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CONFORMATIONAL STUDIES OF TWO ISOMERIC RING-EXPANDED PURINE NUCLEOSIDES AND THEIR 5'-MONO- AND -DIPHOSPHATE DERIVATIVES

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SUMMARY: The nucleosides **Ia** and **IIa** exist in syn and anti conformations, respectively, both in solid state and solution. Compound **Ia** undergoes significant conformational change, accompanied by increased population of the anti conformer, upon conversion to the corresponding 5'-mono- and-diphosphate derivatives, whereas conformation of **IIa** remains reasonably constant between nucleoside and nucleotides. While **Ia** possessed the C2'-endo-C3'-exo geometry, **IIa** had the opposite C2'-exo-C3'-endo conformation. The C5' of the two nucleosides bore axial and equatorial conformations, respectively.

It has recently been reported (1) that a correlation exists between preferred sugar conformation and activity of nucleoside analogues against the human immunodeficiency virus (HIV) (2), the causative agent of the dreadful acquired immunodeficiency syndrome (AIDS) (3). The compounds chosen for the study belong to the general category of nucleic acid chain terminators (4). Chain terminators inhibit HIV reverse transcriptase, the enzyme responsible for the viral replication. Chain terminating nucleoside analogues have been gaining increased scientific attention in light of inherent problems associated with the development of AIDS vaccines (5a-c), although some progress (5d) is reported on that front recently.

Chain terminating property of a majority of known anti-AIDS drugs such as AZT, ddC, ddI, ddA, and CS-87 (3b) has been attributed to the lack of their sugar 3'-hydroxyl group. Chain termination may also be brought about by base mis-pairing accompanied by considerable conformational deviation of the 3'-hydroxyl group from the natural array (6). The absence or the significant deviation of the 3'-OH group would prevent incorporation of

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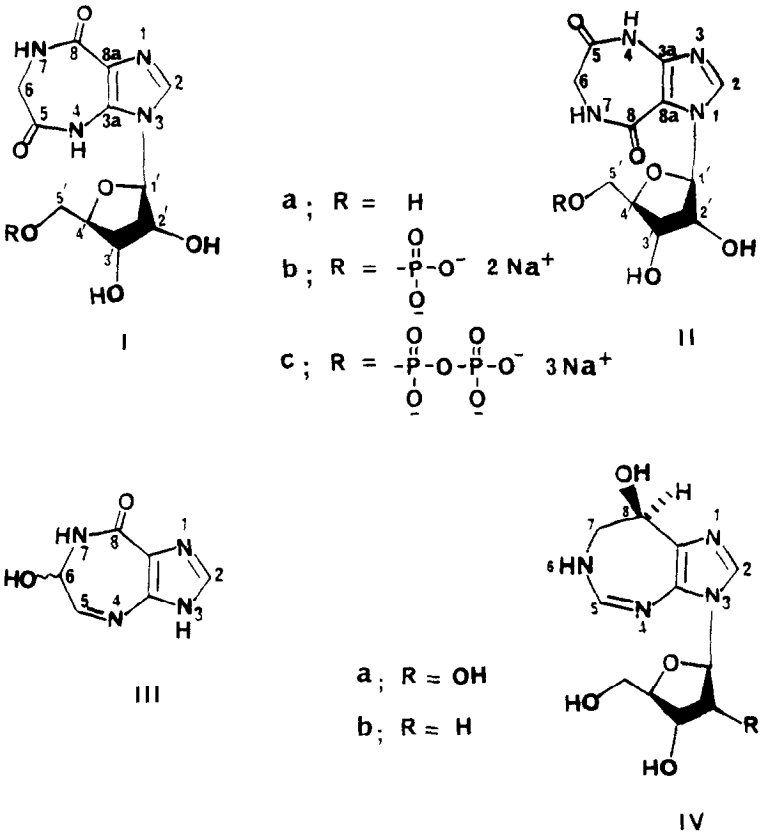
subsequent nucleotides into the growing nucleic acid chain. However, the ease of phosphorylation *in vivo* of the chain terminating nucleoside analogue may also be crucial in its efficacy as a drug (1). In this regard, conformation of the 5'-hydroxyl group--the site of phosphorylation of the nucleoside--would equally play an important role in the overall activity.

Systematic study is warranted for exploring the little understood interrelationships of aberrant base-sugar conformation, base mispairing, sugar pucker, ease of enzymic phosphorylation, and chain termination. In this regard, ring-expanded nucleoside analogues offer excellent scopes to probe such interrelationships by virtue of their unique structural features and steric constraints. Ring-expansion of natural nucleosides is anticipated (7) to affect significantly the base-ribose conformational array, sugar pucker, and *syn/anti* conformational arrangement. Equipped with novel electronic, ionic, and other physico-chemical characteristics which are anticipated to be distinct from those of the natural nucleosides, these "fat" nuclosides are also potentially a rich source of substrates or inhibitors of enzymes of the purine biosynthetic pathway, as well as of those requiring energy cofactors.

We report here the novel base-ribose conformational characteristics of two regioisomeric, ring-expanded analogues of purine nucleosides, **Ia** and **IIa**, in solid state and in solution. In addition, the solution conformations of the corresponding 5'-mono- and -diphosphate derivatives **Ib, Ic** and **IIb, IIc** are discussed. These nucleo(s/t)ides, which have recently been synthesized in this laboratory (8), contain the 5:7-fused heterocyclic ring skeleton, imidazo[4,5-*e*][1,4]diazepine. Recently, the isolation and synthesis of an antitumor antibiotic, called azepinomycin (**III**), which contains the above ring system, has been reported (9). The other widely studied 5:7-fused nucleosides are the naturally occurring synergistic antitumor antibiotics, coformycin (**IVa**) (10a-c) and pentostatin (**IVb**) (10d-h), which contain the imidazo[4,5-*d*][1,3]diazepine ring system, and their analogues containing a modified sugar (10c), modified imidazole (11) or modified both sugar and imidazole (12). While coformycin and pentostatin are known to be the most potent inhibitors of adenosine deaminase (ADA) ($K_i \simeq 10^{-15} - 10^{-17}$) (13), azepinomycin is reported to be an inhibotor of guanine deaminase (9). By virtue of the tetrahedral geometry of the 7-ring carbon bearing the hydroxyl functionality, all three antibiotics are considered to be transition state analogue inhibitors of ADA (13). The "fat" nucleo(s/t)ides I and II, on the other hand, are structurally more comparable to the natural nucleo(s/t)ides, specifically of xanthine. To the best of our knowledge, none of the earlier reports on 5:7 systems has dealt with their potentially novel base-sugar conformation and sugar pucker. (See Scheme 1.)



Scheme 1

MATERIALS AND METHODS

Proton nuclear magnetic resonance spectra were recorded on a GN-500 (500 MHz) spectrometer, using DMSO-d_6 as solvent, and tetramethylsilane (TMS) as internal reference standard. Ultraviolet (UV) spectra were obtained on either a Carey 219 UV/Vis or a Gilford Response UV/Vis spectrophotometer. Circular dichroism (CD) spectra were recorded on a JASCO J-40A automatic recording spectropolarimeter, using pathlength of 1 mm. Single-crystal X-ray diffraction analyses were performed at the Department of Chemistry, Southern Methodist University, Dallas, Texas, on an automatic Nicolet R_{3m}/V diffractometer at room temperature, using graphite monochromated Mo Kα (λ = 0.71073 Å) radiation. The structures were solved by using SHELXTL-PLUS program (14). Values for Rw were 3.94% and 4.35% for **Ia** and **IIa**, respectively. Detailed X-ray data will be published elsewhere.

RESULTS AND DISCUSSION

The ORTEP views of **Ia** and **IIa** are shown in Figures 1 and 2, respectively. Evidently, the seven-membered ring in each is puckered. While the base-ribose conformational relationship in **Ia** is syn ($\Phi_{C,N}$ = 149°) (15), that in **IIa** is anti ($\Phi_{C,N}$ = 19.9°) (15). The syn conformation of **Ia** is apparently stabilized by intramolecular hydrogen bonding between the 5'-hydroxyl group and the N^4-H of the diazepine ring (5'-O...HN = 1.92 Å).

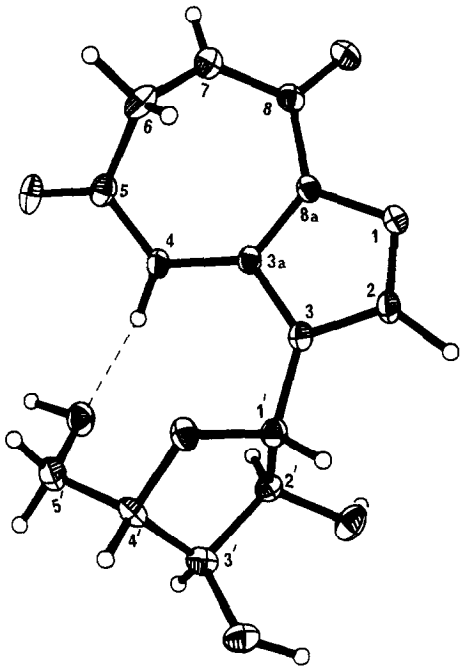


Figure 1 ORTEP view of **Ia** showing thermal ellipsoids at the 30% probability level.

This is unusual since purine nucleosides, including xanthosine, normally assume anti conformation in the crystal lattice (15,16) unless a bulky substituent (17a) such as bromine (17b), tert-butyl (17c) or α-hydroxyisopropyl group (17d) is attached to the imidazole ring at the C-8 position. However, the nucleotide 2'-deoxyguanosine-5'-monophosphate is known to crystallize in the syn form both as a monomer (18a-c) and as a constituent of oligomers (18d) and polymers (18e), thereby forming left-handed Z-DNA double helices. Other exceptions include purine nucleoside

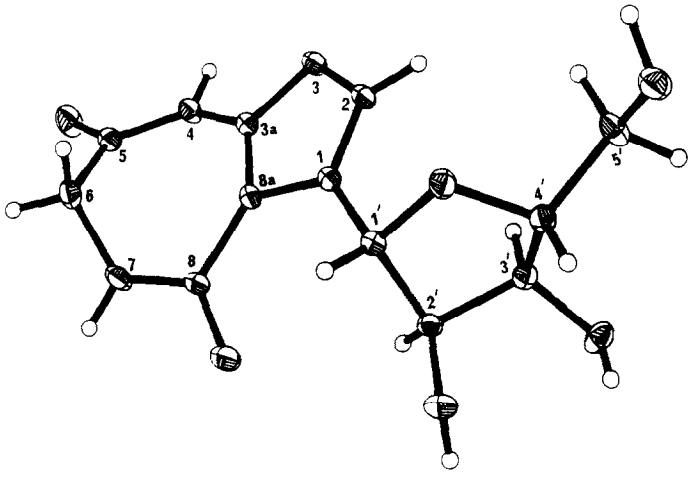


Figure 2 ORTEP view of **IIa** showing thermal ellipsoids at the 30% probability level.

analogues with altered base and/or sugar, for example, a pyrazolo[3,4-b]pyrimidine arabinoside has been recently reported to possess the syn conformation (19).

The presence or absence of intramolecular hydrogen bonding observed in the crystal lattice of **Ia** or **IIa** was also inferred in solution. The ^{1}H NMR spectrum of **Ia** exhibited only one NH signal, a triplet at δ 7.82 ($J_{NH,CH2}$ = 5.4 Hz), corresponding to the N^7-H. The expected fast exchange, between base and sugar, of the hydrogen-bonded N^4-H would make it undetectable on the NMR time scale. By contrast, **IIa** clearly revealed the presence of two NH signals: N^7-H as a triplet ($J_{NH,CH2}$ = 5.2 Hz) at δ 8.06 and N^4-H as a sharp singlet at δ 10.78. All NH signals were confirmed by their disappearance upon D_2O exchange.

Another major distinction between **Ia** and **IIa** lay in their respective sugar pucker: while **Ia** possesssed the C2'-endo-C3'-exo geometry, **IIa** had the opposite C2'-exo-C3'-endo conformation. Consequently, their C5' existed in axial and equatorial conformations, respectively.

The third important structural difference concerned the glycosyl bond length: While it was 1.46 Å in **Ia** and thus was comparable to that in purine nucleosides, the glycosyl bond length of **IIa** with 1.49 Å was closer to the one found in pyrimidine nucleosides (17a).

The unique structural features of **Ia** and **IIa** found in the solid state prompted us to explore their conformational preferences in solution, using CD spectroscopy. The CD and UV spectral data of **Ia** and **IIa**, their corresponding 5'-monophosphates **Ib** and **IIb**, and diphosphates **Ic** and **IIc**, along with those of xanthosine and xanthosine 5'-diphosphate, which were used as controls, are listed in Table I. As can be realized by values of molar ellipticity [θ], there is a marked difference in conformations of **Ia** and **IIa** in solution as is in the solid state. Abundant NMR and CD spectroscopic data exist to suggest that nucleosides undergo rapid syn $\rightleftarrows$ anti equilibration in solution (17a), and that while pyrimidine nucleosides assume predominantly anti conformation, both syn and anti forms prevail for purine nucleosides in approximately equal amounts. However, the presence of intramolecular hydrogen bonding inferred by the ^{1}H NMR spectrum of **Ia** strongly suggests that its conformation is mostly syn in solution also. Intriguingly, as revealed by the abrupt change in the Cotton effect, **Ia** undergoes remarkable change in conformation upon conversion to its nucleotide derivatives **Ib** and **Ic**. By contrast, the conformation of **IIa** does not seem to be significantly affected during the corresponding nucleoside → nucleotide transformation.

The marked CD spectral change observed in the transition **Ia** → **Ib** may be due either to a parallel change in the syn $\rightleftarrows$ anti conformational equilibrium, or simply to a large change in glycosyl torsional angle with no

Table I: CD and UV Spectral Data of I and II, as Contrasted with those of Xanthosine and Xanthosine 5'-Diphosphate

Compound Number	UV λ_{max}(nm)	ε	CD λ_{max}(nm)	θ mdeg	$[\theta]\lambda_{max}$(CD) cm^2 deg/decimole	Δε
Xanthosine	262.5	9000	265	-3.1	-1484	-0.45
Xanthosine 5'-diphosphate	261	9000	262	-5.7	-2892	-0.87
Ia	263.5	7100	253	+40.3	+15,292	+4.63
IIa	267.5	7200	268	+5.6	+5126	+1.55
Ib	264.5	7100	252	-5.6	-4984	-1.51
IIb	268	7200	266	+5.0	+4854	+1.47
Ic	254.5	7100	247	-15.70	-8093	-2.45
IIc	267.5	7200	267	+5.7	+5571	+1.69

All spectra were obtained in distilled water at 25 C.

effect on the overall conformational equilibrium (20). It is more likely, however, that the geometric constraint imposed by the phosphate group leads to the loss of the intramolecular hydrogen-bond upon conversion of **Ia** to nucleotide **Ib** or **Ic**, consequently resulting in an increased population of the anti conformer. On the other hand, the conformation of **IIa**, which is restricted to the anti orientation due apparently to the otherwise severe interaction of its furanose ring oxygen atom with that of the C-8 carbonyl group, does not alter upon conversion to the corresponding nucleotides, and thus, the [θ] values remain reasonably constant between **IIa**, **IIb**, and **IIc**.

The contemplated studies on base-pairing of **I** and **II** with appropriate pyrimidine partners, activity against HIV reverse transcriptase, and polymerization by polynucleotide phosphorylase are anticipated to shed some light on correlation between conformation and activity.

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