Solution Conformation and Relative Acidities of the Sugar Hydroxyls of the O′-Methylated Derivatives of the Antimetabolite 9-β-D-Xylofuranosyladenine†

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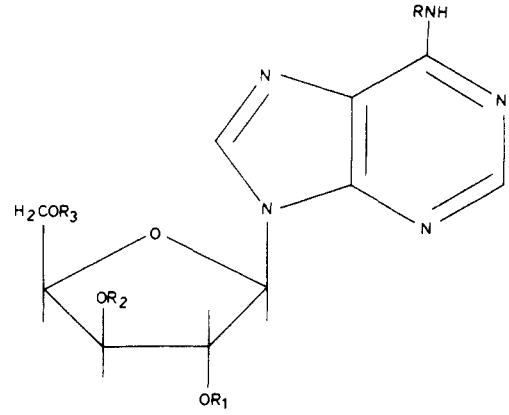
ABSTRACT: Proton magnetic resonance spectroscopy was applied to a conformational analysis of the various *O′*-methyl derivatives of 9-β-D-xylofuranosyladenine, a nucleoside analogue with antimetabolic activity. The free nucleoside and its derivatives with etherified (O′-CH_3) hydroxyls all possess very similar conformations: a distinct preference for the anti configuration about the glycosidic bond, a very pronounced preference for the N-type conformer, C(3′)endo, of the pentose ring, and an unusually low gauche–gauche population of the exocyclic 5′-CH_2OH group relative to that prevailing for ribofuranosyl- and arabinofuranosylnucleosides. In contrast to arabinonucleosides, ionization of the sugar hydroxyl(s) of xylofuranosyladenine does not lead to any major modifications in conformation. The relative acidities of the sugar hydroxyls of xylofuranosyladenine and its O′-methylated derivatives were evaluated from their elution sequence pattern on a strongly basic Dowex (OH^-) column and compared with the elution sequence on the same column of adenosine, 9-β-D-arabinofuranosyladenine, 1-β-D-arabinofuranosylcytosine, and their corresponding O′-methylated derivatives. The relative acidities of the xylofuranosyladenine sugar hydroxyls obtained in this way, together with the ^1H NMR results, argue against the previously proposed formation in the xylofuranosyladenine anion of an intramolecular hydrogen bond between the 5′-OH and the "up" 3′-O^-, i.e., O(5′)–OH···$O(3')^-$, as the source of the known high acidity of the sugar hydroxyls. The most acidic hydroxyl in xylofuranosyladenine appears to be the 2′-OH.

In a continuation of previous studies on the conformational properties of biologically important nucleoside analogues and derivatives of these in which one or more of the sugar hydroxyls are etherified (Remin et al., 1973, 1976a,b; Darżynkiewicz et al., 1975a), we present here the results of an investigation on the conformations of the *O′*-methyl derivatives of 9-β-D-xylofuranosyladenine, *xylo*-A,[1] together with an evaluation of the relative acidities of the sugar hydroxyls and the effect of sugar hydroxyl(s) ionization on nucleoside conformation. The results of this study have, in turn, proven useful in an independent investigation on the conformation of the parent *xylo*-A (in preparation); see Chart I.

xylo-A has been known for some time to exhibit antitumor activity (Ellis and LePage, 1965a,b; Roy-Burman, 1970). It has also been reported highly active against Herpes simplex virus (De Rudder et al., 1967). *xylo*-ATP interferes with the formation of PRPP and is effective as a feedback inhibitor of purine biosynthesis (Roy-Burman, 1970). It has been found to inhibit ribonucleotide synthesis from hypoxanthine and de novo purine biosynthesis (Henderson et al., 1975). It is also of interest that both xylofuranosyl-6-mercaptopurine and its 5′-phosphate inhibit the utilization of guanine for DNA synthesis (Sato et al., 1966; LePage and Naik, 1975), while the nucleotide appears also to possess immunosuppressive activity (LePage and Naik, 1975). However, to our knowledge, no attempts have as yet been made to examine the solution conformation of xylofuranosylnucleosides, which should be of interest not only in relation to their antimetabolic properties but also from the point of view of nucleoside conformation in general and the influence on conformation of the sugar hydroxyls prior to and following their ionization at highly alkaline pH.

CHART I: 9-β-D-Xylofuranosyladenine and Its N^6- and/or *O′*-Methyl Derivatives.[a]



[a] R, R_1, R_2, R_3 = H or CH_3. For example, if R = CH_3, R_1 = H, R_2 = CH_3, R_3 = H, the compound is N^6,3′-m_2-*xylo*-A.

Experimental Section

xylo-A was kindly provided by Dr. Harry B. Wood, Jr., of the Drug Development Branch, National Cancer Institute. The synthesis of the various *O′*-methyl derivatives of *xylo*-A has been elsewhere described (Darżynkiewicz at al., 1977).

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[1] Abbreviations used are: *xylo*-A, 9-β-D-xylofuranosyladenine; *ara*-A, 9-β-D-arabinofuranosyladenine; *ara*-C, 1-β-D-arabinofuranosylcytosine; 2′-m-*xylo*-A, 2′-*O*-methyl-*xylo*-A; 3′,5′-m_2-*ara*-A, 3′,5′-di-*O*-methyl-*ara*-A; with similar connotations for other *O′*-methyl nucleosides, e.g., 2′-mA, 2′-*O*-methyladenosine; ^1H NMR, proton magnetic resonance; DSS, sodium 4,4-dimethyl-4-silapentanesulfonate; N and S, the "northern" and "southern" extremes of the continuum of conformers of the pentose ring, usually corresponding to C(3′)endo and C(2′)endo, as defined by Altona and Sundaralingam (1972, 1973).

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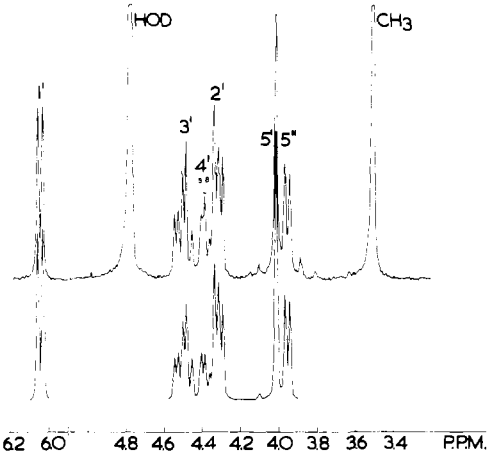

FIGURE 1: ^{1}H NMR spectrum of 2'-*O*-methyl-*xylo*-A (2'-m-*xylo*-A) in neutral medium, pD ~7. Upper spectrum was that obtained experimentally, lower one was obtained by simulation as described under the Experimental Section.

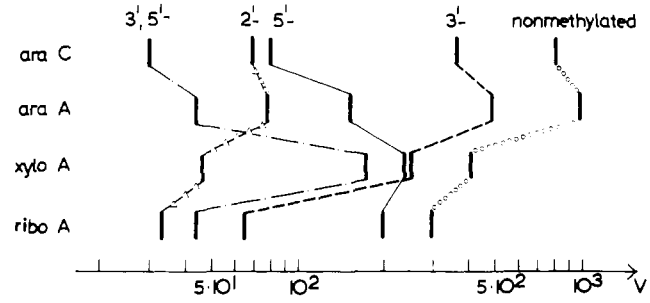

FIGURE 2: Relative elution sequence with 50% aqueous methanol, from a Dowex 1- × 2 (OH^-) column, of various pentose nucleosides and their 2'-, 3'-, and 5'-*O*-monomethyl and 3',5'-di-*O*-methyl derivatives. V indicates the volume of eluent in mL.

^{1}H NMR spectra were recorded with a Bruker-90 spectrometer in the Fourier transform mode locked to the resonance of internal D_2O. Data point densities were 3.4 points/Hz. All signal positions were measured vs. internal DSS (sodium 4,4-dimethyl-4-silapentane-5-sulfonate) at a probe temperature of 27 °C. Spectral analyses were carried out with the aid of the LAOCOON III program. Chemical shifts are uncorrected for concentration effects.

Solutions of increasing alkalinity, in the high pH range where ionization of sugar hydroxyls occurs, were obtained as previously reported (Remin et al., 1976a) by stepwise addition, with the aid of Carlsberg micropipets, of 1 or 10 N NaOD (>99 mol % D, from Merck, Darmstadt, GFR) to 0.4-mL samples of nucleoside solutions.

The elution sequence of methylated nucleosides was determined on a 15 × 1.5 cm column of 200/400 mesh Dowex 1- × 2 (OH^-). A 0.5-mL solution of 2–6 mg of a mixture of two or three nucleosides was deposited on the column. Elution was then carried out with 50% aqueous methanol at a flow rate of 1 mL/min. The effluent was monitored with an LKB 8300 Uvicord II analyzer fitted with a chopper bar point-printing LKB 6520 recorder. Measurements of elution sequence were repeated two or three times and the results averaged.

Results and Discussion

^{1}H NMR Spectra. A typical spectrum for 2'-m-*xylo*-A in neutral medium is exhibited in Figure 1. Several of the analogues embraced in this study exhibited spectra with overlapping signals of various protons which were frequently coupled with each other. Establishment of parameters for such compounds was possible only with the aid of simulation procedures. The final values of chemical shifts and coupling constants are presented in Tables I and II.

Alkali-Induced Changes in Chemical Shifts of Sugar Protons. These results, listed in Table III, show that the changes in chemical shifts of the xylofuranoside protons resulting from hydroxyl ionization are smaller than for the corresponding ribo- and arabinonucleosides (Remin et al., 1976a,b). Hence, in order to establish whether, under the conditions prevailing in these experiments, there was indeed marked dissociation of the *xylo*-A sugar hydroxyl(s), comparisons were made of the behavior of the two nucleoside pairs 3',5'-m_2-*ara*-C and 2',5'-m_2-*xylo*-A, on the one hand, and 2'-m-*xylo*-A and 2'-mA, on the other, following addition of identical quantities of NaOD to solutions of similar concentration.[2] These compounds were selected because of their weaker acidities, based on the sequence of their elution from a Dowex (OH^-) column (see below). For each pair, the one more strongly bound to the resin, hence, presumably more acidic, is the *xylo*-A derivative (Figure 2). Both for 3',5'-m_2-*ara*-C and 2'-mA the changes in chemical shifts accompanying hydroxyl dissociation (0.1–0.2 ppm) are distinctly more pronounced than for 2',5'-m_2-*xylo*-A and 2'-m-*xylo*-A and clearly point to the presence of marked dissociation of the sugar hydroxyls. Consequently, the extent of dissociation of the sugar hydroxyls in the *xylo*-A derivatives would be expected to at least be equal to, if not greater than, this. For 2'-m-*xylo*-A (see below) the changes in chemical shifts were followed as a function of increasing alkalinity of the solution (Table III). It will be noted that the major changes in chemical shifts occur on addition of up to 0.3 mmol of NaOD; further addition of NaOD only minimally affects the chemical shifts. It follows that, with the exception of 2',3'-m_2-*xylo*-A, the extent of dissociation of the sugar hydroxyls in the *xylo*-A derivatives is appreciable.

From the elution sequence on a Dowex (OH^-) column, it follows that the primary 5'-OH is the least acidic, as expected (Gin and Dekker, 1968); from the behavior of the various *O'*-methyl xylonucleosides, the acidities of the 5'- and 3'-OH are clearly lower than that of the 2'-OH. The values for the alkaline-induced changes in proton chemical shifts (Table III) are in agreement with this. For those derivatives with a blocked 2'-OH, viz., 2'-m-*xylo*-A, 2',3'-m_2-*xylo*-A, and 2',5'-m_2-*xylo*-A, the alkali-induced changes in chemical shifts are generally lower than those for compounds with a free 2'-OH. This may be due in part to the weaker acidity and hence lower degree of ionization provoked by the addition of 0.3 mmol of NaOD to 0.4-mL samples of 0.03–0.07 M solutions of the compounds under study. The particularly small changes in chemical shifts for 2',3'-m_2-*xylo*-A (Table III) are fully consistent with the known high p*K* of the primary 5'-OH (Gin and Dekker, 1968; Darżynkiewicz et al., 1975b). For derivatives with a blocked 2'-OH and a free 3'-OH, viz., 2'-m-*xylo*-A and 2',5'-m_2-*xylo*-A, in which dissociation proceeds mainly from the 3'-OH, the nature of the alkali-induced changes in chemical shifts is similar. The influence of 3'-OH ionization is most marked on H(2') and H(4') and only minimal on the geminal H(3'). For derivatives with a free 2'-OH, i.e., 3'- and 5'-m-*xylo*-A, addition of NaOD leads to pronounced, similar,

[2] In separate experiments, to be reported elsewhere, addition of 0.4 mmol of NaOD to a solution of 2'-mA led to changes in chemical shifts of the H(1'), H(2'), H(3'), and H(4') protons of 0.06, 0.23, 0.13, and 0.12 ppm, respectively.

TABLE I. Chemical Shifts (in ppm vs. Internal DSS) of the Protons in *O'*-Methyl Derivatives of *xylo*-A in D_2O, at 27 °C (Accuracies Are ±0.01 ppm, Unless Otherwise Indicated).

Derivative	Concn	Total NaOD added (mmol)	H(1')	H(2')	H(3')	H(4')	H(5')	H(5'')	H(2)	H(8)	2'-OCH_3	3'-OCH_3	5'-OCH_3	N^6-CH_3
2'-m-*xylo*-A	0.08	0.0	6.05	4.32	4.52	4.38	4.02	3.96	8.15	8.30	3.52			
	0.07	0.1	6.05	4.25	4.52	4.32	*a*	*a*	8.18	8.42	3.51			
	0.08	0.3	6.03	4.19	4.54	4.29	4.03[c]	4.02[c]	8.16	8.47	3.51			
	0.07	0.6	6.04	4.19	4.55	4.28	*a*	*a*	8.18	8.51	3.51			
3'-m-*xylo*-A	0.07	0.0	6.02	4.77	4.10	4.56	3.98	3.93	8.13	8.21		3.42[b]		
		0.3	5.88	4.68	3.83	4.57	3.95	3.88	8.18	8.22		3.39		
5'-m-*xylo*-A	0.06	0.0	6.00	4.63	4.37	4.55	3.89[c]	3.82[c]	8.14	8.27			3.45	
		0.3	5.84	4.55	4.15	4.57	3.89	3.79	8.17	8.32			3.44	
2',3'-m_2-*xylo*-A	0.03	0.0	6.15	4.45[c]	4.17	4.42[c]	3.99[c]	3.93[c]	8.20	8.25	3.49	3.42		
		0.3	6.15	*a*	*a*	*a*	3.98[c]	3.91[c]	8.25	8.28	3.52	3.42		
2',5'-m_2-*xylo*-A	0.06	0.0	6.05	4.31	4.48	4.43	3.89	3.78	8.18	8.30	3.52		3.45	
		0.3	6.02	4.22	4.46	4.38	3.88	3.81	8.19	8.43	3.50		3.44	
3',5'-m_2-*xylo*-A	0.04	0.0	6.07	*a*	*a*	*a*	3.84		8.20	8.23		3.39	3.44	
N^6-m-*xylo*-A	0.05	0.0	5.93	*a*	*a*	*a*	*a*		8.04	8.17				2.99
N^6,2'-m_2-*xylo*-A	0.03	0.0	6.03	4.29–4.36	4.49	4.29–4.36	3.99[c]	3.94[c]	8.17	8.23	3.49			3.06

[a] No data available. [b] 3.3 ppm in Me_2SO (Robins et al., 1974). [c] Accuracy 0.02 ppm.

TABLE II: Coupling Constants (in Hz) of Vicinal Protons in *O'*-Methyl Derivatives of *xylo*-A.[a]

Derivative	Total NaOD added (mmol)	$J_{1'-2'}$	$J_{2'-3'}$	$J_{3'-4'}$	$J_{4'-5'}$	$J_{4'-5''}$	$J_{5'-5''}$
2'-m-*xylo*-A	0.0	2.3	1.8	4.0	4.3	6.8	−12.3
	0.3	2.3	1.8	3.9	4.7[b]	4.9[b]	−12.1
3'-m-*xylo*-A	0.0	3.2	3.2	5.0	4.4[b]	5.6[b]	−12.0
	0.3	2.4	2.1	4.2	4.6	6.7	−12.0
5'-m-*xylo*-A	0.0	2.2	1.8	3.7	3.4[b]	7.2[b]	−11.2
	0.3	2.0	1.5	3.8	3.2	8.0	−11.3
2',3'-m_2-*xylo*-A	0.0	2.3	*c*	*c*	5.0[b]	5.9[b]	−12.2[b]
	0.3	2.5	*c*	*c*	5.0[b]	5.9[b]	−12.2[b]
2',5'-m_2-*xylo*-A	0.0	2.0	1.1	3.8	3.4	7.4	−11.7
	0.3	2.6	1.7	4.2	3.1	7.3	−11.4
3',5'-m_2-*xylo*-A	0.0	2.7	*c*	*c*	4.6[b]	6.3[b]	*c*
N^6-m-*xylo*-A	0.0	2.5	*c*	*c*	*c*	*c*	*c*
N^6,2'-m_2-*xylo*-A	0.0	2.4	*c*	*c*	3.7	6.3	−12.5[b]

[a] Accuracy ±0.2 Hz, unless otherwise indicated. [b] Accuracy ±0.5 Hz. [c] Not identified.

changes in chemical shifts, due principally to 2'-OH dissociation, and are characterized by marked shifts of the cis vicinal H(1') and H(3') protons and a much feebler effect on the geminal H(2').

Effect of Sugar Hydroxyl Ionization on the Aglycone Protons (Conformation About the Glycosidic Bond). Dissociation of the sugar hydroxyl(s) of 2'-m-*xylo*-A and 2',5'-m_2-*xylo*-A leads to a pronounced deshielding of the purine H(8) (Table IV). This effect is absent in those derivatives where ionization involves primarily the 2'-OH, e.g., 3'- and 5'-m-*xylo*-A. It follows that the deshielding of H(8) is related to dissociation of the 3'-OH. In fact, examination of a CPK model of *xylo*-A shows that, with the conformation anti, the H(8) is in close vicinity to the "up" 3'-OH. Ionization of this group may consequently be profited from for establishing the preferential orientation about the glycosidic bond, as has been done in the case of purine nucleoside 5'-phosphates by following the effect of secondary phosphate hydroxyl ionization on the chemical shift of H(8) (Danyluk and Hruska, 1968) and in arabinofuranosylnucleosides via the effect of ionization of the "up" 2'-OH with formation of an intramolecular hydrogen bond, viz., O(5')-H···O(2')$^-$, on H(6) of a pyrimidine (Remin et al., 1976a) or on H(8) of a purine (Remin et al., 1976b) aglycone.

Since, for purines and purine nucleosides in aqueous medium, both chemical shifts and coupling constants may exhibit some concentration dependence as a result of stacking, measurements were carried out for 2'-m-*xylo*-A at two different concentrations, 0.07 and 0.004 M. The values of $J_{1',2'}$ at these two concentrations, 2.3 and 2.1 Hz, respectively, point to the absence of any marked changes in conformation of the furanose ring over this concentration range. There is, however, the expected concentration dependence of the chemical shifts of H(2) and H(8) (Table IV). Shielding of these protons with an increase in concentration, more pronounced for H(2), is similar to that observed for adenosine (Evans and Sarma, 1974a,b).

TABLE III: Changes in Proton Chemical Shifts Following the Addition of NaOD (in ppm × 10^2).[a]

Derivative	Concn	Total NaOD added (mmol)	H(1')	H(2')	H(3')	H(4')	H(5')	(H5'')	H(2)	H(8)	2'-OCH_3	3'-OCH_3	5'-OCH_3
2'-m-*xylo*-A	0.07	0.1	0	7	0	6	*b*	*b*	−2	−11	1		
	0.08	0.3	1	13	−2	9	−1	−6	0	−17	1		
	0.07	0.6	1	13	−3	10	*b*	*b*	−2	−20	1		
3'-m-*xylo*-A	0.07	0.3	14	9	27	−1	3	5	−5	−2		3	
5'-m-xylo-A	0.06	0.3	16	8	22	−3	1	4	−4	−6			2
2',3'-m_2-*xylo*-A	0.03	0.3	−1	<3	<3	<3	1	2	−5	−2	3	0	
2',5'-m_2-*xylo*-A	0.06	0.3	3	9	2	5	0	−3	−1	−14	2		1

[a] Accuracies are ±0.01–0.03 ppm; results are uncorrected for stacking effects. [b] No data available.

TABLE IV: Chemical Shifts (in ppm vs. Internal DSS) of H(2) and H(8) Protons in 2'-m-*xylo*-A for Neutral Form (pD ~7) and, Following Ionization of Sugar Hydroxyls (pD ~14), at Two Different Concentrations.

	H(2)		H(8)	
	0.004 M	0.07 M	0.004 M	0.07 M
pD ~7	8.25	8.15	8.34	8.30
pD ~14	8.25	8.16	8.51	8.47
Difference	0.00	−0.01	−0.17	−0.17

TABLE V: Chemical Shifts of the Methyl Protons of Various O'-Methyl Nucleoside Derivatives in D_2O.

	2'-OCH_3	3'-OCH_3	5'-OCH_3
ribo-C[a]	3.55 ± 0.01	3.46 ± 0.01	3.45 ± 0.01
ribo-U[b]	3.52 ± 0.01	3.47 ± 0.02	3.44 ± 0.01
Virazole[c]	3.51	3.52	
ribo-A[d]	3.50 ± 0.02	3.56 ± 0.03	3.44 ± 0.01
ara-C[e]	3.37 ± 0.01	3.50 ± 0.01	3.46 ± 0.01
ara-A[f]	3.20 ± 0.02	3.58 ± 0.02	3.47 ± 0.02
xylo-A	3.50 ± 0.02	3.41 ± 0.02	3.45 ± 0.01
lyxo-C[g]	3.33	3.47	

[a] Remin and Shugar, 1973; Kuśmierek et al., 1973; Martin et al., 1968. [b] Remin and Shugar, 1973; Martin et al., 1968. [c] Dudycz et al., 1977. [d] Kazimierczuk et al., 1976. [e] Darżynkiewicz et al., 1972; Darżynkiewicz and Shugar, 1974. [f] Darżynkiewicz et al., 1976. [g] In preparation.

Despite the existence of this concentration effect on chemical shifts, the data in Table IV clearly demonstrate that the ionization-induced changes in chemical shifts of H(2) and H(8) are independent of concentration; the resultant pronounced change in the chemical shift of H(8), and invariance of the chemical shift of H(2), points to a preference for the conformation anti.

The transition from the conformation syn to anti is known to be accompanied by a change in chemical shifts of the pentose protons (Sarma et al., 1974), in particular of H(2'). For the O'-methylated derivatives of *xylo*-A, the observed changes in chemical shifts are readily interpretable in terms of the effects of the O'-methyl substituents (Darżynkiewicz et al., 1977). It is, consequently, most unlikely that the other methylated derivatives, of *xylo*-A exhibit any marked population of the syn conformer.

Chemical Shifts of O'-CH_3 Groups. Table V lists the chemical shifts of the O'-methyl protons in ribo-, arabino-, lyxo-, and xylonucleosides. It will be seen that the chemical shifts of the exocyclic 5'-O-methyl protons are essentially identical for all three classes of nucleosides, while those of the 2'- and 3'-O-CH_3 are dependent both on the nature of the sugar and the aglycone. Particularly marked is the shielding (by 0.1–0.3 ppm) of the 2'-OCH_3 cis to the aglycone in lyxo- and arabinonucleosides relative to the same group trans to the aglycone in ribo- and xylonucleosides. There is a comparable shielding of the protons of the "up" 3'-O-CH_3 in the *xylo*-A derivatives relative to the "down" O'-CH_3 protons in adenosine and araA analogues. It should also be noted that the location of the 3'-O-CH_3 signal differs significantly between pyrimidine and purine nucleosides, reflected by deshielding by about 0.1 ppm in the latter; a similar effect in the reverse direction is observed for the 2'-O-CH_3 protons.

Conformation of Pentose Rings. With the possible exception of the neutral form of 3'-m-*xylo*-A (see below), the coupling constants of the furanose ring protons for the various derivatives are closely similar, $J_{1',2'}$ being in the range 2–3 Hz, $J_{2',3'}$ slightly below 2 Hz, and $J_{3',4'}$ close to 4 Hz. Experimental data for ribo- and arabinonucleosides (in preparation), as well as quantum chemical calculations for ethanol (Jaworski et al., 1976), indicate that dissociation of a hydroxyl group only minimally affects the coupling constants (usually less than 0.5 Hz), assuming that the conformation of the molecules is unchanged following hydroxyl ionization. Methylation of the sugar hydroxyls also affects the coupling constants to only a minor extent (Remin and Shugar, 1973). It follows that all the derivatives possess similar conformations.

The low values of the transoidal coupling constants $J_{1',2'}$ and $J_{2',3'}$ correspond to a maximal torsional angle of 120°, thus excluding the presence of a marked proportion of the S conformer of the sugar ring. The value of $J_{3',4'}$, ~4 Hz, corresponds to a torsional angle of 40–50°.[3] The predominant conformation may therefore be taken to be C(3')endo-C(4')exo, although this might equally be C(3')endo-C(2')exo, for which the values of $J_{1',2'}$ and $J_{2',3'}$, calculated from the Karplus relation, are lower than those observed experimentally. The foregoing results consequently support a conformational

[3] These conclusions are valid with the use of the generally accepted parameters of the Karplus relation (Altona and Sundaralingam, 1973; Davies and Danyluk, 1974, 1975). It should nonetheless be noted that, with a type N conformation, the coupling constant $J_{3',4'}$ must be considerably decreased due to the electronegativity of the two oxygen atoms anti periplanar to the protons in the fragment HC(4')-C(3')H (Booth, 1965; Jaworski et al., in preparation).

TABLE VI: Vicinal Proton–Proton Coupling Constants (in Hz) for Various β-D-Xylofuranosyl Nucleosides from Literature Data.

Nucleoside	Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	Ref
xylo-A	$(CD_3)_2SO$	2.0			*a*
3′-m-*xylo*-A	$(CD_3)_2SO$	2.7	2.5		*a*
9-(3-Fluoro-3-deoxy-β-D-xylofuranosyl)adenine	$(CD_3)_2SO$	2.3	2.3	2.5	*a*
9-(4-*O*-Cyclopropyl-β-D-xylofuranosyl)adenine	D_2O	1.8	1.0	3.7	*b*
	C_5D_5N	1	1	3.0	*b*
1-(2-*O*-Acetyl-3-*O*-tosyl-5-*O*-methyl-β-D-xylofuranosyl)uracil	$CDCl_3$	1.6	1.8	4.4	*c*
9-(3-Azido-3-deoxy-β-D-xylofuranosyl)adenine	$(CD_3)_2SO$	6	6		*a*
9-(3-Amino-3-deoxy-β-D-xylofuranosyl)adenine	$(CD_3)_2SO$	6	6		*a*
xylo-GMP	$(CD_3)_2SO$–D_2O	2.5			*d*
1-(2,5-Di-*O*-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)-N^4-acetylcytosine	$CDCl_3$	1	1		*e*
9-(3-Chloro-3-deoxy-β-D-xylofuranosyl)adenine	$(CD_3)_2SO$	4.2	4.0		*f*
9-(3-Bromo-3-deoxy-β-D-xylofuranosyl)adenine	$(CD_3)_2SO$	4.5	4.5	5	*f*
9-(3-Iodo-3-deoxy-β-D-xylofuranosyl)adenine	$(CD_3)_2SO$	5.5	6.5	6.5	*f*
9-(3-Thio-3-deoxy-β-D-xylofuranosyl)adenine	$(CD_3)_2SO$	6.3	8.3		*g*

[a] Robins et al., 1974. [b] Horton and Tindall, 1971. [c] Akhrem et al., 1975. [d] Revankar and Huffman, 1976. [e] Marumoto and Honjo, 1974. [f] Mengel and Wiedner, 1976. [g] Mengel and Griesser, 1977.

equilibrium shifted strongly in favor of a type N conformer but somewhat less marked for 3′-m-*xylo*-A.

Similar values prevail for the coupling constants in the parent *xylo*-A (in preparation) and the methylated derivatives examined in this study; hence, as for ribo- and arabinonucleosides referred to above, methylation of the sugar hydroxyls in *xylo*-A does not significantly affect the furanose ring conformation. Furthermore, comparison of the coupling constants of 3′-m-*xylo*-A in Me_2SO (Table VI) (Robins et al., 1974) with the corresponding values in D_2O in Table II show that a change in solvent does not affect the sugar ring conformation. It follows that the previously observed pronounced increase in susceptibility to enzymatic deamination of 2′-m-*xylo*-A, relative to *xylo*-A itself (Darżynkiewicz et al., 1977), is not due to conformational changes resulting from methylation but to either steric effects or blockage of a free hydroxyl.

It is of interest to compare the foregoing with results reported for other xylofuranosides presented in Table VI. It will be noted that 4′-cyclopropyl-*xylo*-A (actually 5′-cyclopropyl-*xylo*-A in our notation) and 2′-*O*-acetyl-3′-*O*-tosyl-5′-*m*-*xylo*-A exhibit similar vicinal coupling constants for the sugar ring protons. For 3′-deoxy-3′-fluoro-*xylo*-A the values of the vicinal coupling constants $^3J_{^1H,^1H}$ and $^3J_{^1H,^{19}F}$ (Robins et al., 1974) clearly point to a preference for the conformation C(3′)endo–C(4′)exo, C(3′)endo, or C(4′)exo.

It may therefore be concluded that a strong preference for a type N sugar conformation is characteristic for xylofuranosylnucleosides or at least for those with adenine as the aglycone. Consequently, these differ from ribonucleosides, which exhibit comparable populations of two conformational states, with a slight preference for the type S conformation in the case of purine nucleosides and nucleotides (Davies and Danyluk, 1974, 1975; Lüdemann et al., 1975; in preparation).

Note also from Table VI that replacement of the "up" 3′-OH by $-NH_2$, $-N_3$, –SH, or halogen (chlorine, bromine, iodine, but not fluorine) leads to a marked shift in conformation toward a preference of type S. Such substituents have also been found to result in appreciable modifications in sugar conformation of 2′-deoxynucleosides (Cushley et al., 1968; Blandin et al., 1974; Imazawa et al., 1975).

Conformation of the Exocyclic 5′-CH_2OH Group. On the assumption of the existence of a conformational equilibrium between three classical rotamers (gauche–gauche, gauche–trans, trans–gauche), as in the case of ribonucleosides (Blackburn et al., 1970; Remin and Shugar, 1972) and arabinonucleosides (Remin and Shugar, 1973; Remin et al., 1976a,b), calculations of the rotamer populations were made as elsewhere described (Wood et al., 1974, and references therein), with no modifications for the presence of the "up" 3′-OH cis to the exocyclic 5′-carbinol. The results are listed in Table VII.

TABLE VII: Calculated Conformer Populations of the Exocyclic 5′-CH_2OH Group in the Neutral (pD ~7) and Anionic (pD ~14) Forms of the *O*′-Methyl Derivatives of *xylo*-A.[a]

Compd	pD	Gauche–gauche	Trans–gauche[c] (gauche–trans)	Gauche–trans[c] (trans–gauche)
xylo-A[b]	7	25	35	40
2′-m-*xylo*-A	7	20	30	50
	14	30		
3′-m-*xylo*-A	7	30		
	14	20	30	50
5′-m-*xylo*-A	7	25		
	14	20	15	65
2′,3′-m_2-*xylo*-A	7	20		
	14	20		
2′,5′-m_2-*xylo*-A	7	20	20	60
	14	25	15	60
3′,5′-m_2-*xylo*-A	7	20		
N^6,2′-m_2-*xylo*-A	7	30	20	50

[a] Calculated from the Karplus relation, with $J_{60°}$ = 1.5 and $J_{180°}$ = 11.5 Hz (Wood et al., 1974, and references therein). [b] Values from an independent study on *xylo*-A (in preparation). [c] The gauche–trans and trans–gauche populations were calculated only for those cases where accurate analysis of the exocyclic 5′-CH_2OH protons was possible.

In contrast to the situation for ribonucleosides (Hruska, 1973; Hruska et al., 1977), there is no preference for the gauche–gauche rotamer, the populations of which in the various derivatives are below 30%. This is undoubtedly due to steric hindrance by the "up" 3′-OH, an effect observed in other nucleosides and sugars; e.g., in other xylofuranosylnucleosides (α-*xylo*-U and α-*xylo*-C) the gauche–gauche populations are about 15% and in α-methylxylofuranoside about 30% (unpublished). In lyxofuranosylnucleosides it is 20% for *lyxo*-C and 10% for *lyxo*-U (in preparation). A similar effect of a C(4) hydroxyl cis to a C(5) carbinol has been noted in hexapyranoses (Hall et al., 1969; Lemieux and Brewer, 1973). This pronounced effect of the 3′-OH on the rotamer populations, and probably on the chemical shifts of H(5′) and H(5″), rendered doubtful identification of these protons, as has been achieved in the case of ribonucleosides (Remin and Shugar,

1972) and arabinonucleosides (Remin and Shugar, 1973). This is regrettable inasmuch as there is a marked preference for the gauche–trans or trans–gauche rotamer, particularly when the 5′-OH is etherified. Direct identification in these cases would require selective replacement of one of the methylene protons by deuterium, as has been done by Ritchie and Perlin (1978) for adenosine; the intermediates in this synthesis were 1,2-*O*-isopropylidene-5-*O*-acetyl-α-D-xylofuranose-5-*d* and its 3,5-di-*O*-acetyl and 3-*O*-acetyl-5-*O*-trityl derivatives. The ^{1}H NMR parameters reported for these compounds show that preference for one of the rotamers, gauche–trans or trans–gauche, is markedly dependent on the substituents on the 3′- and 5′-OH; hence, attempts to transpose these results to our compounds were not feasible.

Although there are no data available on the solid-state structures and conformation of β-xylofuranosylnucleosides, it is of interest that both the neutral and protonated forms of 1-α-D-xylofuranosylcytosine in the solid state exhibit the trans–gauche conformation (M. Post et al.; in preparation). By contrast, O^2,2′-anhydro-1-α-D-xylofuranosyluracil in the crystal is gauche–gauche, with an intramolecular hydrogen bond between the O(5′) and O(3′), viz. O(3′)-H···O(5′)-H (Birnbaum et al., 1976).

Possible Intramolecular Hydrogen Bonding in Xylo-A. For arabinonucleosides in strongly alkaline medium, ionization of the 2′-hydroxyl and accompanying modifications in conformation of the sugar ring lead to formation of an intramolecular hydrogen bond, O(5′)-H···O(2′)$^-$ (Darżynkiewicz et al., 1975a).Similar intramolecular hydrogen bonding, O(2′)-H···O(5′)-H, is found for the neutral forms in the solid state (Chwang and Sundaralingam, 1973; Tollin et al., 1973).

One of the purposes of the present study was to examine the possible formation of intramolecular hydrogen bonding in the solution conformer(s) of *xylo*-A, either in the neutral form or following hydroxyl dissociation. Such a bond, O(3′)-H···O-(5′)-H, does in fact exist in the solid state neutral form of 2,2′-anhydro-α-*xylo*-U (Birnbaum et al., 1976), in which the sugar ring has a C(4′) exo pucker, and the conformation of the exocyclic 5′-CH_2OH is gauche–gauche under these conditions. The formation of a similar hydrogen bond in the anionic form in alkaline medium, O(5′)-H···O(3′)$^-$, was advanced as a plausible interpretation for the high acidity of the sugar hydroxyl(s) of *xylo*-A, identical with that for adenosine (Christensen et al., 1966).

The model derivative selected for examining the possibility of such hydrogen bonding in *xylo*-A was 2′-m-*xylo*-A, in which it is virtually only the 3′-OH which ionizes at pH values up to about 14. The conformation of this compound, which changes very little on transfer from neutral to alkaline medium, permits such hydrogen bonding. However, the observed increase in the gauche–gauche population of the exocyclic carbinol group, from 20 to 30% on addition of 0.3 mmol of NaOD, is not very marked by comparison with arabinonucleosides, where sugar hydroxyl dissociation increases the gauche–gauche population to well over 80% (Remin et al., 1976a,b).[4,5] But the gauche–gauche population of 2′,5′-m_2-*xylo*-A, where intramolecular hydrogen bonding is excluded by the blocked O(5′), also increases slightly on ionization of the 3′-OH (Table VII). The low gauche–gauche population of the anionic form of 2′-m-*xylo*-A, which argues against formation of intramolecular hydrogen bonding, does not, however, fully exclude such a possibility. More decisive evidence against such hydrogen bonding is forthcoming from the results of an analysis of the relative acidities of the sugar hydroxyls, based on the sequence of elution of various derivatives from a strongly basic column of Dowex (OH^-), described below.

Relative Acidities of O′-Methyl Nucleosides. It was shown by Dekker (1965) and Gin and Dekker (1968) that *O*′-methyl nucleosides may be readily fractionated on a strongly basic Dowex (OH^-) column, the affinity of a given derivative for the resin being dependent on its acidity. From observations on a number of model compounds, it has also been shown that the acidities of nucleosides and sugars are dependent not only on the dissociation constants of the individual sugar hydroxyls but may be appreciably increased by formation of intramolecular hydrogen bonds and resultant stabilization of the anionic form (Darżynkiewicz et al., 1975b).

An examination was therefore made of the relative affinities toward Dowex (OH^-) of various *O*′-methyl derivatives of *xylo*-A and some other selected nucleosides and their etherified analogues. The sequences of elution of all of these are exhibited in Figure 2. The clearly delineated differences in affinity for the resin of the derivatives of the ribo-, arabino- and xylonucleosides may be correlated with the ability of some of these to form intramolecular hydrogen bonds in the anion.

Arabinonucleosides. The elution sequence of corresponding derivatives of araC and araA is similar, although the affinity of the adenine nucleosides for the resin is higher than that of cytosine nucleosides. The relative acidities in both series of compounds, inversely related to the sequence of elution, correlate with the ability to form an intramolecular hydrogen bond, O(5′)H···O(2′)$^-$, as described elsewhere (Darżynkiewicz and Shugar, 1974), and leading to an increased acidity of the 2′-OH when the 5′-OH is not blocked. Etherification of either the 5′-OH or 2′-OH leads to a very marked reduction in acidity of arabinonucleosides.

Ribonucleosides. Etherification of the 2′-OH or 3′-OH markedly reduces the acidity of adenosine and is consistent with the existence of the proposed intramolecular hydrogen bond in the anion, O(2′)H···O(3′)$^-$ ⇌ O(3′)H···O(2′)$^-$ (Izatt et al., 1965, Kusmierek et al., 1973).

Xylonucleosides. The elution sequence (Darżynkiewicz et al., 1977) is 2′,3′-m_2-*xylo*-A, 2′,5′-m_2-*xylo*-A, 2′-m-*xylo*-A, N^2,2′-m_2-*xylo*-A, 3′,5′-m_2-*xylo*-A, 5′-m-*xylo*-A, 3′-*xylo*-A, N^6-m-*xylo*-A, *xylo*-A. Note the increasing acidity of isolated hydroxyls, in those derivatives with the remaining two hydroxyls etherified, in the order 5′-PH < 3′-OH < 2′-OH. Blocking of the 5′- or 3′-OH affects to only a minor extent affinity for the resin, whereas etherification of the 2′-OH drastically reduces the acidity. From Figure 2 it appears that the isolated 2′-OH in *xylo*-A is appreciably more acidic than in *ribo*-A and *ara*-A. The relatively small effect on the acidity of *xylo*-A of etherification of the 3′-OH and/or 5′-OH clearly argues against the previously postulated (Birnbaum et al., 1976) formation in solution of an anionic intramolecular hydrogen bond of the form O(5′)H···O(3′)$^-$. The high acidity of *xylo*-A, virtually identical with that for adenosine (Christensen et al., 1966), appears to be due to the 2′-OH.

Relevant to the foregoing is the demonstration that a shift in the syn-anti equilibrium about the glycosidic bond, for β-nucleosides, may affect affinity for the ion-exchange resin, the nucleoside with the syn conformation being eluted first, so that it is more acidic than its anti counterpart (Shugar et al., 1976). While this effect may to some extent influence the elution sequences shown in Figure 2, it would not affect the conclusions derived therefrom above. For *xylo*-A, for example, a marked

[4] The gauche–gauche population was calculated as described in Table VII.

[5] This problem has been examined in greater detail in the case of *xylo*-A with the aid of a 300-MHz spectrum, making possible a more accurate analysis of the exocyclic group conformation (in preparation).

reduction in acidity occurs only on etherification of the 2′-OH, but this is unlikely to be associated with any appreciable modification in the syn–anti equilibrium, since the NMR chemical-shift data point to the highly preferred anti conformation of 2′-m-*xylo*-A. This is further supported by the high acidities of all the *xylo*-A derivatives with an unblocked 2′-OH.

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