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SOLUTION CONFORMATIONS OF THE ANTIMETABOLITE
9-β-D-XYLOFURANOSYLADENINE AND ITS 8-BROMO ANALOGUE*

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An analysis has been made, with the aid of $^1$H NMR spectroscopy, of the solution conformation of the known antimetabolite, 9-β-D-xylofuranosyladenine (xyloA), and of its 8-bromo analogue. For xyloA, the results point to a strong preference for the sugar ring of the conformation type N (C(3') endo), a relatively low population of the gauche-gauche rotamer of the exocyclic 5'-CH$_2$OH, and a preference for the conformation anti about the glycosidic bond. For 8-bromo-xyloA, the preference for the type N conformation of the sugar ring is less marked, and the preferred conformation about the glycosidic bond is syn. The conformation of the sugar ring in the foregoing xytonucleosides consequently differs appreciably from that for the corresponding ribonucleosides, which adopt preferentially the type S (C(2') endo) and gauche-gauche conformations. Comparison with previously reported results for O'-methyl derivatives of xyloA points to the similarity in conformational properties of all of these.

In contrast to arabinonucleosides with free 2' and 5' hydroxyls, the conformation of xyloA is relatively unaffected in strongly alkaline medium where the sugar hydroxyls dissociate. Under these conditions, there is no formation of an intramolecular hydrogen bond such as might have been anticipated from X-ray diffraction studies in the solid state.

In an extension of a previous study (Ekiel et al., 1978) on the solution conformation of O'-methyl analogues of xyloA$^1$ (9-β-D-xylofuranosyladenine), we present here a more detailed study of the solution conformation of the parent xyloA, with

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$^1$ Abbreviations employed: xyloA, 9-β-D-xylofuranosyladenine; 2'-mxyloA, 2'-O-methyl-xyloA; 8-bromo-riboA, 8-bromoadenosine; DMSO, dimethyl sulphoxide, (CH$_3$)$_2$SO; DMSO-d$_6$, (CD$_3$)$_2$SO; TSP, 2,2,3,3-tetradecanoic acid, sodium salt.
the aid of $^1$H NMR spectroscopy. With a view to examining more closely the conformation about the glycosidic bond, we have also prepared 8-bromo-xyloA, which is predominantly, but not exclusively (Pless et al., 1978; Dudycz et al., 1979), in the syn conformation, for use as a reference analogue (see Scheme 1).

![Scheme 1](image_url)

Apart from the intrinsic physico-chemical interest of the conformation of xylo nucleosides in relation to those of other nucleosides, such results are of obvious relevance to the extensively characterized antimetabolic activities of xyloA. It exhibits both antitumour (Ellis & LePage, 1965; Roy-Burman, 1970) and antiviral (De Rudder et al., 1967) activities. The 5'-triphosphate is known to interfere with formation of PRPP and to be a feedback inhibitor of purine biosynthesis (Roy-Burman, 1970). It inhibits ribonucleotide synthesis from hypoxanthine and de novo purine biosynthesis. An interesting recent finding is that xyloA effectively inhibits nuclear RNA methylation, to a greater extent than it inhibits RNA synthesis (Glazer & Peale, 1978), and that such inhibition of methylation is linked to inhibition of S-adenosyl-L-methionine synthesis (Glazer & Peale, 1979). The potential antimetabolic properties of other xylofuranosyl nucleosides have been less extensively studied, but xylofuranosyl 6-mercaptopurine and its 5'-phosphate have been reported to inhibit the utilization of guanine for DNA synthesis (Sato et al., 1966; LePage & Naik, 1975), and the 5'-phosphate appears also to possess immunosuppressive activity (LePage & Naik, 1975).

It is consequently reasonable to anticipate that a knowledge of the conformational properties of xyloA in solution should be of value in the interpretation of its antimetabolic properties.

**EXPERIMENTAL**

A sample of xyloA was kindly provided by Dr. Harry B. Wood, Jr. of the Drug Development Branch, National Cancer Institute (Bethesda, Md., U.S.A.), while 8-bromo-xyloA was prepared by L. Dudycz in this laboratory by the procedure of Ikehara et al. (1971).

$^1$H NMR spectra were recorded on 0.1 M solutions of the nucleosides in $^2$H$_2$O or DMSO-$d_6$ (>99.9 mol % $^2$H, from Merck, Darmstadt, F.R.G.) with the aid of a Varian HA-300, a Bruker 270, or a Jeol 100 instrument. Several spectra on
samples of low concentration (6 - 7 mm) were run on a Bruker 90. In the case of 8-bromo-xyloA in DMSO-d$_6$, partial overlapping of the H(3') and H(4') signals made a proper analysis difficult, even with the use of the 270 MHz instrument.

![Fig. 1. $^1$H NMR (300 MHz) spectrum of xyloA, 0.1 m in neutral $^2$H$_2$O. Upper spectrum was that recorded experimentally; the lower one was obtained by simulation as described in text.](image)

Simulations were carried out for all the spectra of 0.1 m solutions with the aid of the program LAOCOON III and a graphic plotter. Typical spectra, experimental and simulated, are exhibited in Fig. 1 for xyloA in neutral aqueous medium. Errors in vicinal coupling constants ($J$) from the simulated spectra were of the order of 0.1 - 0.2 Hz.

RESULTS AND DISCUSSION

The parent xyloA was examined in aqueous medium and in DMSO. However, the poor solubility of 8-bromo-xyloA in aqueous medium limited measurements to DMSO as the solvent system. Nonetheless, it did prove possible, with a relatively low concentration of 8-bromo-xyloA (6 mm) in $^2$H$_2$O, to obtain an experimental value for $J(1',2') \sim 3.9$ Hz. This was virtually identical to the value in DMSO, testifying to the small effect of solvent on coupling constants and, consequently, on conformation.

The experimentally determined values of chemical shifts and coupling constants for xyloA and 8-bromo-xyloA are listed in Table 1. A comparison of the values of $J(1',2')$ for xyloA at a concentration of 0.1 m (Table 1) and 6 mm (Table 2) demonstrates that the conformation of the ribose ring is not essentially affected over this concentration range.

Conformation of the sugar ring. For xyloA, both in $^2$H$_2$O and in DMSO, the values of the three vicinal coupling constants of the sugar ring are relatively low, and very similar to those previously reported for the various O'-methyl derivatives of this nucleoside (Ekiel et al., 1978). This simply reflects the similarities in conformation, and once again underlines the relatively small effects of O'-methylation.
on the puckering of the sugar ring in nucleosides (Hruska et al., 1973, 1977; Ekiel et al., 1979a).

For xyloA in neutral and alkaline aqueous medium, as well as in DMSO, a classical analysis of the transoidal coupling constants J(1',2') and J(2',3'), with the use of the Karplus relation in the form $^3J = A \cos^2 \varphi + B \cos \varphi + C$, and the generally accepted parameters for this equation (e.g. Davies & Danyluk, 1974), points to the existence of the sugar ring in a conformational equilibrium $N \leftrightarrow S$ [C(2') endo $\leftrightarrow$ C(3') endo] but strongly shifted in the direction of the state of type N. This is fully supported by theoretical calculations of the dependence of the value of J(3',4') on the conformation of the xylofuranosyl ring, which predict a pronounced difference in the values of J(3', 4') for states of the type N and S, as shown in Fig. 2 (Jaworski et al., 1978). This marked difference in the values of J(3',4') for the N and S type states in xylofuranosyl nucleosides, assuming the sugar ring to exist in a two-state

### Table 1

**Chemical shifts (in ppm vs internal TSP) and vicinal coupling constants (in Hz) for xyloA and 8-bromo-xyloA**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>Chemical shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H(1')</td>
</tr>
<tr>
<td>XyloA</td>
<td>$^2$H$_2$O, p$^3$H~7</td>
<td>5.98</td>
</tr>
<tr>
<td>XyloA</td>
<td>$^2$H$_2$O, p$^3$H~14</td>
<td>5.85</td>
</tr>
<tr>
<td>XyloA</td>
<td>(C$_2$H$_5$)$_3$SO</td>
<td>5.87</td>
</tr>
<tr>
<td>8-Br-xyloA</td>
<td>(C$_2$H$_5$)$_3$SO</td>
<td>5.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coupling constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>J(1',2')</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>XyloA</td>
</tr>
<tr>
<td>XyloA</td>
</tr>
<tr>
<td>XyloA</td>
</tr>
<tr>
<td>8-Br-xyloA</td>
</tr>
</tbody>
</table>

* Accuracy about 0.5 Hz.

### Table 2

**Chemical shifts (in ppm vs internal TSP), and coupling constants J(1',2') in Hz, for xyloA and 8-bromo-xyloA in aqueous neutral and strongly alkaline media**

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Concentration (mm)</th>
<th>Amount of added NaO$^3$H (mmol)</th>
<th>Chemical shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H(2)</td>
</tr>
<tr>
<td>XyloA</td>
<td>7</td>
<td>0.0</td>
<td>8.24</td>
</tr>
<tr>
<td>XyloA</td>
<td>7</td>
<td>0.4</td>
<td>8.25</td>
</tr>
<tr>
<td>8-Br-xyloA</td>
<td>6</td>
<td>0.0</td>
<td>8.22</td>
</tr>
<tr>
<td>8-Br-xyloA</td>
<td>6</td>
<td>0.4</td>
<td>8.20</td>
</tr>
</tbody>
</table>

* Accuracy 0.03 ppm, due to partial overlapping of the H(2') signal by a water side band.
equilibrium, is to be expected since the observed range of variation of the foregoing coupling constant in various analogues, e.g. the O'-methyl derivatives of xyloA (Ekiel et al., 1978) is comparable to the experimentally observed ranges for J(1',2') and J(2',3'). The theoretically calculated curve in Fig. 2 fulfils this requirement.

![Diagram](image)

**Fig. 2.** Dependence of vicinal coupling constants $^3J$ on conformation of β-D-xylofuranosyl nucleosides. The curves denoted $J(1',2')$, $J(2',3')$ and $J(3',4')$ were obtained from the standard Karplus relation (as described in text); that denoted by $J(3',4')$, is a theoretically calculated curve taken from Jaworski et al. (1978). $P$ is the parameter of pseudorotation.

From the observed dependence of the values of the coupling constants on the pseudorotational parameter $P$, shown in Fig. 2, the population of the N type state of the sugar ring in xyloA may be evaluated as about 75% in neutral aqueous medium, and about 80% in DMSO. The values of all three coupling constants in aqueous medium are decreased on alkaliization (Table 1), and at $p^\text{H}>14$ the population of the state N is consequently increased by about 5%. It is consequently clear that, under all conditions employed in this study, xyloA exhibits a very marked preference.

**Table 3**

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Solvent</th>
<th>% N</th>
<th>% gauche-gauche</th>
</tr>
</thead>
<tbody>
<tr>
<td>XyloA</td>
<td>$^3\text{H}_2\text{O}$, p$^\text{H}$ 7</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>XyloA</td>
<td>$^2\text{H}_2\text{O}$, p$^\text{H}$ 14</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>XyloA</td>
<td>(C$^2\text{H}_2$)$_2$SO</td>
<td>80</td>
<td>15</td>
</tr>
<tr>
<td>8-Bromo-xyloA</td>
<td>(C$^2\text{H}_2$)$_2$SO</td>
<td>65*</td>
<td>25</td>
</tr>
</tbody>
</table>

* The N-type state for this nucleoside is somewhat different from that for the parent xyloA.
for the N type state. Introduction of the 8-bromo substituent reduces the population of this state by 10 - 20\%, possibly linked with a shift of this state in the direction C(4') exo (see Table 3).

**Conformation of exocyclic 5'-CH₂OH.** As in the case of the O'-methyl derivatives of xyloA (Ekiel et al., 1978) and other pentofuranosyl nucleosides in general with a 3'-OH in the configuration cis relative to the exocyclic 5'-carbinol group (Ekiel et al., 1979b), the value of $\Sigma = J(4',5') + J(4',5'')$ for xyloA is relatively high, pointing to a relatively low population of the rotamer gauche-gauche. Quantitatively, from the relation $% (\text{gauche-gauche}) = \frac{13 - \Sigma}{10}$, we find for both xyloA and 8-bromo-xyloA values for the gauche-gauche populations of the order of 20 - 30\% (Table 3). It should be noted that the lowest gauche-gauche population is observed for xyloA in DMSO, under which conditions the preference of the sugar ring for the type N state is most pronounced.

**Comparison with ribonucleosides.** It is of some interest to compare the conformations of the foregoing nucleosides with those of the corresponding ribonucleosides. For adenosine, in aqueous medium at pH 8, the population of the N type state is 33\%, and for 8-bromoadenosine 22\% (Sarma et al., 1974), hence markedly different from the analogous xylono nucleosides (70 - 80\%). Equally different are the gauche-gauche populations of the exocyclic 5'-CH₂OH, 66\% for adenosine and 74\% for 8-bromoadenosine, as calculated from appropriate literature data on coupling constants, in comparison with 27\% for the xylono nucleosides (see above). The low value for the gauche-gauche population in xylono nucleosides is undoubtedly due to interaction of the "up" 3'-OH with the exocyclic 5'-CH₂OH. In cordycepin (3'-deoxyadenosine), which has no "up" 3'-OH, and the sugar ring conformation is similar to that in xylono nucleosides, about 75\% of the type N state (Westhof et al., 1977), the gauche-gauche rotamer is predominant, as in ribonucleosides. Preliminary theoretical calculations, by the force field method (in preparation), indicate that the interaction between the "up" 3'-OH and the exocyclic 5'-CH₂OH in xylono nucleosides is largely of a steric nature, as might be anticipated from an examination of molecular models.

The foregoing is also of possible interest in relation to the correlation proposed by Hruska et al. (1977) between the conformation of the exocyclic 5'-CH₂OH group and that of the sugar ring for purine ribonucleosides. We have, in fact, examined the possible applicability of this relationship to a number of xyloA analogues, viz. O'-methyl and 2'(3')-deoxy-2'(3')-halogeno derivatives (unpublished results). Plotting the sum $J(4',5') + J(4',5'')$ against $J(1',2')$, it was found that an approximate straight line could be drawn through the points for purine ribonucleosides and the xyloA analogues; and, as for the purine ribonucleosides (Hruska et al., 1977), the gauche-gauche rotamer populations for the xyloA derivatives decrease to a very low value with the N-type puckering of the sugar ring. However, a closer examination, including that of solvent effects, shows that this apparent linear relationship is not unique (cf Davies, 1979), and clearly requires a more detailed analysis.
Possible intramolecular hydrogen bonding of xyloA in alkaline medium. In strongly alkaline medium, where the sugar hydroxyl(s) undergo ionization, the possibility exists for formation of an intramolecular hydrogen bond of the form 5'-OH···O(3')⁻⁻. This was initially shown by the demonstration that, in the solid state, O²⁻,2'-anhydro-1-α-D-xylofuranosyluracil possesses the conformation C(4') exo of the sugar ring, while the exocyclic 5'-CH₂OH is in the gauche-gauche conformation; the geometry of this system is such that there is formation of an intramolecular hydrogen bond O(3')-H···O(5')-H (Birnbaum et al., 1976). In solution the conformation of the sugar ring of xyloA is very similar in both neutral and strongly alkaline medium (Table 1), but the low gauche-gauche population decreases even further on alkalization of the medium, arguing against intramolecular hydrogen bonding in solution with a conformation of the molecule similar to that observed in the solid state. Geometrically, formation of the intramolecular hydrogen bond O(5')-H···O(3') is feasible also with a conformation of the type N, i.e. C(3') endo or C(4') exo, for values of the angle φOC C(3')-C(4')-C(5')-O(3') about −20° and the angle C(4')-C(5')-O(5')-H(5') about 60°. Such a conformation represents, however, a marked departure from those normally encountered in nucleosides (gauche-gauche, gauche-trans and trans-gauche) and is, furthermore, excluded by the experimentally observed values of the coupling constants J(4',5') and J(4',5''). For an angle φOC = −20°, these values should be about 8 Hz and 7 Hz. Formation of an intramolecular hydrogen bond should therefore be accompanied by a marked increase in the values of these couplings, contrary to what is actually observed. Hence, in contrast to β-arabinofuranosyl nucleosides, where ionization of the “up” 2'-OH leads to a marked change in sugar ring conformation, with formation of the intramolecular hydrogen bond O(5')-H···O(2')⁻⁻ (Darżynkiewicz et al., 1975), the possible analogous hydrogen bond in xylonucleosides, viz. O(5')-H···O(3')⁻⁻, is apparently not formed.

Conformation about glycosidic bond. Proton chemical shifts were profitied from to evaluate the conformations about the glycosidic bonds of xyloA and 8-bromo-xyloA. It has been shown that, amongst the various protons of the furanose ring, the chemical shift of H(2') best reflects the transition between the forms syn and anti, the transition from the former to the latter being accompanied by shielding of this proton (Ikehara et al., 1972; Sarma et al., 1974).

Table 2 exhibits the chemical shifts of some selected protons in xyloA and 8-bromo-xyloA. It will be noted that the transition from the former to the latter in neutral aqueous medium is accompanied by a downfield shift of H(2') of 0.23 ppm. The corresponding downfield shift of H(2') in the transition from adenosine to 8-bromo-adenosine is 0.27 ppm (Sarma et al., 1974). Since in both ribo- and xylo-nucleosides the H(2') proton is located cis relative to the aglycone, the foregoing values point to similar conformations about the glycosidic bond of xyloA and riboA on the one hand, and 8-bromo-xyloA and 8-bromo-riboA on the other. It should be noted that, bearing in mind the appreciable difference in sugar conformation, and the possibility of a somewhat different location of the energy minima for rotation about the glycosidic bond, in xylo- and ribo-nucleosides, the effect
of the conformation about the glycosidic bond (in terms of the populations syn and anti) on the chemical shifts of H(2') may not be strictly identical in both classes of compounds. Since riboA is known to be predominantly anti, and 8-bromo-riboA predominantly, but not exclusively, in the syn conformation (Dudycz et al., 1979), it follows that in neutral aqueous medium xyloA is predominantly anti and 8-bromo-xyloA predominantly syn.

In DMSO-d_6 the difference in chemical shifts of H(2') between 8-bromo-xyloA and xyloA amounts to 0.43 ppm. This value is very close to that for the difference between 8-bromo-adenosine and adenosine, 0.48 ppm (Jordan & Niv, 1977), pointing to the similarities in conformation about the glycosidic bond of adenosine and xyloA on the one hand, and 8-bromo-adenosine and 8-bromo-xyloA on the other. These results also indicate an apparent increase in population of the syn forms of the 8-bromo derivatives in DMSO-d_6 as compared to H_2O. However, the difference in chemical shifts of H(2') between 8-hydroxyisopropyladenosine (a model compound unequivocally in the syn conformation) and adenosine is only 0.36 ppm (Stolarski et al., in preparation), i.e. less than that between 8-bromo-adenosine and adenosine. It is consequently conceivable that the values of the chemical shifts of H(2') depend, at least in part, on factors other than the syn and anti populations, such as solvent effects, or differences in the value of the glycosidic torsion angle. Solvent effects, in particular, may be especially significant, e.g. it has been reported that the difference in chemical shifts of H(2') between 8-bromo-adenosine and adenosine in liquid N_2H_3 is as high as 0.62 ppm (Westhof et al., 1975). Similar solvent-dependent effects have been noted in the case of guanine nucleosides (Jordan & Niv, 1977; Davies, 1979), and to a lesser extent for pyrimidine nucleosides (Davies, 1979). It is clear that the role of solvent in conformational analysis of nucleosides requires more detailed examination.

Bearing in mind that in xylo nucleosides, including the various O'-methyl derivatives, the most acid hydroxyl is the 2'-OH (Ekiel et al., 1978), it is to be expected that this hydroxyl will be predominantly ionized at p^2H > 14. Hence one would anticipate that the effect of dissociation of the 3'-OH on the chemical shift of H(8) would be somewhat limited, even though these are in the vicinity of each other in the conformation anti. In fact, it will be seen from Table 2 that the difference in chemical shifts of H(8) in going from p^2H 7 to 14 is only 0.07 ppm. In the case of 2'-mxyloA, where only the 3'-OH is predominantly ionized, this difference amounts to 0.17 ppm (Ekiel et al., 1978), consistent with the conformation anti.

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REFERENCES


KONFORMACJA ANTYMETABOLITU, 9-β-β-o-KSYLOFURANOZYLOADENINY I JEJ 8-BROMO POCHODNEJ W ROZTWORZE

Streszczenie

Metodą spektroskopii 1H NMR przeprowadzono analizę konformacji w roztworze antymetabolitu, 9-β-o-ksylofuranosyladeniny (ksyloA) i jego 8-bromo pochodnej. Dla ksyloA wyniki wskazują na dominację konformacji typu N (C(3')endo) pierścienia cukrowego, stosunkowo niską populację rotameru gauche-gauche grupy egzocyclicznej 5'-CH₂-OH i preferencję konformacji anti wokół wiązania glikozydowego. Dla 8-bromo-ksyloA preferencja konformacji typu N jest słabiej zaznaczona, natomiast obserwujemy znaczną udział konformacji syn wokół wiązania glikozydowego. Konformacja części cukrowej przebadanych ksylokolezóydów różni się istotnie od konformacji odpowiednich rybonukleozóydów, dla których obserwuje się uprzywilejowanie stanu S (C(2') endo, gauche-gauche. Porównanie wyników dla ksyloA i dla O'-etylowanych pochodnych ksyloA wskazuje na podobieństwo konformacji.

W odróżnieniu od arabinokolezóydów z wolnymi grupami hydroksylowymi 2' i 5', konformacja ksyloA prawie nie zmienia się w silnie alkalickim środowisku, gdy dysocjują grupy hydroksylowe cukru. W tych warunkach nie tworzy się wewnątrzcząsteczkowe wiązanie wodorowe, którego powstawania można było oczekiwać na podstawie badań rentgenograficznych.

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