Ribose Conformations of Adenosine Analogs Modified at the 2′, 3′ or 5′ Positions

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The solution conformation of adenosine(β)ribosides modified at the 2′, 3′ or 5′ position is derived from the analysis of the HRNMR spectra of the ribose protons. The conformational equilibria of the furanoside rings are described by the two state $N \leftrightarrow S$ model introduced by Altona and Sundaralingam. The new compounds studied are: 2′-thiobenzoyl-2′-deoxyadenosine, 3′-thio-3′-deoxyadenosine, 2′-chloro-2′-deoxyadenosine, 3′-chloro-3′-deoxyadenosine, 2′-bromo-2′-deoxyadenosine, 3′-bromo-3′-deoxyadenosine, 2′-O-methyladenosine, 3′-O-methyl-adenosine, 2′-deoxy-3′-O-methyladenosine, 5′-amino-5′-deoxyadenosine, 5′-acido-5′-deoxyadenosine, and 5′-chloro-5′-deoxyadenosine.

The emphasis in this work is to study systematically the influence of the different substituents upon the conformational equilibria of the sugar. It is found that any substitution at the 2′ position stabilizes the *S*-conformer. An even more pronounced stabilization of the *N*-conformer in the 3′ substituted analogs is observed. The equilibrium changes in these classes of compounds can neither be correlated quantitatively with electronegativity differences nor with sterical differences between the various substituents. Substitution at the 5′ position influences the $N \leftrightarrow S$ equilibrium only slightly, but has significant effects upon the conformational preferences of the exocyclic 5′-CH_2R_3 group.

Introduction

The natural purine(β)nucleosides and many of their synthetic analogs exhibit a wide variety of physiological activities [1]. Several analogs of this class of compounds are undergoing clinical testing for their applicability as antiviral, antibiotic, or antitumor agents [2]. The continued interest of preparative chemists in the synthesis of new analogs [1, 3] results from the therapeutic potential that this class of compounds has demonstrated.

The biological activity of nucleoside analogs can arise from their different behaviour in the complex purine(β)riboside metabolism resulting from substituent structural effects. In addition, the activity might be influenced by geometrical constraints introduced by the new substituents. Two major causes for the conformational effects observed have been discussed in the literature. It has been postulated that either bulkier substituents at the sugar moiety or the base force the nucleoside into one dominating conformation [4] or that differences in the electronegativity of the hydroxyl group and the newly introduced substituent stabilize a specific conformer [5, 6].

In this paper the effect of chemical modifications at the 2′, 3′ or 5′ positions upon the conformational preferences of the ribose moiety of purine(β)ribosides is studied. It is obvious that a crucial physiological effect of any of the 3′ or 5′ modified analogs may result from the inability of this derivative to form proper phosphoester bonds.

The emphasis in the present work was to study systematically the influence of a chemical modification at one of the 2′, 3′ or 5′ carbon atoms upon the conformational equilibria of the ribose moiety. This would allow critical evaluation of the two published explanations given above for the shifts in the conformational equilibria.

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Experimental

Sample preparation

2′-thiobenzoyl-2′-deoxyadenosine (2′-BSA), 3′-thio-3′-deoxyadenosine (3′-HSA) [7]; 2′-chloro-2′-de-

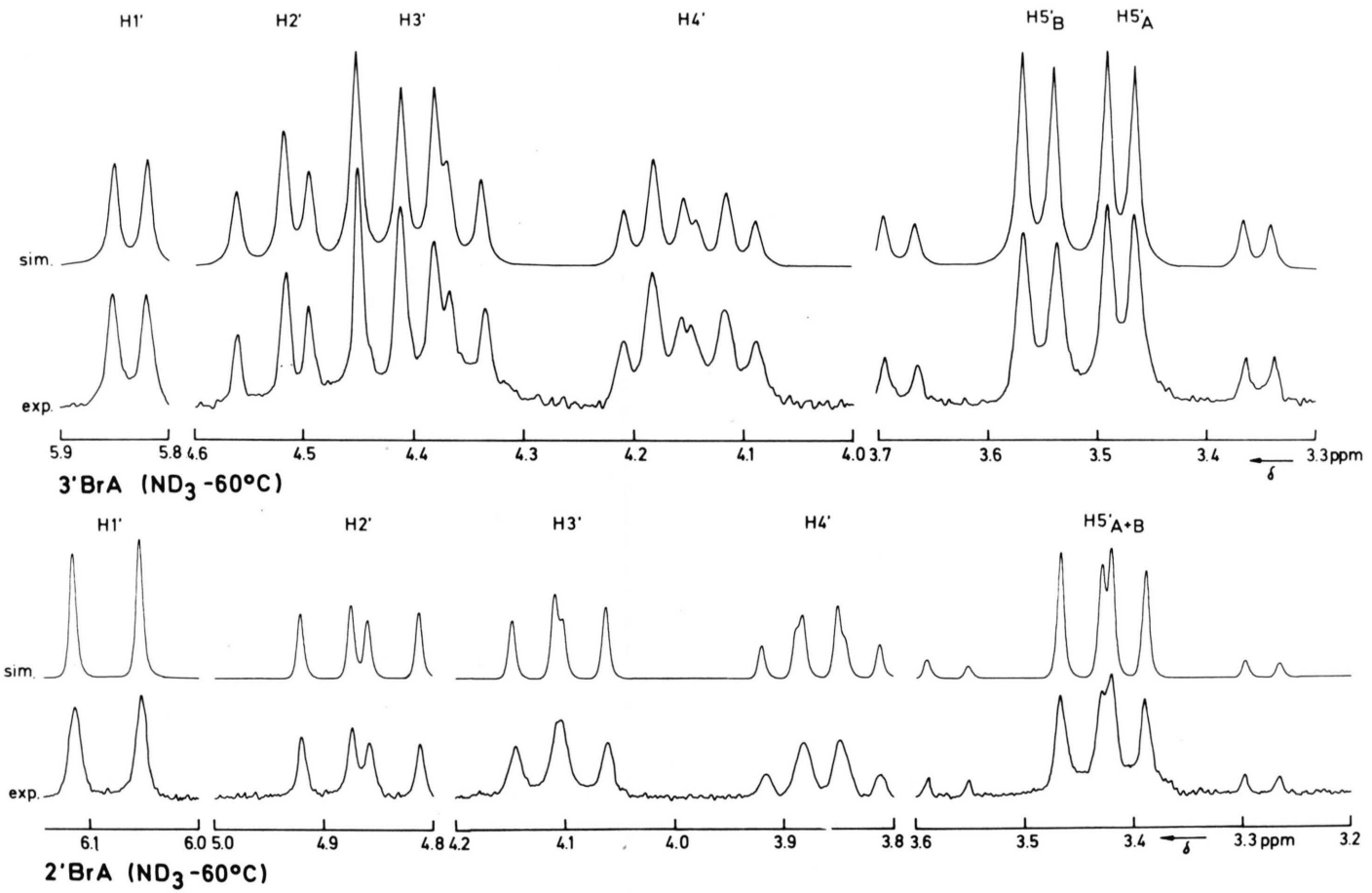

Fig. 1. Experimental proton high resolution spectra of a solution of 2′-BrA and 3′-BrA in ND_3 at − 60 °C covering the region of the ribose protons compared with the simulated spectra.

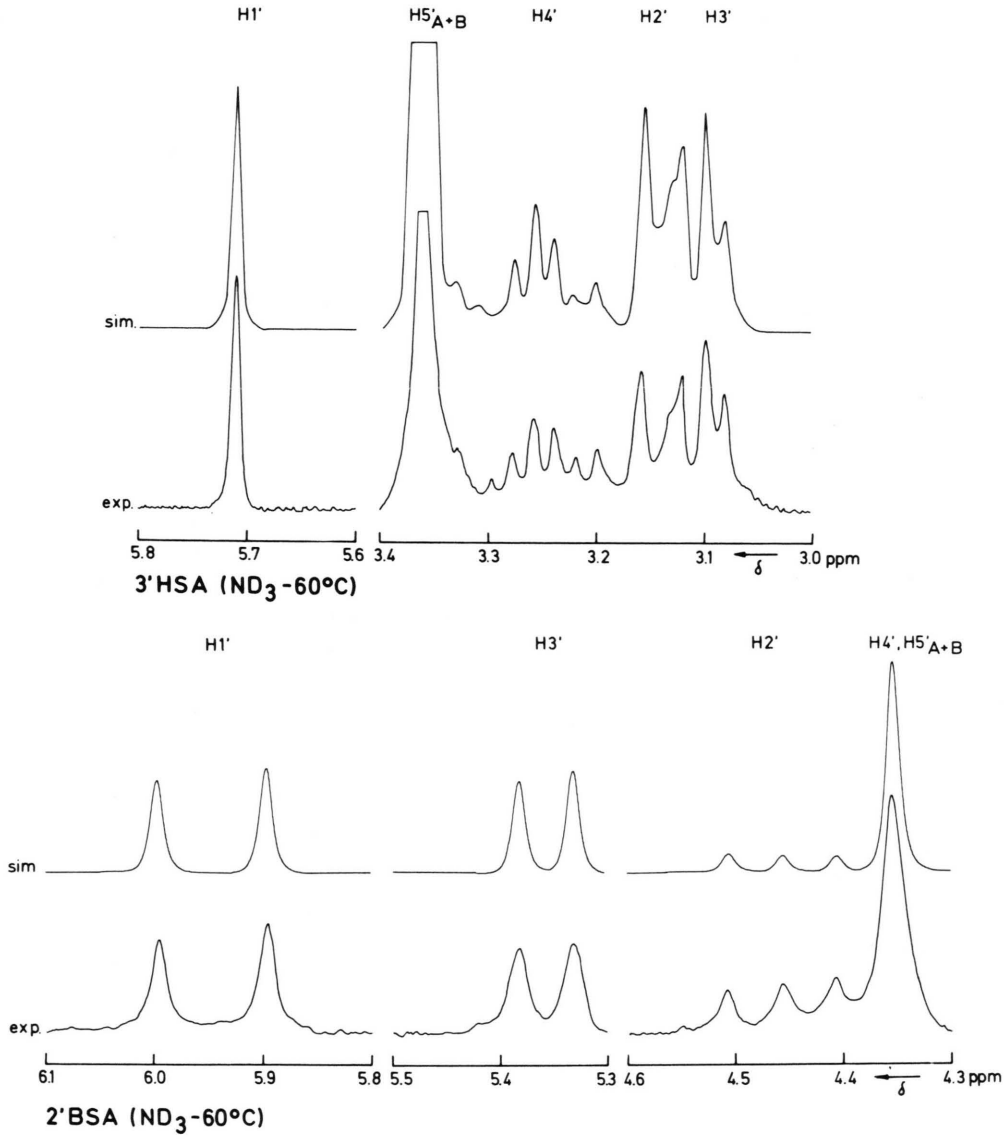

Fig. 2. Experimental proton high resolution spectra of a solution of 2′-BSA and 3′-HSA in ND_3 at − 60 °C covering the region of the ribose protons compared with the simulated spectra.

oxyadenosine (2′-ClA), 3′-chloro-3′-deoxyadenosine (3′-ClA) [8]; 2′-bromo-2′-deoxyadenosine (2′-BrA), 3′-bromo-3′-deoxyadenosine (3′-BrA) [9]; 2′-O-methyladenosine (2′-OCH_3A), 3′-O-methyladenosine (3′-OCH_3A) [10]; 2′-deoxy-3′-O-methyladenosine (2′-d-3′-OCH_3A) [11]; 5′-amino-5′-deoxyadenosine (5′-NH_2A), 5′-acido-5′-deoxyadenosine (5′-N_3A) [12]; and 5′-chloro-5′-deoxyadenosine (5′-ClA) [13] were prepared as described in previous publications.

Exchangeable hydrogens were removed by three lyophylisations from D_2O. Alll substances were finally dried at the high vacuum line at 5×10^{-5} torr for at least 24 hours.

5 mg of each nucleoside were dissolved in 0.5 ml trideuteroammonia in 5 mm tubes. The trideuteroammonia was dried over potassium deuterooxide prior to use. Details of the sample preparation have been described [14].

Spectra

The spectra were obtained in the FT mode at 100.1 MHz on a Varian XL-100-15 FT spectrometer equipped with a 16 k 620/l 100 computer and disk accessory. The digital resolution of the spectra was 0.1 Hz. The temperatures given are accurate to ± 0.5 K.

Chemical shifts and coupling constants were determined from the experimental spectra by application of the computer programme LAME (QCPE no. 111). The simulations were considered successful if the deviations between experimental and simulated spectra were ≦ 0.1 Hz. The chemical shifts given in Table I are referenced to an external standard of 1% TMS dissolved in CS_2. No attempts were made to correct for bulk magnetic susceptibility effects.

Results

Figs. 1 and 2 show the spectra of 2′-BrA and 3′-BrA and of 2′-BSA and 3′-HSA at a temperature of −60 °C. The simulated spectra were obtained with the values of the chemical shifts and coupling constants from Tables I and II. The chemical shifts for the sugar protons of the various derivatives are listed in Table I for + 40 °C and − 60 °C. Table II contains the vicinal proton-proton coupling constants for the temperatures + 40 °C and − 60 °C. They are compared to the unmodified adenosine. Where the solubility and stability of the substances permitted, the data in neutral aqueous solution are included. Only minor differences can be seen comparing the data obtained in the two solvents.

The data show that replacing the 2′-hydroxyl group in the ribose ring by any other substituent increases $J_{1'2'}$ and decreases $J_{3'4'}$. The same substitutions at the 3′ position lead to the opposite behaviour: $J_{1'2'}$ decreases, while $J_{3'4'}$ increases. In both 2′ and 3′ substituted derivatives $J_{2'3'}$ does not show any significant changes. This finding is in agreement with results in 2′ and 3′ ribonucleotides obtained by Davies and Danyluk [18]. The influence of the methylation of the 2′ or 3′ hydroxyl group upon the vicinal coupling constants is very small ($\lesssim$ 0.2 Hz). Comparably small effects have been observed for the corresponding pyrimidine nucleosides [19, 20].

2′-d-3′-OCH_3A represents an exception in this series. Compared to 2′-dA $J_{1'2'}$ is changed by ~ 2 Hz. Moreover, the temperature dependence is considerably more pronounced than in the other O′-methylated derivatives and in A.

In all 2′ and 3′ modified derivatives, the coupling constants between the 4′ and 5′ protons are similar.

The effect of the replacement of the 5′ hydroxyl group by another substituent upon the ribose ring couplings is small. $J_{4'5'A}$ and $J_{4'5'B}$, however, do show relatively large effects.

Table 1. Chemical shifts of the ribose protons in ppm of the various nucleosides dissolved in ND_3 at +40 °C and −60 °C.

Compound	2′BSA		2′BrA		2′ClA		2′OCH_3A		2′d3′OCH_3A	
T[°C]	+40	−60	+40	−60	+40	−60	+40	−60	+40	−60
H1′	dec.	5.945	5.997	6.080	5.895	5.989	5.754	5.874	6.048	6.159
H2′	dec.	4.438	4.801	4.863	4.738	4.816	3.956	4.006	2.472	2.639
H2″									2.213	2.363
H3′	dec.	5.356	4.044	4.101	4.140	4.218	4.071	4.173	3.883	3.966
H4′	dec.	4.356	3.803	3.862	3.800	3.869	3.716	3.824	3.783	3.889
H5′A	dec.	4.356	3.427	3.483	3.436	3.501	3.434	3.520	3.342	3.404
H5′B	dec.	4.356	3.325	3.370	3.334	3.383	3.318	3.397	3.298	3.356

Table I. (continued)

Compound	3′HSA		3′BrA		3′ClA		3′OCH_3A		5′NH_2A		5′N_3A		5′ClA	
T[°C]	+40	−60	+40	−60	+40	−60	+40	−60	+40	−60	+40	−60	+40	−60
H1′	5.454	5.711	5.753	5.838	5.478	5.585	5.664	5.776	5.626	5.682	5.670	5.788	5.685	5.762
H2′	3.000	3.113	4.350	4.377	4.232	4.324	4.406	4.494	4.401	4.452	4.364	4.588	4.373	4.474
H3′	2.936	3.133	4.413	4.499	4.101	4.235	3.597	3.702	3.948	3.899	3.922	4.026	3.953	4.012
H4′	3.116	3.298	4.081	4.149	3.968	3.811	3.782	3.887	3.735	3.729	3.827	3.977	3.907	4.003
H5′A	3.130	3.364	3.512	3.598	3.232	3.325	3.428	3.521	2.594	2.609	3.365	3.536	3.694	3.840
H5′B	3.130	3.364	3.378	3.439	3.093	3.173	3.302	3.373	2.519	2.546	3.355	3.472	3.589	3.758

Table II. Vicinal proton-proton coupling constants of the ribose protons in Hz of the various nucleosides dissolved in ND_3 or D_2O at + 40 °C and − 60 °C. a) this work, b) ref. 15, c) ref. 16, 2′NH_2A = 2′-amino-2′-deoxyadensone, 3′NH_2A = 3′-amino-3′-deoxyadenosine, d) ref. 17.

Compound	A[b]			2′dA			2′BSA[a]		2′NH_2A[c]		2′BrA[a]		2′ClA[a]	
Solvent	ND_3		D_2O	ND_3[b]		D_2O[d]	ND_3		ND_3		ND_3		ND_3	
T[°C]	+40	−60	+10	+40	−60	+60	+40	−60	+40	−60	+40	−60	+40	−60
J(1′2′)	5.0	5.1	5.9	6.7	6.9	7.3	dec.	10.05	7.5	8.25	6.7	6.2	6.3	5.8
J(1′2″)	–	–	–	6.3	6.2	6.3	–	–	–	–	–	–	–	–
J(2′3′)	5.0	4.8	5.0	6.0	6.0	6.4	dec.	5.0	5.3	5.0	4.9	4.7	4.9	4.8
J(2″3′)	–	–	–	3.9	3.6	3.5	–	–	–	–	–	–	–	–
J(3′4′)	4.4	3.9	3.4	3.4	3.4	2.9	dec.	0.0	2.1	1.5	3.4	3.9	3.7	4.1
J(4′5′A)	3.4	3.8	3.0	3.2	3.9	4.7	dec.	n.r.	3.6	4.3	3.4	3.8	3.4	3.6
J(4′5′B)	3.5	3.0	3.4	4.8	4.1	3.5	dec.	n.r.	4.1	4.1	3.5	3.2	3.5	3.1

Table II. (continued)

Compound	2′OCH_3A[a]		2′d3′OCH_3A[a]		5′dA[b]			5′NH_2A[a]		5′N_3A[a]		5′ClA[a]	
Solvent	ND_3		ND_3		ND_3		D_2O	ND_3		ND_3		ND_3	
T[°C]	+40	−60	+40	−60	+40	−60	+40	+40	−60	+40	−60	+40	−60
J(1′2′)	4.75	5.0	8.2	8.8	4.1	4.8	4.8	5.3	6.3	4.6	5.1	4.8	5.4
J(1′2″)	–	–	6.4	6.0	–	–	–	–	–	–	–	–	–
J(2′3′)	4.9	4.7	6.3	5.5	5.0	5.0	5.5	5.2	4.9	4.8	4.7	4.7	4.5
J(2″3′)	–	–	2.8	2.0	–	–	–	–	–	–	–	–	–
J(3′4′)	4.4	4.2	2.4	1.6	5.7	5.2	5.2	4.8	3.5	5.3	4.9	4.8	4.3
J(4′5′A)	3.2	3.4	4.5	4.8	6.2	6.2	6.2	3.8	3.6	4.4	5.4	4.5	4.3
J(4′5′B)	3.4	3.0	4.5	4.8	6.2	6.2	6.2	6.0	6.1	4.4	3.9	5.5	5.2

Table II. (continued)

Compound	3′dA[b]			3′HSA[a]		3′NH_2A[c]			3′BrA[a]		3′ClA[a]		3′OCH_3A[a]	
Solvent	ND_3		D_2O	ND_3		ND_3		D_2O	ND_3		ND_3		ND_3	
T[°C]	+40	−60	+60	+40	−60	+40	−60	+10	+40	−60	+40	−60	+40	−60
J(1′2′)	1.7	0.5	2.4	1.4	0.0	1.8	0.6	2.1	3.4	3.2	3.9	3.7	5.4	5.3
J(2′3′)	5.6	4.9	6.0	5.0	5.0	5.3	5.1	5.3	4.8	4.4	5.5	5.3	5.0	4.7
J(2′3″)	2.4	1.2	2.5	–	–	–	–	–	–	–	–	–	–	–
J(3′4′)	9.6	10.8	8.8	8.9	10.0	8.0	8.9	7.8	6.4	6.9	5.6	5.3	4.3	4.5
J(3″4′)	5.8	5.3	6.7	–	–	–	–	–	–	–	–	–	–	–
J(4′5′A)	3.45	2.6	4.7	3.0	2.0	2.5	2.7	2.2	3.0	3.0	3.1	3.4	3.5	3.9
J(4′5′B)	3.4	2.7	3.0	3.0	2.0	3.5	2.0	3.4	3.0	2.5	3.15	2.8	3.5	2.9

Conformational Analysis

Excluding conformationally flexible hydroxyl and amino groups, the mobility of the nucleosides can be described by three modes of internal motion (Fig. 3):

i) Rotation of the base around the glycosidic bond: *syn* ↔ *anti* equilibrium;
ii) ribose puckering;
iii) conformation of the exocyclic 5′-CH_2R_3 group.

The ribose puckering and the conformational equilibrium of the exocyclic 5′ group can be determined quantitatively from the analysis of the vicinal proton-proton coupling constants.

The position of the *syn* ↔ *anti* equilibrium is derivable from the analysis of the intramolecular proton-proton relaxation rates [16] and from the study of the long range carbon proton couplings [21].

In the following only the conformations of the ribose moiety will be discussed.

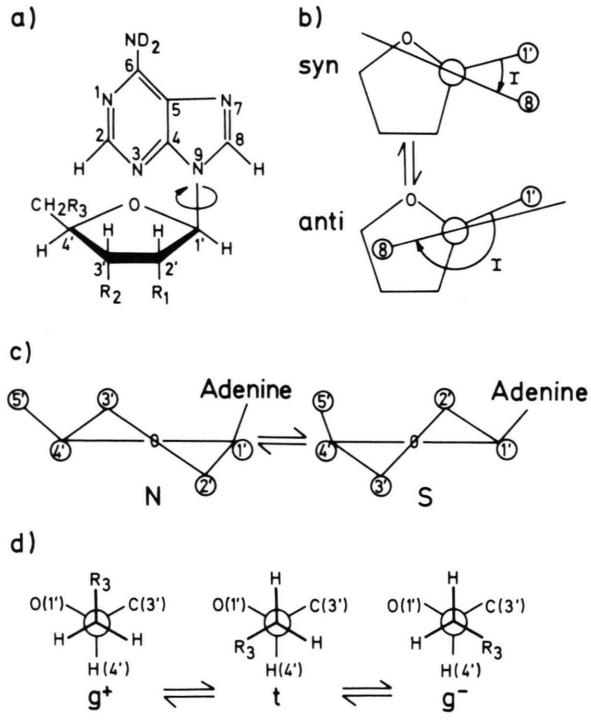

Fig. 3. Structural formula and possible internal motions of the nucleoside analogs studied: a) Chemical structures: A, $R_1=R_2=R_3=OD$; 2′ derivatives of A: $R_1=H$ (2′-dA), BS (B=PhCO), NH_2, Br, Cl, OCH_3, $R_2=R_3=OD$; 3′ derivatives of A: $R_1=R_3=OD$, $R_2=H$ (3′-dA), HS, NH_2, Br, Cl, OCH_3; 5′ derivatives of A: $R_1=R_2=OD$, $R_3=H$ (5′-dA), NH_2, N_3, Cl; b) rotation around the glycoside bond; c) pseudorotation of the ribose ring; d) Newman projections along the C(4′)-C(5′) bond showing the three clasical rotamers.

The furanose ring pucker is described in the two state $N \leftrightarrow S$ model of Altona and Sundaralingam [22], based on the concept of pseudorotation. In solution, a rapid equilibrium exists between the two states N and S. Thus the experimental vicinal proton-proton coupling constants are averaged values over the coupling constants in each state:

$$J_{ij}^{obs} = J_{ij}^{N}[N] + J_{ij}^{S}[S] \qquad (1)$$

where $[N]$ and $[S]$ are the mole fractions for each state with $[N]+[S]=1$. Each coupling constant $J_{ij}^{N,S}$ is given by a Karplus relation [23]:

$$J_{ij}^{N,S} = A\cos^2\Phi_{ij}^{N,S} + B\cos\Phi_{ij}^{N,S}. \qquad (2)$$

$\Phi_{ij}^{N,S}$ being the dihedral angle between the coupling protons i and j in the N or S state, respectively. The values of A and B depend on the molecule investigated and are regarded as adjustable parameters. The best values for the purine(β)ribosides with unmodified sugar moiety derived from the analysis of the ND_3 solutions are:

$A = 10.0$ Hz, $B = -0.95$ Hz [24]. In modified ribose rings changes of the Karplus parameters with substituent electronegativities have to be taken into account. Necessary conditions for the applicability of the present $N \leftrightarrow S$ calculation are that $J_{2'3'}$ and the sum $(J_{1'2'}+J_{3'4'})$ should be independent of the position of the conformational equilibrium [22]. The coupling constants $J_{2'3'}$ and $(J_{1'2'}+J_{3'4'})$ for the 2′ and 3′ substituted compounds are plotted in Fig. 4 *versus* the electronegativity of the substituents. It can be seen that $J_{2'3'}$ and $(J_{1'2'}+J_{3'4'})$ vary only weakly with electronegativity. Variations of this magnitude are in accordance with the effects observed for electronegativity changes in simple ethylene and vinyl derivatives [25, 26] and are also found theoretically for the corresponding ethane fragment [23].

Deriving the Karplus parameters from the coupling constants given in Fig. 4 results in changes of the parameter $A \lesssim 0.4$ Hz and of $B \lesssim 0.1$ Hz for the range of electronegativities considered. A change of this magnitude, however, is within the uncertainty of the Altona Sundaralingam two state model. In the analysis given in the following, therefore, the parameters of the unmodified ribosides given above were applied to all of the compounds studied.

The conformation of the exocyclic 5′-CH_2R_3 group is described as an equilibrium between the three classical staggered rotamers g^+, t, and g^- [27]. From the Karplus equations for the three rotamers the mole fractions can be derived:

$$[g^+] = 1.46 - \frac{J_{4'5'A} + J_{4'5'B}}{8.9}, \qquad (3)$$

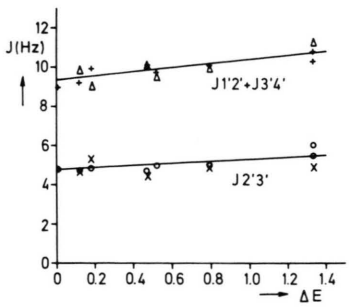

Fig. 4. Plot of the observed vicinal coupling constants vs. the electronegativity $\Delta E = E_{OH} - E_R$ of the substituents R at −60 °C. (o: $J_{2'3'}$ of the 2′ derivatives; +: $J_{1'2'}+J_{3'4'}$ of the 2′ derivatives; ×: $J_{2'3'}$ of the 3′ derivatives; Δ: $J_{1'2'}+J_{3'4'}$ of the 3′ derivatives).

$$[t]\,([g^-]) = \frac{J_{4'5'A(B)}}{8.9} - 0.23. \quad (4)$$

Changes of the coupling constants due to different electronegativities give an error in the evaluation of the mole fractions of $\leq 5\%$.

The determination of the mole fractions $[t]$ and $[g^-]$ requires an unequivocal assignment of the two 5′ protons. Though this assignment has been achieved for adenosine by stereospecific partial deuteration [28], it is uncertain whether this result can be used for a general assignment in all modified nucleosides.

Discussion

2′ and 3′ modified derivatives

The results of the conformational analysis are contained in Table III.

All compounds modified at the 2′ position show a stabilization of the *S* state, the effect being most

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

Solvent	ND_3				D_2O			
	T[°C]	[N]	[g⁺]	[t]	*T*[°C]	[N]	[g⁺]	[t]
A	+40	0.45	0.68	0.15	+10	0.35	0.74	0.15
	−60	0.43	0.70	0.20				
2′dA	+40	0.36	0.56	0.13	+60	0.30	0.54	0.30
	−60	0.36	0.56	0.21				
2′BSA	+40	dec.	dec.	dec.				
	−60	0.0	n.r.	n.r.				
2′NH_2A	+40	0.20	0.60	0.17				
	−60	0.13	0.52	0.25				
2′BrA	+40	0.30	0.68	0.15				
	−60	0.37	0.67	0.20				
2′ClA	+40	0.34	0.68	0.15				
	−60	0.39	0.71	0.17				
2′OCH_3A	+40	0.52	0.72	0.13				
	−60	0.44	0.74	0.15				
2′d3′OCH_3A	+40	0.16	0.45	0.28				
	−60	0.08	0.38	0.31				
3′dA	+40	0.82	0.68	0.16	+60	0.75	0.59	0.30
	−60	0.95	0.86	0.06				
3′HSA	+40	0.89	0.79	0.11				
	−60	1.0	1.0	0.0				
3′NH_2A	+40	0.82	0.79	0.05				
	−60	0.94	0.92	0.07	+10	0.80	0.83	0.15
3′BrA	+40	0.65	0.79	0.11				
	−60	0.68	0.84	0.11				
3′ClA	+40	0.56	0.76	0.12				
	−60	0.61	0.76	0.15				
3′OCH_3A	+40	0.43	0.67	0.16				
	−60	0.43	0.70	0.21				
5′dA	+40	0.58			+40	0.49		
	−60	0.51						
5′NH_2A	+40	0.45	0.36	0.44				
	−60	0.33	0.37	0.46				
5′N_3A	+40	0.53	0.47	0.26				
	−60	0.47	0.42	0.38				
5′ClA	+40	0.50	0.34	0.28				
	−60	0.44	0.39	0.25				

pronounced in the thiobenzoyl derivative, which, within the accuracy of the two state model is found in the *S* state exclusively.

All substitutions at the 3′ position lead to a preference of the *N* conformer. Compared to adenosine and the 2′ modified analogs these effects are significantly larger. The introduction of the thio group at the 3′ position again leads to the most drastic effect; in 3′-HSA at − 60 °C the mole fraction of the nucleoside in the *N* state equals one.

2′ or 3′ O-methylation does not produce any significant conformational changes when compared to adenosine. This result is in accordance with conformational energy calculations on 2′-O-methyladenosine by Prusiner *et al.* [29]. Conformational changes induced by 2′ O-methylation are observed only in nucleotides [30].

In all 2′ and 3′ substituted compounds the g^+ rotamer with the hydroxyl above the sugar ring dominates the conformational equilibrium of the exocyclic 5′-CH_2OH group. Table III shows that the 3′ modified derivatives have a stronger preference for g^+ than the 2′ analogs. This effect is most pronounced in 3′-thio-3′-deoxyadenosine, where $J_{4'5'A} = J_{4'5'B} = 2.0$ Hz and consequently $[g^+] = 1.0$. The equality of $J_{4'5'A}$ and $J_{4'5'B}$ can only be achieved when the g^+ state corresponds to the classical staggered rotamer with symmetrical angles of 60°. It has been argued that nonclassical rotamers with dihedral angles significantly deviating from 60° also have to be taken into account [31, 32]. The results obtained with 3′-HSA, however, are compatible with the g^+ rotamer in a symmetrically staggered arrangement. In general the results suggest a correlation between g^+ and *N*. In Fig. 5 the mole fractions of g^+ and *N* are plotted. The figure shows an increase of $[g^+]$ with $[N]$. This finding has to be compared with the position of the *syn* ↔ *anti* equilibrium. Measurements of the longitudinal proton relaxation rates of 2′- and 3′-amino-2′- and 3′-deoxyadenosine carried out previously [16] lead to the conclusion that the 3′ modified nucleoside is essentially restricted to the anti range and that the 2′ substituted analog shows at least a strong preference for the *syn* range.

Finally, as g^+ is always the dominating rotamer, this would lead to a stringent correlation *anti-N-*g^+ for the 3′ modified derivatives and a less strict preference *syn-S-*g^+ for the 2′ analogs.

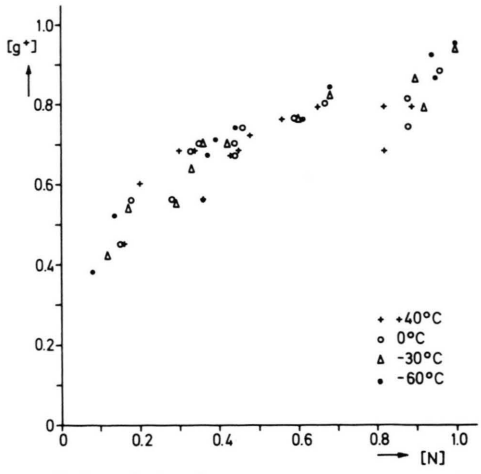

Fig. 5. Correlation between the rotamer population $[g^+]$ of the exocyclic 5′-CH_2OD group and the mole fraction $[N]$ of the furanoside ring.

It has been suggested that the *syn-S-*g^+ correlation could be stabilized by hydrogen bonding between *N* (3) and *O* (5′) [15]. The correlation *anti-N-*g^+ has been observed in many solid state structures [33].

No simple explanation can be found for the fact that 3′ modified compounds prefer the *N* state and 2′ compounds the *S* state. In Fig. 6a the changes of *N* compared to adenosine are plotted *versus* the diferences between the electronegativities of the substituents and the hydroxyl group. The results given refer to − 60 °C and ND_3 solutions. The temperature of − 60 °C was chosen, since the conformational preferences are most clearly seen at the lowest temperatures. Obviously, there is no simple correlation between these two quantities. The same correlative failure holds for a postulated relationship between the size of a substituent, described by the van der Waals' radius, and the change of the population of the *N* or *S* state (Fig. 6b). This observation for the purine(β)ribosides is in contrast to the results obtained by Cushley *et al.* [34] for pyrimidine(β)-ribosides and -arabinosides, modified at the sugar moiety. In this class of compounds a correlation between $J_{1'2'}$ and the electronegativity of the sugar substituent could be established.

The temperature dependence of the position of the $N \leftrightarrow S$ equilibrium was derived from the data. In Fig. 7 the Van't Hoff plots of the mole fractions of the 2′ and 3′ modified derivatives are shown. Within the accuracy of this analysis, a linear dependence of $\ln([S]/[N])$ *versus* $1/T$ is found. At first

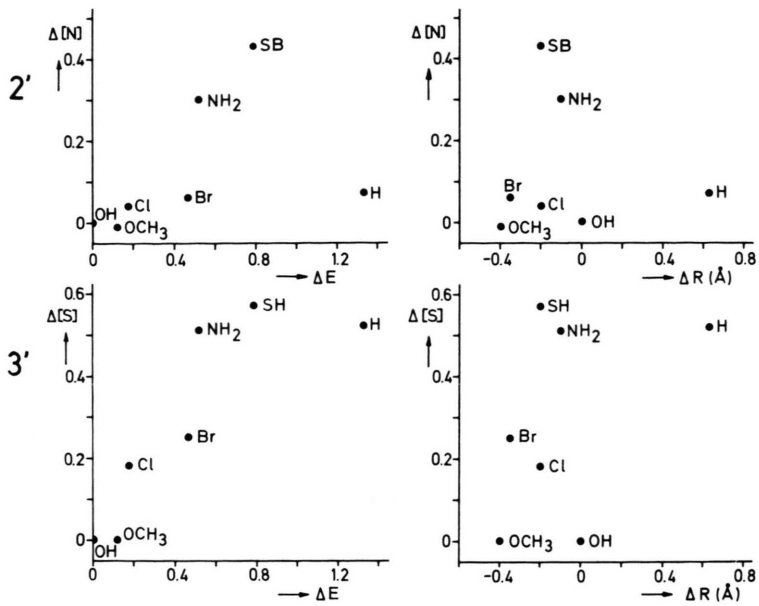

Fig. 6. a) Plot of the mole fractions $\Delta[N] = [N_{OH}] - [N_R]$ and $\Delta[S] = [S_{OH}] - [S_R]$ *vs.* the difference of the electronegativities $\Delta E = E_{OH} - E_R$ of the substituents for the 2′ and 3′ derivatives at − 60 °C. b) Plot of the mole fractions *vs.* the difference of the van der Waals' radii $\Delta R = R_{OH} - R_R$.

sight this could be taken as evidence for a simple two state equilibrium. The results, however, do not all converge for high temperatures to $\ln([S]/[N]) = 0$. In some compounds (2′-dA, 2′-BrA, 2′-ClA) with $\ln([S]/[N]) \lesssim 1$ this quantity even increases with rising temperature. This behaviour has been found previously for nucleosides with an unmodified ribose moiety [24] and leads to the reasonable conclusion that the sugar conformation cannot be fully described by a simple two state model. The effects of intramolecular hydrogen bonding, the rotameric range of the base in the *syn* ↔ *anti* equilibrium, and the interaction of the nucleosides with solvent molecules obviously have nonnegligible influence. As well, other modes of pseudorotational puckering of the furanose ring are possible.

5′ modified derivatives

The results obtained for the 5′ analogs are given in Table III. The conformation of the sugar ring is similar to that of adenosine, except for 5′-dA in which the *N* state is more highly populated.

However, the conformation of the exocyclic 5′-CH_2R_3 group changes drastically. Upon replacement of the 5′ hydroxyl group, the population of the g^+ rotamer is reduced and no preference for any specific rotamer can be seen. Hydrogen bonding between *N* (3) and *O* (5′) which could contribute to stabilizing g^+ in adenosine is not possible in the 5′

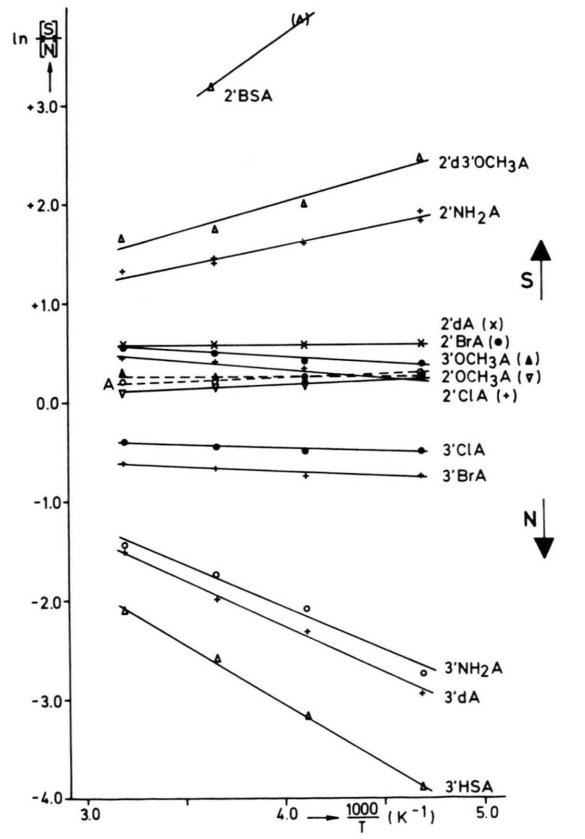

Fig. 7. Temperature dependence of the mole fractions in the *N* and *S* state for the 2′ and 3′ derivatives.

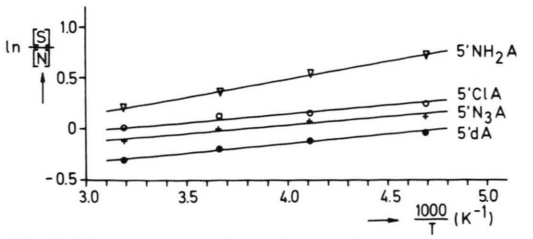

Fig. 8. Temperature dependence of the mole fractions in the *N* and *S* state for the 5′ derivatives.

substituted compounds. The dominance of the *S* state ($[S] = 0.67$) at $-60°$ measured with 5′-amino-5′-deoxyadenosine would suggest a preference for the *syn* range by the base. However, stabilization of the g^+ rotamer *via* putative *N* (5′) to *N* (3) hydrogen bonding does not occur since 5′-NH_2A behaves like the other 5′ analogs. Steric, polar, and solvation effects may result in the observed destabilization of g^+ in these compounds.

The temperature dependence of the 5′ modified analogs on the $N \leftrightarrow S$ equilibrium is shown in Fig. 8. It is similar to the 2′ and 3′ derivatives so the same arguments apply.

In summary it is seen that substitution at 2′ results in a preference for the *S* conformation range, while substitution at 3′ stabilizes *N*. In all 2′ and 3′ modified derivatives the g^+ rotamer of the exocyclic 5′-CH_2OH group predominates. Subsitution at 5′ has only a small effect on the sugar ring conformation, but reduces the g^+ population significantly. The effects observed in these analogs show a systematic influence of substitution upon the conformational equilibria. The two explanations suggested in the literature, namely differences in electronegativity and differences in the size of the substituents cannot explain the experimental results quantitatively.

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