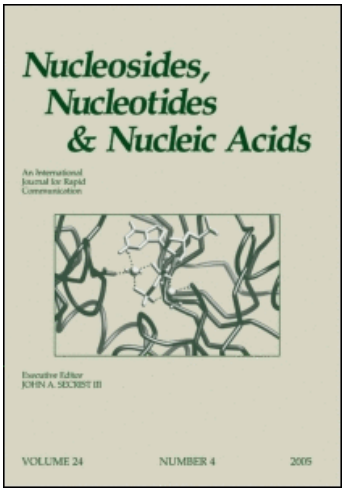

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CONFORMATIONAL ANALYSIS OF 3'-DEOXYRIBONUCLEOSIDES USING 1D-NOE DIFFERENCE SPECTROSCOPY

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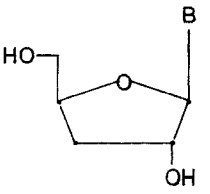
ABSTRACT : The results of PMR studies on 3'Deoxyribo nucleosides (**1a-d**) reveals that the sugar puckering is predominantly in **N** state with **g+** conformation of the 5'-CH_2OH group. Except in **1a**, nucleobases in other nucleosides favour **anti** conformation.

INTRODUCTION

Recently we have reported the synthesis and antifungal activity of four 3'-deoxyribonucleosides (**1a-d**)[1]. These nucleosides were synthesized by glycosilation of a 3'-deoxy sugar derivative with the corresponding silylated bases using $SnCl_4$ 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 these nucleosides.

Earlier Rosemeyer and Seela[2-5] 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 *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'. Inspection of the **n.O.e.** data (**table 1**) of these nucleosides (**1a-d**), clearly indicates **β-D** configuration at *C*1', *viz.* Irradiation of *H*-1' produces an **n.O.e.** on *H*-4' and vice versa while there is no effect between



(1a-d)

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

H-1' and *H*-3'. Besides 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 *C*4'-*C*5' bond (**g+/g-/t**).

RESULTS AND DISCUSSION

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 we observed relatively more **n.O.e.** effect on H_{β}-3' and H_{β}-2' and less effect 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 graph[5]. It is observed that the nucleosides **1b-d** prefer **anti** population (75-80%) while 3'-deoxyadenosine (1a) exists in **syn/anti** equilibrium (50%).

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

TABLE 1 : Results of ^1H-^1H 1D NOE Difference Spectroscopy Experiments

Nucleosides	Proton Irradiated	n.O.e.(% Enhancement)
1a	*H*-1'	*H*-4'(2.3), *H*-2'(3.4), 2'-O*H*(4), *H*-8(5.2)
	H-8	H_β-3'(2.7), *H*-2'(2.9), 5'-O*H*(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), *H*5'a(1.3), *H*5'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'-O*H*(1.0), 2'-O*H*(1.4), *H*-1'(2.4)
	H-6	H_β-3'(3.0), *H*-2'(3.3), 5'-O*H* (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'-O*H*(3.1), *H*-1'(2.4)

constants ($J_{1'2'}$) provides information regarding the sugar puckering. It has been observed that the **N** form ($^{3'}T_{2'}$) corresponds to $J_{1'2'}$ value of 1.2 Hz, whereas the **S** form ($_{3'}T^{2'}$) 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 literature[6]. The results presented in **table 2** indicate that the major population of the furanose ring in 3'-deoxyribonucleosides corresponds to the **N** conformation. This is in contrast to the 2'deoxyribonucleosides where the sugar puckering is predominantly in the **S** form. On the other hand

TABLE 2 : Results of the Conformational Analysis of 3'-Deoxyribonucleosides.

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

common ribonucleosides show a definite preference for the **N** conformation.

The conformation around the *C*4'-*C*5' bond can have three possible staggered arrangements and their distribution is dependent mostly on the sugar pucker. Each rotamer can be quantitated from the *H*-4'-*H*-5' coupling constant. In the native spectra of nucleosides **1a-d** signals due to *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. It was found 3.94, 3.91, 3.47 and 3.34 Hz in nucleosides **1a**,**1b**,**1c** and **1d** respectively. Following Hruska *et al*[7], the conformation around the exocyclic *C*4'-*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'-deoxyribonucleosides 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+** rotamer[8].

The present findings may be summarized as follows : The 3'-deoxyribonucleosides 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 *C*4'-*C*5' bond is mostly in the **g+** rotamer form. It is apparent that there are striking differences in the conformation of 3'-deoxyribonucleosides versus 2'-deoxyribonucleosides 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 compounds (**1a-d**) have been synthesized[1] and additionally characterised by ^{13}C NMR spectroscopy (Table 3).

All NMR measurements were performed on AC-250 (Bruker, FRG) at 298°K with $(CD_3)_2SO$ (99.5%) as solvent using it's 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 N_2 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. An irradiation time of 1.5 s with an irradiation power of 40 dB below 0.2 W yielded a saturation of $\geq$95%. The analysis of spectral data was performed in two different ways : (1) sequential exponential multiplication (line broadening : 0.25) and Fourier transformation of two FID's (one entry for a desired irradiation point plus one off-resonance control value) and then substraction of the two spectra; (2) substraction of the two FID's followed by exponential multiplication and fourier transformation of the differential

TABLE 3 : Results of ^{13}C NMR spectroscopy

Carbon atom	Nucleosides 1a	1b	1c	1d
C-1'	90.9	93.2	93.1	91.0
C-2'	74.7	75.8	75.2	74.5
C-3'	38.6	39.1	33.2	33.5
C-4'	80.8	82.1	80.7	80.3
C-5'	62.7	61.2	61.7	61.7
C-2	152.2	137.6	155.2	108.5
C-4	149.0	153.1	165.6	150.5
C-5	140.6	108.4	92.3	163.6
C-6	156.1	154.0	141.0	136.3
C-8	139.2	137.7	-	-
CH_3				12.15

FID. All n.O.e. value (∩, %) were obtained by repeated integration of the peaks of the difference spectra.

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