Structure and Conformation of Pyrimidine Lyxofuranosides. 1-β-D-Lyxofuranosyluracil, 1-β-D-Lyxofuranosylcytosine, and Some O'-Methyl Derivatives[1]

Irena Ekiel,[2a] Edward Darzynkiewicz,[2a] George I. Birnbaum,*[2b] and David Shugar*[2a,c]

Contribution from the Department of Biophysics, Institute of Experimental Physics, University of Warsaw, 02-089, Warsaw, Poland, Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6, and Institute of Biochemistry and Biophysics, Academy of Sciences, 02-532 Warsaw, Poland. *Received November 20, 1978*

Abstract: ^{1}H NMR spectroscopy has been applied to a study of the conformation, in aqueous medium, of 1-β-D-lyxofuranosyluracil (*lyxo*-U), 1-β-D-lyxofuranosylcytosine (*lyxo*-C), and some O'-methyl derivatives of the latter. For the neutral molecules, as well as the protonated forms in the case of the *lyxo*-C derivatives, the pentose rings exhibit an $N \rightleftharpoons S$ type equilibrium, while the gauche-gauche rotamer populations of the exocyclic 5'-CH_2OH are relatively low (10–20%), as previously found for the corresponding xylofuranosyl nucleosides. For all the compounds, the three coupling constants for the pentose ring protons were interrelated, the resulting correlation being that expected for a two-state equilibrium, and implying a linear dependence of each of the three coupling constants on the conformer populations. In the solid state conformation of *lyxo*-U, established by X-ray diffraction, the pentose ring is in the rarely encountered twist form $_3T^2$, the exocyclic carbinol group is gauche-trans with no intramolecular hydrogen bonding, and the position of the aglycone about the glycosidic bond is anti (χ_{CN} = 27°). In aqueous strongly alkaline medium (pD ~14), where the 2'-OH and/or 3'-OH are ionized, the conformation of 3'-*O*-methyl-*lyxo*-C becomes predominantly C(2')endo and gauche-gauche, stabilized in this form by strong intramolecular hydrogen bonding, viz., O(5')-H···O(2')$^-$. Similar alkali-induced modifications occur with *lyxo*-C and *lyxo*-U, but to a lesser extent, probably because of simultaneous ionization of the 3'-OH. The conformational changes in 2'-*O*-methyl-*lyxo*-C in strongly alkaline medium argue against formation of an intramolecular hydrogen bond of the form O(5')-H···O(3')$^-$.

In a continuation of investigations on the structure and conformation of nucleoside analogues with potential antimetabolic activities,[3] we present here the results of a study, by means of ^{1}H NMR spectroscopy, of the solution conformations of 1-β-D-lyxofuranosyluracil (*lyxo*-U)[4] and *lyxo*-C, and of several analogues of the latter in which one or two of the sugar hydroxyls are blocked by methylation. Of particular interest are the conformations of the carbohydrate moieties and the possibility of intramolecular hydrogen bonding in a sugar ring where all three hydroxyls are in the "up" position. Both *ara*-C and *ara*-U in the solid state are C(2')endo and gauche-gauche, with such an intramolecular hydrogen bond, O(2')-H···O(5')-H, as in Figure 1a,[5] not observed in neutral aqueous solution.[3c,6] But in strongly alkaline medium, where ionization of the sugar hydroxyl(s) occurs, dissociation of the 2'-OH leads to a striking change in conformation from C(3')endo ⇌ C(2')endo to predominantly C(2')endo, with a simultaneous pronounced increase in the gauche-gauche population, readily interpretable in terms of stabilization of such a conformation by intramolecular hydrogen bonding of the form O(5')-H···O(2')$^-$, as in Figure 1b,[3c,7] also displayed by other β-arabinonucleosides in solution.[3d]

With xylofuranosylnucleosides, where the 3'-OH is in the "up" position, intramolecular hydrogen bonding between the 5'- and 3'-hydroxyls exists in the solid state for the neutral form of 1-α-D-2,2'-anhydroxylofuranosyluracil, which is C(3')endo and gauche-gauche,[3a] but not for other xylofuranosylnucleosides in solution, even at alkaline pH.[3e]

With lyxofuranosylnucleosides, intramolecular hydrogen bonding is theoretically feasible between the 5'-OH on the one hand, the 2'-OH and/or the 3'-OH on the other, the requisite geometrical conformations being C(2')endo, gauche-gauche and C(3')endo, gauche-gauche, respectively.

The present study consequently includes an X-ray analysis of the crystal structure of neutral *lyxo*-U. Lyxofuranosylnucleosides have not previously been subjected to such studies;[8] only two crystal structures have been reported for compounds containing a single, unfused, lyxofuranose ring.[9,10]

The biological activity of lyxofuranosylnucleosides has hitherto received rather limited attention. Although *lyxo*-A was found to exhibit only marginal inhibitory effects against L1210 leukemic cells, it was both deaminated and phosphorylated by murine tissue extracts.[11] The survival time of mice bearing the Ehrlich ascites carcinoma was increased by more than 50% on administration of 9-β-D-lyxofuranosyl-6-mercaptopurine.[11]

Experimental Section

lyxo-U was synthesized as described by Fox and Fescher.[12] The preparation of *lyxo*-C and its O'-methyl derivatives is to be described elsewhere.[13] D_2O (>99.9 mol % D), NaOD, and DCl were products of Merck (Darmstadt, GFR).

^{1}H NMR spectra were recorded on a Varian-100, or on a Bruker-90 operating in the Fourier transform mode, at 22 °C. Solutions were made up to concentrations of 0.05–0.15 M in D_2O at pD >6 to give the neutral forms. In a number of instances, in order to improve signal resolution for appropriate analyses of the spectra, it was necessary to use the DCl salts, so that pD ~4. Comparisons between spectra at pD values of about 6.5 and 4 demonstrated that this only minimally affected sugar ring and exocyclic group conformations.

The anionic forms with the sugar hydroxyl(s) ionized were obtained by addition to the neutral samples of 15 M NaOH with a Carlsberg micropipet as previously described.[3c]

The spectra were simulated with the aid of the program LAOCOON III, and the final calculated coupling constants are within an accuracy of 0.1–0.2 Hz.

Suitable crystals of *lyxo*-U for X-ray diffraction were obtained by slow evaporation of a neutral aqueous solution at room temperature. Precession photographs established the space group uniquely as $P2_12_12_1$. A prismatic crystal, 0.45 × 0.20 × 0.20 mm, mounted along the *a* (prism) axis on a card-controlled Picker diffractometer provided the following data: a = 5.022 (1), b = 10.794 (1), c = 18.734 (1) Å, V = 1015.5 Å^3, D_x = 1.597, D_m = 1.592 g cm^{-3} (flotation in bromobenzene–iodobenzene), Z = 4 (22 °C, Cu Kα_1, λ 1.54051 Å; Cu Kα_2, λ 1.54433 Å), F (000) = 524, μ (Cu Kα) = 11.2 cm^{-1}.

The moving-crystal/moving-counter method ($\theta/2\theta$ scan) was used to collect the intensity data. Reflections with net counts <50 or <8% of background were categorized as unobserved. There were 1035 unique reflections accessible to the diffractometer ($2\theta \leq 130°$), of

0002-7863/79/1501-4724$01.00/0

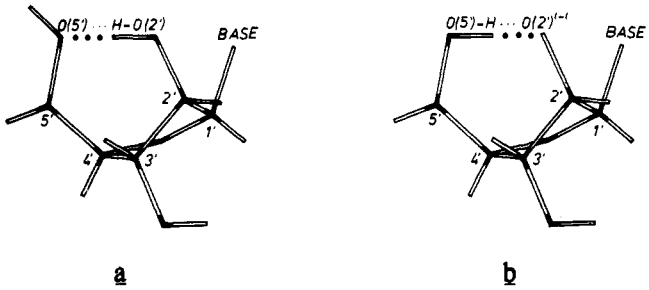

Figure 1. Intramolecular hydrogen bonding in (a) the neutral forms of *ara*-C and *ara*-U in the solid state; (b) the ionic forms of these nucleosides with the 2′-*OH* ionized.

which 1021 had intensities above threshold values. The intensities were corrected for Lorentz and polarization factors; absorption corrections were considered unnecessary.

The structure was determined by the multisolution approach to direct methods, and the atomic parameters refined by block-diagonal least squares. All scattering factors were taken from the "International Tables for X-Ray Crystallography",[14] and the oxygen curve was corrected for anomalous dispersion. All hydrogen atoms were located on difference Fourier maps and their parameters refined isotropically. Throughout the refinement, the function $\Sigma w(|F_o| - |F_c|)^2$ was minimized and a factor of 0.8 applied to all shifts. The following weighting scheme was used during the final stages: $w = w_1 w_2$, where $w_1 = 1$ for $|F_o| \leq 12$, $w_1 = 12/|F_o|$ for $|F_o| > 12$; and $w_2 = \sin^2\theta/0.7$ for $\sin^2\theta < 0.7$ and $w_2 = 1$ for $\sin^2\theta \geq 0.7$. After the final cycle the average parameter shift equalled 0.13σ and the largest 0.48σ. The conventional residual index R is 0.036 and the weighted index R' is 0.044. A final difference Fourier map was featureless.

Results and Discussion

Crystal Structure of *lyxo*-U. The final coordinates, together with their standard deviations, are listed in Table I. A stereoscopic view of the molecule is exhibited in Figure 2, and all conformational details are shown in Newman projections (Figure 3).

The pucker of the sugar ring is C(3′)exo-C(2′)endo, or ${}_3T^2$, corresponding to a phase angle of pseudorotation, P, calculated[15] to be 183.5°. C(3′) is displaced "downward" by 0.356 Å from the plane defined by C(1′), C(4′), and O(1′), and C(2′) is displaced in the opposite direction by 0.251 Å (Table II). Such a "twist" conformation of the carbohydrate moiety is rather rare, e.g., in a recent compilation of 98 crystal structures of nucleosides and nucleotides,[16] only 3 exhibited values of P in the range 180–198°, all with a deoxyribose moiety, only one of which was a pyrimidine nucleoside.[17]

Although *inter*molecular hydrogen bonds are formed preferentially in carbohydrates in the solid state,[18] the structure of *lyxo*-U is such that *intra*molecular hydrogen bonding would be facilitated by a gauche-gauche conformation of the exocyclic side chain. However, one might expect some destabilization of the gauche-gauche form by the presence of an "up" 3′-hydroxyl (as also in xylofuranosides). In fact, the observed

Table I. Final Coordinates (×10⁴)

atom	*x*	*y*	*z*
N(1)	3087 (5)	4599 (2)	4175 (1)
C(2)	3664 (6)	4441 (2)	4890 (1)
O(2)	5315 (5)	3676 (2)	5081 (1)
N(3)	2270 (6)	5167 (2)	5349 (1)
C(4)	310 (8)	6012 (3)	5181 (2)
O(4)	−746 (8)	6618 (3)	5652 (1)
C(5)	−330 (7)	6070 (3)	4428 (1)
C(6)	1073 (6)	5391 (2)	3970 (1)
C(1′)	4573 (5)	3812 (2)	3675 (1)
O(1′)	4534 (4)	4394 (2)	2993 (1)
C(2′)	3392 (5)	2520 (2)	3576 (1)
O(2′)	571 (4)	2523 (2)	3648 (1)
C(3′)	4157 (5)	2260 (2)	2800 (1)
O(3′)	2578 (4)	1325 (2)	2483 (1)
C(4′)	3853 (5)	3516 (2)	2432 (1)
C(5′)	1145 (5)	3812 (2)	2132 (1)
O(5′)	1310 (5)	4988 (2)	1794 (1)

Table II. Least-Squares Planes and Deviations of Atoms from Them[a]

plane 1[b]		plane 2	
atom	Δ, Å	atom	Δ, Å
N(1)	0.025	C(1′)	
C(2)	−0.022	C(4′)	
N(3)	−0.002	O(1′)	
C(4)	0.023	C(2′)*	−0.251
C(5)	−0.022	C(3′)*	0.356
C(6)	−0.001	C(5′)*	−1.319
C(1′)*	−0.004		
O(2)*	−0.094		
O(4)*	0.062		

[a] Atoms marked with an asterisk were not included in the calculation of the plane. [b] Plane 1: $0.6781X + 0.7295Y - 0.0894Z = 3.9475$. Plane 2: $0.9690X - 0.2157Y - 0.1208Z = 0.5059$.

5′-CH_2OH conformation is gauche-trans (Figure 3), as is also the case for methyl α-D-lyxofuranoside in the solid state.[9] In a study recently completed,[19] it was noted that for both the neutral and protonated forms of α-*xylo*-U, in which the 3′-OH is "up", the exocyclic group conformation in the solid state is trans-gauche. Only for 2,2′-anhydro-α-D-*xylo*-U was a gauche-gauche conformation observed, with concomitant formation of an *intra*molecular hydrogen bond, O(3′)-H···O(5′)-H.[3a] The crystallographic data are consequently consistent with the conclusions from *solution* data for xylo-[3e] and lyxonucleosides (see below), viz, that the presence of an "up" 3′-OH (hence cis to the 5′-CH_2OH) destabilizes the gauche-gauche rotamer.

The conformation about the glycosidic bond in *lyxo*-U is anti, χ_{CN} for O(1′)-C(1′)-N(1′)-C(6) being 27.0°, a some-

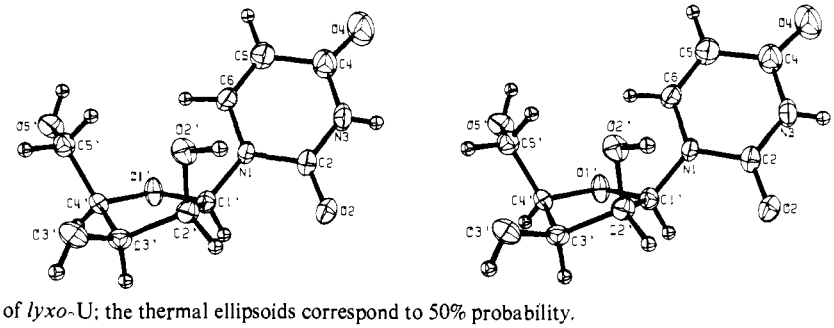

Figure 2. Stereoscopic view of *lyxo*-U; the thermal ellipsoids correspond to 50% probability.

Table III. Distances and Angles for the Hydrogen Bonds

D-H···A		distances, Å			angles, deg	
		D···A	H···A	H···A_{corr}	D-H···A	H-D···A
N(3)-H···O(5′)	$(\frac{1}{2}-x, 1-y, \frac{1}{2}+z)$	2.805	2.03	1.76	174	4
O(2′)-H···O(2)	$(-\frac{1}{2}+x, \frac{1}{2}-y, 1-z)$	2.714	1.85	1.77	165	10
O(3′)-H···O(1′)	$(1-x, -\frac{1}{2}+y, \frac{1}{2}-z)$	2.691	1.88	1.73	173	5
O(5′)-H···O(3′)	$(\bar{x}, \frac{1}{2}+y, \frac{1}{2}-z)$	2.780	1.92	1.85	165	10

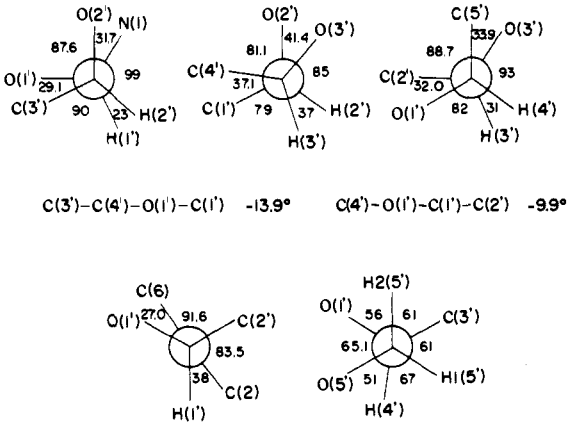

Figure 3. Newman projections along C(1′)-C(2′), C(2′)-C(3′), C(3′)-C(4′), N(1)-C(1′), and C(4′)-C(5′).

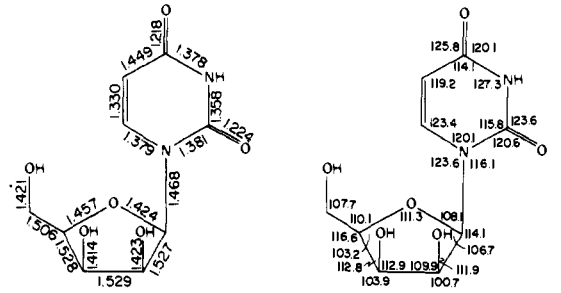

Figure 4. Molecular geometry: left, bond lengths in angstroms (all esd's are 0.003–0.004 Å); right, bond angles in degrees (all esd's are 0.2–0.3°).

what low value by comparison with the range 40–65° for C(2′)endo pyrimidine ribo- and deoxyribonucleosides,[16] but consistent with the range 26.7–34.0° observed for C(2′)endo arabinonucleosides.[20] These data may be rationalized on the basis of repulsion between C(6) and the 2′-OH when the latter is cis to the base, as in lyxo- and arabinofuranosides. A decrease in the value of χ_{CN} leads to an increase in the distance between C(6) and O(2′), which is 3.164 (3) Å in *lyxo*-U, corresponding to van der Waals' contact.

The observed bond lengths and bond angles, shown in Figure 4, are not unusual. However, it is interesting to examine what effect the "up" hydroxyl groups have on bond angles. The O(2′)-C(2′)-C(3′) angle is significantly smaller than the adjacent angle C(2′)-C(3′)-O(3′). Furthermore, C(1′)-C(2′)-O(2′) is somewhat smaller than C(4′)-C(3′)-O(3′). This situation is reversed in methyl α-D-lyxofuranoside,[5] which adopts the C(3′)endo, rather than C(3′)exo, conformation. In uridine and in other C(3′)endo ribonucleosides,[21] the angles are related in the same manner as in *lyxo*-U. The following general pattern emerges from these observations: in lyxofuranosides in C(3′)exo (or C(2′)endo) conformation, and ribofuranosides in C(3′)endo conformation, O(2′)-C(2′)-C(3′) < C(2′)-C(3′)-O(3′) and C(1′)-C(2′)-O(2′) < C(4′)-C(3′)-O(3′); in lyxofuranosides in C(3′)endo (or C(2′)exo) conformation, and ribofuranosides in C(2′)endo conformation, O(2′)-C(2′)-C(3′) > C(2′)-C(3′)-O(3′) and C(1′)-C(2′)-O(2′) > C(4′)-C(3′)-O(3′). However, more lyxofuranoside structures will have to be examined to confirm these tentative rules.

The six atoms of the pyrimidine ring are significantly nonplanar (Table II), with deviations up to 0.025 Å. In contrast to many other nucleoside structures, C(1′) lies in the plane of this ring.

No *intra*molecular hydrogen bonding, such as discussed above, is observed in the crystal structure of *lyxo*-U, and all four available protons are involved in *inter*molecular hydrogen bonds. Details of the geometry are given in Table III. As commonly observed in X-ray analyses, the O-H and N-H bonds appear shorter than their real values of 0.97 and 1.04 Å, respectively, which are obtained from neutron diffraction studies. By expanding the covalent bond lengths in the direction of the bond up to their real values, one obtains corrected H···O distances that express more accurately the strength of the hydrogen bond.[22] On this basis, it can be seen that all hydrogen bonds are quite strong. Figure 5 shows a packing diagram; as is evident, the bases are not mutually hydrogen bonded and they are not stacked.

Solution Conformation of Sugar Rings. The vicinal proton-proton coupling constants for the sugar rings of *lyxo*-U, *lyxo*-C and some *O′*-methyl derivatives of the latter, obtained from analyses of the simulated spectra (Figure 6; see paragraph regarding supplementary material), are exhibited in Figure 7. This method of presentation of the results was selected when it was found by trial that linear relationships existed between the coupling constants of the individual derivatives, and is discussed further, below.

It is proposed that the coupling constants for each compound represent an equilibrium between the states N and S. The state N would be ${}^3E$ or ${}^3_4T$, for which one would anticipate $J(1',2') \simeq 7$–8 Hz, $J(2',3') \simeq 5$ Hz, and $J(3',4') < 4$ Hz (Figure 8). Of all possible conformations of the sugar ring, the foregoing is in best agreement with the interdependences of the three sets of coupling constants shown in Figure 7. Actually, the experimentally observed value for $J(3',4')$ in this state (≤ 2.9 Hz) is unusually low for a cisoidal coupling constant in a furanose ring.

Theoretical calculations (in preparation), as well as simple quantitative considerations, indicate that the value of $J(3',4')$ should be minimal for the conformations ${}^3E$ and ${}^3_4T$. By contrast, the value of $J(3',4')$ should be considerably higher for the conformations ${}_3E$ and ${}^4_3T$, symmetrical about the eclipsed form. This follows from the conformational dependent effects of the electronegative 3′-OH and ring oxygen on the value of the cisoidal coupling constant $J(3',4')$, as has been demonstrated in the case of xylofuranosylnucleosides.[3e,f]

The state S falls within the pseudorotational region ${}^2_1T \cdots {}^2_3T$ (see below), and the conformation of the neutral form of *lyxo*-U in the solid state (see above) is within this region (Figure 8). For such a conformation, the expected values for the coupling constants are $J(1',2') \simeq 4.5$–5 Hz, $J(2',3') \simeq 4.5$–5 Hz, and $J(3',4') \simeq 6.5$ Hz (Figure 8). The value of $J(3',4')$ is, in fact, appreciably higher than that observed experimentally (3.8 Hz), while $J(1',2')$ is well below the observed value (6.3 Hz), showing that in solution we are dealing with an equilibrium conformation.

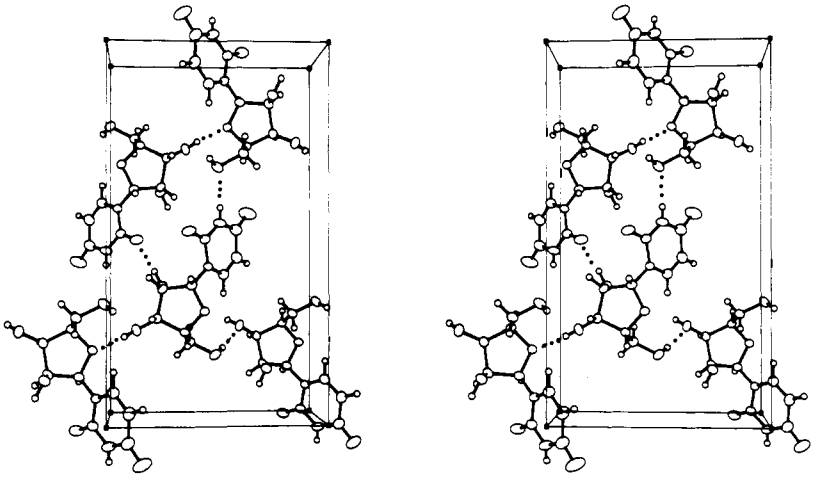

Figure 5. Stereoscopic view along x of the contents of a unit cell. The directions of the axes are $y\rightarrow$ and $z\uparrow$. Dotted lines indicate hydrogen bonds.

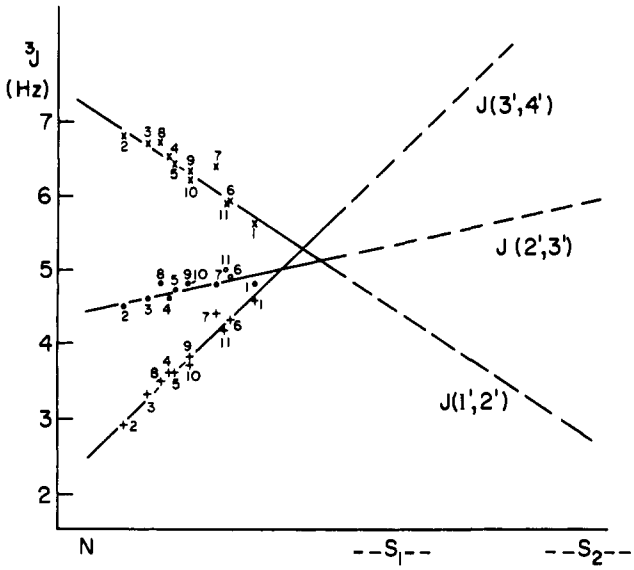

Figure 7. Interdependence of the values of the coupling constants in β-D-lyxofuranosylnucleosides. For each of the compounds, the three experimental points corresponding to the three coupling constants are located on a vertical line, the interaction of which with the abscissas is determined by the relative conformer populations in the two-state conformational equilibrium model for the furanose ring. The abscissa scale is only relative, since the location of the S state (S_1 or S_2) is not uniquely known. Individual points correspond to either neutral or partially protonated (DCl salts) forms, or the anions (addition of NaOD) involving sugar hydroxyl(s) dissociation (but excluding those cases, discussed in the text, where hydroxyl ionization is accompanied by intramolecular hydrogen bonding): (1) 3′-m-*lyxo*-C·DCl; (2) 2′-m-*lyxo*-C·DCl; (3) 2′-m-*lyxo*-C + 0.3 mmol of NaOD; (4) 2′-m-*lyxo*-C + 0.7 mmol of NaOD; (5) 2′-m-*lyxo*-C + 0.75 mmol of NaOD; (6) 3′,5′-m$_2$-*lyxo*-C·DCl; (7) 3′,5′-m$_2$-*lyxo*-C + 0.3 mmol of NaOD; (8) 2′,5′-m$_2$-*lyxo*-C; (9) *lyxo*-U; (10) *lyxo*-C·DCl; (11) *lyxo*-C.

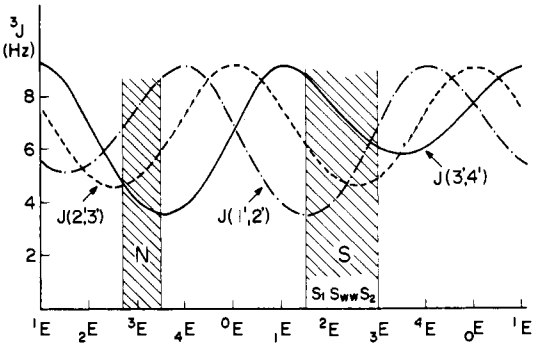

Figure 8. Schematic representation of the dependence of ^{1}H,^{1}H vicinal coupling constants on the sugar ring conformation of lyxofuranosylnucleosides on the basis of the Karplus relation with $J(0°)$ = 9.2 Hz (actually in the range 8.9–9.3 Hz for the most frequently employed parametrization of the Karplus relation for ribonucleosides) and $J(90°)$ = 0.0 Hz. The conformation-dependent effects of the aglycone were taken into account[3f] to a first approximation, assuming a 20% decrease in the values of the cisoidal coupling constants due to the -OH group or ring oxygen in that conformation in which these are *quasi*antiperiplanar relative to the proton involved in the coupling, and a 10% decrease due to the aglycone. The hatched areas correspond to the proposed N and S states in equilibrium with each other; S_{ww} refers to the proposed conformation stabilized by intramolecular hydrogen bonding, O(5′)-H···O(2′)$^-$, while S_1 and S_2 are two different versions of the state S for those derivatives without intramolecular hydrogen bonds. More accurate theoretical calculations of these curves are underway.

The foregoing analysis does not allow us to exclude the possibility of the existence in solution of a single state, of the form N. The energy minimum in this pseudorotational range would then shift from ^3_4T for the protonated form of 2′-m-*lyxo*-C to ^3_2T for the protonated form of 3′-m-*lyxo*-C. However, such a possibility appears to us most unlikely.

Interdependence of Coupling Constants. The three coupling constants for the pentose rings are interrelated, as shown by the calculated correlation coefficients, which are: $\rho(J(1',2'), J(2',3')) = -0.72$; $\rho(J(2',3'), J(3',4')) = 0.78$; and $\rho(J(1',2'), J(3',4')) = -0.90$. The latter value is closely similar to that for ribonucleosides. Such a correlation is expected for a two-state equilibrium of the sugar ring in solution, which implies a linear dependence of each of the three coupling constants on the conformer populations. Such an interdependence exists for ribofuranosyl nucleosides, as shown in Figure 9 (see paragraph regarding supplementary material). The appropriate experimental dependences for lyxofuranosides are shown in Figure 7, where the continuous lines are based on orthogonal regression lines in three-dimensional space of the coupling constants for the experimental points 1–11.

From Figure 7 one may attempt to determine the values of the coupling constants for the two equilibrium states. It is relatively simple to do this for one of the two states, that denoted as N (see discussion below). Since $J(3',4')$ = 2.9 Hz for 2′-m-*lyxo*-C DCl (point 2 in Figure 7), a very low value for a cisoidal coupling constant in a pentose nucleoside, this derivative must exhibit a very pronounced preference for the form N. On the assumption that $J(3',4')$ is not less than 2.5 Hz, the three coupling constants for the state N may be formulated as: $J(1',2')$ = 7–7.5 Hz, $J(2',3') \simeq$ 4.5 Hz, $J(3',4')$ = 2.5–3 Hz.

Establishment of the analogous coupling constants for the second state is somewhat more complex and requires com-

Table IV. Gauche-Gauche Rotamer Populations (in Percent)[a] of the 5′-CH_2OH Exocyclic Group of Lyxonucleoside Derivatives, and *ara*-C and *ara*-U, at pD 7 (Neutral Forms) and/or pD 4 (Partially Protonated Forms),[b] and at pD ~14, Where the 2′-OH and/or 5′-OH Are Ionized

nucleoside	pD ~4	pD ~7	pD ~14
lyxo-U		15	45
lyxo-C	20	20	45
2′-m-*lyxo*-C	10	20	30[c]
3′-m-*lyxo*-C	20	*b*	75
3′,5′-m_2-*lyxo*-C	15		15
ara-C[d]	40	45	70
ara-A[e]	50	55	70

[a] Calculated from % g-g = {13 − [J(4′,5′) + J(4′,5″)]}/10. [b] Values are for the protonated forms, because in some instances, e.g., 3′-m-*lyxo*-C, overlapping of signals in the spectrum of the neutral forms rendered calculations difficult. [c] Sugar hydroxyl ionization in this instance was incomplete. [d] Values from ref 3c. [e] Values from ref 3d.

parison with Figure 8, which indicates that this state should be in the range S. In the S_1 state in this range, J(3′,4′) is much higher than J(1′,2′), and J(2′,3′) exhibits intermediate values, in agreement with the trend shown in Figure 7. Bearing in mind the approximations in Figure 8, another possibility is the appearance of the state S_2 (in place of S_1), for which the three coupling constants exhibit similar values. While the states S_1 and S_2 correspond to closely similar conformations, it is nonetheless clear from Figure 7 that selection of one or the other will drastically affect calculations of the relative proportions of the conformers N and S in solution.

Conformation of the Exocyclic 5′-CH_2OH Group. The conformations of the exocyclic 5′-CH_2OH for the neutral and partially protonated forms of the various compounds are exhibited in Table IV. As in the case of the corresponding xylonucleosides,[3e] it will be seen that the gauche-gauche populations are relatively low as compared to ribonucleosides and arabinonucleosides (see data for *ara*-C and *ara*-A in Table IV). This is probably due to steric and electrostatic effects due to the 3′-OH, which is cis to the exocyclic 5′-CH_2OH. Similar low values for the gauche-gauche populations of the exocyclic 5′-CH_2OH have been recently reported for the α- and β-xylofuranosides of oxidized and reduced nicotinamide.[23] The values for the gauche-gauche populations at pD ~14 will be discussed in the next section.

Effects of Sugar Hydroxyl(s) Ionization. Intramolecular Hydrogen Bonding. As pointed out in the introductory statement, intramolecular hydrogen bonding in lyxofuranosylnucleosides is theoretically possible between the 5′-OH on the one hand, and the 3′-OH or 2′-OH on the other, the requisite geometrical conformations being C(3′)endo, gauche-gauche and C(2′)endo, gauche-gauche, respectively (Figure 10). However, the low gauche-gauche populations of the neutral forms of *lyxo*-U and *lyxo*-C in solution argue against formation of such bonds. The situation might be expected to differ in strongly alkaline medium, where the 2′-OH and/or the 3′-OH undergo dissociation,[24] as observed for arabinonucleosides.[3c,d,7] It consequently appeared of interest to compare the exocyclic 5′-CH_2OH gauche-gauche populations of the neutral forms with those for the same compounds in strongly alkaline medium.

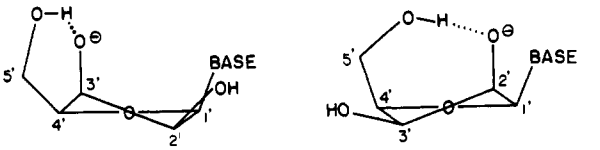

Figure 10. Possible intramolecular hydrogen bonding in the ionized forms of lyxonucleosides.

With the foregoing in mind, it was first necessary to establish the alkaline conditions necessary for dissociation of the sugar hydroxyls. This was done by following the changes in chemical shifts of the sugar and base protons (measured vs. internal DSS) accompanying stepwise addition of increasing quantities of NaOD. It was found in this way that, as in the case of arabinonucleosides,[3c] the maximal changes in chemical shifts occurred on addition of 0.2–0.3 mmol of NaOD to 0.5-mL solution samples. The alkali-induced changes in chemical shifts are appreciable (see data for 3′-m-*lyxo*-C in Table V). The occurrence of these changes in chemical shifts as a consequence of sugar hydroxyl(s) ionization is further testified to by the fact that, when both the 2′-OH and 3′-OH are etherified (as in 2′,3′-m_2-*ara*-C), the alkali-induced changes in chemical shifts are small (<0.05 ppm). Finally, it has been shown that ^1H NMR titration of the sugar hydroxyls of arabinosides in this alkaline region leads to pK_a values close to those obtained by spectrophotometric methods.[25]

Spectral analyses were then carried out for *lyxo*-U, *lyxo*-C, 2′-m-*lyxo*-C, 3′-m-*lyxo*-C, and 3′,5′-m_2-*lyxo*-C at neutral pD (or at pD 4, where the *lyxo*-C nucleosides are partially protonated, but spectra exhibit better resolution) and in strongly alkaline medium (pD ~14). The transition from neutral to alkaline medium was accompanied by changes in coupling constants for all the compounds, most marked with 5′-m-*lyxo*-C, and least so with 2′-m-*lyxo*-C. The results for the gauche-gauche populations of the exocyclic 5′-CH_2OH are listed in Table IV.

It will be noted that, for 3′-m-*lyxo*-C, where only the 2′-OH ionizes and where there is a free 5′-OH, the gauche-gauche population is relatively low at neutral pH and increases in alkaline medium at least as markedly, if not more so, than for the corresponding arabinosylnucleosides.[3c] For 2′-m-*lyxo*-C, where only the 3′-OH ionizes, the increase in gauche-gauche population in alkaline medium is relatively low, while the increases with *lyxo*-C and *lyxo*-U are intermediate in value.

Interpretation of the foregoing results must necessarily take account of any changes in conformation of the pentose rings accompanying sugar hydroxyl(s) dissociation. From the coupling constants of the sugar ring protons, it is clear that the S type conformer population increases with increasing alkalinity, and is maximal for 3′-m-*lyxo*-C (Figure 7). The values of the coupling constants for this derivative at pD ~14 (J(1′2′) = 3.6 Hz, J(2′,3′) = 3.6 Hz, J(3′,4′) = 7.4 Hz) do not fall on the linear plots in Figure 7, possibly because of a small difference in the type S conformations of molecules with and without intramolecular hydrogen bonding. From Figure 8 the most likely conformation of the sugar ring of 3′-m-*lyxo*-C in alkaline medium appears to be of the form ^2E, whereas in those derivatives without intramolecular hydrogen bonding, there is rather an equilibrium which includes the conformation ^2_1T (S_1) or ^2_3T (S_2).

Table V. Chemical Shifts[a] of the Protons of 3′-m-*lyxo*-C at pD ~7 and at pD ~14 Where the 2′-OH Is Ionized

pD	H(1′)	H(2′)	H(3′)	H(4′)	H(5′)	H(5″)	H(5)	H(6)
7	6.13	4.69	4.10	4.30	3.99	3.92	6.03	7.84
14	5.98	4.40	4.25	4.41	3.71	3.58	5.99	8.01

[a] In aqueous medium, in parts per million vs. internal DSS.

It is hardly coincidental that the high C(2′)endo conformation of 3′-m-*lyxo*-C in alkaline medium is accompanied by an increase in the gauche-gauche population from about 20% (in neutral medium) to at least 75%. It may reasonably be concluded that the anion of 3′-m-*lyxo*-C is stabilized by formation of the intramolecular hydrogen bond O(5′)-H···O(2′)$^-$, as in the case of arabinosides,[3c] further supported by the fact that this does not occur when the 5′-OH is etherified, i.e., in 3′,5′-m_2-*lyxo*-C (Table IV). The minor alkali-induced changes in conformation of 2′-m-*lyxo*-C (Figure 7 and Table IV) argue against intramolecular hydrogen bonding in this instance. With the parent *lyxo*-C and *lyxo*-U there is only partial formation of such hydrogen bonding, probably because of parallel ionization of the 3′-OH.

Acknowledgments. This work profited from the support of the Polish Academy of Sciences (Project 09.7.1) and the Polish National Cancer Research Program (Project PR-6/1701). All crystallographic computations were carried out with programs written by Ahmed et al.[25] Figures 2 and 5 were drawn with the ORTEP program.[26]

Supplementary Material Available: Listing of observed and calculated structure factors for *lyxo*-U as well as hydrogen atom parameters and anisotropic temperature parameters of the nonhydrogen atoms; also, Figure 6 showing ^{1}H NMR spectra of *lyxo*-C at pD 4 and 14 and Figure 9 showing the interdependence of the values of the coupling constants in β-D-ribofuranosylpurine and pyrimidine nucleosides (10 pages). Ordering information is given on any current masthead page.

References and Notes

(1) Issued as NRCC No. 17605.
(2) (a) University of Warsaw; (b) National Research Council, Ottawa; (c) Institute of Biochemistry and Biophysics, Warsaw.
(3) (a) G. I. Birnbaum, J. Giziewicz, C. P. Huber, and D. Shugar, *J. Am. Chem. Soc.*, **98**, 4640 (1976); (b) M. L. Post, G. I. Birnbaum, C. P. Huber, and D. Shugar, *Biochim. Biophys. Acta*, **479**, 133 (1977); (c) M. Remin, E. Darzynkiewicz, A. Dworak, and D. Shugar, *J. Am. Chem. Soc.*, **98**, 367 (1976); (d) M. Remin, E. Darzynkiewicz, I. Ekiel, and D. Shugar, *Biochim. Biophys. Acta*, **435**, 405 (1976); (e) I. Ekiel, E. Darzynkiewicz, L. Dudycz, and D. Shugar, *Biochemistry*, **17**, 1530 (1978); (f) A. Jaworski, I. Ekiel, and D. Shugar, *J. Am. Chem. Soc.*, **100**, 4357 (1978).
(4) Abbreviations employed are: *lyxo*-U, 1-β-D-lyxofuranosyluracil; *lyxo*-C, 1-β-D-lyxofuranosylcytosine; *xylo*-C, 1-β-D-xylofuranosylcytosine; *ara*-C and *ara*-U, 1-β-D-arabinofuranosylcytosine and -uracil; 2′-m-*lyxo*-U, 2′-*O*-methyl-*lyxo*-U; 2′,5′-m_2-*lyxo*-U, 2′,5′-di-*O*-methyl-*lyxo*-U; and similar connotations for other *O*′-methyl derivatives of nucleosides; DSS, sodium 4,4-dimethyl-4-silapentane-5-sulfonate.
(5) (a) A. K. Chwang and M. Sundaralingam, *Nature (London), New Biol.*, **243**, 78 (1973); (b) P. Tollin, H. R. Wilson, and D. W. Young, *Acta Crystallogr., Sect. B*, **29**, 1641 (1973); P. Tougard and O. Lefebvre-Soubeyran, *ibid.*, **30**, 86 (1974).
(6) J. G. Dalton, A. L. George, F. E. Hruska, T. N. McGaig, K. K. Ogilvie, J. Peeling, and D. J. Wood, *Biochim. Biophys. Acta*, **478**, 261 (1977).
(7) E. Darzynkiewicz, M. Remin, A. Dworak, and D. Shugar, *Cancer Biochem. Biophys.*, **1**, 85 (1975).
(8) During the course of this investigation, we learned of a comparable study of the ^{1}H NMR spectrum of the neutral form of *lyxo*-U by M. MacCoss (personal communication).
(9) P. Groth and H. Hammer, *Acta Chem. Scand.*, **22**, 2059 (1968).
(10) W. Depmeier and O. H. Jarchow, *Acta Crystallogr., Sect. B*, **31**, 945 (1975).
(11) G. A. LePage, *Handb. Exp. Pharmakol.*, **38**, Part 2, 426 (1975).
(12) J. J. Fox and R. Fescher, *J. Am. Chem. Soc.*, **83**, 1889 (1961).
(13) E. Darzynkiewicz, I. Ekiel, and D. Shugar, manuscript in preparation.
(14) J. A. Ibers and W. C. Hamilton, Ed., "International Tables for X-Ray Crystallography", Vol. IV, Kynoch Press, Birmingham, England, 1974.
(15) C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **94**, 8205 (1972).
(16) B. Pullman and A. Saran, *Prog. Nucleic Acid Res. Mol. Biol.*, **18**, 215 (1976).
(17) D. W. Young, P. Tollin, and H. R. Wilson, *Acta Crystallogr., Sect. B*, **25**, 1423 (1969).
(18) G. A. Jeffrey, *Adv. Chem. Ser.*, **No. 117**, 177 (1973).
(19) M. L. Post, G. I. Birnbaum, C. P. Huber, and D. Shugar, manuscript in preparation.
(20) G. I. Birnbaum, E. Darzynkiewicz, and D. Shugar, *J. Am. Chem. Soc.*, **97**, 5904 (1975).
(21) W. Saenger and F. Eckstein, *J. Am. Chem. Soc.*, **92**, 4712 (1970).
(22) G. A. Jeffrey and S. Takagi, *Acta Crystallogr., Sect. B*, **33**, 738 (1977).
(23) N. J. Oppenheimer, *J. Carbohydr. Nucleosides, Nucleotides*, **5**, 251 (1978).
(24) Under the alkaline conditions employed here (pD ~14), ionization of the 5′-OH is minimal, since its p*K* is higher than 14.5.
(25) E. Darzynkiewicz, H. Sierakowski, and D. Shugar, *Z. Naturforsch. C*, **30**, 565 (1975).
(26) F. R. Ahmed, S. R. Hall, M. E. Pippy, and C. P. Huber, *J. Appl. Crystallogr.*, **6**, 309 (1973).
(27) C. K. Johnson, ORTEP Report ORNL-3794 (2nd revision), Oak Ridge National Laboratory, Oak Ridge, Tenn., 1970.