
Nucleoside conformation is determined by the electronegativity of the sugar substituent

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ABSTRACT

The proton and ^{13}C NMR spectra of uridine, deoxyuridine and four 2'-substituted uridines (dUn, dUz, dUcl and dUfl) are reported. A linear relationship between the electronegativity of the 2'-substituent and the carbon-13 chemical shift of C_2 , is observed. Taking into account the effect of electronegativity by using the correction proposed by Karplus or by Jankowski, the proton-proton coupling constants have been used to compute the conformational equilibria of the six uridines. It is shown that the contribution of the N form (3'-*endo*-2'-*exo*) increases with the electronegativity of the 2' substituent. Thus dUfl contains some 85 % N form in solution. - Applying similar corrections to published data in the adenosine series, a similar correlation is observed. This observation, that the most polar substituent pulls the pucker to its side, holds also for 3'-substituted compounds, like cordycepin(3'dAdo) and 3'-deoxy-3'-amino-adenosine. It is suggested that the influence of the electronegativity could be the dominating effect in nucleoside conformations and would also hold for arabinosides and xylosides. This effect should therefore also be the principal force which determines the differences between DNA and RNA.

The chemical difference between DNA and RNA, i.e. H vs. OH, is now considered not to be the determining factor in the vast biochemical and structural differences of these two nucleic acids. The demonstration that 2'-O-methyl polynucleotides showed great similarities with polynucleotides^{1,2} brought about a great interest in 2'-substituted nucleosides³⁻⁹ and polynucleotides⁹⁻¹³. These studies have established that the nature of the 2'-substituent has an important, if not determining influence on the structure and conformation of polynucleotides.

Before attempting to analyse in detail the structure of such polynucleotide analogues, the conformation of the monomers in solution has to be established in terms of the equilibrium of the canonical N and S conformers^{14,15}. The main tool for the study of nucleoside conformations is high resolution Nuclear Magnetic Resonance (NMR). The differences in electronegativities have,

however, well-known effects on chemical shifts and coupling constants.

In the present report we have analyzed a series of 2'-deoxy-2'-substituted uridines by ^1H -NMR and ^{13}C -NMR and have attempted to account for the large differences of the polarities of the 2'-substituents. We have found a linear correlation between the electronegativity of the substituent and the ^{13}C chemical shift of the C_2 , carbon atom to which the substituent is attached. More detailed analysis of the proton NMR spectra revealed a very strong dependence of the conformational equilibria on the electronegativity of the 2'-substituent.

When this work was terminated, a similar study on 2'-substituted adenosines appeared¹⁶, the conclusions of which concord perfectly with the present results.

MATERIAL AND METHODS

Nucleosides: 2'-Deoxy-uridine (dU), uridine (rU) and 2'-deoxy-2'-azido-uridine (dUz) were purchased from Sigma, Fluka and Boehringer-Mannheim, respectively. 2'-Deoxy-2'-amino-uridine (dUn) was obtained by catalytic reduction of dUz¹⁷; 2'-deoxy-2'-chloro-uridine (dUcl) was synthesized according to Greenberg and Moffatt⁵; 2'-deoxy-2'-fluoro-uridine (dUfl)⁷ was obtained either by deamination of dCfl¹⁸ or by the method of Coddington *et al.*¹⁹. The purity of the compounds was checked by NMR and by mass spectrometry (Hitachi RM50 GC-MS system).

NMR measurements: ^1H -NMR spectra were recorded on a CAMECA TSN 250 spectrometer in the FT mode; spectra in $^2\text{H}_2\text{O}$ were measured with DSS (2,2-dimethyl-2-silapentane-5-sulfonate) as internal reference, spectra in dimethylsulfoxide (DMSO) with TMS (tetramethylsilane) as internal reference. ^{13}C -spectra were recorded in $^2\text{H}_2\text{O}$ on a Varian XL-100 or CFT-20 spectrometer with TMS as internal standard. All proton spectra were simulated using LAOCOON III.

RESULTS

Proton and ^{13}C NMR Spectra of 2'-substituted Uridines

The high resolution (250 MHz) proton NMR spectra of the six 2'-substituted uridines were measured in $^2\text{H}_2\text{O}$ and DMSO. As an example, the ^1H -NMR spectrum of the sugar protons of dUcl in DMSO is shown in figure 1, together with its simulation by LAOCOON III. The chemical shifts and coupling constants are summarized in Table I. The data (for dU, rU and dUfl) compare well with those in the literature^{3,7,20-23}. Particularly noteworthy are the

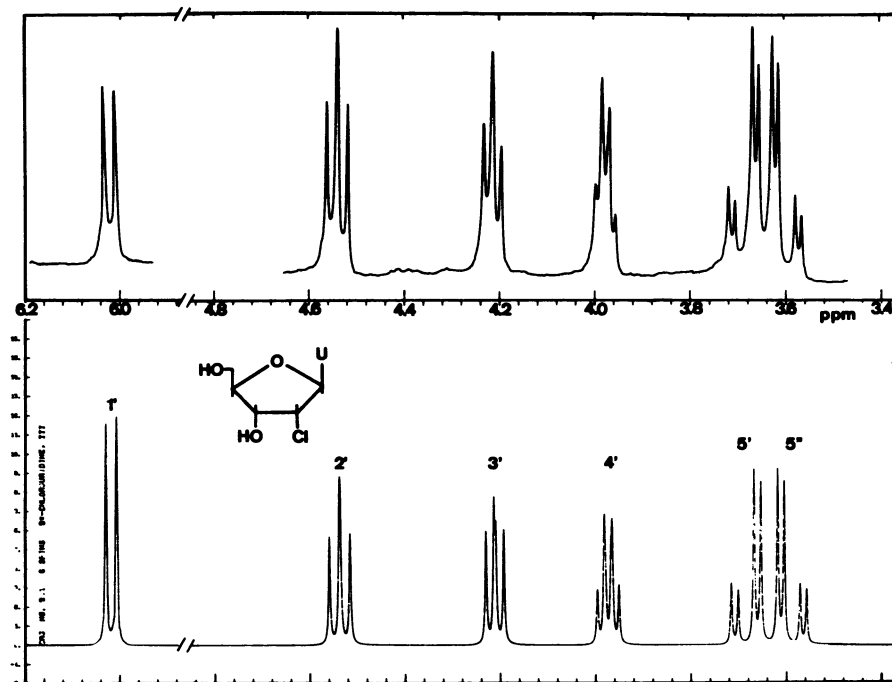


Figure 1: 250 MHz proton NMR spectrum of the sugar protons of dUcl in DMSO (internal reference TMS). LAOCOON III simulation (lower part) is also included.

large changes in the conformation dependent coupling constants^{14,15}, $J_{1,2'}$, and $J_{3,4'}$. The chemical shifts of the $H_{2'}$ protons show large variations too. Both these parameters follow roughly the electronegativity of the 2'-substituent, although large scatter is observed (fig. 2). The data available in the literature have also been included in fig. 2 and do not show any better agreement as those from the present study. It can be seen that the scatter in fig. 2a does not follow the scatter in fig. 2b. This is not surprising, since the coupling constants $J_{1,2'}$ are primarily conformation dependent^{14,15}, while the chemical shifts of $H_{2'}$ are largely influenced by the shielding (or deshielding) by the neighbouring base²⁴, which in turn is dependent on the *syn-anti* equilibrium.

The ^{13}C -NMR data are summarized in Table II. The chemical shifts of uridine agree reasonably well with those of Jones *et al.*²⁵, except that we find an inversion of the C_2 and C_3 resonances. The chemical shifts and

TABLE I: ^1H Chemical shifts and $^3J_{\text{HH}'}$ (or $^3J_{\text{HF}}^{\text{a}}$) coupling constants of 2'-substituted uridines.

	Spectra in $\text{D}_2\text{O}^{\text{b}}$						Spectra in DMSO^{c}			
	dU	dUn	dUz	rU	dUcl	dUfl	dU	rU	dUcl	dUfl
δ										
H^6	7.839	7.845	7.874	7.866	7.965	7.819	7.829	7.882	7.874	8.369
H^5	5.875	5.907	5.888	5.883	5.900	5.862	5.615	5.651	5.888	6.103
H_1	6.272	5.893	5.926	5.901	6.129	6.006	6.141	5.918	6.018	6.392
H_2	2.363	3.561	4.344	4.339	4.650	5.229	2.063	4.357	4.537	5.506
$\text{H}_{2''}$	2.404						2.114			
H_3	4.448	4.206	4.448	4.215	4.424	4.391	4.224	4.229	4.211	4.634
H_4	4.035	4.145	4.094	4.120	4.216	4.134	3.783	4.136	3.973	4.369
H_5	3.821	3.802	3.915	3.900	3.934	4.008	3.565	3.916	3.679	4.230
$\text{H}_{5''}$	3.744	3.788	3.795	3.795	3.829	3.825	3.537	3.804	3.595	4.068
J										
$5-8$	8.1	8.0	8.1	8.0	8.1	8.1	8.2	8.2	8.1	8.1
$1'2'$	6.25	7.6	4.6	4.2	4.8	1.5	7.2	5.0	5.7	1.75
$1'2''$	6.35					19.7^{a}	6.5			17.4^{a}
$2'2''$	-14.2					52.7^{a}	-12.9			53.0^{a}
$2'3'$	6.25	5.6	5.7	5.3	5.7	5.1	5.2	5.5	5.2	5.05
$2''3'$	4.3					21.6^{a}	3.8			20.1^{a}
$3'4'$	4.0	2.7	6.0	5.7	4.9	8.7	3.1	4.7	4.25	7.5
$4'5'$	3.4	3.0	3.0	3.1	2.9	2.3	3.7	3.4	3.7	2.4
$4'5''$	5.1	4.0	4.2	4.3	3.9	4.4	3.7	4.6	3.35	3.15
$5'5''$	-12.6	-13.0	-12.9	-12.9	-13.1	-12.9	-11.9	-12.1	-11.8	-12.4

^b downfield from internal DSS; ^c downfield from internal TMS.

coupling constants are essentially the same between the different compounds, except those involving C_2 . If the C_2 chemical shifts are plotted as a function of electronegativity, an excellent linear relation is obtained (fig. 3a), while the correlation with the $\text{C}_2\text{-H}_2$ couplings is less good.

All these variations appear to be correlated with the electronegativity ϵ_{R} of the 2'-substituent. Taking the electronegativities of hydrogen ($\epsilon_{\text{H}}=2.2$)

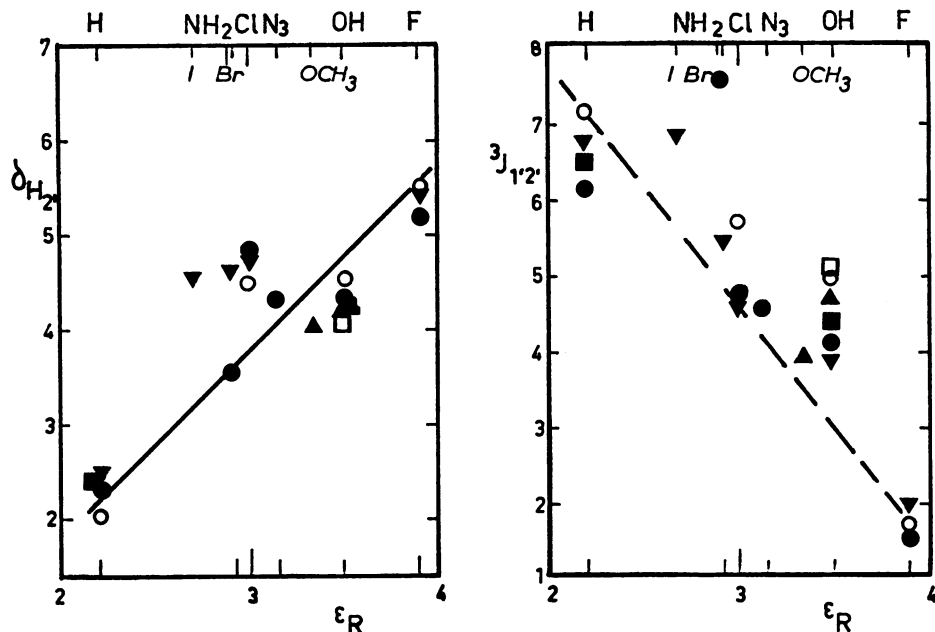


Figure 2: Dependence of proton NMR parameters on electronegativity (ϵ_R) of the 2'-substituent of 2'-substituted uridine analogues: left: left: chemical shifts of $\text{H}_{2'}$; right: coupling constants $J_{1'2'}$. Closed symbols: in $^2\text{H}_2\text{O}$, open symbols: in DMSO. ● this work, ▲ data of Hruska *et al.* (ref. 21), ▼ data of Cushley *et al.* (ref. 3), ■ data of Deslauriers and Smith (ref. 22).

and of fluorine ($\epsilon_F=3.9$) of Huggins²⁶ as references, "apparent electronegativities" of the four other substituents have been computed from the various NMR data. These results are summarized in Table III and are compared with the Huggins and Pauling electronegativities, as well as with those determined by Cavanaugh and Dailey²⁷ and Muller²⁸ from methyl shifts of substituted hydrocarbons. By far the best agreement is obtained from ^{13}C chemical shifts of C_2 , while the proton shifts and ^{13}C coupling constants give much less satisfactory results. The empirical relationship between the electronegativity ϵ_R and the ^{13}C chemical shifts δ_2 , is given as follows

$$\epsilon_R = 0.034 \cdot \delta_2 + 0.84 \quad (1)$$

Conformational Analysis of substituted Uridines.

In order to compare the proton coupling constants of Table I in detail and extract from them the conformational parameters, the electronegativities

Table II: ^{13}C chemical shifts and $^1J_{\text{CH}}$ coupling constants of 2'-substituted uridines.

		dU	dUn	dUz	rU	dUc1	dUf1
δ	C ⁶	142.9	144.4	144.1	143.5	139.8	142.9
	C ⁵	103.0	105.4	105.0	104.4	102.0	102.6
	C ⁴	167.0	168.9	168.6	168.8	162.9	166.7
	C ²	152.4	154.9	154.0	154.3	150.5	151.7
	C _{1'}	87.7	91.3	90.4	92.0	87.9	90.6
	C _{2'}	39.7	59.7	68.2	76.4	61.9	90.2
	C _{3'}	71.4	73.8	72.6	72.2	69.1	68.2
	C _{4'}	87.4	88.4	87.1	86.9	84.9	83.1
	C _{5'}	62.2	64.2	63.0	63.5	60.1	60.5
J	C ⁶	183.7	183.7	185.7	181.2	185.0	180.0
	C ⁵	178.7	178.0	178.4	178.0	173.0	178.0
	C _{1'}	170.0	168.0	173.1	169.3	173.0	171.0
	C _{2'}	135.0	144.0	152.4	154.2	151.3	169.1
	C _{3'}	150.0	149.4	149.9	149.0	148.8	150.2
	C _{4'}	150.0	150.7	151.2	149.5	150.0	152.0
	C _{5'}	142.5	143.2	143.8	142.0	145.0	144.2

Spectra in $^2\text{H}_2\text{O}$, internal reference TMS; chemical shifts in ppm, coupling constants in Hz.

of the 2'-substituents have to be taken into account.

Since the vast majority of the NMR data of nucleosides and nucleotides has been obtained for ribosides^{29,30}, we have chosen to refer to the ribosides as the standard. In this case the average values of coupling constants found are in a very narrow range: $(J_{1',2'} + J_{3',4'}) = 9.7 \pm 0.2$ Hz, $J_{2',3'} = 5.3 \pm 0.2$ Hz. Inspection of Table I shows that these values are well respected in the "middle range", but deviate considerably for the extremes, i.e. dU, dUn, dUf1.

The original Karplus equation³¹ has been modified to include two angular terms^{15,32} and is mostly used in the form

$$J_{\text{HH}'} = A \cdot \cos^2 \phi_{\text{HH}'} + B \cdot \cos \phi_{\text{HH}'} + C \quad (2)$$

For ribonucleosides and nucleotides the optimal values determined from a

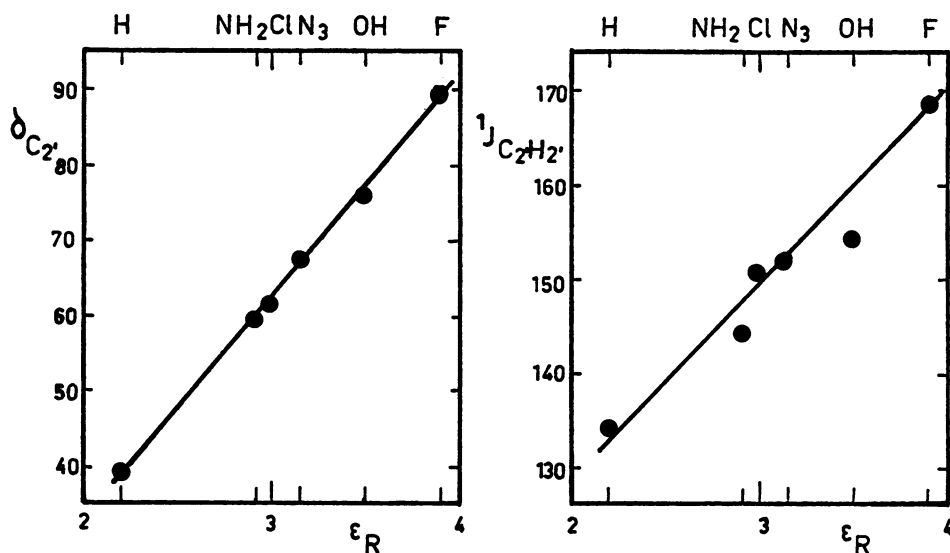


Figure 3: Dependence of ^{13}C -NMR parameters of 2'-substituted uridines on electronegativity (ϵ_R) of the substituent. Left: chemical shifts of C_2' ; right: coupling constants $J_{\text{C}_2'\text{H}_2'}$.

Table III: Electronegativities of various substituents determined by different methods

Substituent:	H	NH_2	N_3	OH	Cl	F
Pauling	2.1	3.0	3.0	3.5	3.0	4.0
Huggins ²⁶	2.2	3.05	3.05	3.5	3.15	3.9
Cavanaugh & Dailey ²⁷	2.2	2.91		3.43	3.25	3.9
Muller ²⁸		2.96-3.05		3.42	3.20	3.76
This work:						
a)	2.2	2.87	3.16	3.44	2.95	3.9
b)	2.2	2.64	3.07	3.10	3.05	3.9
c)	2.2	2.93	3.33	3.33	3.42	3.9

a) From ^{13}C chemical shift of C_2' , of 2'-substituted uridines (fig. 3a).

b) From ^{13}C coupling constants $\text{C}_2'-\text{H}_2'$, of 2'-substituted uridines (fig. 3b).

c) From ^1H chemical shifts of H_2' , of 2'-substituted uridines (fig. 2a).

large number of NMR data of ribonucleosides are $A = 10.0$, $B = -1.2$, $C = 0$.

Several possibilities exist to take into account the effects of electronegativity or substitution effects. Karplus³² had proposed a multiplicative correction term

$$J_{HH'}^{obs} = J_{HH'}^{corr} \cdot (1 - \alpha \cdot \Delta\epsilon_R) \quad (3a)$$

$$\text{and } J_{HH'}^{corr} = J_{HH'}^{obs} / (1 - \alpha \cdot \Delta\epsilon_R) \quad , \quad (3b)$$

where α is a constant and $\Delta\epsilon_R$ is the difference between the electronegativity of the substituent and that of hydrogen, $\Delta\epsilon_R = \epsilon_R - \epsilon_H$. Several values for α have been suggested, an average value $\alpha = 0.07$ appears the most justified in systems where rapid blends of conformations occur, and where therefore the protons under consideration can be equatorial or axial.³² We have, however, used in eq. (3) $\Delta\epsilon_R = \epsilon_R - \epsilon_{OH}$, since the coefficients A , B and C have been established for the riboside system.

An alternative mode consists of varying the additive term C ^{33,34} in equ. (2):

$$J_{HH'}^{obs} = A \cdot \cos^2 \phi_{HH'} + B \cdot \cos \phi_{HH'} + C \cdot (J_{CH} + J_{C'H'}) \quad (4a)$$

$$\text{or } J_{HH'}^{obs} = A \cdot \cos^2 \phi_{HH'} + B \cdot \cos \phi_{HH'} + C \cdot (\delta_C + \delta_{C'}) \quad , \quad (4b)$$

i.e. by using the sum of the chemical shifts of the connecting carbon atoms or their $^1J_{CH}$ coupling constants as the monitoring parameters. As can be seen from Table II and fig. 3 the chemical shifts and coupling constants are highly sensitive to electronegativity effects, being on the same time dependent on the hybridization.

These corrections have been applied to the data in Table I; using the computational or graphic procedure described elsewhere³⁰, the conformational equilibria of uridine and its derivatives were computed. The results obtained when using the electronegativity correction of Karplus (eq. 3) are shown in Table IV and compared with the relevant x-ray data from the literature. Use of the additive corrections (eq. 4) gave consistently the same conformational equilibria x_N , but frequently considerably higher τ_m values. The last possible monitoring parameter, δ_H , is also giving high τ_m values:

$$J_{HH'}^{obs} = A \cdot \cos^2 \phi_{HH'} + B \cdot \cos \phi_{HH'} + C \cdot (\delta_H + \delta_{H'}) \quad (4c)$$

The use of δ_H as correction parameter has been previously studied and its limited applicability to other than sp^2 - sp^3 systems was concluded^{35,36}.

The consistency of the pucker amplitude of the solution data compared with the x-ray results (Table IV) is noteworthy, as well as the similarity of the phase angles, $^N P$ and $^S P$, respectively. The increase of the contribution of the N conformer with the increasing electronegativity of the 2'-substituent

Table IV: Conformational equilibria and parameters of 2'-substituted Uridines in solution compared with conformations in crystals

Compound	ϵ_R	solution				ref.	x-ray (b)			
		solvent	τ_m	N_P	S_P		τ_m	P	conformation	ref.
dU	2.2	^2H_2O	37	18	162	0.40	(a)	38.6	173.0	$^2T_3$ 37
		DMSO	42	9	171	0.32	(a)	36.5	177.9	$^2T_3$ 37
		^2H_2O	35	24	156	0.38	20			
		pyridine - ^2H_2O	37	17	163	0.31	24			
dUn	2.9	^2H_2O	39	19	161	0.25	(a)			
dUz	3.2	^2H_2O	40	24	156	0.58	(a)			
rU	3.5	^2H_2O	39.5	18	162	0.58	(a)	40.4	3.7	$^3T_2$ 38
		DMSO	38	18	162	0.48	(a)	42.4	13.8	$^3T_2$ 38
		^2H_2O	41	21	159	0.53	21			
		DMSO	40	18	162	0.56	22			
dUcl	3.0	^2H_2O	38	14	166	0.51	(a)	39.0	157.0	^2E 39
		DMSO	41	12	168	0.44	(a)			
dUfl	3.9	^2H_2O	41	21	159	0.87	(a)	38.2	70.8	$^0T_4$ 40
		DMSO	38	12	168	0.81	(a)			
		^2H_2O	42	18	162	0.80	3			
dUfl(ac) ₂	3.9	DMSO	37	12	168	0.82	7	34.9	38.2	$^3T_4$ 6
dUom	3.4	^2H_2O	39	18	162	0.60	21			

(a) this work. (b) conformations computed using programme CONFOR¹⁵.
 Solution conformations have been computed using the electronegativity correction (eq. 3).

is also apparent and is plotted in fig. 3. It is apparent that this correlation is not a simple one, since solvent effects are important for dU, but are less so for the other uridine derivatives. Other effects but conformation are also suggested by the data in figs. 2 and 3.

Analogous Systems.

Only few other NMR data on 2'-substituted nucleosides exist in the literature, all of them on adenosine and its analogues^{8,16,41}. These data

Table V: Conformational equilibria and parameters of 2'- and 3'-substituted Adenosines.

Compound	ϵ_R	solvent	τ_m	N_P	S_P	x_N	ref.
dA	2.2	ND ₃	38	15	165	0.35	41
dAbr	2.95	DMSO	43	3	177	0.25	16
dAn	2.9	ND ₃	39	9	171	0.22	8
dAcl	3.0	DMSO	42	6	174	0.27	16
rA	3.5	DMSO	39	6	174	0.34	16
		ND ₃	40	9	171	0.47	41
dAfl	3.9	DMSO	44	3	177	0.75	16
3'dA	2.2	ND ₃	42.5	24	156	0.92	41
3'dAn	2.9	ND ₃	39	15	165	0.83	8

have been analyzed using the same electronegativity corrections as for uridines (eq. 3). The results are summarized in Table V. Here again, as in the case of uridines, the 2'-amino-analogue is the one with the highest preference for the S-form, while the 2'-fluoro-analogue prefers the N-form. This con-

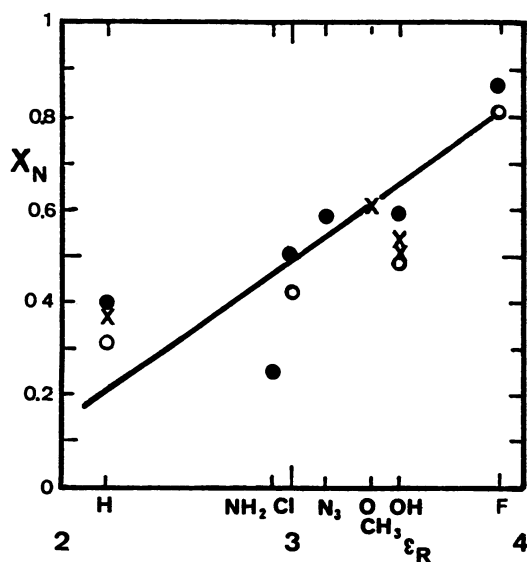


Figure 4: Mole fraction of N conformation (x_N) of 2'-substituted uridines as a function of the electronegativity (ϵ_R) of the substituent. O in DMSO; ● in ^2H_2O ; X data from the literature²⁰⁻²².

clusion has been reached in a simplified manner by Uesugi *et al.*¹⁶.

Most important are also the data on two 3'-substituted adenosine analogues which apparently follow the opposite trend: both 3'-deoxy-adenosine (cordycepin) and 3'-deoxy-3'-amino-adenosine have been shown to be preferentially in the 3'-*endo* (N) form by a German group^{8,41}.

DISCUSSION

The results of the present paper, as well as those in the literature (ref. 3,8,16,41) show without ambiguity the dominant influence of the polarity of the sugar substituent on the conformation of the pentose ring. As a corollary we can conclude that the most electronegative substituent pulls the pucker towards its side. This is apparently true for carbons C₂, and C₃. As a matter of fact, cordycepin is the nucleoside with the highest N conformer contribution observed so far.

In this line of reasoning, one would expect that the arabinosyl nucleosides should be preferentially in the S conformation, a prediction which appears to be verified, both in single crystal studies⁴² and in solution³. Here again the substitution of the 2'-OH group by fluorine should and does increase the S-conformer contribution³. In a similar line of thought, xylosides should prefer the N-form. It would be evidently desirable to investigate the theoretical basis of the results obtained.

From the data presented here it is also clear that the electronegativity is not solely responsible for the conformational effects observed. The consistent deviation of the 2'-deoxy and 2'-amino-analogues, dU and dUn (fig. 4) and dA and dAn (Table V), respectively, clearly indicate additional influences, for which only suggestions can be offered at present. The work of Bolton and Kearns^{43,44} which demonstrated that in polynucleotide structures the 2'-OH and 2'-OCH₃ groups can accept a water hydrogen bond, may be of interest in this connection. All substituents, except H, can in principle accept a water hydrogen bond. Although the dynamics of such a process will be extremely fast, the water binding could be conceivably quite different for the various substituents. Besides OH and OCH₃, F, N₃ and eventually NH₂ could be candidates for such binding.

The analysis of the nucleoside conformations gives a rationale to the polynucleotide studies¹⁰⁻¹³, in particular the finding that the poly-2'-fluoro-nucleotides form structures with the same or higher stabilities than polyribonucleotides, but did not at all resemble polydeoxynucleotides. This

clearly shows that steric effects are of little or no importance in polynucleotide structure. It could thus well be that the difference in electronegativity of H and OH is the dominating force in the differences between DNA and RNA.

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