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CONFORMATIONAL ANALYSIS OF 3'-DEOXY- β -D-RIBONUCLEOSIDES
USING 1D-NOE DIFFERENCE SPECTROSCOPY

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ABSTRACT : The results of PMR studies on 3'-deoxy- β -D-ribo-nucleosides (1a-d) reveals that the sugar puckering is predominantly in N state with g⁺ conformation of the 5'-CH₂OH group. Except in 1a, nucleobases in other nucleosides favour anti conformation.

INTRODUCTION

Recently we have reported the synthesis and antifungal activity of four 3'-deoxy- β -D-ribonucleosides (1a-d)¹. These nucleosides were synthesized by glycosilation of a 3'-deoxy-D-ribosugar derivative with the corresponding silylated bases using SnCl₄ as a Lewis acid. During coupling the glycosilic bond can have α or β configuration. Thus necessitating unambiguous assignment of configuration at the anomeric carbon in these nucleosides. In the present study we have extended the use of 1D-nuclear Overhauser enhancement (n.O.e.) spectroscopy to study the configuration and conformation of these nucleosides.

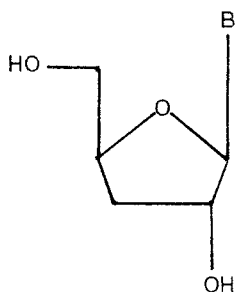
Earlier Rosemeyer and Seela²⁻⁵ have successfully used n.O.e. spectroscopy to assign the stereochemistry at the anomeric center in nucleosides. In this technique α -D and β -D anomers can be easily identified by saturating

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$H-1'$ and measuring the n.O.e. factor at $H-4'$ and $H-3'$. Because of their spatial proximity in β -D anomers, $H-1'$ and $H-4'$ exhibit mutual enhancement upon irradiation. While in the case of α -D nucleosides, irradiation of $H-1'$ produces an n.O.e. on $H-3'$ but not on $H-4'$. Besides anomeric configuration, the n.O.e. difference spectroscopy has been used to obtain semiquantitative information about the nucleoside conformations⁵.

RESULTS AND DISCUSSION

Inspection of the n.O.e. data (table 1) of these nucleosides (1 a-d), clearly indicates β -D configuration



- 1a, B= Adenine
1b, B= Guanine
1c, B= Cytosine
1d, B= Thymine

(1a-d)

at $C1'$, viz., Irradiation of $H-1'$ produces an n.O.e. on $H-4'$ and vice versa while there is no effect between $H-1'$ and $H-3'$. Beside such stereochemical assignments, this technique also gives information regarding the orientation of the heterocyclic base around the N -glycosilic bond (syn/anti), the sugar puckering (N/S) and the conformation around the $C4'-C5'$ bond (g+/g-/t).

Since $H-8$ (in purines) and $H-6$ (in pyrimidines) are not spin coupled to any other proton but their orientation with respect to sugar protons allows differentiation, it is an ideal probe for measuring the conformation around the N -glycosyl bond. Accordingly in the present study upon irradiation of the $H-8$ / $H-6$, the combined

TABLE 1 : Results of ^1H - ^1H 1D n.O.e. Difference Spectroscopy Experiments

Nucleosides	Proton Irradiated	n.O.e.(% Enhancement)
1a	H-1'	H-4'(2.3), H-2'(3.4), 2'-OH(4), H-8(5.2)
	H-8	H β -3'(2.7), H-2'(2.9), 5'-OH (2.1), H-1'(5.6)
1b	H-1'	H-4'(4.5), H-8(2.8), H-5'(1.9)
	H-8	H α -3'(0.5), H β -3'(2.1), H5'a(1.3), H5'b(1.4), H-2'(3.3), H-1'(3)
1c	H-4'	H α -3'(5.7), H-5'a + H-5'b(8.3), 5'-OH(1.0), 2'-OH(1.4), H-1'(2.4)
	H-6	H β -3'(3.0), H-2'(3.3), 5'-OH (2.8), H-5 + H-1'(14.3)
1d	H-1'	H-4'(5.6), H-6(2.5)
	H-6	CH $_3$ (6.1), H β -3'(3.5), H-2'(5.1), 5'-OH(3.1), H-1'(2.4)

n.O.e. effect on H β -3' and H β -2' was found to be more than on H-1' (Table 1). This leads to the suggestion that the nucleobases are anti oriented. Furthermore, a fairly accurate population of anti and syn conformers can be estimated from the standard calibration graph⁵. It is observed that the nucleosides 1b-d prefer anti population (75-80%) while 3'-deoxy- β -D-riboadenosine (1a) exists in syn/anti equilibrium (50%).

TABLE 2 : Results of the Conformational Analysis of 3'-Deoxy- β -D-ribonucleosides.

Nucleosides	%anti conformer	%N conformer	%g+ rotamer
1a	50	91.43	64.61
1b	74	97.94	66.97
1c	75	100	70.15
1d	80	91.7	76.52

The orientation of the aglycone moiety has a significant effect on the overall geometry of the nucleoside including the sugar puckering. Analysis of the vicinal proton coupling constants ($J_{1,2'}$) provides information regarding the sugar puckering. It has been observed that the N form (3T_2) corresponds to $J_{1,2'}$ value of 1.2 Hz, whereas the S form (${}_3T_2'$) has $J_{1,2'} = 8.8$ Hz. In the present study $J_{1,2'}$ was found to be 2.4, 2.38, 1.94, 1.8 Hz in 1a, 1b, 1c and 1d respectively. These values were used to estimate the percentage of N / S conformers using the formula reported in the literature⁶. The results presented in table 2 indicate that the major population of the furanose ring in 3'-deoxy- β -D-ribonucleosides corresponds to the N conformation. This is in contrast to the 2'-deoxy- β -D-ribonucleosides where the sugar puckering is predominantly in the S form⁷. On the other hand common ribonucleosides show a definite preference for the N conformation.

The conformation around the C4'-C5' bond can have three possible staggered arrangements and their distribution is dependent mostly on the sugar puckering. Each rotamer can be quantitated from the H-4'-H-5' coupling constant. In the native spectra of nucleosides 1a-d, signals of H-5'a/b appeared as multiplets. However, upon irradiation of the 5'-OH proton, this region of the spectra was simplified resulting in H-5' methylene protons appearing as double doublets. The coupling constants were then easily calculated. The $J_{4',5',a}$ was found 3.29, 3.12, 3.27 and 2.84 Hz whereas $J_{4',5',b}$ 3.94, 3.91, 3.47 and 3.34 Hz in nucleosides 1a, 1b, 1c and 1d respectively. Following Hruska *et al*⁸, the conformation around the exocyclic C4'-C-5' bond were calculated from the equation given below.

$$Pg+ = 1.46 - \frac{J_{4',5',a} + J_{4',5',b}}{8.9}$$

All the 3'-deoxy- β -D-ribonucleosides exhibited predominantly the g+ rotamer population (table 2). This is consistent with the earlier observation that the high population of the N conformer is linked to a high population of the g+ rotamer⁹.

The present findings may be summarized as follows : The 3'-deoxy- β -D-ribonucleosides exhibit preponderance of the anti configuration around the N-glycosyl bond, except 1a which has equal distribution of syn/anti conformers. The furanose ring in all these nucleosides exhibits a high population of the N state. The orientation around the C4'-C5' bond is mostly in the g+ rotamer form. It is apparent that there are striking differences in the conformation of 3'-deoxy- β -D-ribonucleosides versus 2'-deoxy- β -D-ribonucleosides and the former resemble the common ribonucleosides. This information

would be useful in structure analysis of 2'-5' linked oligonucleotides *vis a vis* 3'-5' linked oligonucleotides.

EXPERIMENTAL

All NMR measurements were performed on AC-250 (Bruker, FRG) at 298°K with (CD₃)₂SO (99.5%) as solvent using its deuterium for internal lock. Only decoupling studies were carried out on Bruker DRX-300 spectrometer.

For the n.O.e. measurements the solutions (0.1 M) were degassed by bubbling N₂ through it, followed by sonication. All compounds were measured under identical spectral and processing conditions applying the NOEDIFF pulse sequence of the Bruker software package (release version 1992) applying its recommendations for steady-state n.O.e. measurements. The parameters were as follows : D3 (to set O2): 0.1 s; S3:40dB below 0.2 W yielding a saturation of ≥ 95%; D1:3 s; D2:1.5 s; RD:0 s; PW:9.8 μs (=90° pulse); NS (per each loop): 32; dummy scans: 4; NE (number of experiments): 12. Analysis of spectral data was performed by subtraction of two FID's (one off-resonance irradiation) followed by exponential multiplication (line broadening: 0.25) and fourier transformation of the differential FID. All n.O.e. values (n, in %) were obtained by repeated integration of the peaks of the difference spectra.

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