Ribose Conformations of 8-Azapurine Nucleosides in Solution

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(Z. Naturforsch. **31 c**, 135–140 [1976]; received February 5, 1976)

8-Azapurine (β) ribosides, Trideuteroammonia, Deuterium Oxide, Pyridine, S ⇌ N Equilibrium

The ribose conformations of 8-azaadenosine, 8-azaguanosine, and 8-azainosine have been studied using proton magnetic resonance in ND_3 solutions, in D_2O solutions, and in pyridine solutions. The temperature was varied between −60 and +40 °C in ND_3 and between +10 and +60 °C in D_2O solutions. The analysis is based on the two state S ⇌ N model of the ribose moiety proposed by Altona and Sundaralingam. In D_2O, the 8-aza substitution destabilizes the *gg* rotamer and simultaneously diminishes the population of the S state of the ribose. It is deduced that the *anti* population of the base is greater in the 8-azapurine (β) ribosides than in the common purine (β)-ribosides.

1. Introduction

The importance of nucleoside analogs in biology and chemotherapy has grown during the past quarter of a century because of their effectiveness as antibacterial, antiviral, and antitumor agents [1]. The determination of the mode of action of a given nucleoside analog is extremely difficult; for nucleosides can undergo extensive metabolic conversion, thereby effecting a variety of cell constituents. In order to be useful as therapeutic agents, correlations between a given structural substitution and the corresponding biological effects are needed. The knowledge of the conformations adopted by such analogs in solutions may help to find such correlations and to elucidate their mode of action. This can be seen in the following examples. If a nucleoside analog presents identical conformations with those of the corresponding nucleoside, it can normally be incorporated into DNA and RNA. This incorporation can then interfere with enzymatic production or activity. This is the case with the highly cytotoxic nucleoside tubercidin [2]. In a previous paper [3], we have shown that tubercidin possesses the same conformational properties as the common nucleosides. On the other hand, the biochemical properties of the antibiotic formycin have been attributed to its adoption of the unusual *syn* conformation [4, 5]. In support of this hypothesis, there is evidence that formycin and formycin B exist in solution in the *syn* conformation [3, 6].

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Our studies on the conformations of purine (β) ribosides in solution [3, 7, 8] are here extended to some 8-azapurine (β) ribosides because several azapurine nucleosides have shown cytotoxicity to cells in culture [9–11]. In relation to the present considerations, it is more interesting to study nucleosides since, contrary to the nucleotides, they can penetrate membranes.

2. Experimental

The 8-azapurine (β) ribosides were kindly supplied by Dr. Montgomery from the Southern Research Institute (Birmingham, Alabama). The compounds were used without further purification.

The PMR-spectra were obtained in thickwalled 5 mm tubes in the FT-Mode at 100.1 MHz on a Varian XL-100.15 FT spectrometer equipped with a 8K 620i-computer. The temperatures given are accurate to ±0.5 °C. During the experiments the spectrometer was locked to the deuteron resonance of the solvent.

The chemical shifts of the aqueous solutions were referenced to internal DSS (sodium 2,2 dimethyl, 2 silapentanesulfonate) and those of the pyridine solutions to internal TMS (tetramethylsilane). For technical reasons, the solutions in liquid ammonia were referenced to an external standard (2% TMS dissolved in CS_2) contained in a coaxial outer tube. No attempts were made to correct for bulk magnetic susceptibility effects. The rather large differences observed between the chemical shifts of the various protons in water and in liquid ammonia may partly be due to the influence of the magnetic susceptibility and its temperature dependence. However, only the relative differences of the resonance frequencies within each set of coupled protons enter into the calculation of the simulated spectra, and

these differences are determined by the accuracy of the Fourier-transform spectra (± 0.1 Hz).

The simulation of the spectra was considered successful if the deviations between experimental and simulated spectra were $\leq \pm 0.1$ Hz. The values for the chemical shifts given in the tables were rounded off to ± 0.01 ppm (~ 1.0 Hz). The original results obtained from the computer analysis together with the values found at intermediate temperatures (0 °C and -30 °C in ammonia and $+40$ °C in heavy water) have been omitted from the paper, but are available upon request.

3. Results

The abbreviations used are the following: 8-azaadenosine (aA), 8-azainosine (aI), 8-azaguanosine (aG) and adenosinie (A).

The conformation analysis of the ribose ring was made according to the concept of pseudorotation of Altona and Sundaralingam [12, 13] as explained in more detail in a previous paper [3]. The Karplus equation, determined from results obtained with purine nucleosides, has the form:

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

The mole fractions of each rotamer of the exocyclic hydroxymethyl group were obtained from:

$$P_{gg} = 1.46 - (J^{4'5'_A} + J^{4'5'_B})/8.9 ,$$
$$P_{gt} = J^{4'5'_A}/8.9 - 0.23 ,$$
$$P_{tg} = J^{4'5'_B}/8.9 - 0.23 .$$

The equations were derived following the procedure of Blackburn *et al.* [14] and Hruska *et al.* [15].

3.1. Chemical shifts

The chemical shifts of the three above mentioned azanucleosides dissolved in three different solvents are collected in Table I.

Table I. Chemical shifts in ppm at two different temperatures for aA, AI, and aG dissolved in ND_3 and in D_2O. The chemical shifts for aA, aG and A dissolved in pyridine are also given.

D_2O	Compound	aA		aI		aG	
δ	°C	+60	+10	+60	+10	+60	+10
1′		6.40	6.41	6.33	6.35	6.15	6.17
2′		5.05	5.08	5.00	5.05	4.95	4.99
3′		4.63	4.70	4.59	4.66	4.55	4.60
4′		4.32	4.36	4.29	4.32	4.26	4.29
5′A		3.88	3.92	3.86	3.89	3.86	3.89
5′B		3.78	3.81	3.75	3.78	3.75	3.78

ND_3	Compound	aA		aI		aG	
δ	°C	+40	−60	+40	−60	+40	−60
1′		5.94	5.92	5.75	5.84	5.66	5.66
2′		4.58	4.64	4.45	4.56	4.46	4.44
3′		4.04	4.02	3.91	4.00	3.94	3.96
4′		3.77	3.77	3.65	3.76	3.68	3.71
5′A		3.36	3.33	3.29	3.35	3.34	3.33
5′B		3.25	3.20	3.16	3.21	3.22	3.20

Pyridine	Compound	aA	aG	A
δ	°C	+40	+40	+40
1′		7.14	6.91	6.67
2′		5.74	5.65	5.47
3′		5.21	5.17	5.03
4′		4.85	4.82	4.74
5′A		4.32	4.28	4.29
5′B		4.18	4.18	4.12

3.2. Coupling constants

The coupling constants in ND_3 and in D_2O are contained in Table 2. Figs 1 and 2 show that the spectra simulated with the values of the chemical shifts and of the coupling constants contained in Tables I and II are in good agreement with the experimental ones.

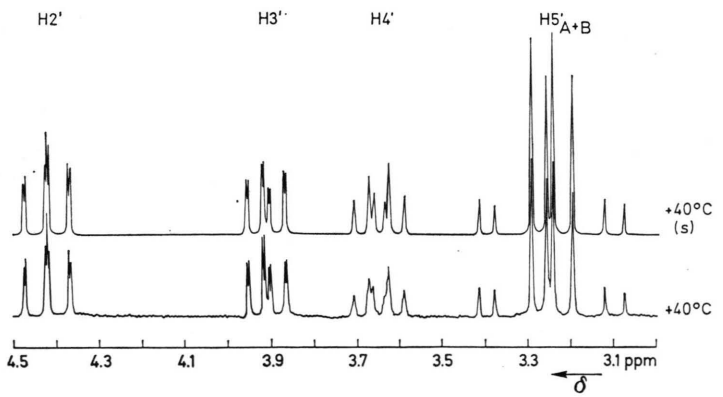

Fig. 1. Experimental proton high resolution spectrum of a solution of 8-azaguanosine in ND_3 (2 mg/ml) at +40 °C covering the region of the protons H(2′) to H(5′A) and H(5′B) compared with the simulated spectrum.

Table II. Coupling constants in Hz at two different temperatures for aA, aI and aG dissolved in ND_3 and in D_2O.

D_2O	Compound	aA		aI		aG	
J	°C	+60	+10	+60	+10	+60	+10
1′2′		4.4	4.2	4.5	4.5	4.7	4.7
2′3′		5.2	5.1	5.1	5.1	5.2	5.1
3′4′		4.9	4.9	5.1	4.8	4.7	4.5
4′5′$_A$		3.4	2.7	3.3	3.1	3.3	3.5
4′5′$_B$		5.0	4.7	4.9	5.0	5.1	5.0
5′$_A$5′$_B$		−12.6	−12.7	−12.6	−12.8	−12.5	−12.8

ND_3	Compound	aA		aI		aG	
J	°C	+40	−60	+40	−60	+40	−60
1′2′		5.2	5.6	5.5	5.5	5.5	5.5
2′3′		5.2	4.9	4.9	4.8	5.1	4.8
3′4′		4.2	3.6	3.7	3.5	3.7	3.5
4′5′$_A$		4.5	5.6	3.8	5.0	3.5	4.5
4′5′$_B$		5.5	5.7	4.9	5.6	4.7	5.6
5′$_A$5′$_B$		−12.0	−11.8	−12.1	−12.1	−12.2	−12.2

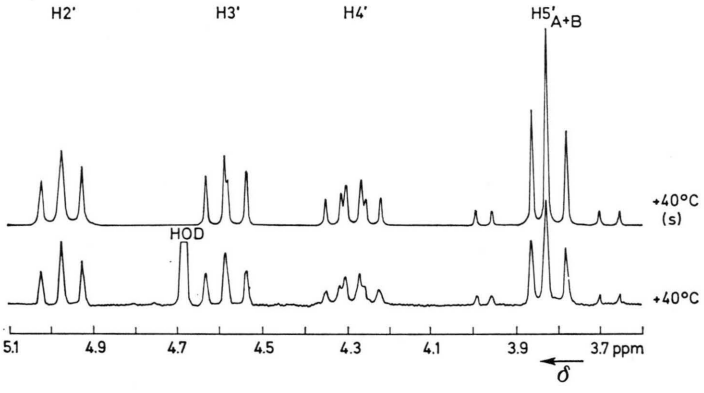

Fig. 2. Experimental proton high resolution spectrum of a solution of 8-azaguanosine in D_2O (2 mg/ml) at +40 °C covering the region of the protons H(2′) to H(5′$_A$) and H(5′$_B$) compared with the simulated spectrum.

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

Solvent	Compound	T[°C]	P_N	[N]	P_S	[S]	P_{gg}	P_{tg}
ND_3	aA	+40	25	.43	175	.57	.34	.38
		−60	10	.38	175	.62	.19	.41
	aI	+40	25	.38	175	.62	.48	.32
		−60	10	.37	175	.63	.27	.40
	aG	+40	25	.38	175	.62	.54	.30
		−60	10	.37	175	.63	.32	.40
D_2O	aA	+60	10	.51	175	.49	.52	.33
		+10	3	.53	175	.47	.63	.30
	aI	+60	25	.52	175	.48	.54	.32
		+10	10	.49	175	.51	.55	.33
	aG	+60	25	.48	175	.52	.52	.34
		+10	10	.47	175	.53	.51	.33

The mole fractions of the two states of the ribose, together with the angles of pseudorotation, and the mole fractions of the two rotamers of the exocyclic group have been calculated using the method applied previously to the common purine(β)ribosides [3]. The results are presented in Table III.

From this table, the following main conclusions about the ribose conformations of 8-azanucleosides in ND_3 solutions can be drawn:

1. as in the common purine ribosides, the S state dominates with a mole fraction around .60 in the 8-azapurine ribosides;
2. the strongest effect of the aza substitution is observed on the mole fractions of the exocyclic group. In the purine ribosides, the *gg* conformer dominates (.75) and there is a small temperature dependence. However, in the 8-azapurine ribosides, the mole fraction of the *gg* conformer is only .45 at +40 °C and falls to .26 at −60 °C, where the *tg* conformer dominates over the *gt* conformer.

From Table III, it can also be seen that in D_2O solutions:

1. the S and N state are equally populated. In contrast to the behaviour of the common purine nucleosides in ND_3 and in D_2O solutions, the azapurine nucleosides in D_2O solutions present therefore a decrease in the S state population;
2. the *gg* conformer has the highest mole fraction ($\sim$ .55), followed by the *tg* conformer ($\sim$ .33). As in the common purine ribosides, there is a small temperature dependence of the mole fractions of the three rotamers of the exocyclic group.

On the other hand, the following properties are common to both solvents:

1. The S $\rightleftharpoons$ N equilibrium is not significantly altered by the temperature in both kinds of compound;
2. the angles of pseudorotation of the S state do not vary with the temperature and are the same as in the common purineribosides. The temperature dependence of the angle of pseudorotation of the N state is stronger in the purine ribosides than in the azapurine ribosides.

These observations are in agreement with previous results obtained by Lee *et al.*[16] for aqueous solutions of 8-azapurine nucleosides and nucleotides. It is also interesting to note that the 6-azapyrimidine derivatives favour slightly the N state of the ribose and strongly the *gt*, *tg* rotamers of the exocyclic group[17]. Since the base is considered to be predominantly in the *anti* range for common purine derivatives, the observed destabilizing effect of the aza substitution on the *gg* rotamer is thought to be due to repulsive electrostatic interaction in the *anti* conformation between the aza nitrogen and the hydroxyl (or phosphate) group at the 5′ position. In order to accommodate these electrostatic repulsions the base moves to the *syn* range and the group at the 5′ position to the *gt* and *tg* conformations[16].

However, it is now apparent that a dynamic equilibrium exists between the *syn* and *anti* conformations of the base in the common nucleosides[7, 8, 18–20]. Furthermore we have shown that, in the purine ribosides, the *gg* rotamer is correlated with the S state of the ribose and stabilizes the *syn* conformation of the base through the formation of an intramolecular hydrogen bond between 05′ and N3 of the base[3]. It is therefore possible to interpret the experimental results differently. Instead of showing a shift toward the *syn* range, the *syn* $\rightleftharpoons$ *anti* equilibrium could be displaced toward the *anti* region in the 8-aza analogs; thereby reducing the population of the *gg* rotamer. For, in the *anti* range, there is no possibility for an intramolecular hydrogen bond between the base and 05′. Also, in the *anti* range, electrostatic repulsions occur between N8 and 05′.

Since the 8-azapurinenucleosides behave in a peculiar way in ND_3 solutions, we looked for another solvent which would dissolve the purine nucleosides as well as their aza substituted derivatives. Tables IV and V contain the coupling constants and the results of the theoretical analysis for aA and aG together with A in pyridine solutions.

Table IV. Coupling constants in Hz for aA, aG, and A dissolved in pyridine.

Pyridine	Compound	aA	aG	A
J	°C	+40	+40	+40
1′2′		5.5	4.9	5.9
2′3′		5.0	5.1	4.9
3′4′		3.5	4.1	3.0
$4'5'_A$		3.5	3.2	2.6
$4'5'_B$		4.2	4.6	2.7
$5'_A5'_B$		−12.3	−12.1	−12.4

Table V. Results of the theoretical analysis for aA, aG, and A dissolved in pyridine. For comparison, the results for A and I dissolved in D_2O are also given.

Solvent	Compound	*T*[°C]	P_N	N	P_S	S	P_{gg}	P_{tg}
Pyridine	aA	+40	10	.38	175	.62	.60	.24
	aG	+40	10	.45	175	.55	.58	.29
	A	+40	3	.34	175	.66	.87	.06
D_2O	A	+80	3	.40	161	.60	.66	.22
		+10	25	.35	175	.65	.74	.15
	I	+80	25	.43	161	.57	.57	.28
		+10	25	.39	175	.61	.68	.21

Compared to aqueous solutions, the pyridine solutions provoke an increase both in the S state of the sugar and the population of the *gg* rotamer for the 8-azaderivatives (Table III) and A (Table V). However, in A the increase of the *gg* rotamer is greater than in aA and aG; whereas, in aA and aG, the increase of the S state is much greater than in A. As a result, the S/N ratio is the same in A as in aA and aG, but the population of the *gg* rotamer is still less in aA and aG than in A. This means that, in

aqueous solutions of A, the stabilizing effect of the *gg* rotamer on the S state of the ribose is already complete and, consequently, the main effect of the 8-aza substitution is to reduce the population of the *gg* rotamer. In other words, the decrease in the population of the S state of 8-aza nucleosides in aqueous solutions is a consequence of the smaller population of the *gg* rotamer. This conclusion agrees with the correlation, proposed in a previous paper [3], between the S state of the ribose and the *gg* rotamer of the exocyclic group in purine nucleosides.

4. Discussion

We have shown that the main difference between the 8-azapurine nucleosides and the common purine nucleosides is that the former have a lower population of the *gg* rotamer. This conclusion is true for all the temperatures and all the solvents studied here. The depopulation of the *gg* rotamer is therefore a property of the 8-aza substitution in purine nucleosides. The molecular explanation for this effect is however not straight forward. The problem is complicated by the fact that we have no direct experimental evidence about the location of the *syn* $\rightleftharpoons$ *anti* equilibrium. The conclusions about the orientation of the base relative to the ribose have to be deduced from indirect arguments.

In the solid state, the 8-azapurine nucleosides lie outside the usual *syn* and *anti* ranges and adopt the so-called "high-*anti*" conformation [21, 22]. At this orientation of the base relative to the ribose, electrostatic repulsion between the negatively charged nitrogen atom N(8) and the ribose O(5′) atom occurs [23]. This repulsion can be lessened if the exocyclic group assumes the *gauche-trans* or *trans-gauche* conformation. Since we have shown that the main effect of 8-aza substitution is to destabilize the *gg* rotamer, we are tempted to extend to the liquid state the conclusions reached in the solid state. This would mean that in dissolved 8-azapurine ribosides the *syn* $\rightleftharpoons$ *anti* equilibrium is shifted toward the *anti* range.

However, in the solid state, the C-nucleosides formycin and formycin B behave similarly to the 8-aza derivatives. This is certainly not the case in the liquid state. Indeed, in all solvents studied, both compounds present a high population of the *gg* rotamer together with a high population of the S state of the ribose [3, 6]. Therefore, the populations of the *gg* rotamer and of the S state of the ribose increase in the order 8-azanucleosides < common nucleosides < C-nucleosides. Moreover, the C-nucleosides have a similar behaviour to the 8-bromonucleosides, which are known to be restricted to the *syn* range. This implies that in the C-nucleosides the *syn* $\rightleftharpoons$ *anti* equilibrium is shifted toward the *syn* region.

It is possible to understand these results qualitatively if one assumes that the repulsion between O(5′) and the atoms at position 8 [N(8) or C(8)-H] increases in the order C-nucleosides < common nucleosides < 8-azanucleosides. We use this assumption in the following way. The experimental results have shown that there exists a dynamic equilibrium between the *syn* and *anti* conformations and that the *gg* rotamer of the exocyclic group is the most stable one. After the previous considerations, one can assume that the ribose conformations depend mainly on the orientation of the exocyclic group. Now, if a molecule in the *syn-gg* conformation switches to the *anti* conformation, the exocyclic group will keep the *gg* orientation or switch to either *tg* or *gt* depending on the interactions between positions 8 and 5′. Thereby, the *gg* population is reduced. With the assumption stated above, the probability for such a switch should be greater in the 8-azanucleosides than in the C-nucleosides, with the common nucleosides between both of them. Further, because of the interrelations between the various degrees of freedom, these changes should shift the *syn* $\rightleftharpoons$ *anti* equilibrium toward the left in the C-nucleosides and toward the right in the 8-azanucleosides. In these complicated correlations, it is difficult and perhaps unrealistic to distinguish cause and effect. Alternatively, one could conceive that it is the orientation of the exocyclic group which is dependent on the conformation of the ribose. In this case, repulsion between the atoms at position 8 and some ribose atom [for example C(2′)-H] could be the factor which determines when the molecule switches from the *syn* conformation to the *anti* conformation. Indeed, with the ribose in the S state and the base in the *anti* range, interactions between C(2′)-H and N(8) occur in the 8-azanucleosides [22].

We wish to thank Dr. Montgomery for his kindness in supplying us with the 8-azanucleosides.

This research was supported by grants from the Fonds der Chemischen Industrie and the Deutsche Forschungsgemeinschaft.

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