CONFORMATIONAL STUDIES OF NUCLEOSIDE DERIVATIVES USING DIFFERENCE NUCLEAR OVERHAUSER EFFECTS

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ABSTRACT

The *syn/anti* equilibrium about the C–N glycosidic bond between the sugar and the base of two isopropylidene nucleoside derivatives has been investigated in the solution phase by difference nuclear Overhauser effect measurements. It has been found that these nucleoside derivatives adopt a predominently *syn* conformation, in keeping with the single crystal x-ray studies of these compounds.

INTRODUCTION

THE *syn/anti* equilibrium about the C–N glycosidic bond between the sugar and the base in nucleosides and nucleotides is of considerable importance in structural studies of nuclic acids. Most models of DNA structure, including the Watson-Crick B-DNA model, have the *anti* conformation[1-3] and a preference for this conformation has also been observed in the crystal structures of nucleosides and nucleotides[4]. However, in the crystal structures of the oligonucleotides dCGCG[5] and dCGCGCG[6], the guanine bases are *syn* with respect to the sugar.

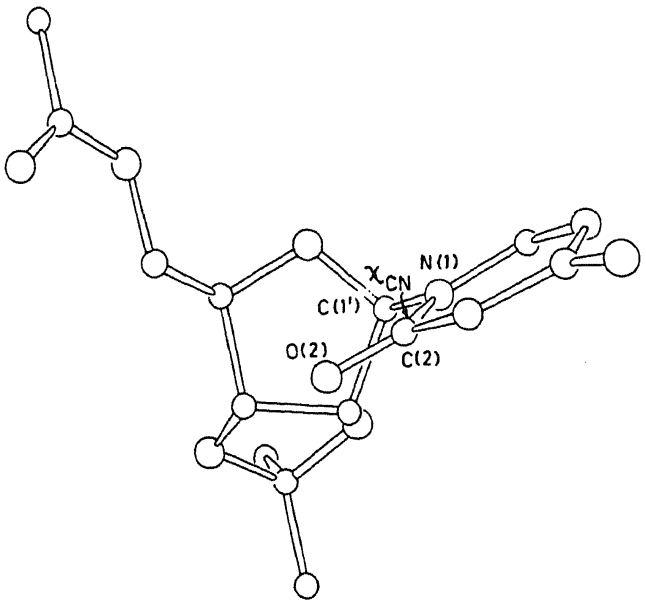

Figure 1. ORTEP drawing of II showing the *syn* conformation about the glycosidic bond with $\chi_{CN} = -103.9°$, as obtained in the single crystal structure. The oxygen atom O(2) is above the ribose ring in this conformation as opposed to *anti* where it is away from the ring.

The *syn/anti* equilibrium in the solution state has been investigated by a variety of NMR techniques[7] of which the nuclear Overhauser effect (NOE)[8] is perhaps the most useful. These studies show that most nucleosides and nucleotides exist in the *anti* conformation in solution also[7]. However, nucleosides which have an isopropylidene group attached to the 2′ and 3′ positions of the sugar exist predominantly in the *syn* orientation[8-10].

As part of a systematic programme of x-ray single crystal structure analysis of 2′,3′-*o*-isopropylidene nucleoside derivatives, we recently obtained the structures of 5′-*o*-(4″-methylphenylsulphonyl)-2′,3′-*o*-isopropylideneuridine[11] (I) and 5′-*o*-monoacetate-2′,3′-*o*-isopropylideneuridine[12] (II). The glycosidic torsion angle in these two cases was $\chi_{CN} = -116.0°$ and $-103.9°$ respectively; that is, both molecules are *syn* about the glycosidic bond (figure 1). This is an unusual conformation especially for pyrimidine nucleosides. It would be of interest to ascertain if this uncommon conformation is preserved in the solution phase. In this paper we report the solution conformation studies of (I) and (II) using difference NOE techniques.

MATERIALS AND METHODS

Compound I was kindly supplied by Dr S. A. Salisbury of the University of Cambridge, UK. Compound II was purchased from the Sigma Chemical Company. USA 12 mM solutions of the compounds were prepared in DMSO-d_6 (Stohler Isotope Chemicals) and used without degassing. The spectra were recorded on a WH-270 Bruker FT-NMR spectrometer, equipped with BNC-12 computer with 20K memory.

The resonances were identified by comparison with the spectrum of 2′,3′-*o*-isopropylideneuridine which had been earlier reported[8].

The difference NOE experiment was performed by first obtaining the normal spectrum for each of the compounds (figures 2a, 3a) and then subtracting this from the spectrum obtained after saturating the H(6) resonance. The signal-to-noise ratio of both normal and difference spectra was enhanced by exponential multiplication of 1 Hz.

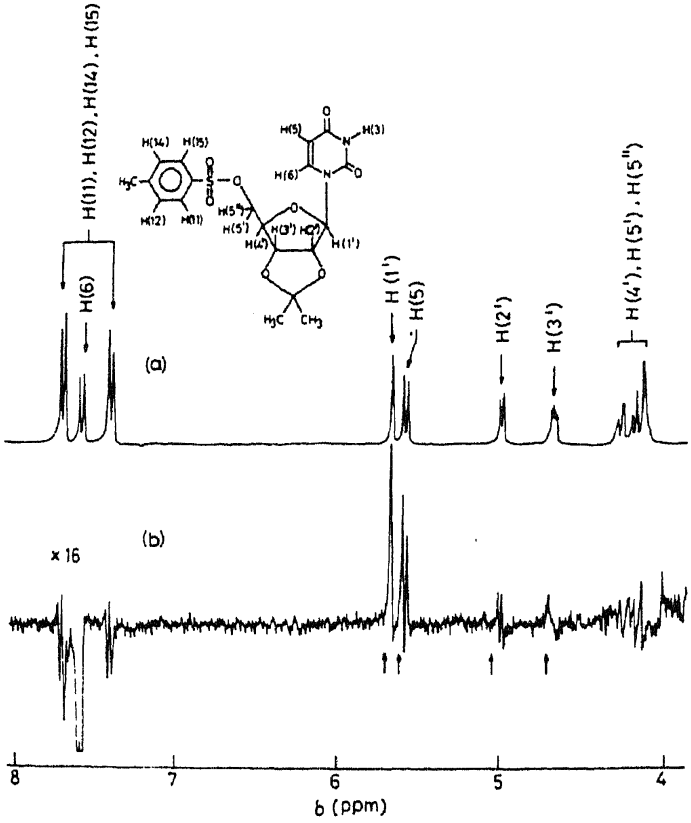

Figure 2. 270 MHz proton NMR spectra of compound I, (a) normal spectrum, (b) difference NOE spectrum magnified 16 times with respect to (a) The "normal" spectrum obtained by placing the saturating radio frequency off-resonance while keeping all other parameters identical to the on-resonance saturated spectrum case. The difference spectrum was obtained by taking the difference between the "normal" spectrum and the on-resonance saturated spectrum. The "block-averaging" subroutine of the BNC-12 computer of the Bruker WH-270 was utilized for separate collection of the two spectra and for taking the difference.

RESULTS AND DISCUSSION

The difference NOE spectra of compounds I and II are given in figures 2b and 3b and the results summarized in table 1. In both these compounds saturation of H(6) proton gives rise to significant NOEs at H(1') and H(5) protons and little NOEs at the H(2'), H(3') and other protons. A reference to table 2, where

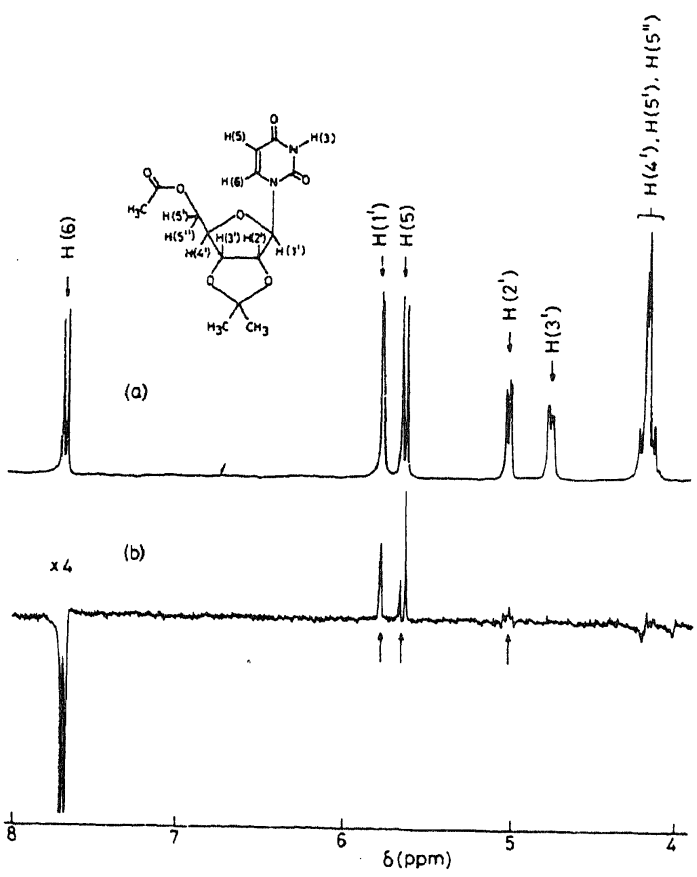

Figure 3. 270 MHz proton NMR spectra of compound II, (a) normal spectrum (b) difference NOE spectrum magnified 4 times with respect to (a). All other details same as in figure 2.

the typical distances between various protons in *syn* and *anti* conformations in such systems are summarized, indicate *syn* conformation for both these compounds based on the above observations.

As a cross-check, a similar difference NOE experiment, was performed on uridine (III), a compound

Table 1. *Percentage NOE observed. Nucleus saturated-H(6)*

Compound No.	Nuclei observed			
	H(5)*	H(1')	H(2')	H(3')
I	10	12	Small†	Small†
II	15	10	Small†	Not observed
III	19	9	10	Small†

I: 5'-*o*-(4'' Methylphenylsulphonyl)-2',3',-*o*-isopropylideneuridine.

II: 5'-*o*-Monoacetate-2',3'-*o*-isopropylideneuridine.

III: Uridine

† Small NOE: 2–4% with S/N of 2:1

* These figures are averages of NOEs observed on the two peaks of H(5) which, on account of small inequality in the saturation of the two J-coupled peaks of H(6), show unequal enhancement.

Table 2 *Approximate distances* (Å) *between the protons for an unpuckered sugar ring and glycosidic torsion angle* $\chi_{CN}^{syn} \simeq -120°$ *and* $\chi_{CN}^{anti} \simeq 20°$

From H(6) to	H(5)	H(1′)	H(2′)	H(3′)
syn	2.5	2.4	4.0	6.0
anti	2.5	3.9	2.5	3.5

reported to exist in the *anti* orientation in the crystal structure[13]. This compound showed significant NOEs at H(5), H(1′) and H(2′) protons (table 1), with nearly identical NOEs at H(1′) and H(2′) protons. This observation indicates that uridine equilibrates between *anti/syn* conformations in the solution phase at room temperature. While no serious attempt is made to obtain *syn/anti* equilibrium constants from these figures, it seems, by reference to tables 1 and 2, that compounds I and II are indeed entirely in the *syn* conformation while compound III is a mixture of both *syn* and *anti*.

The results obtained for compounds I and II are in keeping with both the crystal structure conformations as well as with the solution conformations of other isopropylidene nucleoside derivatives[8-10]. They are in marked contrast to both crystal and solution conformations of most non-cyclized nucleosides and nucleotides. The *syn* orientation in the present compounds may be a result of the reduction in the base-sugar interaction due to cyclization. It may be noted however that, in general, the crystal structures of isopropylidene nucleoside derivatives do not show any significant deviation from the known propensity of nucleic acid monomers to be *anti*[14].

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