing for an appreciable fraction of time. A quantitative analysis of the longitudinal relaxation rates of this proton appears not to be justified since other relaxation paths than dipole-dipole interactions may contribute considerably to $\langle R^1 \rangle$. The ϱ^* calculated above amounts to more than 10% of R_2^1 and is certainly not negligible.

In our calculations of the xanthosine conformation in liquid deuteroammonia we used Eq. (2) and (3) to derive $\langle \varrho_{8z} \rangle$ values, from which the distances between the pentose protons and H(8) were determined. Considering the large contributions from the two 5′ protons to (2) and the relative uncertainty in localizing these protons we will not use this method in the following but we shall apply the R^1 values only in connection with Eq. (1) and the experimentally determined NOE of the next section.

Nuclear Overhauser Enhancements

In order to determine intramolecular distances from NOE values given in Table 4 one has to accept the two state model of Altona and Sundaralingam as a quantitative description of the ribose moiety. In this model the $\langle R_d^1 \rangle$ values of the individual protons are given by

$$\langle R_d^1 \rangle = [N] \sum_{n \neq d} \varrho_{dn}^N + [S] \sum_{n \neq d} \varrho_{dn}^S \,. \tag{7}$$

From the high resolution spectra all $\langle \varrho_{dn} \rangle$ involving only sugar protons can be calculated by application of (3) and the distances obtained from the analysis of the high resolution spectra. The small relaxation rates of H(2) allow to neglect the contribution of this proton. Eq. (1) for the three cases $s = 8$ and $d = 1'$, $2'$ and $3'$ contains only the "unknown" variable $\langle \varrho_{d8} \rangle$ which in the model of Altona and Sundaralingam is determined by

$$\langle \varrho_{d8} \rangle = [N] \, \varrho_{d8}^N + [S] \, \varrho_{d8}^S \,. \tag{8}$$

In Fig. 3.3 and 3.4 the distances between the ribose protons 1′ to 4′ in the N and S state and $H(8)$ are given as a function of the glycosyl torsion angle Y. Inspection of these diagrams reveals that only in the N state H(3′) and H(8) can approach each other close enough to produce a measurable dipole-dipole-interaction. $\langle \varrho_{3'8} \rangle$ accordingly is determined by the contribution of the N state only and the second term in (8) can safely be neglected. With the assumption

$$(r_{3'8}^N)^{-1} \approx \sqrt[6]{\langle (r_{3'8})^{-6} \rangle} \tag{9}$$

one obtains for all three nucleosides $r_{3'8}^N = 0.27 \pm 0.01$ nm. Two regions in Fig. 3 fit the result obtained. One around 100° and a second around 210° to 220°. The first of these possibilities is excluded for the N state, since it would force H(2′) and H(8) to approach each other to a distance considerably smaller than their van der Waals radii. The distances $r_{1'8}^N$ and $r_{2'8}^N$ corresponding to Y 210° to 220° are taken from diagram 3.3, with $\varrho_{1'8}^N$ and $\varrho_{2'8}^N$ calculated using Eq. (3). Insertion of these results

Table 4. Nuclear Overhauser enhancements between $H(8)$ and the ribose protons of A, G, and I at various temperatures

Solvent	Concentration (molal)	NOE / Temperature (° C)	$f_8(1')$	$f_8(2')$	$f_8(3')$	$f_8(4')$	$f_8(5'_{A+B})$	$f_{1'}(8)$	$f_{2'}(8)$	$f_{3'}(8)$	$f_{4'}(8)$	$f_{5'_{A+B}}(8)$
			Adenosine									
ND_3	0.12	+ 25	0.198	0.126	0.070	0.002	0.016	0.182	0.073	0.039	0	0
		0	0.189	0.127	0.067	0.003	0.029	0.170	0.082	0.035	0	0
		− 30	0.189	0.135	0.066	0.003	0.029	0.168	0.084	0.031	0	0
		− 45	0.185	0.142	0.061	0	0.027	0.162	0.090	0.036	0	0
		− 60	0.187	0.158	0.063	0	0.023	0.162	0.094	0.036	0	0.008
D_2O[a]	0.025	+ 38	0.197					0.207				
			Guanosine									
ND_3	0.12	+ 25	0.231	0.096	0.034	0.004	0.044	0.207	0.084	0.021	0	0
		0	0.245	0.112	0.045	0	0.036	0.205	0.096	0.018	0	0
		− 30	0.241	0.110	0.044	0	0.045	0.173	0.088	0.023	0	0.004
		− 45	0.214	0.109	0.053	0	0.036	0.168	0.093	0.028	0	0
		− 60	0.187	0.128	0.058	0	0.025	0.143	0.096	0.033	0.004	0.005
			Inosine[b]									
ND_3	0.12	+ 25	—[c]	—[c]	—[c]	—[c]	—[c]	0.230	0.075	0.029	0	0.007
		0	0.223	0.099	0.043	0	0.032	0.232	0.079	0.027	0	0.006
		− 30	0.218	0.097	0.045	0	0.034	0.225	0.083	0.034	0	0.011
		− 45	0.217	0.124	0.053	0	0.033	0.221	0.088	0.039	0	0.008
		− 60	0.221	0.135	0.055	0.004	0.040	0.210	0.095	0.039	0	0.010
D_2O[a]	0.025	+ 38	0.198					0.229				

[a] Results in D_2O taken from Lüdemann and v. Goldammer (1973).

[b] In the case of I only the $f_x(8 + 2)$ enhancements could be determined because of the proximity of H(8) and H(2) resonances. The low R^1 of H(2) (see Table 3) allows the assumption that the contributions of H(2) to $f_x(8 + 2)$ are negligible.

[c] Not measurable, resonances of H(8) and H(2) coalesce at this temperature.

Table 5. Average proton-proton distances and glycosyl torsion angle Y of the purine nucleosides

Nucleoside	Adenosine		Guanosine		Inosine		Xanthosine[a]	
Temperature (° C)	−60	−45	−60	−45	−60	−45	−60	−45
$r^N_{3'8}$ (nm)	0.27	0.26	0.27	0.26	0.26	0.27	0.28	0.28
$Y^N(3')$					210 − 220°			
$r^N_{1'8}$ (nm)					0.38 ± 0.01			
$r^S_{1'8}$ (nm)	0.28	0.27	0.27	0.27	0.28	0.27	0.28	0.29
$Y^S(1')$					30−50°			
$r^N_{2'8}$ (nm)					0.41 ± 0.01			
$r^S_{2'8}$ (nm)	0.25	0.24	0.24	0.24	0.24	0.25	0.26	0.26
$Y^S(2')$					80−90°			

[a] results taken from Lüdemann *et al.* (1974).

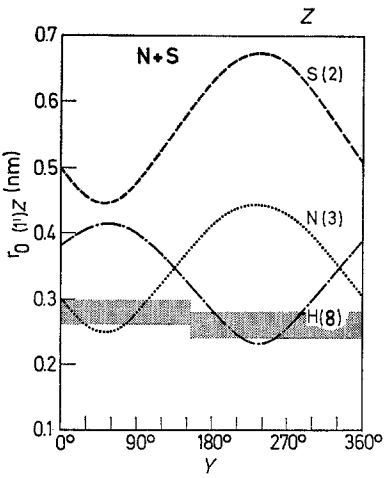

Fig. 4. Distances between O(1′) and some base atoms as a function of the glycosyl-torsion angle Y. S(2) stands for the substituent in position 2. Pseudorotation of the base does not influence these distances. The upper limit of the shaded zone between 0.2 and 0.3 nm represents the sum of the van der Waals radii of the atoms involved, while the lower limit gives the shortest distance possible

together with $\langle \varrho_{1'8} \rangle$ and $\langle \varrho_{2'8} \rangle$ obtained from combining (1) and (8) for the three nucleosides yields the data presented in Table 5. For comparison the values found previously for xanthosine are included.

For a solution of X in deuteroammonia we deduced a correlation between the syn$\rightleftarrows$ anti equilibrium and the $N\rightleftarrows S$ state of the ribose moiety. This correlation seems to hold for all four purine nucleosides investigated. There is one puzzling discrepancy in the glycosyl-torsion angles found for the S state. The results for Y derived from H(1′) concentrate around 30° to 50° while the H(2′) values suggest a Y near 90°. Apart from the explanation that Eq. (9) is only approximative one can speculate on the path of the syn$\rightleftarrows$ anti conformational change. The energy of activation for the syn$\rightleftarrows$ anti equilibrium has been determined by ultrasonic relaxation studies to be 6.2 kcal · mole^{-1} (Rhodes and Schimmel, 1971). We have found no experimental determination of the activation energy for the pseudorotation. Theoretical reasoning favours a value considerably below 1 kcal · mole^{-1} for the unsubstituted tetrahydrofurane (Bastiansen, Seip and Boggs, 1971), while for the furanoside ring of the pentose values of 2 to 4 kcal · mole^{-1} (Durette and Horton, 1971) are given in the literature.

The conclusions to be drawn are that the pseudorotation is activated more frequently than the syn$\rightleftarrows$ anti equilibrium. As can be seen from diagram 3.3 and 3.4 the distance between H(1′) and H(8) is independent of the pseudorotation as long as the glycosyl torsion angle remains constant, whereas the distance between H(2′) and H(8) is affected by the pseudorotation. The value of Y derived from the H(1′) to H(8) interaction is accordingly the more reliable value for the fraction of time where neither the syn$\rightleftarrows$ anti motion nor the pseudorotation is occurring. The low $r^N_{2'8}$ found is taken to show that collisions between H(2′) and H(8) in the process of pseudorotation are much more frequent than rotation of the base from one allowed orientation to the other. Comparison of the energy found experimentally for the syn$\rightleftarrows$ anti equilibrium together with the energy profiles calculated theoretically allows one to speculate about the path of this rotation. In the energy profile given for instance by Jordan (1973) and Kang (1973) as well as from the

diagrams presented in Figs. 3 and 4 it is obvious that only the jump across the barrier presented by H(2′) to H(8) in the vicinity of $Y = 120°$ can account for the effects observed. This assumption would even explain the great deviation between the Y^S found for H(2′) and that for H(1′). The close contact between H(2′) and H(8) during rotation will, due to the r^{-6} dependence, contribute observably to the overall $\langle \varrho_{2'8} \rangle$ of H(2′). Further support for these details of the syn $\rightleftarrows$ anti transition can be obtained from an inspection of the distances between O(1′) and the base atoms as a function of Y together with the distances between N(3) and H(2′) or H(3′), presented in Figs. 3.1, 3.2, and 4. It is obvious that between $240° \lesssim Y \lesssim 330°$ a broad region exists, where contacts between H(8) and O(1′), and especially between H(2′) and N(3) constitute severe obstacles for the rotating base.

5. Conclusions

The conformational equilibria of the four purine ribosides investigated exhibit a very similar behaviour. In A, G, I, and X the furanoside ring of the ribose moiety shows a slight bias towards the S conformation, C(2′)-endo, expressed by a mole fraction of S equal to 0.60 ± 0.05. The temperature dependence of the $N \rightleftarrows S$ equilibrium is slight and is determined by the base. The mole fraction of the N state in solutions of A, G, and I decreases parallel to the temperature, while in X (Lüdemann *et al.*, 1974) the reverse is found. Aqueous solutions of A and I show the same temperature dependence as in deuteroammonia. Davis and Hart (1972) report NOE measurements for A, I, G, and X in DMSO, where I and G yield $f_8(2')$ values significantly higher than $f_8(1')$. On the contrary, the enhancements found in deuteroammonia are very similar for all four nucleosides. When the results obtained for A and I (Table 4) are compared with our previous data found in aqueous solutions (Lüdemann and v. Goldammer, 1973) they show close agreement, proving that the conformations of the nucleosides in the two solvents are indeed very similar. We failed to detect any concentration dependence of the chemical shifts of the base protons in liquid deuteroammonia. Taken together with the observed low proton relaxation rate of the H(2) proton in A and I, this shows that the nucleosides do not associate in this solvent permitting the observation of isolated molecules even at concentrations which are higher than that of a saturated aqueous solution.

The C-13 relaxation rates show that only at temperatures below — 40° C the internal mobility of the nucleosides is slowed down sufficiently to allow the description of the diffusional motion of all carbon atoms by a single correlation time. The analysis of the proton relaxation data at this temperature establishes for all nucleosides a dynamic correlation between the $N \rightleftarrows S$ equilibrium and the glycosyl-torsion angle. In the N conformation the H(8) is found above the ribose-ring with a glycosyl-torsion angle Y around 210° to 220°. The pseudorotation of the sugar moiety into the S position pushes the base into the syn range. The S sugar is correlated to an angle Y of about 30° to 50°. The deviation of Y derived from the $f_8(2')$ and $f_8(1')$ results indicates the path of the syn $\rightleftarrows$ anti transition. The high value of $\langle \varrho_{8\,2'} \rangle$ leading to a Y-value of 80° to 90° shows that during the rotation around the glycosydic bond close contacts between H(8) and H(2′) occur — but only if the movement crosses the region around $Y = 120°$.

If the correlation derived for the glycosyl torsion angle of the nucleosides is also valid for the structure of the ordered polynucleotides then the glycosyl torsion angles of all nucleosides in the two states must be identical. Otherwise no periodical helical structure with the bases stacked parallel on top of each other and bound coplanar to the complementary base by hydrogen bonds can be constructed. The angles derived here are within experimental error independent of the nature of the base.

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Dr. H.-D. Lüdemann
Lehrstuhl für Physik, Fachbereich Biologie
Universität Regensburg
D-84 Regensburg, Postfach 397
Federal Republic of Germany