Xylose Conformations of 9-β-D-Xylofuranosyladenine Analogues Modified at the 2′,3′ or 5′-Positions and Lyxose Conformations of 9-β-D-Lyxofuranosyladenine

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Proton magnetic resonance studies of 9-β-D-xylofuranosyladenine (xyloA), its 2′-amino-2′-deoxy, 2′-azido-2′-deoxy, 2′-bromo-2′-deoxy, 3′-thio-3′-deoxy, 3′-amino-3′-deoxy, 3′-azido-3′-deoxy, 3′-chloro-3′-deoxy, 3′-fluoro-3′-deoxy, 3′-O-methyl, 3′,5′-diazido-3′,5′-dideoxy analogues and 9-β-D-lyxofuranosyladenine (lyxoA) have been carried out to study the effect of chemical modifications at the sugar moiety on the solution conformational equilibria in these classes of nucleosides. Analogously to previous studies the xylose pucker can be described in the two-state *N* ⇔ *S* model of Altona and Sundaralingam. For the xylosides, however, a somewhat different *N* state (C3′-endo-C4′-exo) has to be used than for the ribosides and arabinosides (C2′-exo-C3′-endo).

The results of the conformational analysis are that xyloA exists almost exclusively as an *N* conformer. The effect of the substitutions in the modified compounds is to destabilize the *N* state. This decrease in the mole fraction of *N* is accompanied by an increase in the population of the g^+ rotamer of the exocylic 5′-CH_2OD group. Thus for the xylosides a correlation *N-t*/g^- or *S*-g^+, respectively, can be derived. Proton relaxation rate measurements on 2′-azido-2′-deoxyxylofuranosyladenine indicate that in the xylosides the standard syn or anti ranges do not represent stable positions for the adenine base, but that a glycosyl torsion angle ($\chi \sim 160°$, $\Upsilon \sim 80°$) between syn and anti is preferred. LyxoA behaves similar to the xylosides and also favours the *N* state of the sugar pucker.

In a summarizing discussion the conformational equilibria of the different modified pentosides – ribose, arabinose, xylose and lyxose – are compared. It is shown that generally intramolecular hydrogen bonding does not yield an important contribution to the stabilization of conformational equilibria in solution. It is also not possible to derive a quantitative relationship between such parameters as Van-der-Waals' radii or electronegativity of the substituents and the mole fractions of the different conformers. It can, however, be seen that in all cases, where the hydroxyl groups at C2′ or C3′ are substituted by a more voluminous atom or group, steric effects become dominant and allow a qualitative explanation of the changes of the conformational equilibria. Only for the smallest and most electronegative substituents, like fluorine, other interactions (*e.g.* electrostatic) may become important. It is thus suggested that the purine(β)nucleoside conformation is essentially determined by steric interactions between the different parts of the molecule.

Introduction

Chemical modifications of the pentose moiety of nucleosides lead to a variety of physiologically active analogues of the naturally occurring β-D-nucleosides.

In previous papers we have discussed the influence of chemical modifications of the sugar upon the solution conformation of adenosine analogues [1, 2] and arabinosyladenine analogues [3].

Correlations between the three major conformational equilibria describing the nucleoside framework were observed. In the case of the ribosides a strong preference for the *N-anti-*g^+ conformation was found. The S-range of ribose conformations is associated with the syn conformation of the base, but does not reveal a preference for any of the three rotamers of the exocyclic 5′CH_2OD group. For the

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arabinosyladenine analogues a strong conformational preference for *anti-N-g*$^+$ was derived. Where comparable data exist, it is found that the *N-g*$^+$ conformation is generally more stable in the arabinose analogues than in the ribose derivatives.

Lack of a compound with suitable chemical shifts precluded the proton relaxation analysis of an arabinonucleoside with an *S*-range sugar conformation.

In this paper xylofuranosyladenine (xyloA) analogues and lyxofuranosyladenine are discussed. Again, the investigation includes only adenosine analogues in order to keep the base properties constant. This permits a definite assignment of the conformational changes observed to the chemical modifications of the pentose moiety. Previous studies of xylonucleosides have included X-ray investigations on some cyclic xyloA analogues [4, 5], extensive HRNMR studies on methylated xyloA derivatives [6] and theoretical studies [7] concerning the applicability of the Karplus equation to the analysis of the vicinal coupling constants.

The results presented here include several new xyloA analogues and apply proton relaxation rate studies to a xylo-nucleoside, 2′-azido-2′-deoxyxylofuranosyladenine, for the first time.

Experimental

Sample preparation and spectroscopic techniques have been described in the previous paper [3]. The compounds included in this study, 9-*β*-D-xylofuranosyladenine (xyloA) [8], and its 2′-amino-2′-deoxy (2′ND_2xyloA) [9], 2′-azido-2′-deoxy (2′N_3xyloA) [10], 2′-bromo-2′-deoxy (2′BrxyloA) [11], 3′-thio-3′-deoxy (3′DSxyloA) [12], 3′-amino-3′-deoxy (3′ND_2xyloA) [8], 3′azido-3′-deoxy (3′N_3xyloA) [8], 3′-chloro-3′-deoxy (3′ClxyloA) [11, 13], 3′-fluoro-3′-deoxy (3′FxyloA) [8], 3′-O-methyl (3′OCH_3xyloA) [8], 3′,5′-diazido-3′,5′-dideoxy (3′,5′diN_3xyloA) [14] analogues plus 9-*β*-D-lyxofuranosyladenine (lyxloA) [15], were synthesized as published. The general structure formula for the xylo analogues is given in the insert of Fig. 1. The procedures used in the simulation of the spectra are as described in the first two papers of this series [2, 3].

Results

An example of the quality of spectra obtainable at −60 °C in ND_3 is illustrated by the experimental xylose proton spectrum of 3′DSxyloA given in Fig. 1.

Vicinal coupling constants derived from the simulation are collected in Table I and may be compared with those of the unmodified xyloA in the first column. The data for 3′OCH_3xyloA dissolved in D_2O were taken from Ekiel *et al.* [6]. The chemical shifts obtained from the analysis of the proton spectra are available from the authors upon request.

The coupling constants $J_{1'2'}$, $J_{2'3'}$ and $J_{3'4'}$ of xyloA, 3′OCH_3xyloA and 3′FxyloA are very small. All other 2′ or 3′ substituted compounds studied show a significant increase of these three constants. Parallel with this increase goes a significant reduction of $J_{4'5'A}$ and $J_{4'5'B}$. Comparing 3′N_3xyloA and 3′5′diN_3xyloA, it is found that the introduction of

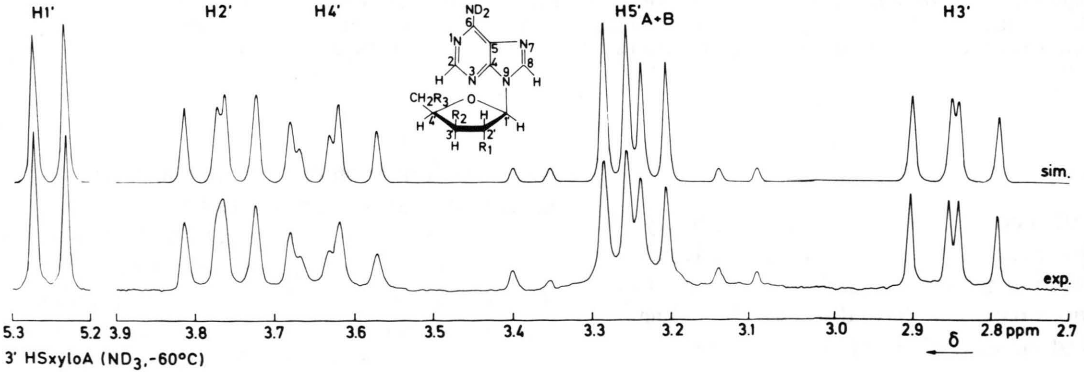

Fig. 1. Experimental proton high resolution spectrum of a solution of 3′DSxyloA in ND_3 at −60 °C covering the region of the xylose protons compared with the simulated spectrum. The insert shows the structural formula of the xylosyladenine analogues studied: XyloA: $R_1 = R_2 = R_3 = OD$; 2′-derivatives: $R_2 = R_3 = OD$; $R_1 = ND_2, N_3, Br$; 3′ derivatives: $R_1 = R_3 = OD$, $R_2 = ND_2, N_3, Cl, F, OCH_3, DS$; 3′,5′di$N_3$xyloA: $R_1 = OD$, $R_2 = R_3 = N_3$.

Table I. Vicinal proton-proton coupling constants of the xylose protons in Hz, of the various nucleosides dissolved in ND$_3$ at + 40 °C and − 60 °C.

Compound	xyloA		2'ND$_2$xyloA		2'N$_3$xyloA		2'BrxyloA [a]	3'DSxyloA		3'ND$_2$xyloA		3'N$_3$xyloA	
T[°C]	+ 40	− 60	+ 40	− 60	+ 40	− 60	− 60	+ 40	− 60	+ 40	− 60	+ 40	− 60
solvent	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$
$J_{1'2'}$	1.4	0.7	2.8	1.8	3.6	3.4	2.5	5.0	4.1	5.2	5.6	3.6	3.6
$J_{2'3'}$	1.6	1.0	2.5	1.6	3.7	3.5		6.4	5.1	6.1	6.7	3.8	3.6
$J_{3'4'}$	3.6	3.0	4.2	3.5	4.8	4.7		7.1	6.1	7.0	7.3	5.5	5.5
$J_{4'5'A}$	5.3	5.8	5.1	5.9	5.2	5.0		4.7	4.3	3.9	3.5	5.1	4.9
$J_{4'5'B}$	6.3	6.6	6.2	6.1	5.2	5.5		4.3	5.5	3.9	3.5	5.1	5.2

Compound	3'ClxyloA		3'FxyloA [b]		3'OCH$_3$xyloA			3'5'diN$_3$xyloA		lyxoA	
T[°C]	+ 40	− 60	+ 40	− 60	+ 40	− 60	+ 27	+ 40	− 60	+ 40	− 60
solvent	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$	ND$_3$	D$_2$O [c]	ND$_3$	ND$_3$	ND$_3$	ND$_3$
$J_{1'2'}$	2.7	2.3	1.6	1.0	1.7	1.25	3.2	4.4	5.5	6.9	7.4
$J_{2'3'}$	2.5	2.3	1.3	1.0	1.7	1.2	3.2	4.8	5.7	4.7	4.5
$J_{3'4'}$	4.45	4.4	2.8	2.4	3.9	3.6	5.0	6.0	7.3	3.7	3.1
$J_{4'5'A}$	5.0	5.0	6.4	6.8	5.9	6.0	4.4	7.2	7.5	4.8	5.2
$J_{4'5'B}$	5.6	5.6	6.4	6.9	6.3	6.5	5.6	4.4	3.5	6.7	7.0

[a] Partial decomposition.

[b] $|J_{\mathrm{HF}}|$ couplings in Hz derived from the simulation, first entry: + 40 °C, second entry: − 60 °C: $|J_{1'F}| = 0.6/0.4$; $|J_{2'F}| = 14.9/13.4$; $|J_{3'F}| = 51.8/50.9$; $|J_{4'F}| = 30.9/32.3$; $|J_{5'F}| = 0.4/0.0$.

[c] Data from ref. [6].

the 5'-substituent significantly increases the vicinal coupling constants between the ring protons and introduces a marked difference between $J_{4'5'A}$ and $J_{4'5'B}$.

In the context of the substances studied here, 3'-deoxyadenosine [16] can be treated as a xyloA analogue.

Conformational Analysis

Conformation of the furanoside moiety

The analysis of the experimental results is based on the formalism defined in the previous papers [2, 3]. In Fig. 2 the measured vicinal proton coupling constant $J_{1'2'}$ is plotted *versus* $J_{2'3'}$. As in the case of the corresponding couplings of the ribo- and arabinonucleosides a linear relation with slope ~ 1 exists between these two constants indicating that the sugar ring conformation of the xylonucleosides can also be described in the two state $N \Leftrightarrow S$ model of Altona and Sundaralingam based on pseudorotation. The Newman projections along the three C−C-bonds of the xylose ring (Fig. 3) show that the dihedral angle $|\Phi_{1'2'}|$ nearly equals $|\Phi_{2'3'}|$ in the N as well as the S state. Therefore the coupling constants $J_{1'2'}$ and $J_{2'3'}$, calculated with the Karplus equation (Eqn (2) of ref. [3]) should not differ by more than ± 1 Hz in agreement with experiment. For the usual ranges of the pseudorotation angle P found in ribosides and arabinosides, the dihedral angle $\Phi_{3'4'}$ should adopt values of 20°−40° leading to coupling constants $J_{3'4'}$ of 5−8 Hz. These couplings vary by only ~ 2 Hz from the N to the S states. In xyloA and 3'OCH$_3$xyloA, however, the $J_{3'4'}$ couplings are much smaller than these calculated values. These two compounds are expected to exist mainly in the N state owing to their low values of $J_{1'2'}$ and $J_{2'3'}$. It is thus apparent that xylose does not adopt a standard N state pentose ring conformation, and the N conformation of xylose must differ significantly from those found for ribo- and arabino derivatives. Within the dihedral angle range of $\Phi_{3'4'} \sim 20° - 40°$ observed in the latter

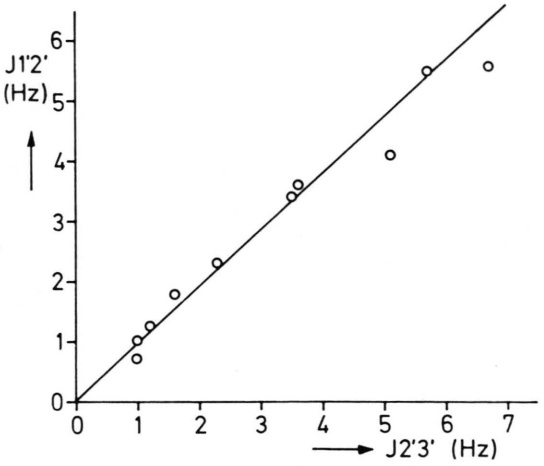

Fig. 2. Plot of the observed vicinal coupling constants $J_{1'2'}$ *vs.* $J_{2'3'}$ of the different analogues studied, showing a linear relation between these two constants.

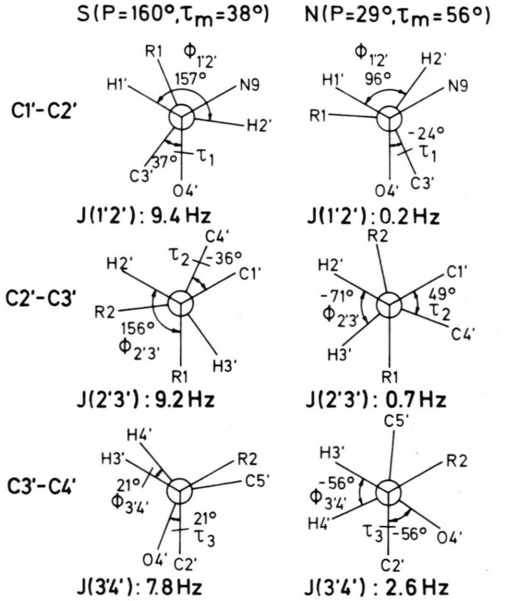

Fig. 3. Newman projections of the *S*- and *N*-xylose conformers along the three C-C bonds of the furanoside ring. The coupling constants given below the projections were calculated with Eqn (2) of ref. [3].

nucleoside families, the 3'hydroxyl group or any other substituent of comparable volume would approach the 5'hydroxymethyl group within distances shorter than their Van der Waals contact radii. Therefore we have incrementally increased $\Phi_{3'4'}$ by rotation about the C3'−C4' bond and calculated the distance between the 5'CH$_2$OD and 3'OD groups with the aid of the computer program COORD (QCPE no. 136) until this value became equal to the Van der Waals contact distance. This occurs at $|\Phi_{3'4'}| \sim 50°$.

The dihedral angle $\Phi_{3'4'}$ in the *N* conformation can also be estimated from the coupling constants. Since xyloA and 3'OCH$_3$xyloA are almost exclusively in the *N* state, their values of $J_{3'4'}$ should yield directly $\Phi_{3'4'}$. When $J_{3'4'} \sim 3$ Hz the Karplus equation gives $|\Phi_{3'4'}| \sim 55°$. Thus the angles determined by both arguments correspond to an *N* conformation with a pseudorotation angle $P \leq 36°$, *i.e.* in the ${}^3_4T$-C3'endo region. A C3'endo-C4'exo conformation for the xylofuranosides was calculated also by Jaworski *et al.* [7].

The dihedral angles resulting from an *N* xylose conformation with these parameters are given in the Newman projections of Fig. 3 together with the corresponding coupling constants.

All xylo nucleosides studied hitherto show a preference for the *N*-state. The experimental data are thus not sufficient to allow a detailed discussion of the pseudorotational characteristics of the *S*-xylose state. It appears reasonable to use the same dihedral angles for the *S*-xylose state that were derived for the ribo- and arabino analogues. Inspection of $J_{3'4'}$ of 3'ND$_2$xyloA and 3'DSxyloA allows a restriction for the range of $\Phi_{3'4'}$ possible in the *S* state. These two compounds have *S* populations of 50% or more as indicated by their values of $J_{1'2'}$ and $J_{2'3'}$. The value $J_{3'4'} \sim 7$ Hz yields a consistent description of the conformational equilibria only if $\Phi_{3'4'} \lesssim 25°$. An X-ray investigation of lyxofuranosyluracil [17] indicated a lyxose conformation in the *S* range with a dihedral angle $\Phi_{3'4'} \sim 30°$. Since lyxose and xylose have similar steric requirements with respect to the Newman projections along C3'−C4', this study is in harmony with our conclusions.

The steric arguments discussed above have to be incorporated for all xyloA analogues except 3'dA. This compound has no steric restrictions beyond those of the ribosides and thus can be treated with the standard pseudorotational parameters. The position of the two state equilibrium and the pseudorotational parameters of the xylose moiety for the compounds studied are collected in Table II. The results derived for lxyoA, the only lyxo compound available for this study, are given in Table II also.

As discussed by Ekiel *et al.* [17] lyxofuranosyluracil has a fairly distorted ${}_3T^2$ sugar conformation in

Table II. Results of the conformational analysis of the compounds studied.

Compound	T[°C]	P_N	τ_m^N	P_S	τ_m^S	[N]	[g^+]	[t]
XyloA	+ 40	29	56	144	40	0.87	0.16	0.37
	− 60	29	56	144	40	0.95	0.07	0.42
2′-ND$_2$xyloA	+ 40	29	56	144	40	0.75	0.19	0.34
	− 60	29	56	144	40	0.86	0.11	0.43
2′-N$_3$xyloA	+ 40	29	56	144	40	0.64	0.29	0.35
	− 60	29	56	144	40	0.66	0.28	0.33
2′-BrxyloA	+ 40			decomposition				
	− 60	29	56	160	40	0.75 ± 10%	decomposition	
3′-dA [a]	+ 40	25	40	144	40	0.82	0.68	0.16
	− 60	25	40	144	40	0.95	0.86	0.06
3′-DSxyloA	+ 40	29	56	144	40	0.35	0.45	0.30
	− 60	29	56	144	40	0.49	0.36	0.25
3′-ND$_2$xyloA	+ 40	29	56	144	40	0.36	0.58	0.21
	− 60	29	56	144	40	0.30	0.67	0.16
3′-N$_3$xyloA	+ 40	29	56	144	40	0.59	0.31	0.34
	− 60	29	56	144	40	0.59	0.33	0.32
3′-ClxyloA	+ 40	29	56	144	40	0.74	0.27	0.33
	− 60	29	56	144	40	0.78	0.27	0.33
3′-FxyloA	+ 40	29	56	144	40	0.85	0.13	0.43
	− 60	29	56	144	40	0.91	0.04	0.48
3′-OCH$_3$xyloA	+ 40	29	56	144	40	0.84	0.09	0.43
	− 60	29	56	144	40	0.89	0.06	0.44
3′,5′-diN$_3$xyloA	+ 40	29	56	144	40	0.50	0.16	0.58
	− 60	29	56	144	40	0.34	0.22	0.61
LyxoA	+ 40	36	50	180	50	0.70	0.17	0.31
	− 60	36	53	180	50	0.85 ± 15%	0.09	0.35

[a] Ref. [16].

the solid state. This distortion is to be expected for a furanoside ring with four bulky substituents located on one side of the ring. The crowding of these substituents makes it very probable that the pseudorotational parameters of the lyxose ring will depend strongly on the nature of the base. Therefore the results given by Ekiel *et al.* [17] for lyxo-pyrimidine nucleosides can be compared only qualitatively with our lyxoA data.

The characteristic pseudorotational parameters τ_m and P of the lyxose ring were determined with limited accuracy. The steric interference of the 2′ and 3′ hydroxyl groups demands a rather large torsion angle between the C2′−OD2′ and C3′−OD3′ bonds. (Experimentally, 41° was observed for this angle in S-lyxoU in the solid state.) Moreover, the same arguments discussed for the xyloA analogues hold for the torsion angles occurring in the Newman projections along the C3′−C4′ bond of lyxoA. Thus a rather distorted furanoside ring is expected and more data from a variety of lyxoA analogues are needed for a reliable description of the N lyxose state. The mole fractions of N given in Table II are thus reliable to no more than ± 0.15. The same corrections to the experimental proton-proton coupling constands for 3′FxyloA have to be taken into account that were discussed for 3′FaraA in the previous paper [3].

Inspection of the F−H distances within the xylose moiety as derived from the COORD calculations and inspection of the experimental vicinal coupling constants show that the electronegativity correction and the "through space" interaction can have but a small influence ($\Delta J \geq \pm 0.2$ Hz) on $J_{1'2'}$ and $J_{2'3'}$. Both of these contributions can be neglected in the calculation of the $N \Leftrightarrow S$ equilibrium. The situation, however, is different for the "through space" interaction between 3′F and the two 5′ protons. These two protons can approach the fluorine to a contact distance of ~ 2.5 to 3.0 Å for an *N*-xylose state in the *t* or g^- conformation respectively. According to the proposed magnitude for the "through space" interaction [18, 19] this distance could increase the parameters *A* and *B* of the Karplus equation by ~ 10%. Application of Eqns (6) and (7) of ref. [3] (derived with the standard parameters) to the experimental $J_{4'5'A} = 6.8$ Hz and $J_{4'5'B} = 6.9$ Hz found for 3′FxyloA at -60 °C leads to a negative mole fraction for g^+. Therefore, in this case a correction of the Karplus parameters is necessary. Increasing A and B by 10% yields the data given in Table II for the mole fractions g^+, *t*, g^- in 3′FxyloA.

Syn ⇔ anti equilibrium of the base

Among the compounds studied here, only 2′-N$_3$xyloA shows a xyloseproton spectrum sufficiently resolved to study the individual relaxation rates of the protons. The conformational equilibrium of the xylose ring for this substance, however, is characterized by comparable mole fractions of *N* and *S*. In addition, all three C5′ rotamers, g^+, *t*, and g^-, are almost equally populated. For the analysis of the relaxation rates one must therefore consider a blend of conformers. In previous studies on the ribosides [20, 21] we were able to analyse a series of compounds and thus attempt to separate the contributions of the different conformers to the observed relaxation rates. This allowed correlation of the *syn* ↔ *anti* equilibrium of the base with the corresponding conformational preferences of the ribose ring. With results obtained by a single method on just one compound, this approach is not justified. We have thus attempted to fit the experimental data according to the procedures described by Eqns (8) and (9) of ref. [3] with a weighted blend of the ring conformers and one glycosyl torsion angle.

The proton-proton distances within the *N*- or *S*-xylose rings, as well as the distances between H8 and the sugar protons and the 3′hydroxyl group as a function of the glycosyl torsion angle, were calculated with the dihedral angles for the furanoside ring as given in Fig. 3. The results are compiled in Figs. 4 and 5. As can be seen in Fig. 4, only H1′, H2′ and to a lesser extent H3′ can approach H8 sufficiently close to lead to an observable dipole-dipole-interaction in an *N*-xylose conformer.

Moreover, the main contributions to the overall relaxation rate of H8 stem from H1′ and H2′. This results in a very similar dependence on the glycosyl torsion angle for both *N*- and *S*-xylose conformers. The experimental relaxation rates are thus rather insensitive to the position of the *N* ⇔ *S* equilibrium.

Fig. 5 shows that steric interference between the 3′hydroxyl group and the base atoms excludes wide

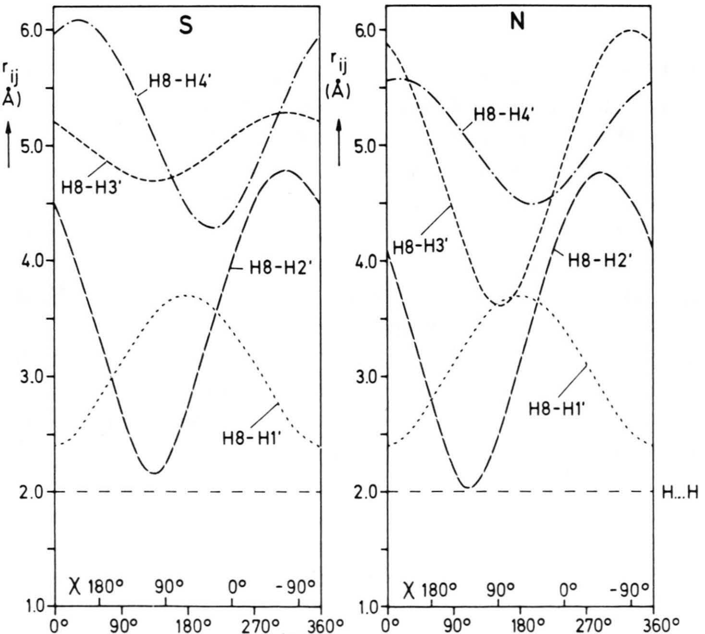

Fig. 4. Distances between the base protons H8 and the four xylose protons H1′ to H4′ in the *S* and *N* states, respectively, as a function of the glycosyl torsion angle Υ. The horizontal broken line is the Van der Waals' constant distance between two hydrogens. (Definition of Υ according to Davis and Hart [22], χ is defined as proposed by Sundaralingam [23].)

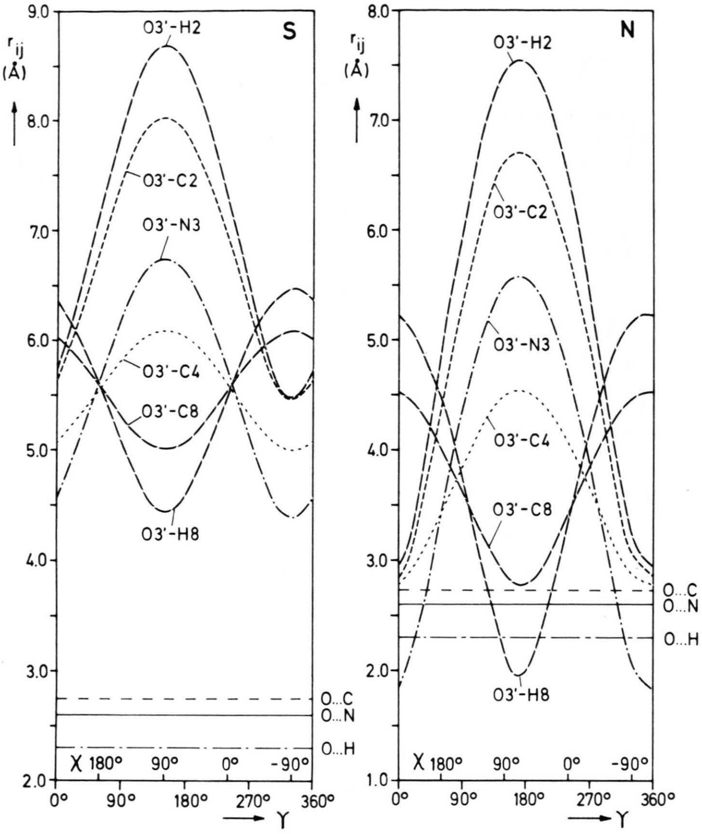

Fig. 5. Distances between atoms of the adenine moiety and the 3′-hydroxylgroup of XyloA in the *S*- and *N*-states, respectively, as function of the glycosyl torsion angle Υ. The horizontal lines are the Van der Waals' contact distances between the different atoms. (Definition of the glycosyl torsion angle Υ according to Davis and Hart [22]; χ is defined as proposed by Sundaralingam [23].)

ranges of the glycosyl torsion angle for *N*-xylose conformers. In contrast this group does not approach any of the adenine ring atoms within Van der Waals contact distances in the *S*-xylose conformation.

In Fig. 6 the reduced longitudinal relaxation rates of H1′ to H3′ and H8 in the *S*- and *N*-states are given as defined by Eqn (8) of ref. [3]. These results allow calculation of the ratios of the different rates $R_d/R_{d'}$ for the blend of ring populations ($[N]=0.66$, $[S]=0.34$) observed at -60 °C. These ratios are plotted in Fig. 7 together with the experimental ratios (thin horizontal lines) derived from the results collected in Table III.

It can be seen that all experimental curves intersect the corresponding theoretical ratios around $\Upsilon \approx 80°$. The intersections common to several experimental ratios $R_d/R_{d'}$ in other ranges of the glycosyl torsion angle can be excluded for at least the *N*-xylose conformer for the following reasons: $\Upsilon = 0° \pm 30°$ is forbidden owing to close contacts between O3′ (or the 3′N$_3$-substituent) and N3. Repulsive distances in the *N*-state between O3′ and H8 rule out the range $\Upsilon \approx 180 \pm 10°$. The range $\Upsilon = 240° \pm 10°$ must be energetically unfavorable in both the *N*- and *S*-states, because of the small distance between O4′ and H8.

Table III. Longitudinal relaxation rates of the single protons of 2′N$_3$xyloA dissolved in ND$_3$ at -60 °C.

Proton, *d*	H2	H8	H1′	H2′	H3′	H4′	H5′
R_d [1/sec]	0.036	0.29	0.30	0.42	0.60	0.60	1.85

For the N-xylose state a fairly wide range of the glycosyl torsion angle Υ is thus forbidden, resulting from the close contact interactions between the pentose and base atoms.

For the *S*-xylose state there appears to be much more conformational flexibility for the glycosyl rotamer equilibrium of the base. It is probable that the *S*-xylosides can have stable conformers in several ranges of the glycosyl torsion angle.

The best fit between the experimental relaxation rates and the curves of Fig. 6 is found for $\Upsilon = 80°$ and $\tau_c = 80$ ps. The reduced experimental relaxation

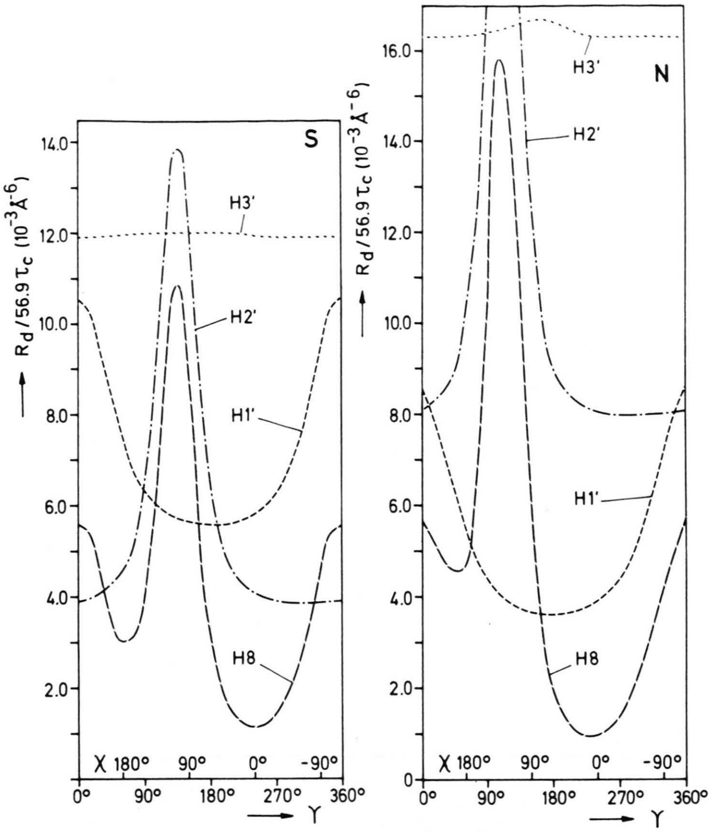

Fig. 6. Calculated proton relaxation rates as function of the glycosyl torsion angle for the xylose protons H1′, H2′ and H3′ and the base proton H8 for the *S*- and *N*-states, respectively.

Table IV. Comparison between experimental and calculated reduced longitudinal proton relaxation rates $R_d/(56.9\ \tau_c)$ of $2'N_3$xyloA.

Proton, *d*	$R_d/56.9\ \tau_c$ [Å^{-G}]	
	Experimental for $\tau_c = 80$ psec	Calculated for $\Upsilon = 80$ °
H8	0.0064	0.0064
H1′	0.0066	0.0053
H2′	0.0092	0.0106
H3′	0.0132	0.0148

rates are compared to the rates calculated for $\Upsilon = 80$° in Table IV.

The results of the analysis of the relaxation rates obtained suggest that $2'N_3$xyloA has a pronounced preference for the syn region of the glycosyl torsion angle and that the data are compatible with a base glycosyl torsion angle $\Upsilon = 80$°.

Discussion

XyloA, in contrast with riboA and araA, is found almost exclusively in an *N* conformer population. Further stabilization of the *N*-state by chemical modification would be difficult to observe. The influence of all chemical modifications, when observable, is consequently a shift of the $N \Leftrightarrow S$ equilibrium towards *S*. Comparing the two pairs 2′- and $3'ND_2$- and 2′- and $3'N_3$xyloA leads to the conclusion that substitution at 3′ results in a greater shift of the $N \Leftrightarrow S$ equilibrium relative to the 2′ analogues.

In contrast with all the other pentosides studied, introduction of the 5′-azido group to give 3′,5′-diN_3xyloA leads to a further significant increase in the *S*-conformer population.

Whether this is typical for the xylosides or a peculiarity of this one example can not be determined with the results available. In Fig. 8 the mole fraction [*N*] of the ring conformers is plotted against the populations of the two rotamers g^+ and *t*. From steric considerations it is predicted that an *N*-xylose cannot form a stable g^+ conformer if the 3′-substituent of the sugar has a Van der Waals radius greater than that of the hydrogen atom. The negative correlation between *N* and g^+ was thus ex-

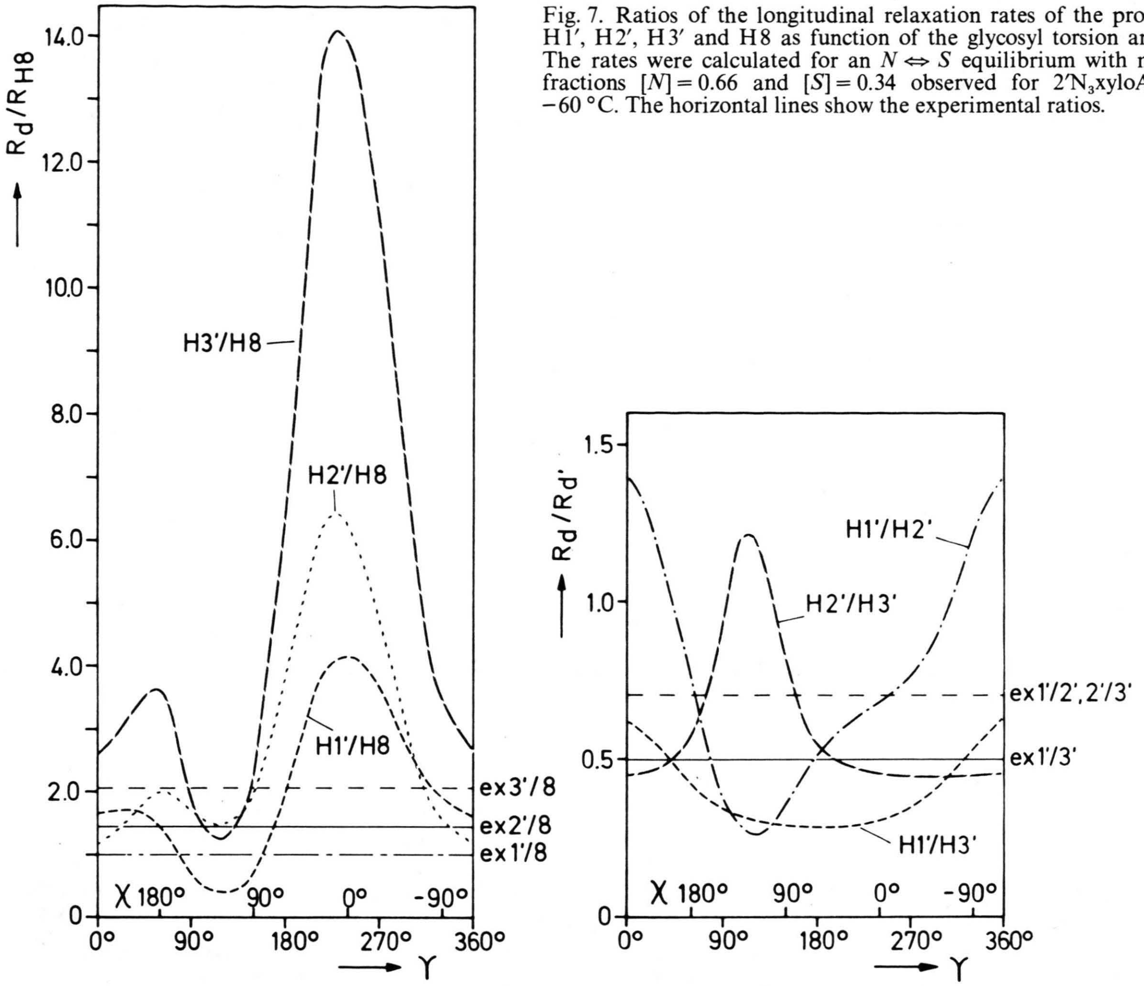

Fig. 7. Ratios of the longitudinal relaxation rates of the protons H1', H2', H3' and H8 as function of the glycosyl torsion angle. The rates were calculated for an $N \Leftrightarrow S$ equilibrium with mole fractions $[N] = 0.66$ and $[S] = 0.34$ observed for 2'N$_3$xyloA at −60 °C. The horizontal lines show the experimental ratios.

pected. It may be noted that the experimental points for 3'dA in this graph do indicate a strong preference of this substance for the N-g^+ conformer. This is in harmony with the steric explanation.

For the N-xylose state the two rotamers t and g^- are equally stable. Thus as $[N] \rightarrow 1.0$, $[g^-] = [t] = 0.5$.

The data obtained for lyxoA suggest that the pentose conformation is similar to that of xyloA. The *syn* ⇔ *anti* equilibrium has not been studied for lyxoA. It is clear that wide ranges of the glycosyl torsion angle are forbidden because of steric interference between 2' and 3'OD and the base.

As observed for the ribosides and arabinosides, the influence of chemical modifications on the $N \Leftrightarrow S$ equilibrium of xylosides cannot be explained quantitatively by differences in electronegativity of the substituents. Differences in the Van der Waals radii likewise do not correlate with changes in this equilibrium. This lack of correlation is most clearly seen in the diagrams of Fig. 9 which illustrate the data obtained for these 3'modified nucleosides.

The general conclusions from the data presented here are that the xylosides show a preference for a *syn*-N-(t/g^-) conformation. If the results obtained for two xylosides (3'DSxyloA, 3'ND$_2$xyloA) with $[S] \gtrless 0.5$ can be generalized, a positive correlation between S and g^+ would be expected. It can be seen from an inspection of the intramolecular distances between the xylose and adenine moiety that the purine base of an S-xyloside can be accommodated in wide ranges of the *syn* and *anti* regions.

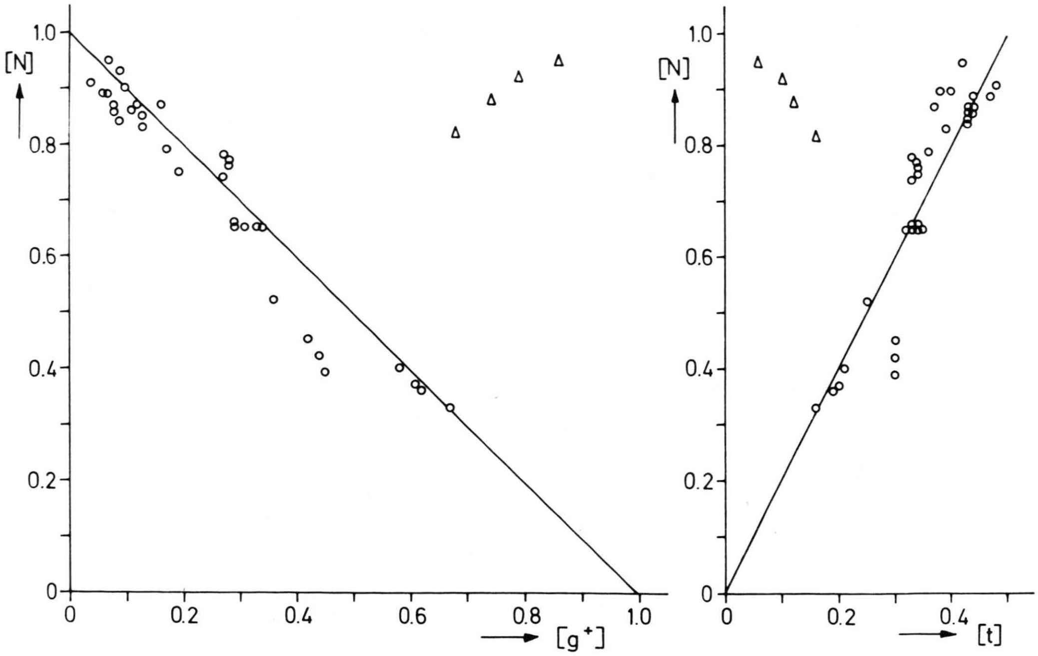

Fig. 8. Correlation between the mole fraction $[N]$ of the furanoside ring and the rotamer population $[g^+]$ (left) and $[t]$ (right) of the exocylic $5'CH_2OD$ group of the xylosyladenine analogues. (Δ: values for 3′dA).

General discussion of the conformational equilibria of ribose, arabinose, xylose and lyxose adenosine analogues

The possible conformers of the nucleoside analogues possess only small energy differences. The influence of a specific chemical modification in the pentose moiety can thus be evaluated only if all other parameters are kept constant. In the systematic approach taken here, only (β)-pentose-adenine analogues were studied in order to exclude the influence of changing the base moiety on the sugar equilibria. Furthermore, concentration, temperature, and solvent were kept constant during this series of experiments. Finally it should be emphasized again that the use of liquid ammonia as a solvent was advantageous in this investigation for the following reasons:

1. The ND_3-molecule can participate in hydrogen bonds as an acceptor and a donor like the biological solvent, water.
2. Self-association of nucleosides, which is known to occur in water and known to influence conformational equilibria, is virtually absent in ND_3.
3. In contrast with neutral water, liquid ND_3 is an excellent solvent for all the nucleoside analogues studied. This permitted the determination of the HRNMR spectra of all substances at a standard concentration of 5 mg per 0.5 ml of ND_3.

The overall energy differences between conformers contributing a mole fraction observable by NMR (*i. e.* $[X] > 0.05$) are in the range of a few $kJ \cdot mol^{-1}$. It is thus difficult to predict the absolute position of the conformational equilibria of a given compound. A multitude of intra- and intermolecular interactions *i.e.* steric repulsion, electrostatic interactions, such as dipolar or inductive forces, hydrogen bonds, etc., contribute to the overall energy of each conformation. As the shortest distances possible between non-bonded atoms or groups (given by the Van der Waals contact distances) are approached, the steric repulsions would provide the dominating contribution to the interaction energy between these groups. Some conformers can be excluded when they demand too small a Van der Waals contact distance.

Over larger distances electrostatic interactions, repulsive as well as attractive, may become important especially for strongly electronegative and polar substituents. The most spectacular among these interactions, the hydrogen bond, has been observed in several crystal studies and yields pronounced

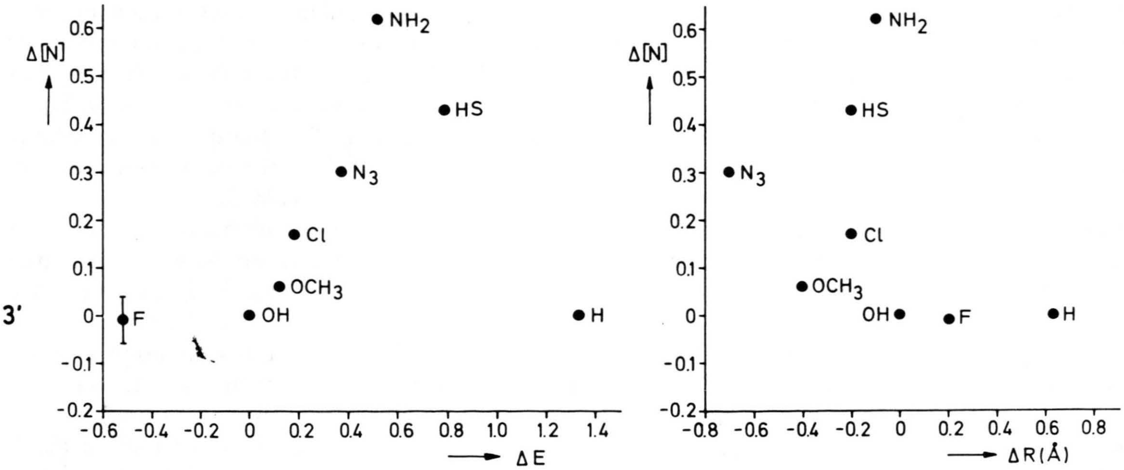

Fig. 9. Left side: Plot of the mole fractions $\Delta[N] = [N_{OH}] - [N_R]$ *vs.* the difference of the electronegativities $\Delta E = E_{OH} - E_R$ of the substituents for the 3′-derivatives of xyloA at −60 °C. Right side: Plot of the mole fractions $\Delta[N]$ *vs.* the difference of the Van der Waals' radii $\Delta R = R_{OH} - R_R$.

energy minima in theoretical calculations. The solution data in neutral D_2O and ND_3, however, are ambivalent as indicated in the following three examples:

1. In crystals of adenosine analogues [24] the *S-syn* conformer appears to be stabilized by a hydrogen bond between OH5′ and N3. This interaction is possible only in the g^+ rotamer. Indeed, in cases of the *S* ribose state a slight preference for the g^+ rotamer is seen in solution [16].
2. Energy calculations and solid state data indicate a hydrogen bond between the 2′ and 5′ hydroxyl groups in arabinosides that stabilizes the *S*-g^+ conformer [25]. In solution, however, only the *N*-g^+ conformer is observed in any degree of prominence.
3. In the solid state a hydrogen bond between OH-3′ and OH-5′ has been found with an accompanying C4′-exo pucker of the xylose ring and a g^+ rotamer for 2,2′anhydro-α-xyloU [26]. However, in solution g^+ is strongly destabilized thus excluding such a hydrogen bond.

Owing to the variety of interactions present, a quantitative dependence of the conformational equilibria on one of the properties of the substituents like volume (size) or electronegativity is not to be expected and cannot be derived from the experimental results. Linear correlations found previously between these properties and the sugar conformation would appear to be fortuitous. A recent attempt by Guschlbauer and Jankowski [27] to predict the $N \Leftrightarrow S$ equilibria of arabinose and xylose nucleosides on the basis of electronegativity effects leads in the case of the arabinosides to a stabilization of the *S*-conformer, while for the xylosides a stabilization of the *N*-state is predicted. Though the results presented here do reveal an *N*-stabilization of the xylosides, the arabinosides are found also with a strong preference for the *N*-pentose, thus invalidating the predictive power of the electronegativity concept.

Some general qualitative predictions concerning the influence of the size of the substituent upon the conformation, however, can be made. The following table shows the "pseudoorientation" of the substituent or hydroxyl group at C2′ and C3′ in the *N* and *S* conformations for the different sugars with respect to the furanoside ring:

		N	*S*
Ribose	2′	axial	equatorial
	3′	equatorial	axial
Arabinose	2′	equatorial	axial
	3′	equatorial	axial
Xylose	2′	axial	equatorial
	3′	axial	equatorial
Lyxose	2′	equatorial	axial
	3′	axial	equatorial

Generally the distances between the 2′ or 3′ substituent and the ring atom three bonds away are longer for the pseudoequatorial than for the pseudoaxial orientation. Therefore it could be expected that the unmodified pentosides would prefer the ring conformation in which the hydroxyl groups occupy the equatorial positions. However, arabinose, xylose, and lyxose, show the opposite trend; especially xyloA exists mainly in the *N* state with both OH groups in axial positions. Apparently simple steric constraints cannot explain the conformational differences between the four pentosides. If, however, the conformations of the unmodified sugars are taken as given by experiment, some common characteristics can be derived for the changes induced by substitutions on the furanoside ring.

For example the introduction of an amino group, which is more voluminous than a hydroxyl, at any of the four orientations at C2′ or C3′ always changes the conformational equilibrium in the direction of that state where the amino group acquires an equatorial position. In the ribosides 2′ND_2A shows a pronounced stabilization of the *S*-conformer, while 3′ND_2A is found in an *N* ribose state. With 2′- and 3′ND_2araA the *N* arabinose is stabilized, whereas in 2′- and 3′ND_2xyloA the conformational equilibrium is shifted towards *S* when compared with the unmodified xyloside. This general trend is most clearly seen in 2′, 3′diND_2araA, which at low temperatures exists exclusively in the *N* state, where both amino groups are pseudoequatorial to the ring.

This correlation can be extended to the chloro-, bromo-, azido- and thiopentosides. Therefore it appears justified to postulate that substitution of the 2′ or 3′ hydroxyl group by a more voluminous atom or group shifts the conformational equilibrium towards the state where the substituent acquires an equatorial-like orientation. This finding is in accordance with a rule established for the stable conformations of cyclohexane derivatives [28, 29].

For substituents with a volume comparable to or smaller than a hydroxyl group the arguments given above obviously no longer apply. The experimental results indicate that with 2′dA and 3′dA as well as 3′FaraA and 3′FxyloA that conformation is stabilized in which the substituents are in an axial orientation with respect to the sugar ring. The data, however, are too limited for a confident generalization and additional deoxy- and fluoro compounds are needed. Further information on the validity of the above considerations can also be obtained by employing additional analogues with identical substituents at C2′ and C3′.

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