Ribose Conformations in the Common Purine(β)ribosides, in Some Antibiotic Nucleosides, and in Some Isopropylidene Derivatives: A Comparison

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Purine (β) nucleosides, Trideuteroammonia, PMR, S ⇌ N Equilibrium

With the use of PMR the ribose conformations have been studied in the temperature range −60 to +40 °C in ND_3 solutions of adenosine (A), guanosine (G), inosine (I), xanthosine (X), purineriboside (PR), 2-aminopurineriboside (2amPR), N6-isopentenyladenosine (N6ipA), 8-bromoadenosine (8-BrA), 8-bromoguanosine (8-BrG), formycin B (F), tubercidin (T), isopropylideneadenosine (iA), and isopropylideneguanosine (iG). The analysis is based on the two-state S ⇌ N model of the ribose moiety proposed by Altona and Sundaralingam. The compounds studied can be classified into two groups: 1. A, I, G, X, PR, 2amPR, N6ipA, and T show a small temperature dependence of the S ⇌ N equilibrium and [S] ∼ 0.6; 2. 8-BrA, 8-BrG, and F have a stronger temperature dependence and [S] ∼ 0.8. Within these two groups the similarities observed are greater than observed in the solid state. Some thermodynamic conclusions about the S ⇌ N and the *syn* ⇌ *anti* equilibria are presented. The results support the previously proposed correlation of the S state of the ribose with the *syn* conformation of the base and of the N state of the ribose with the *anti* conformation of the base. Furthermore, it is derived that the *gg* rotamer is correlated with the S state of the ribose and therefore stabilizes the *syn* conformation of the base.

1. Introduction

High resolution proton NMR has proved to be a powerful tool in the conformational analysis of nucleosides in solution [1]. Early NMR studies have shown that the ribofuranose ring of the common nucleosides exists in solution in dynamic equilibrium between the 2′- and 3′-*endo* puckered forms [2–5]. These studies have also shown that the preferred conformation of the exocyclic C(4′) – C(5′) bond is the *gg* rotamer [5, 6]. We have found that not only such a dynamic equilibrium exists between the syn and anti conformations of the purine base relative to the ribose but also that the states of the ribose are correlated with the conformations of the base [7, 8]. Similar conclusions have been reached by other workers [9, 10]. Knowledge of the energies of activation for the different transitions support these results. Indeed, the energy of activation for the pseudorotation of the ribose in the common purine nucleosides has been determined by us to be 4.7 ± 0.5 kcal mol^{-1} [11]. A lower limit of 3.5 to 4.0 kcal mol^{-1} can be gained for the rotations around C(4′) – C(5′) from results obtained on substituted ethane fragments [12]. And finally, the energy of activation for the *syn* ⇌ *anti* transition of the purine base is known to be 6.2 kcal mol^{-1} [13]. Thus, in the ensemble average a fraction of approximately 10^{-3} molecules possesses sufficient energy to execute transitions, while the vast majority is locked in one of the different stable conformations. It is, therefore, the distribution of these rigid conformations that shows up in the high resolution spectra.

The present investigations of the high resolution spectra of various nucleosides were undertaken to determine systematically the influence of chemical changes in the purine base on the conformational equilibria of the ribose moiety. Ammonia was chosen as a solvent for the following reasons. Studies of purine nucleosides in aqueous solutions have several experimental limitations. First, only some of the compounds of interest are sufficiently soluble at neutral pH. Secondly, the soluble nucleosides associate at higher concentrations thus changing the conformational equilibria [14]. And thirdly, the high viscosity of water limits the resolution of the spectra. As a solvent with properties similar to water, liquid ammonia proved to be very useful [7, 8, 11]. Like water, the ammonia molecule can participate in hydrogen bonds as acceptor and donor. All nucleosides studied to date are very soluble in this solvent. Self-association is absent

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since no concentration dependence of the base proton chemical shifts is found. Due to the low viscosity of ammonia the resolution of the spectra is much better than in water and well-resolved spectra can be obtained down to $-60\,^{\circ}C$. When a comparison can be made, the spectra of the same nucleosides in water and liquid ammonia are very similar.

2. Experimental

2.1. Substances and sample preparation

The nucleosides were purchased from Papierwerke Waldhof-Aschaffenburg AG, Mannheim, BRD. The antibiotics formycin B and tubercidin were obtained from Cyclo Chemicals, Los Angeles, California, USA. Paramagnetic ions were removed by passing aqueous solutions of the nucleosides successively through a Dowex 50 WX 8 column and a chelating Resin A1 column. Exchangeable protons were removed by at least three recrystallisations of the nucleoside from deuterium oxide (99.7% deuterated). The trideuteroammonia (99% deuterated Merck, Sharp and Dohme Ltd., Pointre Claire, Canada) was kept over solid potassium deuteroxide for at least 24 h before use to remove residual moisture. The solutions were prepared as described previously [7]. All spectra were taken with 0.12 molal solutions of the nucleosides.

2.2. Spectra

The PMR-spectra were obtained in 5 mm tubes in the CW mode at 100.1 MHz on a Varian XL-100-15 spectrometer equipped with an XL-100 variable temperature accessory. Temperatures are accurate to $\pm 0.5\,^{\circ}C$. During the experiments the spectrometer was locked to the deuteron-resonance of the solvent. In preliminary experiments small amounts of 2-propanol-2-methyl (deuterated at the hydroxyl group) or TMS were added to the samples. The solubility of TMS in ND_3 varies considerably in the temperature interval investigated and phase separation was difficult to avoid. Addition of $\sim 5\%$ 2-propanol-2-methyl on the other hand influenced the differences in chemical shifts between the single protons of the nucleoside compared to the neat ND_3 solutions by up to 0.02 ppm. Since it was intended to use the same samples in relaxation measurements, where proton contamination has to be kept as low as possible, the spectra presented here were taken in neat ND_3 and referenced in separate experiments to an external coaxial TMS solution (98% CS_2, 2% TMS). In the Tables presented below no attempts to compensate for bulk magnetic susceptibility effects were made. Accordingly, temperature dependence of the chemical shifts apparent in the Tables may partly be due to susceptibility changes and the δ-values given may have an absolute error of ± 0.05 ppm. However, only chemical shifts differences in frequencies enter into the simulation of the ribose spectra. These relative frequencies have been determined with a V-4410 frequency counter to ± 0.05 Hz. For brevity these more accurate data together with the results obtained at intermediate temperatures ($0\,^{\circ}C$ and $-30\,^{\circ}C$) have been omitted from the paper but are available upon request.

3. Theoretical

3.1. Conformational analysis of the ribose ring

Altona and Sundaralingam [15, 16] have introduced a new description of the furanose ring conformations based on the concept of pseudorotation. According to these authors, each conformation of the furanose ring is exactly determined by two parameters, the phase angle of pseudorotation, P, and the degree of pucker, τ_m. Only two relatively narrow ranges for the phase angle of pseudorotation are found for β ribosides in the solid state. In the type N conformations P ranges from 3° to 23°, whereas in the type S conformations P is found in the range

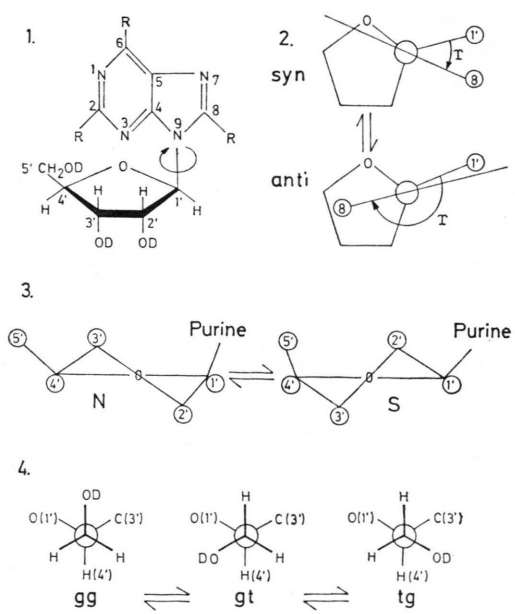

Fig. 1. 1. Schematic representation of the possible internal motions and numbering of the atoms in the purine (β) ribosides. 2. Rotation around the glycosidic bond. 3. Pseudorotation of the ribose moiety. 4. Newman projections along the C(4′) – C(5′) bond showing the three classical rotamers.

$139° - 175°$. Type N conformers include the classical C(3′)-*endo* conformation and type S the classical C(2′)-*endo* (Fig. 1).

In the solid state, the purine ribosides are distributed equally between the N and S conformers. In solution both conformers are present in dynamic equilibrium : $S \rightleftharpoons N$[3-6]. If such an equilibrium is rapid on the time-scale of a high resolution NMR experiment, the observed vicinal coupling constants (J_{obs}) reflect a weighted average value of the respective coupling constants (J_N, J_S) for the various states:

$$J^{ij}_{obs} = [N]J^{ij}_N + [S]J^{ij}_S$$

where the ij represent the ribose protons. If one can determine a Karplus equation relating the vicinal proton-proton coupling constants to the torsion angles between carbon-hydrogen bonds, one can arrive at the three-dimensional geometry of the ribose.

On the basis of crystal structure data and the pseudorotational model, Altona and Sundaralingam [16] have shown that the coupling constant $J^{2'3'}$ as well as the sum $J^{1'2'} + J^{3'4'}$ are nearly independent of the position of the $S \rightleftharpoons N$ conformational equilibrium. Taking values from the literature, they were able to extract a set of Karplus parameters applicable to nucleosides. These parameters were mostly based on results obtained with pyrimidine nucleosides in D_2O and DMSO. Therefore, we recalculated the Karplus parameters using the same method as Altona and Sundaralingam but taking into account only the results obtained with purine nucleosides in ND_3 (A, I, G, X, PR, 2amPR, N6ipA). The Karplus equation has then the form:

$$J^{ij} = 10.0 \cos^2 \varphi^{ij} = 0.95 \cos \varphi^{ij} .$$

With this equation we constructed Table I, which is similar to Table IV of reference 16. It is now possible to calculate the populations of the two conformers

P_N	% N / % S	100 / 0	80 / 20	60 / 40	40 / 60	20 / 80	0 / 100	P_S
3	1′2′	0.0	1.9	3.9	5.8	7.8	9.7	145
	2′3′	4.5	4.8	5.1	5.5	5.8	6.1	
	3′4′	9.2	7.6	5.9	4.3	2.6	1.0	
	Σ	9.2	9.5	9.8	10.1	10.4	10.7	
3	1′2′	0.0	1.9	3.8	5.7	7.6	9.5	161
	2′3′	4.5	4.7	4.8	5.0	5.1	5.3	
	3′4′	9.2	7.4	5.6	3.8	2.0	0.2	
	Σ	9.2	9.3	9.4	9.5	9.6	9.7	
3	1′2′	0.0	1.8	3.6	5.3	7.1	8.9	175
	2′3′	4.5	4.6	4.7	4.8	4.9	5.0	
	3′4′	9.2	7.4	5.5	3.7	1.8	0.0	
	Σ	9.2	9.1	9.1	9.0	8.9	8.9	
10	1′2′	0.0	1.9	3.9	5.8	7.8	9.7	145
	2′3′	4.6	4.9	5.2	5.5	5.8	6.1	
	3′4′	9.5	7.8	6.1	4.4	2.7	1.0	
	Σ	9.5	9.7	10.0	10.2	10.5	10.7	
10	1′2′	0.0	1.9	3.8	5.7	7.6	9.5	161
	2′3′	4.6	4.7	4.9	5.0	5.2	5.3	
	3′4′	9.5	7.6	5.8	3.9	2.1	0.2	
	Σ	9.5	9.5	9.6	9.6	9.7	9.7	
10	1′2′	0.0	1.8	3.6	5.3	7.1	8.9	175
	2′3′	4.6	4.7	4.8	4.8	4.9	5.0	
	3′4′	9.5	7.6	5.7	3.8	1.9	0.0	
	Σ	9.5	9.4	9.3	9.1	9.0	8.9	
25	1′2′	0.3	2.2	4.1	5.9	7.8	9.7	145
	2′3′	5.1	5.3	5.5	5.7	5.9	6.1	
	3′4′	9.8	8.0	6.3	4.5	2.8	1.0	
	Σ	10.1	10.2	10.4	10.4	10.6	10.7	
25	1′2′	0.3	1.9	4.0	5.8	7.7	9.5	161
	2′3′	5.1	5.1	5.2	5.2	5.3	5.3	
	3′4′	9.8	7.9	6.0	4.0	2.1	0.2	
	Σ	10.1	10.0	10.0	9.8	9.8	9.7	
25	1′2′	0.3	2.0	3.7	5.5	7.2	8.9	175
	2′3′	5.1	5.1	5.1	5.0	5.0	5.0	
	3′4′	9.8	7.8	5.9	3.9	2.0	0.0	
	Σ	10.1	9.8	9.6	9.4	9.2	8.9	

Table I. Predicted vicinal coupling constants J^{ij} between the ribose protons in dependence on the $S \rightleftharpoons N$ equilibrium. Σ means the sum of $J^{1'2'}$ and $J^{3'4'}$.

and the phase angles of pseudorotation of the ribose ring.

3.2. *Conformational analysis of the exocyclic hydroxymethyl group*

The exocyclic CH_2OD group may exist in three different classical staggered conformations designated *gauche-gauche*, *gauche-trans*, and *trans-gauche* (Fig. 1). Blackburn *et al.* [5] and Hruska *et al.* [17] have developed a procedure to calculate the rotamer populations. Using the same method and our Karplus parameters, the mole fractions of each rotamer may be obtained from:

$$P_{gg} = 1.46 - (J_A^{4'5'} + J_B^{4'5'})/8.9$$
$$P_{gt}(\text{or } P_{tg}) = J_A^{4'5'}/8.9 - 0.23$$
$$P_{tg}(\text{or } P_{gt}) = J_B^{4'5'}/8.9 - 0.23$$

Without an unequivocal assignment of the protons $H5'_A$ and $H5'_B$ in the NMR spectra, it is not possible to decide between the two possibilities for the populations of *gt* and *tg* rotamers. We have chosen the set of equations where P_{gt} is given by the second equation and P_{tg} by the third because, in this way, the low temperature results give relative concentrations of the conformers in agreement with solid state data [18].

4. Results and Discussion

The abbreviations used are the following: Adenosine (A), inosine (I), guanosine (G), xanthosine (X), purineriboside (PR), 2-aminopurineriboside (2amPR), N6-isopentenyladenosine (N6ipA), 8-bromoadenosine (8-BrA), 8-bromoguanosine (8-BrG), formycin B (F), tubercidin (T), isopropylideneadenosine (iA), isopropylideneguanosine (iG), uridine (U), isopropylideneuridine (iU).

4.1. *Chemical shifts*

Table II collects the chemical shifts determined for all compounds investigated by us. The results given for the ribose moiety were obtained from the computer analysis of the high resolution spectra. The data for the base protons were taken directly from the calibrated plots. Only in T are the protons 7 and 8 *J*-coupled. The experimental values obtained at +40 °C are $J^{78} = 3.7$ Hz and $\delta(7) = 6.13$ ppm; $\delta(8) = 7.02$ ppm. At −60 °C, the value of J^{78} is unchanged, whereas $\delta(7) = 6.29$ ppm and $\delta(8) = 7.23$ ppm. The quotient $J/\Delta\delta \cdot \nu_0$ is ~ 0.05 thus permitting the two protons to be treated as an AX system.

Table II. Chemical shifts as obtained from the computer analysis of the compounds studied at two different temperatures (in ppm). The data at two intermediate temperatures (0 °C and −30 °C) may be obtained upon request.

Compound	A		I		G		X		PR		2amPR		N6ipA	
δ [°C]	+40	−60	+40	−60	+40	−60	+40	−60	+40	−60	+40	−60	+40	−60
1′	5.63	5.67	5.48	5.55	5.36	5.44	5.26	5.33	5.80	5.87	5.58	5.63	5.63	5.68
2′	4.15	4.14	4.18	4.15	4.08	4.05	4.07	3.97	4.19	4.26	4.14	4.15	4.15	4.12
3′	3.86	3.87	3.81	3.84	3.78	3.82	3.74	3.77	3.88	3.93	3.87	3.88	3.85	3.87
4′	3.67	3.71	3.62	3.68	3.58	3.64	3.54	3.59	3.70	3.78	3.66	3.70	3.66	3.71
$5'_A$	3.39	3.40	3.35	3.38	3.31	3.37	3.29	3.33	3.39	3.46	3.37	3.40	3.39	3.41
$5'_B$	3.28	3.29	3.22	3.26	3.20	3.25	3.16	3.20	3.30	3.35	3.28	3.30	3.27	3.30
2	7.88	7.84	7.66	7.67	—	—	—	—	8.59	8.74	—	—	7.88	8.00
6	—	—	—	—	—	—	—	—	8.80	8.98	8.25	8.33	—	—
8	8.08	8.27	7.66	7.85	7.37	7.57	7.18	7.47	8.53	8.86	7.97	8.20	7.99	8.30

Compound	8-BrA		8-BrG		F		T		iA		iG		P	
δ [°C]	+40	−60	+40	−60	+40	−60	+40	−60	+40	−60	+40	−60	+40	−60
1′	5.57	5.62	5.42	5.46	4.62	4.62	5.77	5.89	5.86	5.92	5.59	5.65	—	—
2′	4.77	4.96	4.70	4.71	4.12	4.17	3.97	3.97	5.09	5.17	4.96	5.06	—	—
3′	3.86	3.89	3.86	3.83	3.78	3.78	3.79	3.85	4.69	4.75	4.63	4.71	—	—
4′	3.63	3.70	3.64	3.70	3.55	3.62	3.59	3.66	3.94	4.00	3.85	3.88	—	—
$5'_A$	3.33	3.39	3.39	3.43	3.33	3.36	3.32	3.35	3.24	3.20	3.25	3.25	—	—
$5'_B$	3.20	3.25	3.24	3.27	3.15	3.19	3.42	3.28	3.24	3.20	3.20	3.17	—	—
2	7.76	7.84	—	—	7.48	7.56	7.74	7.81	7.85	7.92	—	—	8.12	8.19
6	—	—	—	—	—	—	—	—	—	—	—	—	8.31	8.40
8	—	—	—	—	—	—	7.02	7.23	8.02	8.27	7.38	7.51	7.64	7.74

In NMR experiments the chemical shifts are the quantities obtainable with the highest accuracy. The quantitative assignments of slight changes of this quantity to specific structural or conformational changes are not straightforward however. Especially for the purine and pyrimidine nucleosides there have been various attempts [19–21] to derive the position of the base in the *syn* ⇌ *anti* equilibrium from minor changes in the base proton chemical shift. In the data presented here the position of all H(8) protons is shifted around 0.2 ppm downfield when the temperature is lowered from +40 °C to −60 °C, whereas the position of H(6) and H(2) is less affected in all compounds. On the other hand, in aqueous solutions, an upfield shift is observed for A and I with decreasing temperature and the H(2) protons are more affected than the H(8) protons [22].

Therefore, in our opinion, these changes should rather be ascribed to the influence of the solvation shell on the chemical shifts than to a variation of the *syn* ⇌*anti* equilibrium with temperature. Similar effects have been discussed for a variety of compounds in aqueous solutions [23]. Qualitatively our attempt at an explanation is corroborated by the data from P, PR, and T. In P, the chemical shifts for the three protons H(2), H(6), H(8) are similar and it seems reasonable to assume a uniform solvation shell around this base. The ribose moiety in the nucleosides will certainly distort this solvation shell. This can be seen in the temperature changes observed in PR, the only nucleoside studied by us lacking a polar substituent, where H(2), H(6), H(8) have a temperature change double that in P. Moreover, the changes of H(2) and H(6) are bigger than those found in any other compound studied. It is obvious that the polar substituents at the positions 2 or 6 have an effect on the temperature dependence of the resonances of 6 or 2 through their solvation shell. In addition the changes of $\delta(7)$ and $\delta(8)$ in T are similar. If the effects observed on H(8) were caused by the interaction of this proton with the ribose moiety, one would expect for all possible conformations of the sugar a considerably smaller influence at H(7).

It is clear that changes in the *syn* ⇌ *anti* equilibrium will influence the chemical shifts of the base protons. However, this is certainly not the only effect to consider and it is difficult to distinguish it from other influences.

4.2. Coupling constants

Adenosine, inosine, guanosine, xanthosine

The coupling constants of these purine ribosides have already been published [7, 8]. For consistency and comparison, we present in Table III the results

Table III. Results of the theoretical analysis for the compounds studied.

Compound	T[°C]	P_N	[N]	P_S	[S]	P_{gg}	P_{gt}
A	+40	25	.45	175	.55	.68	.15
	−60	3	.43	175	.57	.70	.20
I	+40	25	.36	175	.64	.75	.11
	−60	3	.37	175	.63	.77	.14
G	+40	25	.40	175	.60	.73	.13
	−60	3	.37	175	.63	.75	.14
X	+40	25	.39	175	.61	.75	.11
	−60	10	.41	175	.59	.77	.12
PR	+40	25	.44	175	.56	.67	.15
	−60	3	.44	175	.56	.71	.20
2amPR	+40	25	.42	175	.58	.66	.19
	−60	25	.38	175	.62	.66	.23
N6ipA	+40	25	.44	175	.56	.70	.15
	−60	10	.42	175	.58	.70	.22
8-BrA	+40	10	.28	161	.72	.45	.26
	−60	10	.17	175	.83	.22	.47
8-BrG	+40	25	.21	175	.79	.70	.14
	−60	10	.12	175	.88	.72	.14
F	+40	3	.29	145	.71	.76	.08
	−60	3	.16	145	.84	.80	.10
T	+40	10	.43	161	.57	.68	.14
	−60	10	.38	161	.62	.68	.20

for A, I, G, X following the method of chapter 3. From this Table, it can be seen that

1. the S state dominates with a mole fraction around .60;
2. the angle of pseudorotation in the S state is invariant, whereas this is not true for the N state where it decreases with falling temperature;
3. the exocyclic group is to a large extent in the *gg* conformation (.75);
4. the mole fraction of the *gt* conformer increases with decreasing temperature. The mole fraction of the *tg* conformer on the other hand has the opposite variation and has no appreciable population at low temperature, whereas at high temperature it has a higher population than the *gt* conformer. With the other set of formulas for calculating the mole fractions of the *gt* and *tg* conformers, one would have to interchange *gt* and *tg*. It is interesting to note that the *gt* conformer dominates at −60 °C only if the *tg* conformer dominates at +40 °C. Their popula-

tions are equal at +30 °C in A, −2 °C in I and G, and −45 °C in X.

The points 1. and 3. are in agreement with previous results obtained in D_2O and DMSO [15, 2, 1].

Purineriboside, 2-aminopurineriboside, N6-isopentenyladenosine

We have also investigated three less common purine (β) ribosides. The non substituted purine-(β)riboside, also called nebularine, is toxic to animal cells in cultures and tumours [24]. The adenosine substituted at N6 by a isopentenyl group occurs in some tRNAs. The coupling constants are contained in Table IV and the results in Table III. Fig. 2 shows the experimental and simulated spectra of 2amPR at +40 °C. An inspection of these Tables shows that these three compounds have a very similar behaviour to the four preceding ones A, I, G, X. The four conclusions of the preceding paragraph also apply to these three derivatives. Conformational changes cannot therefore explain the toxicity of nebularine. It has indeed been shown that nebularine must be phosphorylated to the 5′-nucleotide before it becomes cytotoxic [24].

The seven purine(β)ribosides already presented show that substitution at C(2) or C(6) does not markedly influence the ribose conformations. It appears that these dissolved nucleosides behave much more uniformly than is observed in the solid state. Since only minor energy differences exist between the conformers, packing effects in the crystalline state can obviously overcome slight bias in the dynamic equilibria present. The effects of the substituents at C(2) and C(6) can only be observed in the slightly different behaviours of the temperature dependence of the S ⇌ N equilibria.

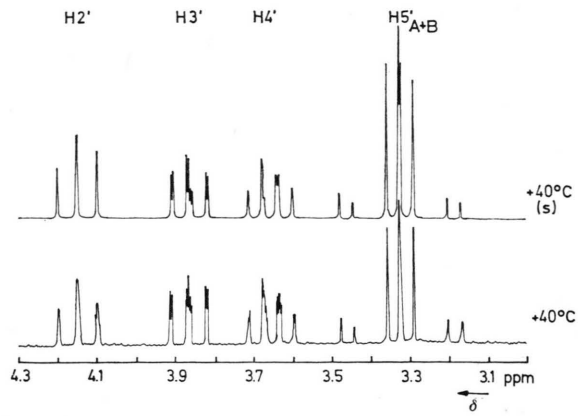

Fig. 2. Experimental proton high resolution spectrum of 0.12 molal solution of 2amPR in ND_3 at +40 °C covering the region of the protons H(2′) to H(5′A) and H(5′B) compared with the simulated spectrum.

Table IV. Coupling constants as obtained from the computer analysis of the compounds studied at two different temperatures (in Hz). Same remarks as for Table II.

Compound	PR [°C] +40	PR −60	2amPR +40	2amPR −60	N6ipA +40	N6ipA −60	8-BrA +40	8-BrA −60	8-BrG +40	8-BrG −60
J										
1′2′	5.1	5.0	5.3	5.6	5.1	5.3	6.8	7.4	7.1	7.8
2′3′	5.0	4.8	5.0	4.7	5.0	4.7	5.1	5.0	5.1	5.0
3′4′	4.3	4.0	4.1	3.7	4.3	4.0	2.8	1.7	2.2	0.8
4′5′A	3.4	3.8	3.7	4.1	3.4	4.0	4.4	6.2	3.3	3.3
4′5′B	3.6	2.9	3.4	3.0	3.4	2.8	4.6	4.8	3.5	3.3
5′A5′B	−12.4	−12.4	−12.2	−12.3	−12.2	−12.3	−12.1	−12.0	−12.4	−12.5

Compound	F [°C] +40	F −60	T +40	T −60	iA +40	iA −60	iG +40	iG −60
J								
1′2′	6.9	8.2	5.4	5.8	2.8	2.9	3.0	2.7
2′3′	5.0	4.8	5.0	4.9	6.2	6.2	6.2	6.4
3′4′	3.4	2.0	4.5	3.7	2.8	2.0	2.5	2.0
4′5′A	2.8	2.9	3.3	3.8	4.6	4.9	4.8	5.5
4′5′B	3.4	3.0	3.6	3.1	4.6	4.9	4.6	4.7
5′A5′B	−12.2	−12.4	−12.1	−12.3	—	—	−11.7	−11.8

8-Bromoadenosine and 8-bromoguanosine

The coupling constants of these two compounds measured at different temperatures are given in Table IV. In Fig. 3 are shown the experimental and simulated spectra of 8-BrA at $+40\,^{\circ}C$. The results of the analysis of chapter 3 are presented in Table III. The main differences between these results and all those above appear in the increased mole fraction of the S state ($\sim .80$) and the strong temperature dependence of the coupling constants. Moreover, in 8-Br-A the population of the *gg* rotamer is significantly lower than in all nucleosides described hitherto. These two compounds have been proved to be mostly in the *syn* state [18, 25]. The high population of the S state of the ribose gives support for our previous hypothesis about a correlation between the *syn* conformation of the base and the S state of the ribose in purine nucleosides [7].

Formycin B and tubercidin

Formycin B, a structural analogue of inosine, is a C-nucleoside antibiotic which is cytotoxic and has been shown to inhibit several aspects of purine metabolism [26]. Tubercidin is the most biologically active of the modified nucleoside antibiotics with selective inhibition of cancer cells [27]. It differs from A by the presence of a C – H group instead of a nitrogen at position 7 of the adenine base. In the solid state they have been found to occur with the sugar in the S state and the CH_2OH group in the *gt* conformation [28–31]. In crystals of Formycin·HBr·H_2O, the base is in the *syn* range. But in crystals of Formycin·H_2O the base adopts the so-called „high-*anti*" conformation (intermediate between *syn* and *anti*), which is typical of the 8-azapurine nucleoside [32, 33]. In crystals of Tubercidin, the base is in the *anti* range [30, 31].

In Fig. 4, experimental and simulated spectra of F taken at two different temperatures are presented. From the chemical shifts and coupling constants of T given in Table II and Table IV, one observes that this antibiotic does not show differences from A, I, G, X. Accordingly, the biological activity of T cannot be explained by conformational changes. On the other hand, the parameters of F have a close similarity to those of 8-BrA and 8-BrG. The mole fractions of the different conformers are also given in Table III. The accuracy of these results may be uncertain, since the C – C glycosyl bond may effect the Karplus parameters calculated with a C – N glycosyl bond. The disagreement between the calculated coupling constants and the experimental ones is particularly great for $J^{2'3'}$ in F and most easily observed for the low temperature coupling constants. A property of the "high-*anti*" region is the existence of an intramolecular interaction between N(8) of the base and C(2′) of the ribose [33]. This can have an effect on the coupling constants of the hydrogen atom located on C(2′). Nevertheless, the comparison with the bromo-substituted purines, which are known to be in the *syn* conformation, shows that in F the base is mostly in the *syn* range. Ward and coworkers [34] accounted for all biochemical properties of poly F by assigning the *syn* conformation to the individual formycin residues in the polymer. Contrary to the solid state results [28–31], the exocyclic group is in both cases in the *gg* conformation.

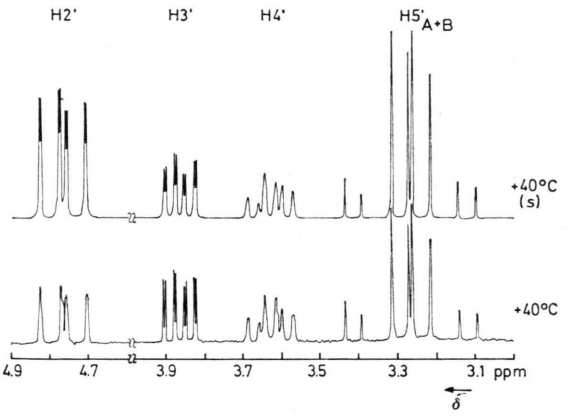

Fig. 3. Experimental proton high resolution spectrum of 0.12 molal solution of 8-BrA in ND_3 at $+40\,^{\circ}C$ covering the region of the protons H(2′) to H(5′A) and H(5′B) compared with the simulated spectrum.

Isopropylideneadenosine and isopropylideneguanosine

In these compounds, the ribose ring is constrained by the isopropylidene group and is therefore less flexible. To our knowledge, no crystal structures of these isopropylidene derivatives have appeared. The only crystal structures to be found in the literature are those of some isopropylidene-cyclonucleosides and cyclonucleotides. For example, in 5′,2-O-cyclo-2′,3′-O-isopropylidene uridine [35] the ribose is in the O(1′)-*exo* conformation as in uridine 2′,3′-O,O-cyclophosphorothioate [36].On the other hand, one molecule of the asymmetric unit of β-cytidin 2′,3′-cyclic phosphate [37] is essentially planar, whereas the other one is in the O(1′)-*endo* conformation.

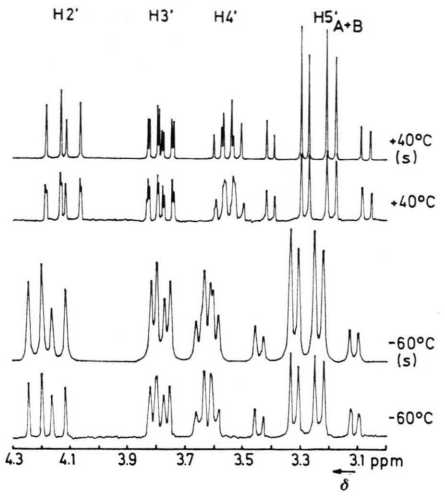

Fig. 4. Experimental proton high resolution spectra of 0.12 molal solution of F in ND_3 at +40 °C and at −60 °C covering the region of the protons H(2′) to H(5′A) and H(5′B) compared with the simulated spectra.

The experimental results are given in Table IV. Fig. 5 shows the spectrum of iG taken at −60 °C. The coupling constants of iA and iG are very similar, whereas between A and G significant differences could be observed. Using the mean values of the coupling constants measured at −60 °C ($J^{1'2'}=2.8$; $J^{2'3'}=6.3$; $J^{3'4'}=2.0$) and assuming the applicability of the Karplus equation proposed in chapter 3 to isopropylidene derivatives, one obtains the following dihedral angles between the ribose protons:

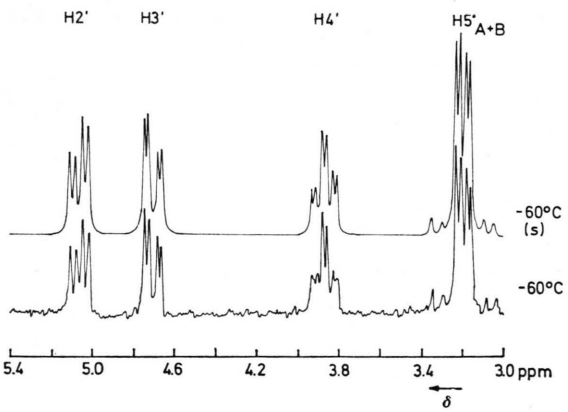

Fig. 5. Experimental proton high resolution spectrum of 0.12 molal solution of iG in ND_3 at −60 °C covering the region of the protons H(2′) to H(5′A) and H(5′B) compared with the simulated spectrum.

φ(H1′−H2′):	119°	and	55°
φ(H2′−H3′):	33°	and	138°
φ(H3′−H4′):	114°	and	60°.

From mechanical models, the following angles are measured for the 0(1′)-*exo* and 0(1′)-*endo* conformations:

	0(1′)-*exo*	0(1′)-*endo*
φ(H1′−H2′):	120°	150°
φ(H2′−H3′):	0°	0°
φ(H3′−H4′):	120°	150°

A comparison between these groups of values shows that the ribose of iA and iG adopts preferably the 0(1′)-*exo* conformation in ND_3 solutions. Nevertheless, from the temperature dependence of $J^{1'2'}$ and $J^{3'4'}$ and the poor fit between experimental and theoretical angles, one must conclude that the substituted sugar ring is interconverting rapidly on the NMR time scale between the 0(1′)-*exo* and 0(1′)-*endo* conformations even at −60 °C. In both derivatives, the population of the *gg* rotamer (∼.40) is considerably lower than in the rest of the compounds, the *gt* and *tg* rotamers having the same populations (∼.30). At low temperature, the populations of the three rotamers are nearly equal. This shows that in these compounds the ΔG_{213} between the three rotamers is approximately zero. But, the other compounds with the exception of 8-BrA should have a ΔG_{213} around 1.0 kcal·mol^{-1}. From mechanical models, it can easily be seen that no hydrogen bond between 0(5′) and N(3) of the purine base is possible in the isopropylidene derivatives. Therefore, we think that the high population of the *gg* rotamer in most nucleosides studied is due to a hydrogen bond between the hydroxymethyl group and the purine base. The following arguments support this hypothesis. Hruska *et al.* [38] have correlated the *gg* conformation of the hydroxymethyl group with the N state of the ribose of pyrimidine nucleosides in aqueous solutions. But, for A, I, G, X, PR, 2amPR, N6ipA, F, and T, in ND_3 solutions, we find rather the opposite correlation, that is the *gg* rotamer is correlated with the S state of the ribose. If the S state is correlated with the *syn* conformation, it follows that the *syn* conformation of base is stabilized by the proposed hydrogen bond between 0(5′) and N(3). This is corroborated by the fact that the highest population of the *gg* rotamer is found in 8-BrG and F, two compounds known to be in the *syn* conformation. In the C^{13}-relaxation experiments [11], contrary to the effects observed in U and iU, no great differences between the longitudinal relaxation rates of C(5′) in A and iA are observed. The value of ΔG_{213} given above can explain this

finding. Though the lowering of the energy of the *gg* rotamer by 1.0 kcal·mol^{-1} is sufficient to raise the equilibrium population of this rotamer, this contribution is too small compared to the activation energy for rotation ($\sim$5.0 kcal·mol^{-1}) to significantly alter the rate of exchange between the three rotamers.

5. Conclusions

The eight nucleosides A, I, G, X, 2amPR, N6ipA, and T show a very similar behaviour in every respect. The small differences observed are most certainly due to effects of the substituents at positions 2 and 6. The temperature dependence of the mole fractions [N] and [S] varies also with the substituents at these two positions.

On the other hand, the 8-bromo substituted nucleosides and the antibiotic formycin B show a strong temperature dependence of the mole fractions of the two ribose states. The same dependence was observed by Schweizer and Robins for 8-BrA in DMSO [20]. Here, reduction of the temperature from 70 to 28 °C increases $J^{1'2'}$ by 0.7 Hz. In these three compounds the bases adopt the *syn* conformation. For 8-BrA and 8-BrG steric hindrance has been invoked as an explanation, since the bulky and moreover strongly solvatized bromine atom cannot take the *anti* position above the ring. Steric factors will not however explain the *syn* conformation in F. In this compound, the replacement of the CH(8) group by a polar nitrogen and the building of a solvation structure around the two neighbouring nitrogens seems sufficient to stabilize the *syn* conformation.

The different temperature dependence of the S $\rightleftharpoons$ N equilibrium observed in the two groups mentioned, together with the similarity within the two groups, tempted us to propose a model for the enthalpy differences between the *syn-anti* conformers and the S – N states. Since the base is restricted to the *syn* conformation in 8-BrA, 8-BrG, and F, the temperature dependence of the coupling constants of the ribose is assumed to reflect the true enthalpy difference between the two states S and N. The purine base in the *syn* position does not sterically interfere with the ribose moiety and thus the latter can adopt the energetically most favourable conformation, which is the S state. Alternatively, one could argue that the bulky substituent at C(8) destabilizes the N conformation, since in this conformation H(2′) comes close to the substituent at C(8) thus reducing the space available for the latter. However, the similarity between the 8-bromonucleosides and formycin B, where sterical arguments can obviously not be invoked, decidedly weakens this argument. The first alternative seems to provide a better interpretation of the experiments so far collected. Indeed, a plot of ln[S]/[N] against $1/T$ (Fig. 6) yields straight lines with equal slopes

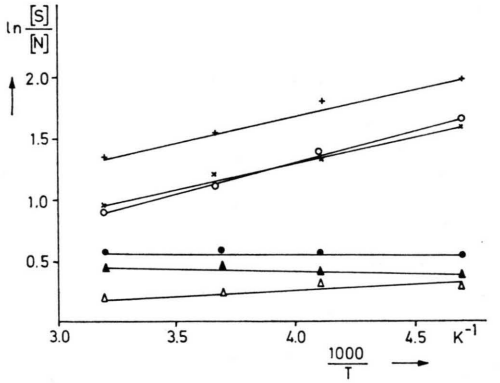

Fig. 6. Van't Hoff plot of the mole fractions of S and N states of the ribose in: 8-BrA (×); 8-BrG (+); F (○); A (△); I (●); X (▲).

for these three derivatives and a ΔH^0 of -0.8 kcal mol^{-1}. Consequently, the considerably smaller temperature dependence of the S $\rightleftharpoons$ N equilibrium in the first group of compounds, where slopes are only slightly positive or negative, indicates that for these compounds the observed ΔH^0 is a difference between the ΔH^0 of the S $\rightleftharpoons$ N and the ΔH^0 of the *syn* $\rightleftharpoons$ *anti* equilibrium, both being roughly equal and of the same sign (~ -0.8 kcal·mol^{-1}):

$$\Delta H^0 = \Delta H^0_{S \rightleftharpoons N} - \Delta H^0_{syn \rightleftharpoons anti}$$

This fact can be rationalized if two conditions are fulfilled. First, the S state of the ribose is correlated with the *syn* conformation of the base and the N state with the *anti* conformation. And secondly, the *anti* conformation is preferred by the base. In terms of free energy differences, this would give at +40 °C a $\Delta G_{S \rightleftharpoons N}$ around -0.7 kcal·mol^{-1} and a $\Delta G_{syn \rightleftharpoons anti}$ around -0.4 kcal·mol^{-1}.

The molecular explanation of the thermodynamic quantities derived above is not straightforward and the separation of the contributions from the two conformational equilibria might be unrealistic. At any rate, the dissolved nucleoside will adopt the con-

formation which minimizes the sum of the free energies from inter- and intramolecular interactions. The largest contribution to the intermolecular terms in liquid ammonia derives certainly from hydrogen bonding interaction between the ammonia molecule and the polar groups of the nucleoside. It is, therefore, difficult to pinpoint the cause of the effects observed from the experimental evidence collected to date. Either the proposed hydrogen bond between N(3) and the 5′-hydroxymethyl group stabilizes the *gg-syn*-S conformation, a model suggested by Pullman and Berthod from PCILO calculations [39]. Or, a difference in the overall interaction between the solvent and the ribose in the two states is responsible for the observations.

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