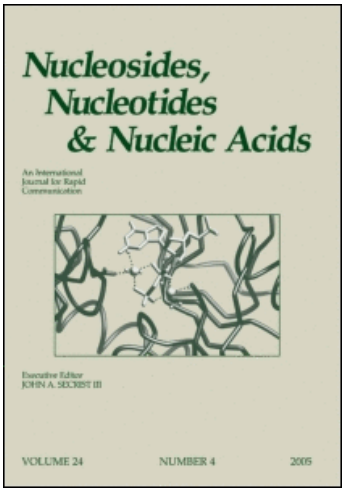

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CONFORMATIONAL STUDIES OF 3'-SPIRONUCLEOSIDES (TSAO-T AND ANALOGUES) BY NMR SPECTROSCOPY

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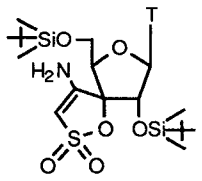
ABSTRACT: The conformational properties in solution of the prototype compound TSAO-T (**1**) and its two analogues **2** and **3** have been determined by ^{1}H and ^{13}C NMR techniques. The three compounds showed a sugar ring conformation rare among HIV-inhibitory nucleosides, probably due to the presence, at the 3'-position of the spiro moiety.

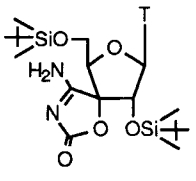
INTRODUCTION

TSAO derivatives represent a unique structural class of highly specific and potent inhibitors of human immunodeficiency virus type 1 (HIV-1) replication, being inactive against HIV-2 or other (retro)viruses.[1] The prototype compound is [1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) designated TSAO-T (**1**). The TSAO-derivatives are specifically targeted at the HIV-1-encoded reverse transcriptase (RT) with which they interact at an allosteric non-substrate binding site.[1,2]

Our current knowledge on the inhibitory potency of the various TSAO analogues comes from experimental cell culture assays. However, only limited data concerning the conformational preferences of these molecules are available.



1 (TSAO-T)



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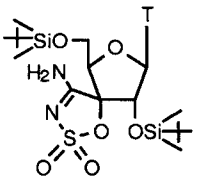

3

TSAO derivatives have a nucleosidic structure, and as such they are flexible molecules. The flexibility and dynamic behavior of molecules are of great importance in

determining their chemical and biological function. NMR studies in nucleosides have proven to be a very useful tool for conformational studies in solution. In order to study the conformational properties in solution of the TSAO molecules we have determined the overall conformation of the prototype compound TSAO-T (**1**), by ^{1}H and ^{13}C NMR techniques. This conformational study has been extended to the new TSAO analogues, modified at the 3'-spiro moiety, **2** and **3**.

RESULTS AND DISCUSSION

The conformation in solution [$(CD_3)_2CO$] of TSAO-T was studied by ^{1}H- and ^{13}C-NMR spectroscopy. The analysis of our data was based on the following features (a) the conformation around the glycosidic bond, (b) the conformation of the furanose ring, and (c) the relative orientation of C4'-C5' side chain.

The conformation around the glycosidic bond was calculated from vicinal $^{3}J_{C2,H1'}$ and $^{3}J_{C6,H1'}$ coupling constants, using the modified Karplus relations.[3] Then, the glycosyl torsion angle (χ = C2-N1-C1'-O4') was derived from these vicinal coupling constants determined from the proton-coupled and low-power selective proton decoupled ^{13}C spectra. The mole fraction of *syn* and *anti* states (n_{syn}, n_{anti}) were determined by generating $^{3}J_{C2,H1'}$ and $^{3}J_{C6,H1'}$ from the dihedral angles ($\phi_{C2,H1'}$ and $\phi_{C6,H1'}$) for each conformer. Then, the average coupling constant values ($^{3}J_{C2,H1'}$ and $^{3}J_{C6,H1'}$) for different n_{anti} values were calculated and compared with the experimental coupling constants. Finally, an optimization was carried out to minimize the rms values for the calculated coupling constants.

By using the concept of pseudorotation we obtained information about the geometry of the furanose ring. The existence in the TSAO molecules of only one interprotonic coupling constant $^{3}J_{H1',H2'}$, precluded the possibility of using the Altona's PSEUROT program.[4] To solve this problem we developed a procedure which relates the vicinal carbon-proton coupling constant values, with the pseudorotational parameters P and τ. Our method assumes the generally accepted two state model N/S, and allows the calculation of the pseudorotational parameters for each conformer (P_N, τ_N, P_S, τ_S and X_N). First we parametrized relationships between H-C-C-C and H-C-O-C exocyclic dihedral angles and the pseudorotational parameters P and τ. Secondly, by using Karplus equations,[5,6] vicinal carbon-proton coupling constants were related with H-C-C-C and H-C-O-C dihedral angles. Thus, we obtained a system of five equations, that relate five observable coupling constants with the pseudorotational parameters P_N, P_S, τ_N, τ_S and X_N.

TABLE 1. Coupling constants (Hz) and conformational parameters for compounds **1**, **2** and **3**.

Fragment	Coupling constants	Coformational parameters	Compound 1	Compound 2	Compound 3
Glycosidic bond	$^3J_{C2,H1'}$		3,2	3,0	4,1
	$^3J_{C6,H1'}$		5,3	3,5	4,6
		ϕ(C2,H1')syn (°)	±140	±165	±165
		χ(C2,H1')syn (°)	20 (100)	45 (75)	45 (75)
		ϕ(C2,H1')anti (°)	±20	±55	±40
		χ(C2,H1')anti (°)	-100 (-140)	-65 (-175)	-80 (-160)
		n_{anti} (%)	0,60	0,60	0,50
		rms	0,11	0,06	0,01
Exocyclic C4'-C5'	$^3J_{H4',H5'a}$		3,61	3,82	5,15
	$^3J_{H4',H5'b}$		3,72	3,82	4,78
		n_{g^+} (%)	0,66	0,58	0,35
		n_{g^-} (%)	0,24	0,30	0,37
		n_t (%)	0,10	0,12	0,28
Furanose	$^3J_{H1',H2'}$		8,0	8,1	7,7
	$^3J_{C4',H2'}$		3,2	<7	<0,7
	$^3J_{C2',H4'}$		1,4	0,9	0,9
	$^3J_{C4',H1'}$		<0,7	<7	<0,7
	$^3J_{C1',H4'}$		1,6	1,7	1,5
		P (°)	86	108	108
		τ (°)	50	51	49

Finally, in order to complete the solution conformational picture of the TSAO compounds under study, the conformation of the exocyclic C4'-C5' bond was analyzed. The conformation around the C4'-C5' bond in nucleosides can be interpreted in terms of rapid rotational averaging among three staggered rotamers gauche+ (g+), trans (t) and gauche- (g-).[7] Information on the conformation of the C4'-C5' bond was obtained on the basis of the experimental vicinal proton-proton coupling constants $^3J_{H4',H5'a}$ and $^3J_{H4',H5'b}$. Since, it is not possible to measure experimentally the corresponding coupling constants for each rotamer, they were calculated using the generalized Karplus equation parametrized for three substituents.[8] Then, from these coupling constants the rotamer populations (g+, g-, t) were calculated.

The results obtained in our studies are sumarized in Table 1. The overall conformation resulting from these studies showed no important differences between the three nucleosides (**1**, **2** and **3**). Thus, regarding the conformation of the glycosidic bond, the three compounds showed a *syn/anti* equilibrium in the orientation of the thymine base relative to the sugar. The geometry of the *syn* conformers suggest the existance of an hydrogen bond between the amino group of the spiro moiety and the 2-C=O of the

thymine base. The geometry of the furanoid ring was also very similar for the three nucleosides, corresponding to an O4'-*endo* envelope (P ≈ 90°) for TSAO-T (**1**), in which the C1'-C2'-C3'-C4' atoms are in the same plane and the O4' atom is located above the plane, and a twist conformation $^{O}T_{1}$ (P=108°) for the spironucleosides **2** and **3**, this geometry is contiguous in the pseudorotational circuit to the one found for TSAO-T and leads to a similar spatial orientation of the base and substituents of the furanose (3'-spiro moiety, 5'-O- and 2'-O-tBDMS groups). The main differences between these compounds were observed in the orientation of the exocyclic C4'-C5' bond. TSAO-T preferentially adopts a g+ conformation, this preference is lower for compound **2**, whereas the calculated data for compound **3** point to a free rotation situation for this C4'-C5' bond, with almost equal populations of the three rotamers.

It is noticeable that the conformation observed for the ribose in all these compounds is rare among HIV-inhibitory nucleosides, its occurrence on the TSAO molecules may be due to the high degree of functionalization and particularly to the presence, at the 3'-position of the ribose, of the spiro moiety.

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REFERENCES

1 For a review see Balzarini, J.; Camarasa, M.J.; Karlsson, A. *Drugs of the Future* **1993**, *18* , 1043-1055. and Camarasa, M.J.; Pérez-Pérez, M.J.; Velázquez, S.; San-Félix, A.; Alvarez, R.; Ingate, S.; Jimeno, M.L.; Karlsson, A.; De Clercq, E.; Balzarini, J. Nucleosides & Nucleotides **1995**, *14*, 585-594.

2 Balzarini, J.; Pérez-Pérez, M.J.; San-Félix, A.; Camarasa, M.J.; Bathurst, I.C.; Barr, P.J.; De Clercq, E. *J. Biol. Chem.*, **1992**, *267*, 11831-11838.

3 Davies, D.B.; Rajani, P.; MacCoss, M.; Danyluck, S.S. *Mag. Res. Chem.* **1985**, *23*, 72-77.

4 (a)Haasnoot, C.A.G.; de Leeuw, F.A.A.M.; de Leeuw, H.P.M.; Altona,C. *Org. Magn. Reson.* **1981**, *15*, 43-52. (b) de Leeuw, F.A.A.M.; Altona,C. *J. Comput. Chem.* **1983**, *4*, 428-437.

5 Aydin, R.; Günter, R. *Mag. Res. Chem.* **1990**, *28*, 448-457.

6 Tvaroska, I.; Hricovini, M.; Petrakova, E.*Carbohydr. Res.* **1989**, *189*, 359-362.

7 Haasnot, C. A. G.; de Leeuw, F. A. A. M.; de Leeuw, H. P. M.; Altona, C. *Recl. Tav. Chim. Pays-Bas.* ***1979***, *98*, 576- 577.

8 Haasnot, C.A.G.; de Leeuw, F.A.A.M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783-2792.