

Arabinose Conformations of 9- β -D-Arabinofuranosyladenine (ARA-A) Analogues Modified at the 2',3' or 5' Positions

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The solution conformations of 9- β -D-arabinofuranosyladenine analogues modified at the 2',3' or 5' positions are derived from the HRNMR spectra and the longitudinal relaxation rates of the protons. The compounds studied are: 9- β -D-arabinofuranosyladenine and its 2'-amino-2'-deoxy, 2'-chloro-2'-deoxy, 2'-azido-2'-deoxy, 3'-amino-3'-deoxy, 3'-bromo-3'-deoxy, 3'-chloro-3'-deoxy, 3'-fluoro-3'-deoxy, 3'-azido-3'-deoxy, 2',3'-diamino-2',3'-dideoxy, 2',5'-diamino-2',5'-dideoxy and 2',5'-diazido-2',5'-dideoxy analogues. It is derived from the data that the conformational equilibria of the furanose rings can be described by the two state $N \rightleftharpoons S$ model of Altona and Sundaralingam.

The emphasis in this work is to study systematically the influence of the different chemical modifications upon the conformational equilibria of the nucleosides. For the arabinosides it is found that substitution of the hydroxyl groups at C2' or C3' by other atoms or groups always stabilizes the N conformation of the arabinose ring. The only exception is fluorine, where S is stabilized. The preference for the N state is correlated with an increasing population of the g^+ rotamer of the exocyclic 5'-CH₂OD group. From the relaxation study of 2'-chloro-2'-deoxy-arabinofuranosyladenine the position of the $syn \rightleftharpoons anti$ equilibrium of the base was estimated to be predominantly $anti$. Thus a preference for the $anti-N-g^+$ conformation was derived for the arabinosides excluding an intramolecular hydrogen bond between the 2' and 5' hydroxyl groups that was found in the solid state. The stabilization of the N conformer in the modified compounds can be qualitatively explained by steric effects.

Introduction

In a previous publication [1] the influence of chemical modifications in the ribose moiety on the conformational equilibria of adenosine analogues was studied by the analysis of the HRNMR spectra of the non-exchangeable ribose protons. Those studies have been extended in this paper to include analogues of 9- β -D-arabinofuranosyladenine (araA) with a variety of substituents at the 2',3' and/or 5' positions. In the following part of this series [2], we will conclude these studies with an investigation of some analogues of 9- β -D-xylofuranosyladenine.

The main emphasis in this series of papers is to investigate the influence of different sugar modifications upon the conformational preferences of the nucleosides under well defined standard conditions. Since minor chemical modifications of the base

moiety exert a significant influence on the conformational equilibria of the sugar moiety [3, 4], only nucleosides with adenine as base were included in this study.

AraA [5, 6] as well as other arabinonucleosides exhibits potent antiviral and antitumor activity, and araA is presently in clinical use. Previous work in the solid state [7, 8], theoretical calculations [9, 10] and analyses of solution conformations by HRNMR [11, 12] have yielded conflicting evidence about the conformational preferences of the arabinose moiety. From an inspection of the X-ray data of araC and araU it was concluded that hydrogen bond formation between the 2' and 5' hydroxyl groups stabilizes the C2'- $endo-g^+$ conformer. However, no evidence to support the occurrence of this hydrogen bond was observed in aqueous or in dimethylsulfoxide solutions [11, 12].

The data presented in this paper include results obtained with several newly synthesized analogues of araA. No preference for the C2'- $endo-g^+$ conformer in these modified compounds was observed.

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Experimental

Sample preparation

9-β-D-Arabinofuranosyladenine (araA) was purchased from Nutritional Biochemicals (Cleveland, Ohio, USA) and used without further purification. The 2'-amino-2'-deoxy (2'ND₂araA) [13], 2'-chloro-2'-deoxy (2'ClaraA) [14], 2'-azido-2'-deoxy (2'N₃araA) [15, 16], 3'-amino-3'-deoxy (3'ND₂araA) [17], 3'-bromo-3'-deoxy (3'BraraA) [18], 3'-chloro-3'-deoxy (3'ClaraA) [18], 3'-fluoro-3'-deoxy (3'FaraA) [18, 19], 3'-azido-3'-deoxy (3'N₃araA) [16, 17], 2',3'-diamino-2',3'-dideoxy (2',3'diND₂araA) [20], 2',5'-diamino-2',5'-dideoxy (2',5'diND₂araA) [20], and 2',5'-diazido-2',5'-dideoxy (2',5'diN₃araA) [20] analogues of araA were synthesized as described in previous publications.

All volatile material was removed from the samples on a high vacuum line at 5×10^{-5} torr. 5 mg of each nucleoside were dissolved in 0.5 ml of trideuteroammonia in 5 mm NMR-tubes. Residual oxygen was removed from the dissolved sample of

2'ClaraA used for the determination of the longitudinal relaxation times by several freeze pump thaw cycles to a final pressure of 5×10^{-5} torr. Freezing was accomplished in liquid nitrogen and thawing in a mixture of acetone and dry ice.

Spectra

The proton spectra were obtained on a Varian XL-100-15 FT-NMR-Spectrometer, interfaced to a 16 K 620/1100 computer with a disk accessory. The digital resolution of the HR spectra was 0.1 Hz. The temperatures were controlled with a miniature thermocouple. They are accurate to ± 0.5 K. The chemical shifts were referenced to an external standard of 2% TMS in CS₂ solution. No attempts were made to correct for bulk magnetic susceptibility effects. The high resolution spectra were simulated by application of the computer program LAME (QCPE no. 111). Coupling constants and relative chemical shifts derived from these simulations are estimated to be accurate to ± 0.1 Hz.

Table I. Vicinal proton-proton coupling constants of the arabinose protons in Hz, of the various nucleosides dissolved in ND₃ at +40 °C and –60 °C.

Compound	AraA			2'ND ₂ araA		3'ND ₂ araA		2',3'diND ₂ araA		2',5'diND ₂ araA		2'N ₃ araA		3'N ₃ araA		2',5'diN ₃ araA	
T[°C]	+40	–60	23	+40	–60	+40	–60	+40	–60	+40	–60	+40	–60	+40	–60	+40	–60
solvent	ND ₃	ND ₃	D ₂ O ^a	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	ND ₃
J _{1'2'}	4.7	5.0	5.5	6.4	6.55	5.9	6.1	6.6	6.65	6.6	6.8	6.4	6.8	6.2	6.5	6.5	7.1
J _{2'3'}	5.1	6.1	5.5	7.45	8.5	7.3	8.2	9.7	10.3	7.2	7.9	7.8	9.2	7.4	8.8	6.8	8.3
J _{3'4'}	5.1	6.0	6.3	6.9	8.0	7.4	8.4	8.4	9.4	7.2	7.65	7.6	9.0	7.6	8.8	7.3	8.5
J _{4'5'A}	4.3	3.9	2.8	2.5	2.4	4.2	3.3	2.5	2.1	3.7	3.4	3.5	2.0	3.8	2.9	2.9	2.2
J _{4'5'B}	4.6	4.4	4.6	3.4	2.6	3.6	3.1	3.1	3.1	5.6	5.7	3.8	2.3	3.7	3.0	6.0	4.9

	2'ClaraA		3'ClaraA		3'BraraA		3'FaraA ^c	
T[°C]	0 ^b	–60	0 ^b	–60	T>–60	–60	+40	–60
solvent	ND ₃	ND ₃	ND ₃	ND ₃	ND ₃	ND ₃	ND ₃	ND ₃
J _{1'2'}	6.2	6.6	6.1	6.3		6.4	4.6	4.3
J _{2'3'}	7.9	9.2	7.6	8.5		9.4	2.9	2.7
J _{3'4'}	7.5	8.4	8.0	8.6	dec.	9.2	2.9	2.4
J _{4'5'A}	3.0	2.4	3.2	2.7		2.8	5.9	6.4
J _{4'5'B}	3.6	2.5	3.2	2.6		3.0	6.0	6.4

^a Ref. [21];

^b decomposition at +40 °C;

^c $|J_{HF}|$ couplings in Hz derived from the simulation, first entry: +40 °C, second entry: –60 °C; $|J_{1F}| = 1.8/2.2$; $|J_{2F}| = 15.6/14.5$; $|J_{3F}| = 52.6/52.4$; $|J_{4F}| = 26.4/27.4$.

The longitudinal proton relaxation times T_1 of the individual protons were determined with a $(5 T_1)$ - 180° -(t)- 90° -AT pulse sequence of the Varian-SYMON-program. " t " was varied between $0.01 T_1$ and $2 T_1$. Within this range no significant deviation from an exponential decay behaviour of the amplitude was found.

The T_1 values given as the decay times were reproducible to $\pm 5\%$.

Results

Fig. 1 includes the experimental and simulated spectra of the non-exchangeable arabinose protons in 2'ClaraA and 3'ClaraA.

The vicinal coupling constants obtained in the simulation of these spectra and of all other substances are collected in Table I. They are compared with the values for the unmodified araA. For araA the vicinal coupling data determined by Remin *et al.* [21] in neutral aqueous solution are included also. Replacement of either the 2' or 3' hydroxyl group by any of the substituents given in Table I leads to a significant increase of $J_{2'3'}$ and $J_{3'4'}$ and a less pronounced increase of $J_{1'2'}$. The only exception to this observation involves 3'FaraA. For this compound $J_{2'3'}$ and $J_{3'4'}$ are ~ 2.5 Hz smaller than the corresponding couplings found in araA. Substitution at the 2' or 3' position has a very minor effect upon $J_{4'5'A}$ and $J_{4'5'B}$. Again 3'FaraA is a notable ex-

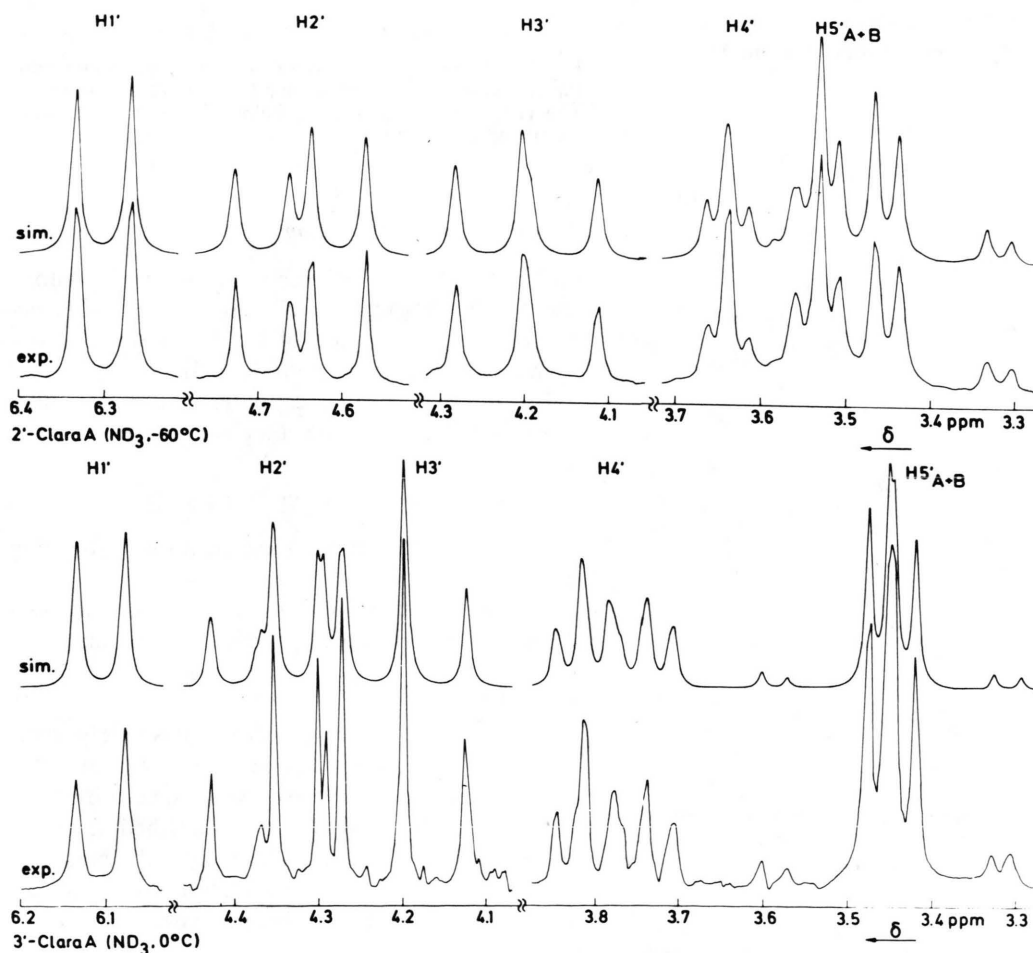


Fig. 1. Experimental proton high resolution spectra of solutions of 2'ClaraA (-60°C) and 3'ClaraA (0°C) in ND_3 , covering the region of the arabinose protons, compared with the simulated spectra.

ception. In this compound both couplings are increased by ~ 2 Hz. Substitution of the 5' hydroxyl group by either N_3 or ND_2 changes the coupling constants between the ring proton marginally, but significant changes are seen for $J_{4'5'A}$ and $J_{4'5'B}$. The chemical shifts obtained from the analysis of the proton spectra are available from the authors upon request.

Conformational Analysis

Fig. 2 summarizes the conformational equilibria of the molecular framework of the nucleosides that were derived by analysis of the NMR-data. The puckering of the furanose ring and the distribution of the three rotamers of the exocyclic 5'-CH₂R₃ group were obtained from the vicinal coupling constants of the arabinose proton spectra. The preferred glycosyl torsion angle of the *syn* \rightleftharpoons *anti* equilibrium was estimated from the longitudinal proton relaxation rates.

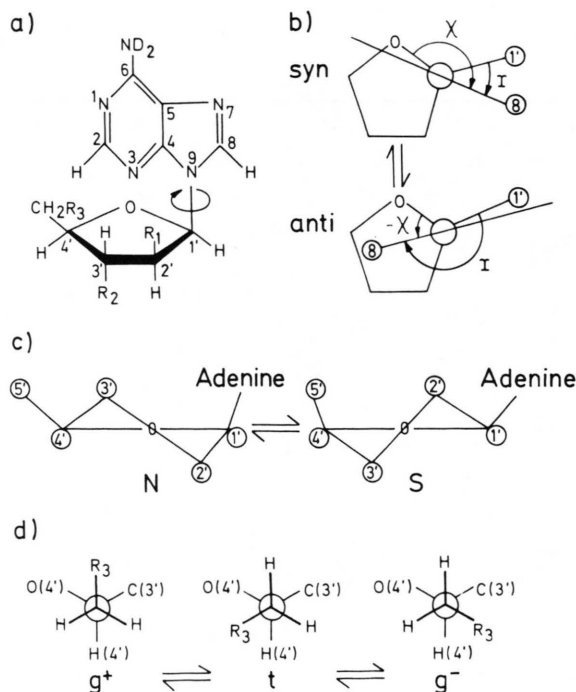


Fig. 2. Structural formula and internal motions of the arabinosyl-adenine analogues studied. a) Chemical structures: araA: $R_1 = R_2 = R_3 = OD$; 2'-derivatives: $R_2 = R_3 = OD$, $R_1 = ND_2$, Cl, N₃; 3'-derivatives: $R_1 = R_3 = OD$, $R_2 = ND_2$, Br, Cl, F, N₃; 2',3'-diND₂araA: $R_1 = R_2 = ND_2$, $R_3 = OD$; 2',5'-derivatives: $R_2 = OD$, $R_1 = R_3 = ND_2$, $R_3 = ND_2$, N₃; b) glycosidic bond rotation (*syn* \rightleftharpoons *anti*); c) pseudorotation of the arabinose ring (*N* \rightleftharpoons *S*); d) rotation of the exocyclic 5'-CH₂R₃ group (*g*⁺ \rightleftharpoons *t* \rightleftharpoons *g*⁻).

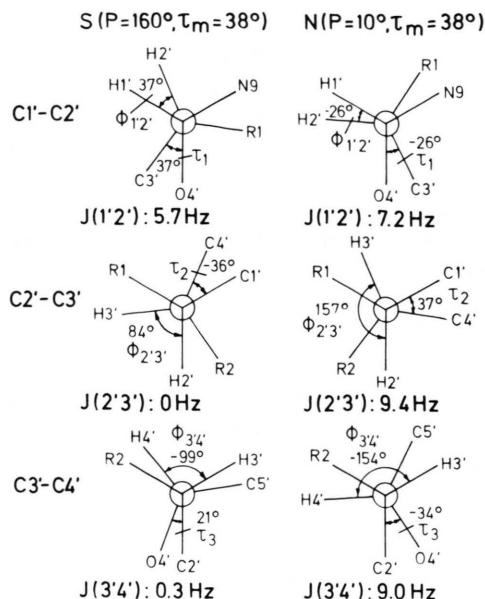


Fig. 3. Newman projections of *N* and *S* arabinose conformers along the three C-C bonds of the furanoside ring. The coupling constants given below the projections were calculated with Eqn (2).

Arabinose ring conformation

The two state *N* \rightleftharpoons *S* model proposed by Altona and Sundaralingam has [22, 23] received wide acceptance for description of the furanose ring conformations of purine(β)ribosides [24].

The observed vicinal coupling constants in this model are assumed to be composite values as given by

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

where *[N]* and *[S]* are the mole fractions of the ring in the two states.

For the ribonucleosides, the vicinal coupling constants are well described by the Karplus equation

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

Here *A* and *B* are regarded as adjustable parameters. For ribonucleosides in ND₃ solutions, *A* = 10.0 Hz and *B* = -0.95 Hz were determined to be the best values [3]. $\Phi_{ij}^{N,S}$ are the dihedral angles between protons *i* and *j* as defined by the Newman projections of Fig. 3. In the pseudorotational model for arabinose the three dihedral angles are interrelated by:

$$\Phi_{1'2'} = \tau_m \cos (P - 144^\circ) \quad (3)$$

$$\Phi_{2'3'} = 120^\circ + \tau_m \cos P \quad (4)$$

$$\Phi_{3'4'} = -120^\circ - \tau_m \cos (P + 144^\circ), \quad (5)$$

where P is the phase angle of the pseudorotation and τ_m is the maximum angle of torsion.

In the previous paper of this series [1] we demonstrated that the description derived for unmodified ribosides can be extended with reasonable accuracy to the chemically modified nucleosides. It also was shown that the effect of changes in electronegativity upon the parameters A and B of Eqn. (2) was smaller than ± 0.5 Hz. An uncertainty of this magnitude will not influence the conclusions derived in this study significantly and it appears reasonable to use the parameters given above in Eqn (2), which were derived for the unmodified ribose, for the arabinosyl analogues. Jaworski *et al.* [25] used INDO calculations to show that application of the Karplus equation for the determination of the dihedral angle Φ_{ij} is prone to error when a hydroxyl group or any strongly electronegative substituent in the ethane fragment under consideration is in an antiperiplanar orientation with respect to one of the coupling protons. As can be seen from inspection of Fig. 3, this could apply only for $J_{1'2'}$ in the S -state. However, $J_{1'2'}$ is the coupling constant that changes least with shifts in $N \rightleftharpoons S$, and thus reveals only limited information about the position of this conformational equilibrium of the arabinose ring. Thus it appears permissible to calculate the mole fractions of N or S by application of Eqn (2), since as is shown below, only $J_{2'3'}$ and $J_{3'4'}$ indicate reliably the position of the $N \rightleftharpoons S$ equilibrium.

The Newman projections along the three ring carbon-carbon bonds given in Fig. 3 have been constructed with the assumption that the projections of the bond angles into the plane perpendicular to the respective C-C bonds are symmetrical and 120° . This approximation was adopted since actual bond angles for the different analogues are unknown. It should not introduce major errors since these angles should vary by only a few degrees. The different J_{ij}^N and J_{ij}^S values calculated with Eqn (2) for the conformers of Fig. 3 are given below the respective projections. It is seen that the change in $J_{1'2'}$ is quite small (~ 1.5 Hz) whereas $J_{2'3'}$ and $J_{3'4'}$ are ~ 0 Hz for the S -state and approach 9 Hz in the N -state.

A plot of the experimental coupling constants $J_{2'3'}$ vs. $J_{3'4'}$ (Fig. 4) yields a linear relationship with a

slope of ~ 1 within the accuracy of this treatment. The description of the arabinose ring conformation by the Altona Sundaralingam model is therefore assumed to be valid.

Further evidence for the applicability of this model arises from inspection of X-ray results for various β -arabinosides [7, 8]. It was found that the conformations of the arabinofuranose rings are very similar to the preferred conformations of the ribosides and can be described in terms of the pseudorotational model.

Table II summarizes the sets of pseudorotational parameters that give the best fit for the three coupling constants for all compounds analysed. In the context of the analogues studied here, 2'-deoxyadenosine (2'dA) can be viewed as a 2'-modified arabinosyladenine. The data for 2'dA are taken from previous work [26].

Conformation of the exocyclic 5' CH_2R_3 -group

Following a procedure proposed by Hruska *et al.* [27] the equilibria between the three classically staggered rotamers can be derived from:

$$[g^+] = 1.46 - \frac{J_{4'5'A} + J_{4'5'B}}{8.9} \quad (6)$$

$$[t] ([g^-]) = \frac{J_{4'5'A(B)}}{8.9} - 0.23. \quad (7)$$

The assignment of the t - or g^- -conformer depends upon an unequivocal assignment of the two

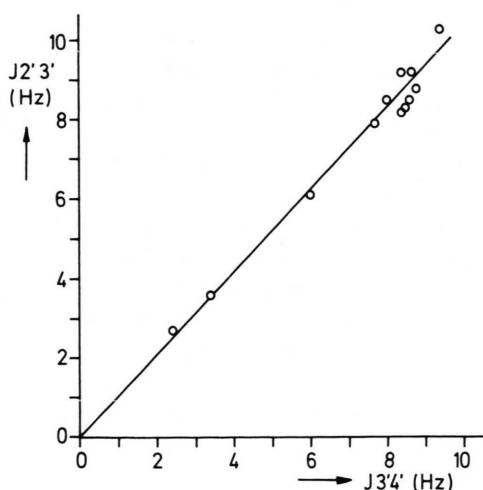


Fig. 4. Plot of the observed vicinal coupling constants $J_{2'3'}$ vs. $J_{3'4'}$ at $T = -60^\circ\text{C}$ for the different analogues studied, showing a linear relation between these two constants.

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

Compound	T [°C]	P_N	P_S	τ_m	$[N]$	$[g^+]$	$[t]$
AraA	+ 40	3	144	38	0.53	0.46	0.25
	- 60	3	144	38	0.65	0.53	0.21
2'-dA	+ 40	10	161	38	0.36	0.56	0.13
	- 60	10	161	38	0.36	0.56	0.21
2'-ND ₂ araA	+ 40	3	144	38	0.76	0.80	0.05
	- 60	3	144	38	0.88	0.90	0.04
2'-ClaraA	+ 40				decomposition		
	- 60	3	161	38			0.04
2'-N ₃ araA	+ 40	6	144	40	0.80	0.64	0.16
	- 60	6	144	40	0.98	0.98	0.00
3'-ND ₂ araA	+ 40	3	144	40	0.77	0.58	0.24
	- 60	3	144	40	0.88	0.74	0.14
3'-BraraA	+ 40				decomposition		
	- 60	3		40			0.08
3'-ClaraA	+ 40				decomposition		
	- 60	3	144	40			0.06
3'-FaraA	+ 40	6	144	45	0.30	0.23	0.38
	- 60	6	144	45	0.25	0.14	0.43
3'-N ₃ araA	+ 40	3	144	40	0.79	0.62	0.20
	- 60	3	144	40	0.96	0.80	0.09
2',3'-diND ₂ araA	+ 40	6	144	40	0.95	0.83	0.05
	- 60	6	144	40	1.00	0.89	0.00
2',5'-diND ₂ araA	+ 40	3	144	40	0.79	0.41	0.19
	- 60	3	161	40	0.88	0.44	0.15
2',5'-diN ₃ araA	+ 40	10	144	40	0.73	0.46	0.10
	- 60	10	144	38	0.91	0.66	0.02

protons H5'A and H5'B. Such an assignment in our opinion is not presently possible for all the analogues studied here [1]. Equations (6) and (7) were derived using the parameters from Eqn (2). The approximations involved with Eqn (2) are thus also introduced into the determination of $[g^+]$, $[t]$ and $[g^-]$.

Considering the approximations underlying Eqns (2), (6), and (7) the mole fractions of the arabinose conformers should be accurate to ± 0.05 .

The most drastic changes in the parameters of the Karplus equation are to be expected with 3'FaraA in which the extremely electronegative fluorine substituent is introduced into the sugar. For fluoroethane Pachler [28] concluded that Eqn (2) has to be modified through the addition of $\sin \Phi$ terms to accommodate the fluoro substituent effects. This modification can lead to changes of +2 to -1 Hz in the vicinal coupling constants depending on the

dihedral angles involved. Analysis of the experimental coupling constants from 3'FaraA with the standard (Eqn (2)) for the modified Karplus equation leads to the conclusion that the $N \rightleftharpoons S$ equilibrium calculated using both sets of parameters is only marginally influenced owing to a fortuitous cancellation of the different contributions.

In addition, a through-space interaction depending only on the distance between a fluorine and a proton can cause significant changes in the vicinal proton-proton coupling constants observed for a fixed dihedral angle [29, 30]. Inspection of the distances between the protons and the fluorine in 3'FaraA indicates that H1' and H5'A,B approach the fluorine closely enough to be subjected to this interaction. It is presently unclear whether this interaction makes a significant contribution to the observed couplings. However, calculations involving changes in the con-

former mole fractions of N , S , g^+ , t and g^- made under the most unfavorable assumptions predict a negligible influence on the $N \rightleftharpoons S$ equilibrium, but indicate an increase in the population of the g^+ -rotamer by $\Delta[g^+] \approx 0.12$. The data included in Table II are calculated with this correction. In Fig. 9 the $[g^+]$ fractions calculated with and without this correction are plotted.

Syn \rightleftharpoons *anti* equilibrium of the base

The position of this internal glycosyl rotamer equilibrium was derived from analysis of the longitudinal relaxation rates R_d of the individual protons. The experimental R_d values are given in Table III. The procedure for this calculation has been described in detail [31, 32]. Among the compounds studied here, 2'ClaraA was chosen for determination of the relaxation rates. Its proton HRNMR spectrum is sufficiently resolved at 100 MHz to make an analysis of the individual R_d values meaningful. AraA and 3'FaraA are the only two compounds that show pronounced differences in the $N \rightleftharpoons S$ equilibrium compared to 2'ClaraA. However, they cannot be analyzed at 100 MHz since the chemical shift differences between the individual protons are too small. The individual proton relaxation rates R_d are given by [31, 32]

$$\frac{R_d}{56.9 \tau_c} [\text{\AA}^{-6}] = \frac{R_d}{\gamma^4 \hbar^2 \tau_c} = \left\{ [N] \sum_{n \neq d} r_{dn}^{N-6} + [S] \sum_{n \neq d} r_{dn}^{S-6} \right\} \quad (8)$$

In the left hand side of Eqn (8) τ_c was evaluated in units of 10^{-10} s. r_{dn}^N and r_{dn}^S are the distances in Ångströms between the individual protons in the N - and S -states. Distances involving only the arabinose protons and the $[N]$ and $[S]$ equilibrium can be determined using the solid state X-ray data in combination with the present analysis of the proton high resolution spectra. The distances between the arabinose protons and the base protons H8 and H2, as well as the other base atoms, can be calculated as a function of the glycosyl torsion angle T from these results. This calculation was effected using the computer program COORD (QCPE no. 136). The results are compiled in Figs. 5 and 6.

It has been shown previously, that the rotational diffusion of the nucleoside can be described at -60°C in ND_3 by a single correlation time τ_c [35].

The relaxation data, then, can be reduced to a function of the distances between H8 and the arabinose protons, and the correlation time τ_c .

Since τ_c is unknown, the ratios of the relaxation rates of the different pairs of protons given by

$$\frac{R_d}{R_{d'}} = \frac{[N] \sum_{n \neq d} r_{dn}^{N-6} + [S] \sum_{n \neq d} r_{dn}^{S-6}}{[N] \sum_{n \neq d'} r_{d'n}^{N-6} + [S] \sum_{n \neq d'} r_{d'n}^{S-6}}$$

were calculated together with the individual $R_d/$ ($56.9 \tau_c$) values as functions of the glycosyl torsion angle. These results are compiled in Figs. 7 and 8. Since 2'ClaraA was found to exist almost exclusively in the $N-g^+$ state ($[N] = 0.97$, $[g^+] = 0.91$), only this conformation was used for evaluation of the relaxation rates. Comparison of the experimental ratios of the different relaxation rates with the curves of Fig. 8 shows that satisfactory agreement is obtained for the ratios R_d/R_8 for only small regions of the *syn* and *anti* ranges. The *syn* range can, however, be excluded because of the poor fit obtained for the three experimental ratios R_1/R_2 , R_1/R_3 and R_2/R_3 . These three ratios are consistent with an assignment of the base to the *anti* range $T \approx 165$ ($\chi \approx 105^\circ$) only. The region of the glycosyl torsion angle between $T \approx 240$ to 360° ($\chi \approx 0$ to -120°) is also excluded for steric reasons. This latter range would demand distances between $\text{O}2'$ or $\text{H}3'$ and $\text{N}3$ in an arabinose N -state that are well below the sum of the Van der Waals radii (cf. Fig. 6). Satisfactory agreement between the experimental and calculated relaxation rates of Fig. 7 is obtained for a correlation time of $\tau_c = 94$ ps. The comparison is given in Table IV.

Table III. Longitudinal relaxation rates of the single protons of 2'ClaraA dissolved in ND_3 at -60°C .

Proton	H2	H8	H1'	H2'	H3'	H4'	H5'
R [1/sec]	0.038	0.36	0.43	0.56	0.56	0.86	1.85

Table IV. Comparison of experimental and calculated reduced longitudinal relaxation rates $R_d/(56.9 \tau_c)$ of 2'ClaraA. The calculations were performed for $T = 165^\circ\text{C}$ ($\chi = 105^\circ\text{C}$) with the arabinose in $N-g^+$.

Proton	$R_d/56.9 \cdot \tau_c [\text{\AA}^{-6}]$	
	Experimental for $\tau_c = 94$ ps	Calculated for $T = 165^\circ$
H8	0.0067	0.0063
H1'	0.0080	0.0093
H2'	0.0105	0.0113
H3'	0.0105	0.0113

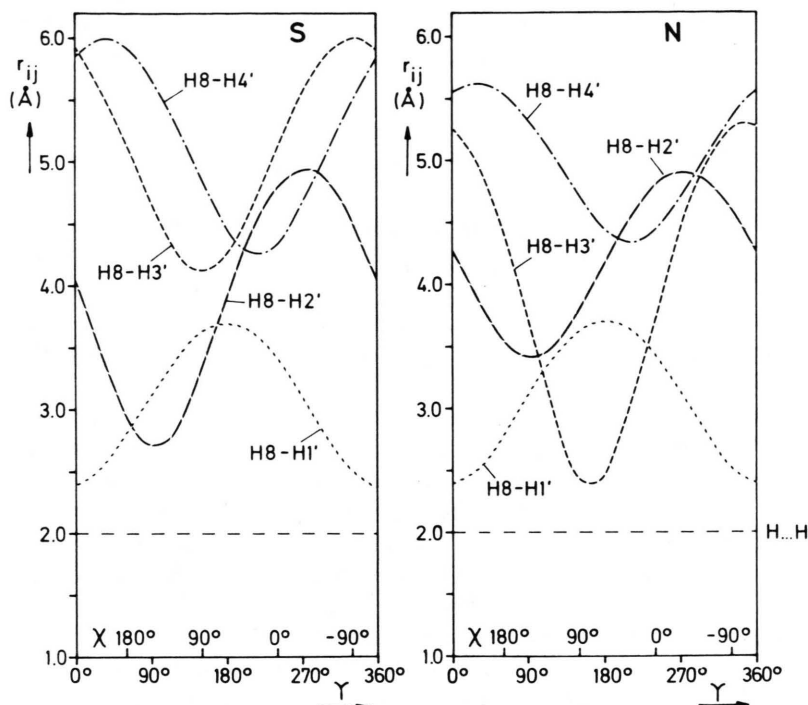


Fig. 5. Distances between the arabinose protons H1' to H4' and the base proton H8 as a function of the glycosyl torsion angle τ in the *N*- and *S*-states. The horizontal broken line at 2.0 Å is the Van der Waals' contact distance between two hydrogens. (Definition of the glycosyl torsion angle τ according to Davis and Hart [33]; χ is defined as proposed by Sundaralingam [34].)

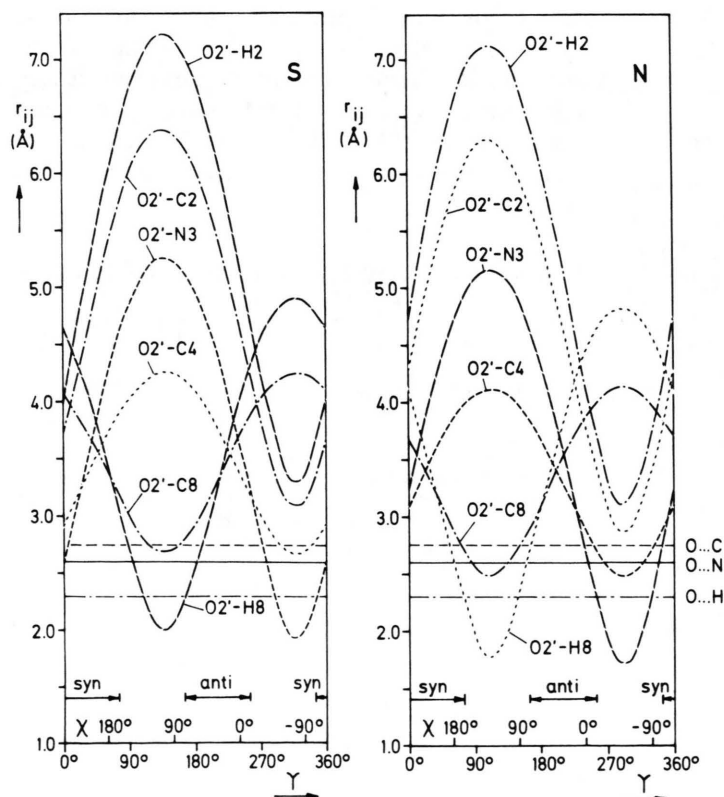


Fig. 6. Distances between atoms of the adenine moiety and the 2'-hydroxyl group of araA in the *N* and *S* states as a function of the glycosyl torsion angle τ . (Definition of the glycosyl torsion angle τ according to Davis and Hart [33]; χ is defined as proposed by Sundaralingam [34].) The horizontal lines are the Van der Waals' contact distances between the different atoms.

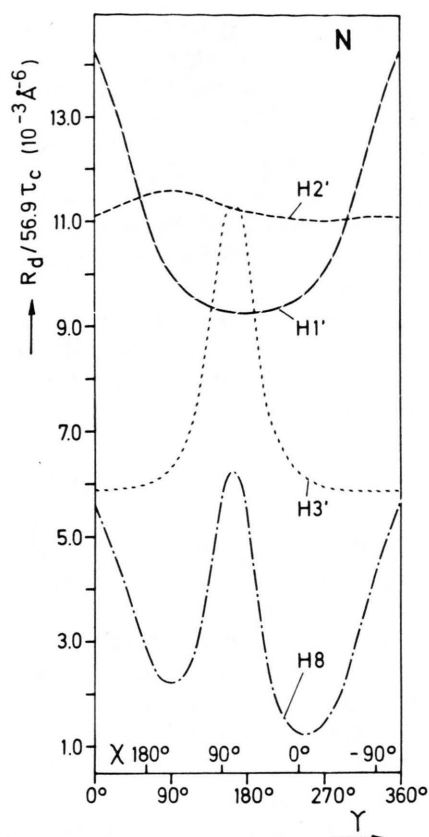


Fig. 7. Calculated reduced relaxation rates as a function of the glycosyl torsion angle for the four different protons used in the determination of the glycosyl torsion angle. Rates are calculated for a N - g^+ conformation of the arabinose ring.

Discussion

In araA at +40 °C, the N and S states are approximately equally populated. Lowering the temperature shifts the equilibrium in the direction of the N -state. With the exception of 2'dA and 3'FaraA, all chemical substitutions lead to a significant stabilization of the N -state regardless of whether the substitution occurs at the 2' or 3' position. This observation is in contrast with the results found for the ribonucleosides [1]. In that series 2' substitution stabilized the S -state whereas all 3' modified analogues showed a preference for the N conformation range.

In general the N -state is markedly preferred in the arabinosides in comparison with the ribosides. This is in accord with theoretical predictions [9, 10].

Fig. 9 illustrates the correlation between the arabinose ring conformation and the conformation of the exocyclic 5'CH₂OD group for all 2' and 3' substituted compounds studied.

It is apparent that all the nucleosides with a large mole fraction of N conformers have a pronounced preference for the g^+ rotamer. This correlation is similar to that observed for the ribosides [1]. The only analogue with a pronounced preference for the S -state, 3'FaraA, shows a greater depopulation of the g^+ rotamer than in the ribosides where for $[S] \rightarrow 1$ $[g^+] \rightarrow 0.4$.

The data clearly indicate that the S - g^+ state is not populated significantly in solution. This

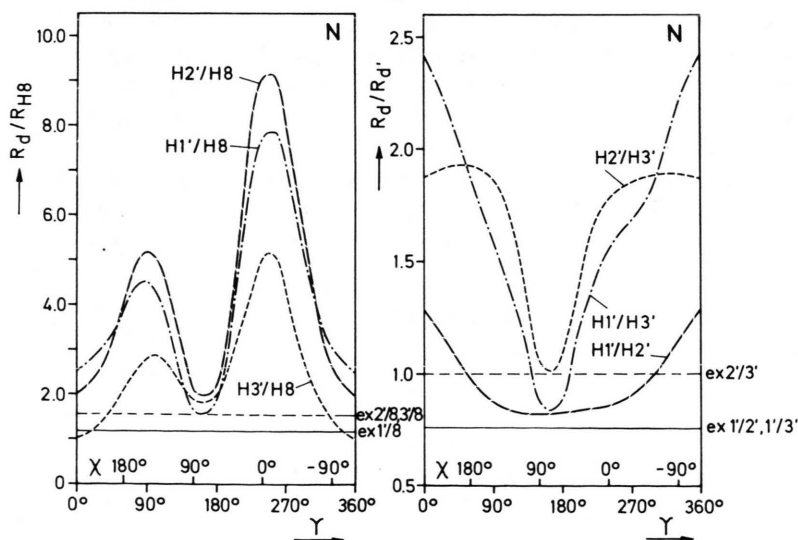


Fig. 8. Ratios of the longitudinal relaxation rates of the protons H1', H2', H3', and H8 as a function of the glycosyl torsion angle. Rates are calculated for a N - g^+ conformation of the arabinose ring. The horizontal lines are the experimental ratios for the different pairs of protons.

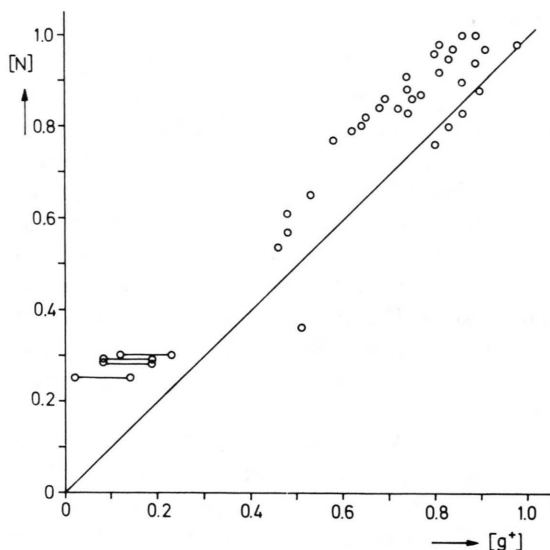


Fig. 9. Correlation between the rotamer population $[g^+]$ of the exocyclic $5'\text{CH}_2\text{OD}$ group and the mole fraction $[N]$ of the furanoside ring of the arabinoside analogues.

undoubtedly results from the fact that in this conformation the distance between the $2'$ and $5'$ hydroxyl groups would become smaller than the sum of their Van der Waals radii. A $S-g^+$ conformation has been observed for some single crystals of arabinonucleosides [7] and also appears as an energy minimum in PCILO [9] and semiempirical calculations [10]. Clearly it must draw its stabilization from a hydrogen bond between $\text{O}2'\text{-H} \dots \text{O}5'$. It can be concluded that in solution the steric repulsion between the two hydroxyl groups must be larger than the attractive interaction of a hydrogen bond. In D_2O and ND_3 the solvent molecules also can compete intermolecularly in hydrogen bonding as donors and acceptors thus preventing the formation of stable intramolecular $\text{O}2'\text{-H} \dots \text{O}5'$ hydrogen bonds.

Summarizing the results of the present conformational analysis, it is concluded that the dissolved arabinosyladenine analogues reveal a clear and strong preference for the anti- $N-g^+$ conformation. Neither araA nor 3'FaraA, the two substances with a high population of the S -conformer, is suitable for an investigation of the longitudinal relaxation rates with our instrumentation since at 100 MHz the spectra are not sufficiently resolved. It is thus impos-

sible to derive any information regarding the glycosyl torsion angle for the S -state analogues from the present data. If the trend observed with 3'FaraA and araA can be generalized, the S -arabinose range will have $[t] \approx [g^-] \approx 0.5$.

The analysis of the data given above does not yield any physical explanation for the pronounced influence that the epimeric arabinose modification exerts on the $N \rightleftharpoons S$ equilibrium. As shown in Fig. 10, the observations can neither be correlated quantitatively with changes in the electronegativity of the substituents nor with their steric requirements. The same lack of correlation was observed with the $2'$ and $3'$ modified ribosides [1].

Changes in the $N \rightleftharpoons S$ equilibrium with different substituents may, however, be explained qualitatively. All substituents, except in 2'dA and 3'FaraA, produce an enhancement of the population of the N -state. This is the conformation in which the substituents are in a quasiequatorial orientation or, regarding the Newman projections, where the substituents lie anti with respect to the relevant ring atoms ($\text{C}4'$ and $\text{O}4'$ for substitution at $\text{C}2'$, and $\text{C}1'$ and $\text{O}4'$ for substitution at $\text{C}3'$). Obviously in this conformation steric interactions are minimized. All the substituents except hydrogen (2'dA) and fluorine (3'FaraA) have larger Van der Waals' radii than the hydroxyl group in unsubstituted araA. It is therefore plausible that the conformational equilibria would be shifted towards N in the analogues. The strong preference of 3'FaraA for the S state may be rationalized as the result of an electrostatic interaction. The $\text{C}3'\text{-F}$ and $\text{C}2'\text{-OH}2'$ bonds form dipoles with the negative ends at F and OH. Evaluation of the interaction energies between these two dipoles in the N - and S -states indicates that the N conformation with F3' gauche to OH2' has an energy several hundred cal/mol higher than the S -state in which F3' is anti to OH2'. This might be sufficient to provide the strong preference for the S state. This qualitative explanation may also be valid for the adenosine (ribo) analogues in which the substituents also prefer the orientation anti to the ribose ring atoms.

A summarizing discussion of the effects observed in the different pentofuranosyladenine nucleosides will be presented at the conclusion of the accompanying paper [2] when the results for the xylo analogues have been analyzed.

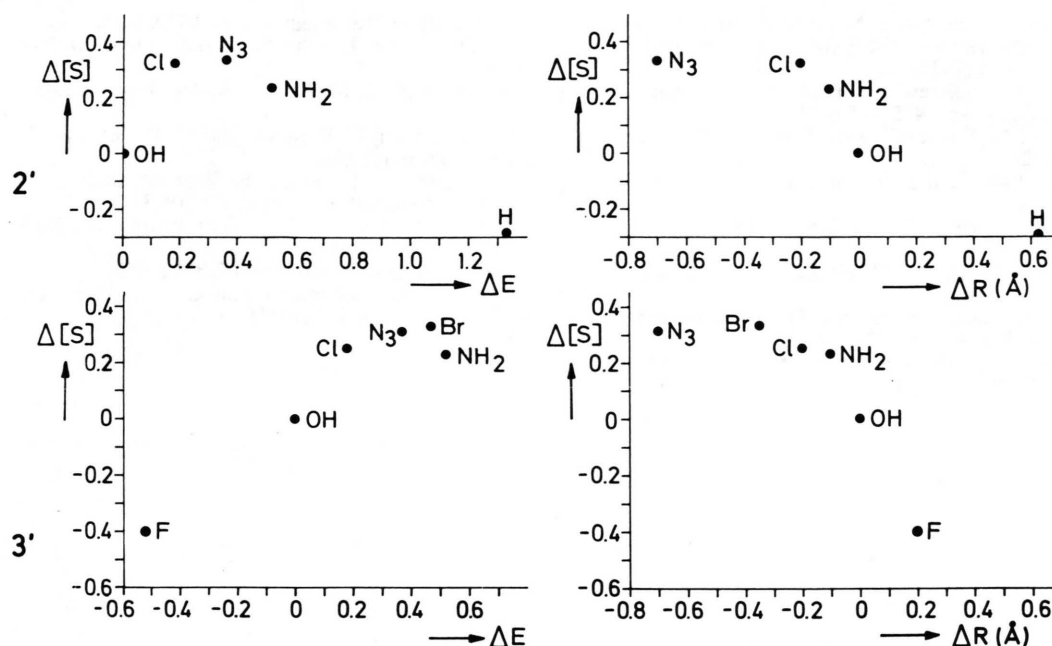


Fig. 10. Left side: Plot of the mole fractions $\Delta[S] = [S_{OH}] - [S_R]$ vs. the difference of the electronegativities $\Delta E = E_{OH} - E_R$ of the substituents for the 2' and 3' derivatives at -60°C . Right side: Plot of the mole fractions $\Delta[S]$ vs. the difference of the Van der Waals' radii $\Delta R = R_{OH} - R_R$.

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neering Research Council (A 5890) is gratefully acknowledged. The calculations necessary for the evaluation of the data were performed at the Computer Center of the Universität Regensburg.

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